

# APPLICATION OF INTERNATIONAL COMMITTEE FOR ANIMAL RECORDING (ICAR) METHODOLOGY IN DAIRY HERD MANAGEMENT IN SOUTH OF RUSSIA

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➤ Supporting Information

**ABSTRACT:** This experiment was conducted to determine the advantages of introducing modern innovative approaches to dairy herd management based on the study and implementation of the methodological of International Committee for Animal Recording (ICAR). This research shows the main directions for introduction to new breeding and the technological model of interaction with breeding farms for breeding dairy cattle. This interaction occurs through the services provision for breeding farms, the control-assistant and expert-boniter services, as well as laboratories for selection control of milk quality and genetic control. The tasks of the control-assistant service included participation in the control milking of cows and individual milk samples picking from each cow and its delivery to the laboratory in a chilled form. Using as the example dairy herd of the black and white breed by studying the dynamics of somatic cells has been showing the effectiveness of different methods for assessing the quality of milk. Implementation of the milk quality regular monitoring during 1-2 months allows bringing the main parameters of milk quality in line with the requirements of national and international ICAR standards. The cow's conformation assessment by animals' linear assessment allows revealing the bull's prepotent abilities and choosing the right strategy for improving the cow's conformation in the herd. Genetic well-being assessment of cows makes it possible to exclude unwanted individuals with genetic abnormalities and use only healthy animals in breeding. The research purpose was studying the possibility of applying the ICAR method in Russian breeding livestock to improve the dairy herd management system. The research results showed that implementation of individual veterinary measures for two months allowed reducing the average somatic cell count by 1.85 times and reaching the level recommended by ICAR. The spread of individual indicators also significantly decreased. Therefore, Using the individual monitoring of dairy raw materials quality assessment makes it possible to study the influence of para-typical (climatic) factors on the content of dairy components, as well as to stabilize the quality parameters in terms of the level of somatic cells not higher than 200 thousand cells/cm<sup>3</sup>.

**Keywords:** Breeding, Dairy cattle breeding, Dairy herd, Genetic resources, Milk quality.

## INTRODUCTION

Dairy cattle breeding is one of the basic branches of agriculture that ensures the national security of the Russian Federation. In most regions of Russia, the main shares of livestock products and milk have been producing in personal subsidiary farms and farms (Galiev and Ahrens, 2018). That leads to the small-scale nature of production processes and the insufficient ability to effectively control the quality and safety of products. Currently, in the domestic Russian market, have been producing about 22.5 million tons of marketable milk, which is approximately 236-237kg/person per year or at the level of 70% of the biological requirement (Melnikova and Bogdanova, 2021). The shortage of raw milk in Russia is almost 100kg per person per year, or 30.7% of the need (Melnikova and Bogdanova, 2021).

The regulatory requirements for food security regulate the production of raw milk in the domestic market for at least 90% of the needs for the population. It could reach by introducing current dairy herd management systems developed based on classical studies (Crowe et al., 2018; Guinn et al., 2019; Cockburn, 2020). To increase the efficiency of the development of national dairy farming, it is necessary to develop and implement an integrated approach to managing a dairy herd, considering the proven accounting practices of the International Committee for Animal Registration (ICAR) for accounting for the milk production of lactating cows (Ferris et al., 2020).

Reproduction of the herd of dairy cattle is one of the major factors that ensure the preservation of the dairy herd and stable milk production. There had been revealing the negative impact of various genetic anomalies in cattle. That often inherited in a recessive form and under certain conditions, upon transition to a dominant form. It causes significant damage to both the health of animals and the reproduction of the herd (Cartwright et al., 2017; Nowicki et al., 2017; Schuster et al., 2020).

An analysis of the official accounting data in the breeding dairy farming of Russia shows that if in 2014 the average age of all breeding dairy cows calving was 2.79, and the withdrawal of animals from the herd for various reasons were at 3.48. Then, there had been observing the so-called "rejuvenation" of cows when they had retired from the herd. Since the average age of all breeding dairy cows calving was 2.52 and disposal occurs already at 3.11 calving. Thus, it could have assumed that during 2014-2019, at the national level, it was not possible to find an organizational and technological solution to ensure a longer period of economic use of highly productive dairy cattle (Yurchenko et al., 2018; Nikitin et al., 2021; Khabipov et al., 2021).

