UDC 636. 4. 082. 12

POLYMORPHISM OF NUCLEOLAR ORGANIZER REGIONS IN DIFFERENT UKRAINIAN CATTLE BREEDS

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Received February 05, 2021 / Received March 05, 2021 / Accepted March 19, 2021

Aim. To study the activity of nucleolar organizer regions (NORs) in different Ukrainian cattle breeds in terms of their apparent activity status in silver stain and possible relation with milk productivity. **Methods**. Chromosome preparations using lymphocytes from the peripheral blood of 90 cows of different breeds were used in the study. NOR activity was determined by visual evaluation of concentrations of silver precipitation on NORs in individual chromosomes. A 50 % silver nitrate solution was used to stain chromosome preparations. NORs were detected as dark spots on telomeres of the corresponding chromosomes. **Results.** The cytological analysis of chromosome preparations from lymphocytes of first lactation cows detected NOR polymorphism in Ukrainian Red-and-Motley dairy cattle (URM), Ukrainian Black-and-White dairy breed (UBW), and hybrid cows, obtained by crossing Ukrainian Red-and-Motley dairy breed and Montbeliarde bulls (URM × M). First lactation cows of URM and UBM had higher or the same incidence of cells with four (29.8 and 30 %) and five (17.1 and 19.5 %) NORs, while in URM × M cows the incidence of cells with the same number of NORs was almost twice lower; cells with 7 and 8 NORs exceeded a similar index for other investigated breeds almost twice (2.5 against 4.5 % and 2.0 against 4.2 %). The highest level of chromosomal aberrations (CA) was observed in the group of animals with medium number (2 to 3 NORs per cell), and the lowest – in the group with a high number of NORs (from 6 to 7) with a reliable intergroup difference ($p < 0.01$). NOR activity was the highest in the group of animals of local origin (URM × M) with a milk yield over 7,000 kg in 305 days of the first lactation and the lowest in the UBW cows with a milk yield of 4–5,000 kg during the first lactation. **Conclusions.** We determined the differences in the activity of nucleolar organizers between the investigated groups of cows of dairy breed. URM × M hybrids reliably ($p \leq 0.05$) exceeded dairy UBW cows by this index. No statistically significant different was found between other investigated groups of animals by this trait. Higher dairy productivity was found in the animals with higher frequency of NORs in the chromosomes of metaphase cells. In our opinion, the number of active NORs demonstrates relative variability between their number and the rate of protein synthesis, required to implement the productivity traits of the investigated animals.

Key words: cows, nucleolar organizer regions (NOR), chromosomal aberrations, dairy productivity.

DOI: https://doi.org/10.15407/agrisp8.01.029

INTRODUCTION

In all animal species, clusters of ribosomal genes, forming nucleolar organizer regions (NORs), are located on some chromosomes. While encoding ribosomal RNA, ribosomal genes control the formation of ribosomes, regulating the synthesis of proteins in the cells of the organism (Howell W et al, 1979). NORs are loops of ribosomal DNA (rDNA) which transcribe to ribosomal RNA (rRNA) and therefore in the end to ribosomes and protein (Dimitrov A et al, 2013).

The method of selective staining of NORs with silver, developed in the 1970s, and the discovery of a new type of chromosomal polymorphism – Ag-polymorphism – made it possible to evaluate the karyotypes of animals by the state of ribosomal RNA (rRNA) genes on the cytological level (Howell W et al, 1975; Howell W et al, 1980). The method is based on the fact that non-histone proteins of NORs are a group of nucleolar proteins, which are remarkable for their specific staining with silver and have a high expression level in the proliferating cells. These proteins are involved in the regulation of RNA-polymerase, DNA transcription, replication, and recombination (Gjerset R, 2006). The association between the proteins and quantitative parameters of argyrophilic structures may be used to evaluate the activity of ribosomal genes, and the proteins are proliferation markers of the cells, where active protein synthesis occurs (Muro E et al, 2010).

