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Participation of individual chromosomes in constitutive cytogenetic abnormalities of cattle

Dzitsiuk V.[1],
Guzevatyi O.[2]
[1] M. Zubets Institute of animal breeding and genetics of NAAS,
Pohrebniak Str., 1, Chubinske, Boryspil area, Kyiv oblast, 08321, Ukraine,
[2] National academy of agrarian sciences of Ukraine, Mykhailo Omelianovych-Pavlenka Str., 9, Kyiv, 01010, Ukraine; e-mail: [1]valentynadzitsiuk@gmail.com, [2] oleg_guzevatiy@ukr.net

The purpose. To study participation of individual chromosomes in constitutive cytogenetic abnormalities of cattle. **Methods.** Cultivation of lymphocytes, preparation of cytogenetic specimens. Grading and the record-keeping aberrations of chromosomes was realized under practical standards. **Results.** The frequency of participation of individual chromosomes in chromosomal rearrangement were studied. It was found that the most frequently happening chromosomal breaks were the following: 20.0% for the first chromosome, 16.0%, for the second one, 8.5% and 7.0% for the thirteenth and the twenty-first chromosomes accordingly. The breaks occurred in places between euchromatin and heterochromatin chromosome segments, as it was displayed by the results of differently colored samples analysis. **Conclusions.** The gained experimental data testify to the following: frequency of participation of individual chromosomes in constitutive cytogenetic abnormalities can be used as an index of non-specific destabilization of the chromosomal apparatus of animals.

Key words*: cattle, karyotype, cytogenetic analysis, lymphocytes, aberrations of chromosomes.*

As early as the 70's and 80's of the last century, cytogenetic studies of farm animals began to develop rapidly [1, 2].

Presently there are the programs of cytogenetic inspection of mammals in the leading economically developed countries of the world, and for tribal economies the cytogenetic monitoring is obligatory.

However, not having regard to sufficiently the necessity of cytogenetic control of agricultural animals set a long ago, first of all cattle, research of them chromosomal vehicle conducted in a limit amount.

One of important tasks of cytogenetics of agricultural animals there are determinations of character of anomalies of karyotype, establishment of possible ways of their origin and their influence on functioning of the systems of organism. Not having regard to an evolutional exhaust mechanism that keeps constancy of physical and chemical and morphological organization of chromosomes of animals, under act of different factors this organization can change. In basis of changes of structure of chromosomes, as a rule, primary violations of her integrity - breaks, that is accompanied by different alterations and that, in turn, cause the change of localization of genes, lie. These processes result in the change of character of functioning of genes, that is accompanied by the change of the genetic program. Obviously, that in structural to aberration, going out the variety of variants of described chromosomal aberrations, any chromosome of karyotype can be attracted. By confirmation to it universally recognized in 80th of past century hypothesis of Nadeau - Taylor [3], in obedience to that chromosomal mutations can arise up in different chromosomes by casual character. A hypothesis was based on the results of analysis of distribution of lengths of homologous antennae blocks (homologous synteny blocks), id est districts of chromosomes of different types of mammals that contain ортологічні genes in an identical sequence. The row of authors educed also, that the breaks of chromosome and their reunion arose up by chance after length of chromosome, and their frequency depends on the amount of DNA in this chromosome [4].

However, the analysis of other literary sources grounds to consider that an unevenness of distribution of individual chromosomes is in chromosomal aberrations [5]. Separate chromosomes are most apt to the

breaks and formation of translocations and it is possible to expect certain specificity of participation of separate chromosomes for chromosomal aberrations animals [6]. Results of researches of Morad, Jonasson [7] also confirm the unchance of appearance of breaks in chromosomes and repressing localization them in the areas of structural heterochromatin or on a limit between heterochromatinous and by euchromatinium material. Questions as far as casual are chromosomal breaks, except theoretical, a practical value takes place. It is known, it is differed a шо euchromatin and heterochromatin of area of chromosomes after molecular and genetic organization of DNA. If in relation to еухроматинових areas obviously all enough - in them there is basic part of genes, necessary for development of organism and functioning cages, then a question about the functional value of heterochromatin to this day remains the article of discussions. Implicitly one - being of heterochromatin in the genomes of almost all kinds testifies to his necessity. Thus, research of conformities to law of appearance of chromosomal aberrations, in particular breaks, and clarification of place of localization in a concrete chromosome, gives an opportunity to envisage to their phenotype consequences for animals.