Considering the fact that the level of reproduction of a herd of dairy cattle, according to official data, is on average at the level of 80%, and the duration of the service period is 130-140 days (for Holstein, Ayrshire, black and white breeds), it will no longer be possible to provide annual replenishment of uterine resources, respectively, and the full reproduction of the dairy herd. Thus, there remains a need to replenish uterine resources in dairy cattle breeding only by importing livestock from abroad. The dairy business is also talking about this with concern.

The current situation in pedigree dairy cattle breeding raises concerns about actually achieving the criteria indicators outlined in the current Food Security Doctrine and causes the search for new breeding and technological solutions (Ananiev et al., 2019; Muzalev and Reshetov, 2020).

In addition, the significant dependence of the national dairy cattle breeding on the import of genetic resources (bull semen, embryos, and heifers) does not allow to fully monitoring the genetic usefulness and well-being of incoming materials. Over the past 40-50 years, in Russian market are being intensive delivered the genetic material from the leading countries of the world. That, of course, led to the creation of a highly productive array of Holsteinized cattle from the leading lines of sires (Hladiy et al., 2018).

Using a limited contingent of Holstein and Ayrshire bulls in the breeding system led to the appearance of newborn calves with various physiological and anatomical abnormalities that have a genetic basis because of gene mutation. There had known about two hundred types of such anomalies, including about 80 in Holstein black and white cattle, and about 20 in Ayrshire cattle. This is physiological abnormalities of organs and tissues because of genetic disorders. These deviations had been inheriting in a recessive form. Therefore, they are an undesirable hidden genetic load in the herd. In the event of an increase in inbreeding under conditions of large-scale selection, a significant change in the dynamics of their inheritance frequencies can occur (Zinovieva, 2016; Gladiy et al., 2018).

Therefore, in order to reduce import dependence on the supply of genetic materials, as well as to develop national breeding resources in dairy cattle breeding, it is necessary to develop a modern innovative dairy herd management system that allows you to consider and meet the needs of dairy cattle, ensure high efficiency of production processes, and purposefully form animals with the desired physique type.

The purpose of the research is to study the possibility of a comprehensive application of the methodological approaches recommended by ICAR to assess phenotypic (yield, milk quality) and genotypic (presence of genetic abnormalities) traits to improve the efficiency of managing a herd of highly productive dairy cattle.

## MATERIALS AND METHODS

For this research, methodological recommendations for the application of the ICAR method was developed in dairy cattle breeding. For this reason had created the control-assistant and expert-boniter services and laboratories for milk selection control quality and genetic control. Cattle phenotypic and genotypic characteristics studying were in herds of breeding black and white (1275 cows) and Holstein (1100 cows) cattle of the South of Russia. Average milk productivity of these herds was 8.2 and 9.8 thousand kg of milk per cow per year. The raw milk quality studying had conducted in the milk quality selection control laboratory. Fluorescence microscopy carried somatic cells determination in milk by a somatic cell analyzer DCC, Delavale. The essence of the method lies in the somatic cells cytoplasmic membrane destruction under the lysogenic buffer, while the cell nuclei become available for the action of a fluorescent dye, which is propidium iodide. This reagent binds to the double-stranded DNA of somatic cells, and forms a fluorescent substance that absorbs green light and emits red, with the help of the latter, the somatic cell is identified. The system gives an image of the cells, and the computer built into the analyzer, using software, counts the number of white dots, which corresponds to the number of somatic cells. Cell analyzer DCC, working on the principle of fluorescence microscopy, equipped with a cassette receiver (removable mandrel), backlit LCD display, complete with cassettes (Nudeo cassette), sample flasks.