Studies, conducted mostly on human lymphocyte chromosomes, determined interchromosomal, interindividual, and population polymorphism of the NOR, and demonstrated its specificity and inheritance pattern (Härtung M et al, 1980; Gall JG, 2019).

Some studies demonstrated that the structure of nucleoli reflected their functional state and indicated active biogenesis of ribosomes. The quantitative determination of NORs therefore enables the assessment of the proliferative activity of interphase and metaphase cells (Buchinskaya LG et al, 2001; Gusk H et al, 2000). It means that when studying the structural specificities of nucleoli, it is possible to assess how actively the transcription of ribosomal genes and the biogenesis of ribosomes in general occur.

Medical literature contains rather detailed description of polymorphism in the activity of ribosomal genes in relation to cancer (Alarcon-Romero LC et al, 2009; Siri V, 2000; van Sluis M, 2019). The study of this trait is promising in terms of applied genetics of livestock as an instrument of evaluating the phenotypic manifestation of the activity of ribosomal genes in relation to protein biosynthesis (Li L, Spradling AC, 2016). It is assumed that NOR activity may serve as a marker of evaluating livestock productivity (Babu KA et al, 1985). In our opinion, NOR state may serve as a reporter system for characterization of the physiological state of animals and evaluation of their productivity potential.

The aim of our study was to investigate NOR polymorphism of chromosomes and their activity in terms of productivity traits in three different Ukrainian cattle breeds, namely Ukrainian Red-and-Motley dairy breed and Ukrainian Black-and-White dairy breed, as well as the cows, obtained from the crossing of Ukrainian Red-and-Motley breed and Montbeliarde bulls.

MATERIALS AND METHODS

The studies were conducted at the Department of Animal Genetics and Biotechnology of the M.V. Zubets Institute of Animal Breeding and Genetics, NAAS.

The materials under investigation were the lymphocytes from the peripheral blood of first lactation cows of Ukrainian Red-and-Motley dairy breed and Ukrainian Black-and-White dairy breed, as well as the cows, obtained from the crossing of Ukrainian Red-and-Motley breed and Montbeliarde bulls (the herd of SE EF “Nyva” of the M.V. Zubets Institute of Animal Breeding and Genetics, NAAS).

To prepare chromosome preparations, whole venous blood (5 ml) was cultivated for 48 h at +37 °C in RPMI 1640 medium (Sigma, USA) with the addition of 0.1 ml/ml PHA (phytohemagglutinin, Sigma, USA), 15 % embryonic calf serum. Colchicine (Serva, Germany, 10 μg/ml) was added two hours prior to the completion of the cultivation period to stop cell division. The precipitate of cells was obtained by centrifugation for 10 min at 1,000 g and subsequent treatment with hypotonic KCl solution (0.075 M) for 20 min. The fixation of cells was conducted in three changes of the methanol-acetic acid mixture in 3 : 1 ratio. The cell suspension of required density, obtained in the last portion of the fixator, was dropped on cooled and wet specimen slides. 50 metaphases from each animal were studied.

Routine staining of chromosomes was conducted with 2 % Giemsa solution (Merk, Germany).

NOR activity was determined by visual evaluation of silver precipitation in individual chromosomes according to the recommendations of Januzzi L. (Januzzi L et al, 1994). For this purpose, to the chromosome preparations 2 drops of 50 % silver nitrate solution ($AgNO_3$) and 2 drops of 1 % formic acid each were applied, whereafter they were covered with a coverslip. Then the preparation was placed into a moistening chamber at +65 °C, protected from light for the period of 3–5 min, until the surface of the specimen slide acquired copper color. NORs were detected as dark spots on telomeres of the corresponding chromosomes.

The digital material obtained was processed using standard methods of variational statistics from the Office Excel 2003 package, determining Student’s t-test.

RESULTS OF STUDIES

The cytological analysis of chromosome preparations from leukocytes of first lactation cows demonstrated NOR polymorphism. The number of active NOR in the investigated animals varied from 1–12 (Fig. 1). We detected up to 12 chromosomes with active NORs in some animals. Similar results were obtained by other researchers as well (Kiseleva TYu, 1985; Novgorodova IP et al, 2020).