The aim of work is research of frequency of participation of individual chromosomes in the constitutive cytogenetic anomalies of cattle.

Materials and methods. Research material were lymphocytes of peripheral blood 50 cows of the Ukrainian redder-pock-marked suckling breed.

Cytogenetic researches conducted on preparations of metaphase chromosomes of lymphocytes of peripheral blood on standard methodology of Moorhead [8].

Raising of culture of lymphocytes of peripheral blood envisaged implementation of row of the stages: cultivation of lymphocytes of peripheral blood, stimulated phytohemagglutinin ("Sigma", the USA), in mixture of nourishing environment of RPMI 1640 ("Sigma", the USA) during 72 hours in a thermostat at 37°C. The stop of mitosis was conducted on 70th time of cultivation bringing of colchicine ("Serva", Germany) in a concentration 0,1 mcg/ml. After hypotonic treatment of KCl("Merck", Germany) during 20 minutes of cage fixed mixture of ethanol and icy ac.a. (3: 1). A cellular suspension was dropped on wet by the chilled subject glasses and dried out. Authentication of chromosomes was conducted on homogeneously painted by dye of Gimza ("Fulka", Switzerland) preparations. At the selection of metaphase plates for a chromosomal analysis followed the generally accepted criteria [9]. The analysis of metaphase plates of chromosomes was conducted by means of binocular microscope of firm Carl Zeiss Jena (Germany).

The differential painting of chromosomes was conducted on methodology of Seabright [10].

The chromosomes of karyotypes of investigational animals subdivided accordingly after their sizes on three groups: A (1-4 with length 5,3-4,0 microns); B (5-20 pairs are with length 2,0-3,02 microns); C (21-29 pairs 1,2-1,9 microns, X). Chromosome У determined as independent. Authentication of G- of the differentially painted individual chromosomes and their alterations came true in accordance with the atlas of Grafodatsky AS "Chromosomes of Agricultural and Labor Mammals" [11].

Biometrical treatment of results of researches was conducted by the method of variation statistics with the use of standard statistical application package.

Results of researches. The results of analysis of metaphase chromosomes for investigational animals educed to aberration of chromosomes chromosomal and chromatid type. From 1085 preparations of metaphase chromosomes in 144 educed to aberration: breaks, fragments, premature divergence of centromeres, aneuploidy, polyploidy (table. 1).

The level of chromosomal aberrations presented 13,2 frequency of aberrations on one cage - 0,131.

The conducted analysis of the differentially painted out chromosomes of animals set that breaks took place in different chromosomes with different frequency (table. 2). Changes in the structure of chromosomes educed in 30 from 58 euchromosomes of karyotype of investigational animals.

1. The cows of the Ukrainian redder-pock-marked suckling breed (n=50) have frequency of chromosomal aberrations

Types of aberrations		Frequency, %
Chromatid type	single fragments	4,20±0,8
Chromosome type	pair fragments	4,20±0,8
	ruptures	3,12±0,1
	premature divergence centromer	4,25±0,3
	aneuploid cells	4,46±0,73
	polyploid cells	0,67±0,18
Total	frequency of chromosomal aberrations	13,2±0,51

Mostly looked after the breaks of chromosomes: first (20,0% of all cases), second (16,0%), thirteenth (8,5%) and twenty first chromosomes (7,0%) that totally present over 50% all detected chromosomal aberrations.