There was monitored a wide range of genetic abnormalities associated with impaired fertility: Leukocyte adhesion deficiency (BLAD); uridine monophosphate synthase deficiency (DUMPS); Complex spinal malformation (CVM); citrullinemia (BC); Brachispin (BY); Blood factor XI (eleven) deficiency (FXID); Axonopathy (DS); Subfertility of bulls (BMS); Chediak-Higashi Syndrome (CHS); Congenital muscular dystonia type 1 (CMD1); Congenital muscular dystonia type 2 (CMD2); Crooked Tail Syndrome (CTS); Bulldog dwarfism (BD); Epidermolysis bullosa (EB); Factor VIII deficiency (eight); hemophilia A (FVIIIID); Idiopathic congenital megaesophagus (ICM),  $\alpha$ -mannosidosis ( $\alpha$ -MAN),  $\beta$ -mannosidosis ( $\beta$ -MAN); Mucopolysaccharidosis (MPSIIIB); maple syrup disease (valinoleucinuria) (MSU); Syndactyly, mule hoof (Mulefoot); Neuronal ceroid lipofuscinosis (NCL), Osteopetrosis (OS); bloated calf syndrome (PCS); Congenital pseudomyotonia (PMT); Congenital erythropoietic protoporphyria (PT); Arachnomyelia and arthrogyposis syndrome (SAA); spinal demyelination (SDM); spinal muscular atrophy (SMA); thrombopathy (TP); Weaver (Weaver Syndrome) (Weaver). Haplotypes associated

with impaired fertility - according to 12 indicators: Holstein haplotype 1 (HH1); Holstein haplotype 2 (HH2); Holstein haplotype 3 (HH3); Holstein haplotype 4 (HH4); Holstein haplotype 5 (HH5); Holstein haplotype associated with cholesterol deficiency (HCD).

Work on monitoring genetic diseases and DNA diagnostics of the productive qualities of dairy cattle were carried out in a certified genetic laboratory. The monogenic diseases carriage studied by selecting biological material as a skin pluck, from which genomic DNA was isolated using Qiagen columns, the Netherlands called QIAamp DNA Blood Mini Kit based on silicon membrane technology under the manufacturer's protocol.

The carrier status of monogenic recessive diseases determination and lethal haplotypes of cattle was using the polymerase chain reaction method with further electrophoretic analysis of the amplicon, the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), the method of sequencing target regions of the bovine genome or the method of whole genome genotyping using technology from Illumina, USA.

Polymerase chain reaction method with further electrophoretic analysis of the amplicon: within the framework of this technique, the polymerase chain reaction was in a volume of 10 ml containing 10 ng of DNA, 2 ml of the 5X MasCFETaqMIX PCR buffer (Dialat, Moscow) and 0.4 ml of primer (concentration 2.5 pmol/100 ml). The polymerase chain reaction was on a C1000™ ThermalCycler instrument (BioRad, USA). PCR products separated on a 2% agarose gel, visualized in an ultraviolet transilluminator, and detected using a gel documentation system. Carrier status was determined based on the identification of bands in the agarose gel, which corresponded to DNA fragments of a certain length.