There was different distribution of cells with a different number of NOR in the groups of animals. For instance, the cows of URM and UBW breeds demonstrated statistically similar and higher incidence of cells with four (29.8 and 30 %) and five (17.1 and 19.5 %) NORs, and hybrid cows had almost twice smaller incidence of cells with five NORs per cell. The incidence of cells with seven and eight NORs per cell was twice higher in 52 cows of URM and UBW and 28 URM × M (2.5 against 4.5 % and 2.0 against 4.2 %) and was significant at the probability threshold of P = 0.95.

We determined a considerable variability of these indices in our study. For instance, in the animals of URM, UBW and URMxM hybrids the variation coefficient by the investigated trait was 2.4%, 0.5% and 28.8 %, respectively. This value demonstrates high level of variability of all investigated groups of animals (Counto MF, 2013)

The examination of three livestock breeds demonstrated statistically considerable differences by the average value of the number of active NORs between the animals from pedigree UBW dairy breed and of animals obtained from crossing cows of URM dairy breed with bulls of Montbeliarde breed (p ≤ 0.01) (Table 1). In addition to higher average value for NOR number, URM×M cows also had the highest NOR polymorphism: their number in the cell varied from 1 to 12.

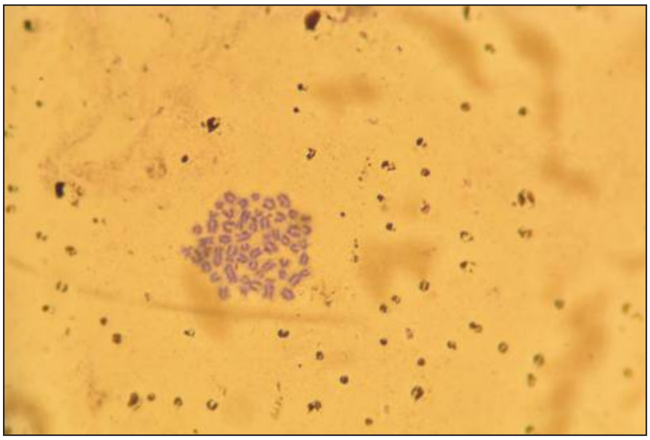

Fig. 1. Metaphase lymphocyte of a dairy URM cow, individual number UA801281092, “Nyva” farm (Giemsa stain, light microscopy, 10 × 100 magnification with active NOR, visible as dark spots, indicated with an arrow

It appeared that nucleolar chromosomes were often involved in chromosomal aberrations (CA) or associations of two chromosomes by one or two chromatids. There was a different incidence of chromosome breaks, their fragments, and premature centromere division of mitotic chromosomes (PCDMC). No statistically significant differences in the incidence of

Table 1. The distribution of nucleolar organizer regions (NOR) in metaphase somatic cells of different livestock breeds

Breed	Number of investigated animals	Number of active NOR per cell	Average number of NOR per cell (n = 4000 metaphases)	Variation coefficient (Cv), %	Number of chromosomal aberrations per cell (n = 4000 metaphases)
Ukrainian Red-and-Motley dairy breed	30	1–10	4.15 ± 0.30	22.4	0.27 ± 0.03
Ukrainian Black-and-White dairy breed	22	1–10	3.8 ± 0.35*	20.5	0.59 ± 0.02
URM × M hybrids	28	1–12	5.33 ± 0.27*	28.8	0.74 ± 0.03

Note. *p ≤ 0.05.