2. Frequency of aberrations is in karyotypes of cows of the Ukrainian redder-pock-marked suckling breed

The metaphase is investigated			Incl. frequency of aberrations of chromosomes in groups by length,%			
total	aberrant,%	including with breaks,%	A	B	C	X,Y
1085	13,2±0,51	8,52±0,33	6,71±0,40	4,62±0,62	1,87±0,09	not found

The got results comport with data of row of scientists [12, 13, 14, 15], what mark that frequency of participation of chromosomes in intracranial damages up to a point associates with an amount in them by DNA and their length. However length of chromosomes is only one of factors that influence on distribution of cytogenetic anomalies in a genome, and far not always has qualificatory character.

Chromosomal and chromatic aberration of group A presented mainly by alterations of the first and second chromosomes with frequency higher, than at the chromosomes of other groups.

In a group B the chromosomal to aberration of sixth and thirteenth chromosomes appeared mostly.

The chromosomes of group C have breaks rarely, but more often than large, attracted in premature divergence of centromeres and aneuploidy.

As a result of researches it is not educed by us chromosomal aberrations in the chromosomes of shallow size. An analogical effect was looked after in researches in a medical cytogenetics [16, 17]. The damage of sexual chromosomes is not educed.

Kochneva M.L. with coauthors [3] analysed frequency of bringing in of chromosomes of karyotype of cattle in a translocation and marked, that euchromosomes from pairs 1, 8, 9 and 11 more often than chromosomes from pairs 2, 4, 20, 21 and sexual chromosomes, participate in such alterations.

It is well-proven researches of karyotype of laboratory rats, that mostly in chromosomal aberrations the involved chromosomes 3, 7, 10, 11 and 13 [18].

3. Participation of individual chromosomes in different types of chromosomal aberrations in animals

Groups of chromosomes	Chromosome	Type of aberration			
		chromosomes		separate chromatids	
		aberration	number	aberration	number
A	1	ruptures	5	ruptures	10
		defects	3	defects	5
		associations with other chromosomes	2	fragmentation	5
	2	ruptures	3	ruptures	6
		defects	2	defects	2
		associations with other chromosomes	3	fragmentation	4
B	6	ruptures	4	ruptures	7
	13	associations with other chromosomes	1	ruptures	2
C	21	premature divergence centromer	6	-	-
	22	associations with other chromosomes	1	not found	0

On results the analysis of the differentially painted preparations of chromosomes set that mostly the breaks of chromosomes take place in places between ey- and by heterochromatinous segments. It testifies that, as a rule, unharmed more often there are districts to the euchromatin, where the arrays of genes are localized. In large acrocentrics breaks were mostly concentrated in the areas of heterochromatin in middle one third of chromosome.

High-frequency of breaks in a structural heterochromatin can be as a result of some physical and chemical features of heterochromatin, that distinguish him from the euchromatinous districts of chromosomes. Yes, in opinion of row of authors the condensed state of heterochromatin stipulates worst availability of places of primary damages for the enzymes of the system of reparation [19, 20]. As a result, if at first pre-mutational events and were placed along a chromosome by chance, eventually breaks appear noncommunicative mainly in a heterochromatin. The timing of replication to the genome in ey- and by heterochromatinous areas can create in places them physical contact certain physical tension and that is why part of breaks arises up on verge of euchromatin and heterochromatin on principle "breaks wherein thinner".

Thomas C.A. [21] considers that to aberration of chromosomes for higher animals rarely enough damage structural genes. It is confirmed by Tartof K.D. et al. [22] and specify, that influence of aberrations chromosomes takes place mainly through the effect of position.

Thus, cytogenetic method of analysis of aberrations chromosomes and presently it remains most accessible and adequate for understanding of molecular processes that determine breaks and other alterations of chromosomes.

Conclusions

The obtained experimental data in relation to participating of individual chromosomes in constitutive cytogenetic anomalies testify that the estimation of their participation can be used as one of indexes of heterospecific destabilization of chromosomal vehicle of animals.

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