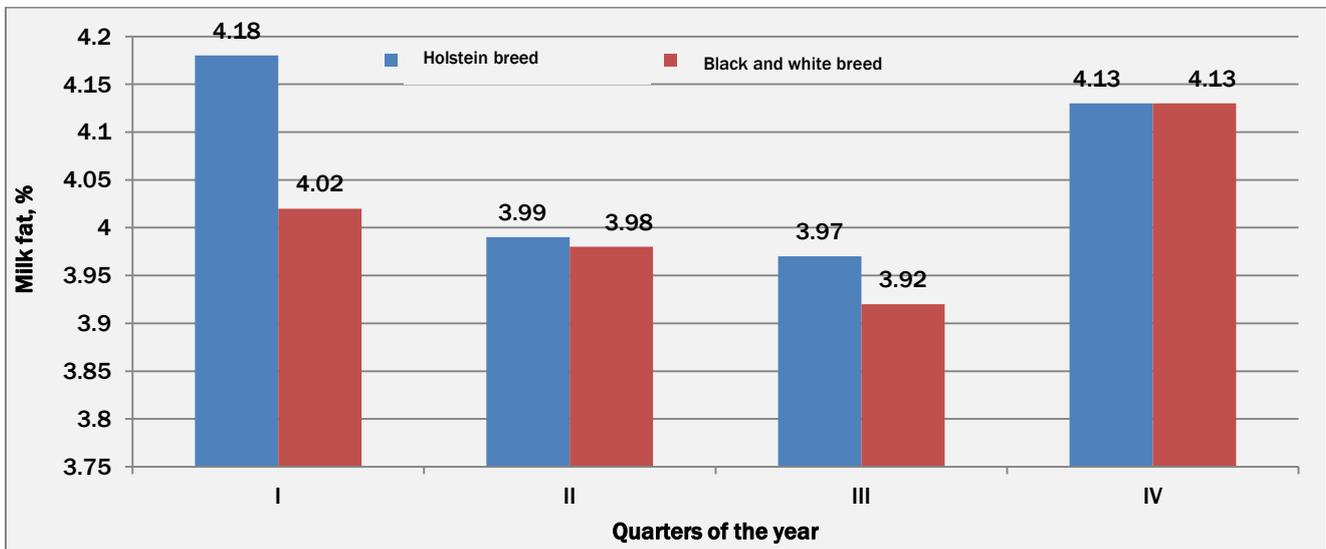
Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The polymerase chain reaction carried out in a volume of 10 ml containing 10 ng of DNA, 2 ml of PCR buffer 5X MasCFETaqMIX (Dialat, Moscow) and 0.4 ml of primer (concentration 2.5 pmol/100 ml). The polymerase chain reaction was carried out on a C1000™ ThermalCycler instrument (BioRad, USA). Next, 10 ml of the restriction mixture containing 0.7 ml of restriction endonuclease, 1.3 ml of 10x buffer for the selected restriction endonuclease, and 8 ml of water was added to 10 ml of the got amplicon. The resulting mixture incubated according to the restriction endonuclease manufacturer's protocol on a C1000™ ThermalCycler instrument (BioRad, USA). The incubated restriction mixture separated on a 4% agarose gel, visualized in an ultraviolet transilluminator and detected using a gel documentation system. The definition of carrier status established based on the identification of bands in the agarose gel, which corresponds to DNA fragments of a certain length. Method for sequencing target sections of the bovine genome: within the framework of this technique, a polymerase chain reaction performed in a volume of 10 ml containing 10 ng of DNA, 2 ml of PCR buffer 5X MasCFETaqMIX (Dialat, Moscow) and 0.4 ml of primer (concentration 2.5 pmol/100 ml). The resulting amplicons were purified by alcohol (ethanol) reprecipitation. Next, the purified amplicon was sequenced on an ABI PRISM 3730 capillary sequencer according to the protocol of the manufacturer. Carrier status was determined based on the determination of the genotype of the casual mutation associated with the disease.

Whole genome genotyping method using technology from Illumina, USA: Whole genome genotyping was performed using standardized protocols from Illumina, USA. Mathematical processing of primary data was by Statsoft and MS Excel. Changes were significant at  $p < 0.05$ .