Table 2. The incidence of chromosomal aberrations (CA) in animals with different numbers of NOR

NOR / CA	Groups of animals by the average number of NOR in the cell					
	2.0–3.0	3.1–4.0	4.1–5.0	5.1–6.0	6.1–7.0	7.1 and above
Incidence of cells with CA, total (n = 4000)	14.03 ± 2.04	19.09 ± 1.53 *	15.7 ± 2.34	16.8 ± 2.56	13.7 ± 1.24 *	8.00 ± 3.01
Incidence of chromosome breaks (n = 4000)	3.5 ± 0.43	2.38 ± 0.39	3.30 ± 0.37	0.96 ± 0.41	2.9 ± 0.46	–
Number of fragments (n = 4000)	6.6 ± 1.57	5.8 ± 1.66	5.9 ± 0.34	1.8 ± 0.40	5.6 ± 0.48	–
PCDMC **	2.7 ± 0.51	1.51 ± 0.33	1.35 ± 0.36	1.65 ± 0.42	1.88 ± 0.44	–

Note. * p ≤ 0.05. ** premature centromere division of mitotic chromosomes.

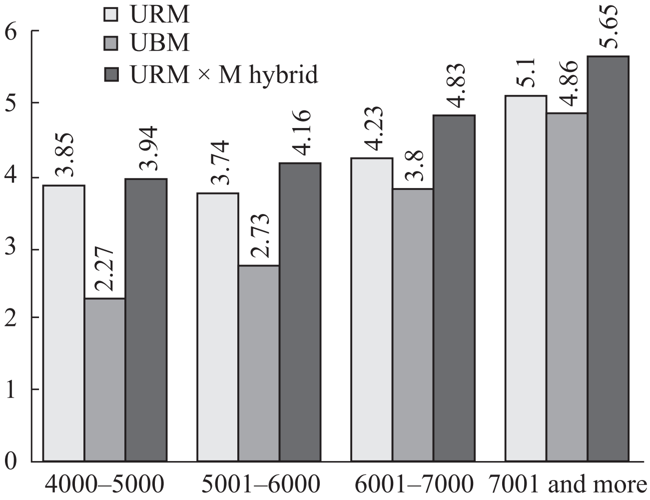

Fig. 2. NOR distribution in the groups of investigated cows of first lactation cows with different productivity levels

chromosome breaks depending on the breed of animals were found.

Statistically significant differences ($p \leq 0.01$) were found in terms of CA incidence between groups of animals with the average number of 3.1–4.0 and 6–7 NOR per cell (Table 2).

To determine a possible connection between NOR activity and the productivity of dairy cattle, each group of cows of the breeds under investigation was divided into two groups: group I up to 7000 kg per first lactation and group II with a production of >7000 kg per lactation. The highest value of active NOR was detected in the group of animals of local origin (URM × M) with a milk yield over 7,000 kg per lactation (Fig. 2). URM × M hybrid cows are notable not only for a higher share of active NOR as compared with other investigated livestock breeds, but also a higher level of rRNA synthesis in cells, as a result.

The average number of NOR's was smaller in cows of all investigated breeds with the productivity of 4,000–5,000 kg of milk with the fluctuations from 2.27 to 3.94 units, as compared with those cows with a milk yield of 7,000 kg and more with the limits of 4.86–5.65. The increased NOR activity of chromosomes is perhaps related to the synthesis of protein, required for the realization of productivity traits. However, the comparison of NOR activity indices within one breed and among breeds is depending on the level of their productivity. Further studies are required to confirm this tendency and determine why the activity of nucleolar organizers increases with the increase in a milk yield in dairy cows.

DISCUSSION

It has been determined that the average number of nucleolar organizer regions (NORs) is constant for each biological breed and is one of the species characteristics (Gerbault-Seureau M, 2017).

The breeds of animals, including agricultural ones, differ considerably in the number of NOR and their localization (Mellink CHM et al, 1994; Loginov SI et al, 2004). There are different opinions in the scientific literature regarding the location of NOR in chromosomes of *Bos taurus* representatives. Usually, NOR are localized on telomeres of five pairs of homologous chromosomes. Italian scientists note that these are pairs 2, 3, 6, 11, and 27 (Di Berardino D et al, 1985), a researcher from the Federal University of Pernambuco, Brazil,) report that in livestock NOR are located on pairs 2, 3, 4, 11, 25 (Melo TC, 2009). There is another opinion that NOR are located on chromosomes 2, 3, 4, 5, 28 (Amancio AP et al, 2019), while there is a communication about their being on chromosome pairs 2, 3, 4, 11, and 28 (Jung W et al, 2003).