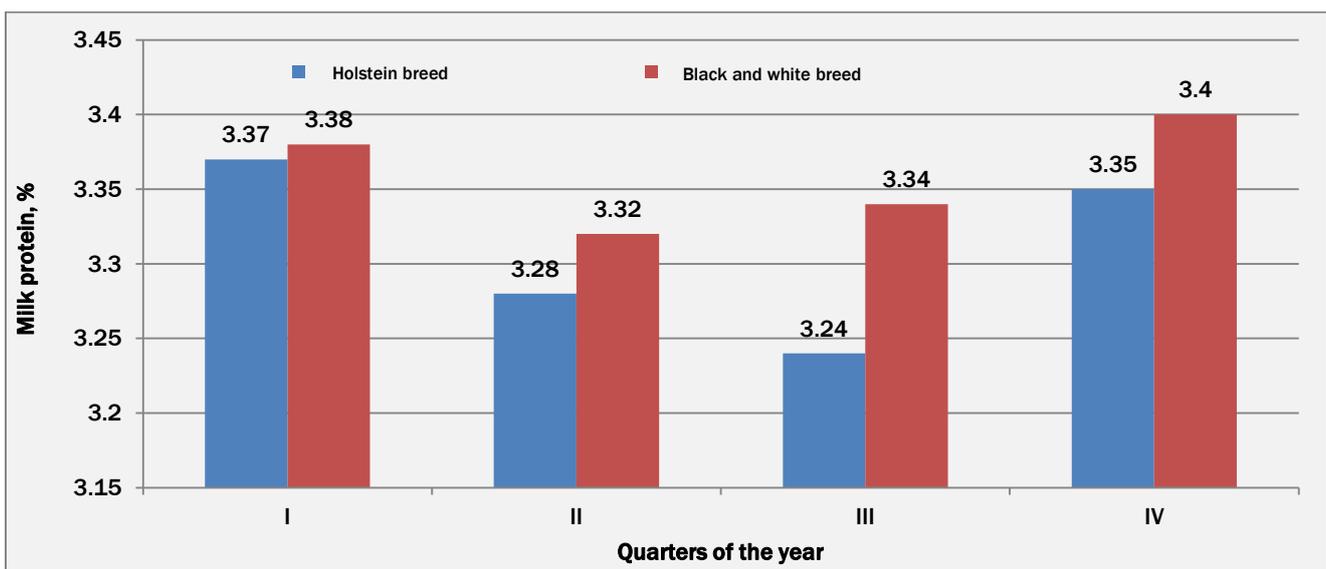
## RESULTS AND DISCUSSION

The organization of individual sampling of raw milk with the involvement of the control-assistant service made it possible to study the seasonal dynamics of the main dairy components, including milk fat, which is the most important element for providing good nutrition to consumers.

Statistical processing of the actual material made it possible to establish a high dependence between the parameters of the temperature factor throughout the seasons of the year and the average concentrations of the main dairy components in cows of the studied breeds - milk fat and protein (Figures 1 and 2). The results showed that in the breeding stock of the Holstein breed, the correlation coefficient between the temperature dynamics and the fat content in milk was  $r = - 0.74$ . Similarly, for protein, this indicator was, respectively,  $r = - 0.77$ . In the breeding stock of the black and white breed, the showed correlative relationship was also high. The correlation index between changes in ambient temperature and the concentration of fat in raw milk was  $r = - 0.71$ , and for protein, respectively,  $r = - 0.80$ . Carrying out individual sampling of milk from breeding cows, prompt delivery of milk samples in compliance with the temperature during transportation not higher than +4...+ 6°C and the study of the content of dairy components using modern methods made it possible to record high negative relationship between changes in the temperature factor and the concentration of milk components - fat and protein. The established high influence of the temperature factor on the indicators of the content of dairy components causes the development of modern feeding and housing systems for highly productive dairy cattle to ensure more stable microclimate parameters throughout the seasons of the year, which is also emphasized by the increasing global climate trends associated with warming. The study of the dynamics of changes in the content of fat and protein in milk over the seasons of the year shows that the studied raw milk meets the established breed requirements and corresponds to the category of high-quality milk, because changes in milk fat were between 3.92...4.18%, and milk protein, respectively, 3.24-3.40%, which correspond to national requirements, and also exceed them by 10-15%.



**Figure 1 - Milk fat average indicators dynamics in cows' milk of Holstein (I) and black and white (II) breeds by seasons**

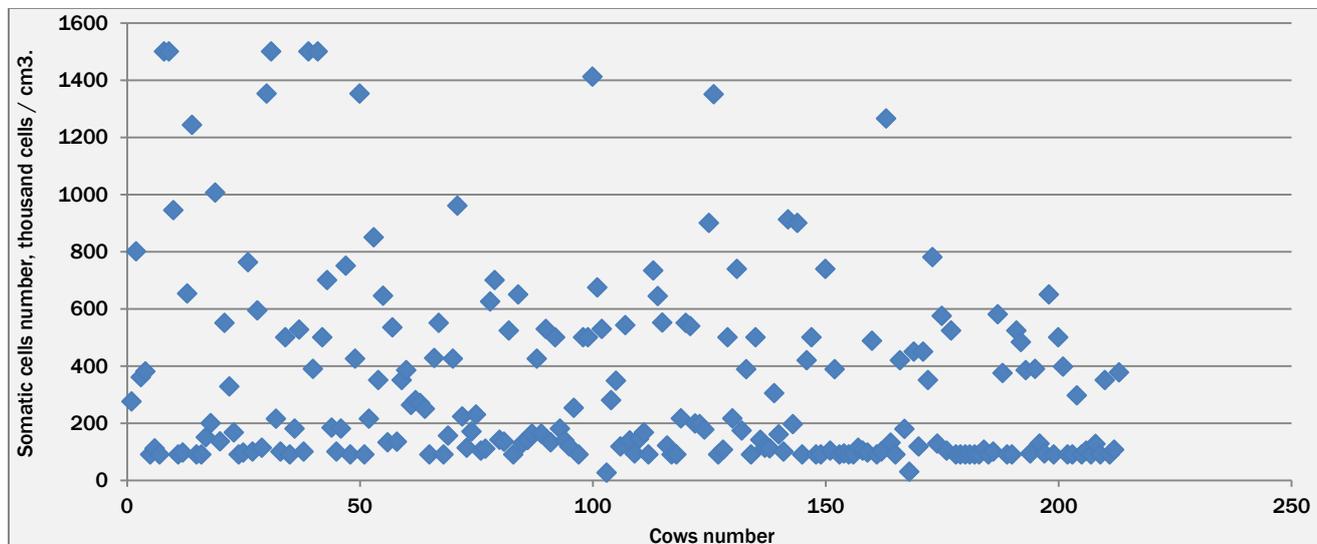


**Figure 2- Milk protein average indicators dynamics in cows' milk of Holstein (I) and black and white (II) breeds by seasons**

The technological factor, the system of keeping animals, of course, affects the parameters of the microclimate and the ability of animals to realize the genetically determined potential of milk production. In the farms under study, the maintenance of black and white breed cows is tethered, and the milking of cows is linear. While the cows of the Holstein black and white breed are kept loose and their milking is carried out in the milking parlour. The dynamics of milk components in cows of the studied genotypes are similar, despite the different systems of feeding and keeping animals in the studied enterprises. There had known that industrial milk production by tethered housing system, animals are in stress. Cows deprived the possibility for free movement and may have reduced immunity (Sawa and Bogucki, 2011). In the conditions of a loose system of keeping animals, labor costs for servicing livestock are significantly reduced. However, because of problems with monitoring the individual condition of the breeding stock, the herd reproduction parameters often deteriorate and the only way out is the widespread use of hormonal stimulation (Lambertz et al., 2014; Kobek-Kjeldager et al., 2020).

Undoubtedly, the achievement of high milk yields in modern dairy cattle genotypes is an important result of selection and breeding work in the breeding herds of dairy cattle (Oitenacu and Broom, 2010). There is obvious fact that massive using the hormonal drugs to ensure the desired herd reproduction indicators cannot be recognized as justified. For example, the duration of the inter-calving period is not over 400 days, as practiced in many countries around the world. It is necessary to conduct an in-depth study of the influence of the genetic characteristics of modern dairy cattle in combination with the development of an adequate system of feeding and keeping animals to minimize the use of veterinary drugs in implementing the natural need of cows - the birth of a calf (Ladyka et al., 2021). This work has begun in the countries of the Eurasian Economic Union. Carrying out individual sampling of milk also allows you to quickly respond to a decrease in the quality of milk because of an increase in somatic cells. Because of the influence of seasonal factors, we studied the possibility of improving the quality of raw milk by interacting with a separate "problem" farm by

monthly monitoring of milk quality for the content of somatic cells. The results of the study of the content of somatic cells in the milk of the controlled group of cows (Table 1) show that in the first period of lactation, there was a slight excess of this parameter compared to the level of somatic cells recommended by ICAR (up to 200 thousand cells/cm<sup>3</sup>), although these parameters correspond to the current regulatory document in the EAEU countries. The individual scatter of data on the content of somatic cells both visually (Figure 3) and mathematical characteristics was quite large. The standard deviation is 6.67% of the mean value. Using individual veterinary measures, there had observed significant improvement in the situation regarding milk quality within two months (Table 1 and Figure 4). Thus, the average value of somatic cells decreased by 1.85 times and reached the level recommended by ICAR. The spread of individual indicators also significantly decreased. The standard deviation was 3.63% of the average value for the herd.