Usually, NORs are localized on telomeres of the respective chromosomes. However, there are reports on their location in pre-centromere sites of chromosomes, which may be a translocation trait (Diamond JR, 1975). It has been found that chromosomes from NOR are most inclined to the formation of aberrations or diseases such as cancer (Yosida TH, 1981. In our study, we found, breaks, and translocations of chromosomes, on which NOR are located. Other researchers also report the participation of NORs in chromosomal breaks, translocations and in the formation of fragile sites in chromosomes (Gerbault-Seureau M et al, 2017). Some authors consider the associations to be the reason for the asynchronous pattern of cell division (Hirai H, 2020). The increase in transcriptional activity of NOR in the cells with a high level of chromosomal anomalies has been reported (Nazarenko SA et al, 1990). Our studies determined the highest number of aberrations in the animals of local origin (0.74 per cell), in which the number of active NORs is also the highest This trait is rather lower in dairy cows of URM and UBW along with their lower frequency of NORs in the cells. .

The localization, number, and sizes of NORs may vary between the representatives of the same species (Zaleśna A, 2017). Usually, the sizes of NOR are in proportion to the number of genes, encoding rRNA Depending on the transcriptional activity, the sizes of NOR may change which may be detected by silver staining (Reeder RH, 1990).

It has been demonstrated that the status of the nucleolar apparatus reflects the functional activity of the cell. Non-histone proteins of the nucleolus are responsible for the activation and control of transcription of ribosomal genes. These proteins are argophilic, can be detected after staining with silver, and their quantitative values may indirectly reflect the activity of ribosomal genes (Gossens G, 1984).

Many studies demonstrated that some diseases and harmful substances of the environment cause the activation of NOR in human and animal lymphocytes (Bilban M, Vaupoti J, 2001; van Sluis M et al, 2020).

Some articles report the increase in NOR sizes as an effect of harmful industrial factors, such as harmful chemical substances (for instance, radon), byproduct cokemaking (Novgorodova IP et al, 2020). It was proven that NOR sizes characterize RNA synthesis and allow evaluating protein synthetic function of the cell in different animals (Wachtler F, 1986; Cooper GM, 2000; Mellink CH et al, 1994).

Recent decades have witnessed the interest of scientists to visualization of metaphase NORs which can reveal the physiological and productive state of the animal organism (Kopytko AS, Kvochko AN, 2014; Buteeva SK, 2014).

The variability in the sizes of NOR, related to the transcriptional activity of rRNA genes in pigs of different breeds, was reported by Mellink CH et al, 1994.

A study demonstrated that quantitatively determined variants of NOR sizes may be viewed as chromosome markers, which characterize the breeds of pigs and, probably, their productivity (Danielak-Czech B et al, 2011).

The possibility of forecasting the productivity of chickens by NOR parameters was studied by Russian researchers (Kopytko AS, Kvochko AN, 2014). The same authors investigated NOR activity in metaphase chromosomes while studying the species diversity of animals. Russian scientists determined the differences between the groups of sheep of different genotypes by the number of NORs in interphase cells and the density of their staining (Klenovitsky PM et al, 2021). The authors believe that these indices characterize the state of argophilic zones of the cell nucleus. There are, however, few articles that report studies on NOR activity in cattle or other representatives of Bovidae family (Mayr B et al, 1987; Mayr B et al, 1989; DzitsiuV et al, 2018; 2020), and the information about the study on the association between the activity of ribosomal genes and the productivity of dairy cattle is presented in few articles (Dai,W et al, 2017)

It is noteworthy that researchers claim the existence of a direct relation between the number of rRNA clusters and the number of NORs present in metaphase chromosomes, when stained with silver nitrate solution (Hernandez-Verdun D et al, 1984; StutsD M, 2008).

This fact demonstrated the activity of RNA synthesis and the level of metabolism in the cell, in particular, and the organism as a whole, which determines the productivity of livestock to some extent. The results of our studies demonstrate that in the group of URM × M hybrids there is an increase in the number and the extension of margins for NOR polymorphism, which, in our opinion, reflects the activity of nucleolar organizers, aimed at maintaining the homeostasis of animals and the increase in their productivity.

CONCLUSIONS

We determined the differences in the activity of nucleolar organizers between the investigated groups of dairy cows. URM × M hybrids reliably ($p \leq 0.05$) exceeded UBW dairy cows by this index. No statistically significant difference between other investigated groups of animals was found by this trait.

Higher dairy productivity was found in the animals with higher frequency of NORs in the chromosomes of metaphase cells.

In our opinion, the number of active NORs demonstrates a relative variability between their number and the level of protein synthesis, required to implement the productivity traits of the investigated animals.

Adherence to ethical principles. All experiments described in this paper were non animal based.

Conflict of interest. Authors declare no conflict of interest.

Financing. This study was not financed by any specific grant from financing institutions in the state, commercial or non-commercial sectors.

Поліморфізм ядерцевих організаторів хромосом у різних українських порід великої рогатої худоби

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Мета. Вивчити активність ядерцеорганізуючих районів хромосом (ЯОР, NOR (Nucleolar Organizer Regions)) у різних українських порід великої рогатої худоби в зв'язку із ознаками їх продуктивності. **Методи**. Для дослідження використали метод приготування препаратів хромосом із лімфоцитів периферійної крові 90 корів різних порід. Активність ЯОР визначали шляхом візуальної оцінки преципітування срібла у індивідуальних хромосомах. Для цього препарати хромосом фарбували 50%-ним розчином азотнокислого срібла. ЯОР виявлялись на теломерних ділянках відповідних хромосом як чорні цятки. **Результати**. Цитологічний аналіз препаратів хромосом із лейкоцитів корів-первісток виявив поліморфізм ЯОР у корів української червоно-рябої молочної (УЧеРМП), української чорно-рябої молочної порід (УЧРМП) і корів-помісей, отриманих від схрещування української червоно-рябої молочної породи з бугаями монбельярдської породи (УЧеРМП×М). У первісток УЧеРМП і УЧРМП частіше і приблизно з однаковою частотою зустрічались клітини з чотирма (29,8 і 30 %) і п'ятьма (17,1 і 19,5 %) ЯОР, а у корів УЧеРМП×М частота клітин з такою ж кількістю ЯОР була майже вдвічі менша, а в той же час клітини із 7 і 8 ЯОР переважали аналогічний показник інших досліджених порід майже вдвічі (2,5 проти 4,5 % і 2,0 проти 4,2 %). Найвищий рівень хромосомних аберацій (ХА) спостерігався у групі тварин із середньою кількістю ЯОР на клітину від 2 до 3 одиниць, а найнижчий – в групі з високою кількістю ЯОР – від 6 до 7 з достовірною міжгруповою різницею (р < 0,01). Активність ЯОР найвища у групі помісних корів з рівнем надою за 305 днів першої лактації більше 7000 кг. **Висновки.** Встановлено відмінності між дослідженими групами корів молочного напряму продуктивності за активністю ядерцевих організаторів. Корови помісного походження достовірно (р ≤ 0,05) переважали за цим показником корів української чорно-рябої молочної породи. Статистично значущої різниці між іншими дослідженими групами тварин за цією ознакою не встановлено. Виявлено вищу молочну продуктивність у тварин з вищою частотою ЯОР у хромосомах метафазних клітин. На нашу думку, число активних ЯОР свідчить про співвідносну мінливість між їх кількістю і рівнем синтезу білку, необхідним для реалізації ознак продуктивності досліджених тварин.

Ключові слова: корови, ядерцеорганізуючі райони хромосом (ЯОР), хромосомні аберації, молочна продуктивність.

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