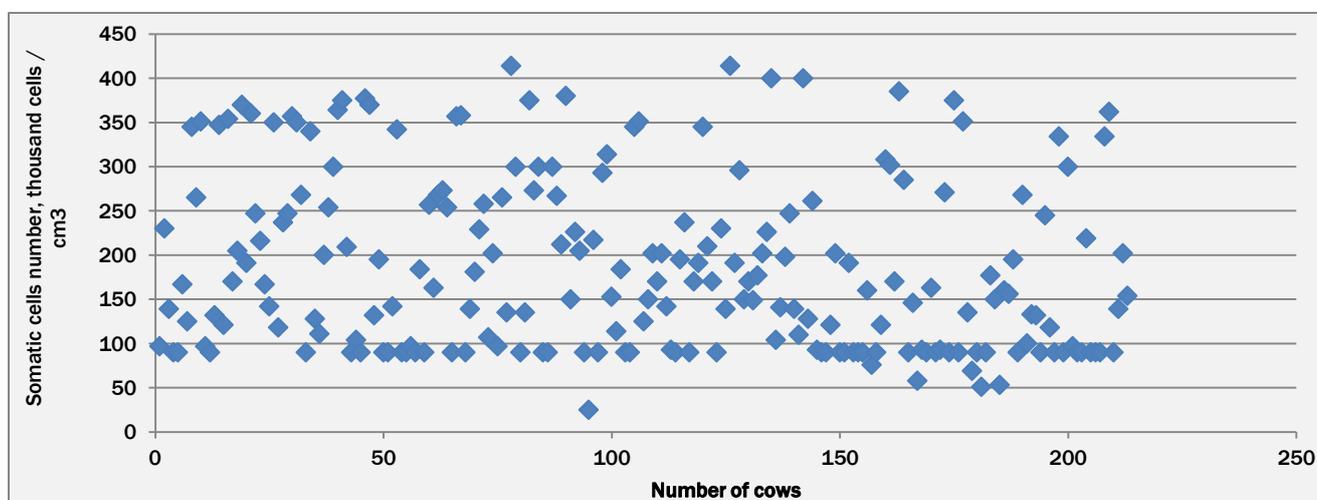


**Figure 3 - Distribution of individual indicators of the content of somatic cells in raw milk of a herd of black and white cows before monitoring**

**Table 1 - Dynamics of the content of somatic cells in raw milk in black and white cows during monitoring**

Control group of cows	Number of cows in the group	The content of somatic cells, thousand cells/cm <sup>3</sup>	Individual minimum level of somatic cells, thousand cells/cm <sup>3</sup>	Individual maximum level of somatic cells, thousand cells/cm <sup>3</sup>
Up to 100 days of lactation	213	346 ± 23.1	26	1500
During the period of 100-200 days of lactation	213	187 ± 6.8*	25	414

**P<0.05**



**Figure 4 - Somatic cells distribution in raw milk of black and white cows after monitoring**

According to the results of genetic studies (65 heads) were determined cows with reproductive problems. There were 2 cows carriers of the monogenic recessive disease BLAD, 1 - carrier of the monogenic recessive disease CVM and 1 - carrier of the recessive monogenic disease BY, 2 - carriers of the lethal HCD haplotype, 1 - carrier of the lethal HH1 haplotype, 2 - carriers of the lethal HH3 haplotype and 1 - carrier of the lethal HH5 haplotype. The isolation percentage of

animals with genetic abnormalities did not exceed 3% of the study group. These genetic abnormalities emphasize the need for widespread genetic monitoring to ensure the effective development of the dairy cattle industry. The results of the research allow us to conclude that implementing the ICAR method in the practical field of livestock breeding in Russia is promising. As mentioned above, the organization of breeding work in dairy farming was under the Order of the Ministry of Agriculture of the Russian Federation of October 28, 2010 No. 379. These documents regulate the selection and breeding work in breeding enterprises, mainly with the involvement of full-time veterinary specialists of these enterprises. The modern practice of organizing dairy herd management, developed in ICAR member countries and presented in the public domain, involves the involvement of specialists from an independent control-assistant service. Also, it should conduct milk quality assessment in an independent, specialized laboratory. It should notice that, since 2016, a regulatory document has also come into force in the breeding dairy cattle breeding of Russia. This document recommends conducting research on the quality of dairy raw materials in independent laboratories for selection control of milk quality. It is certainly a progressive step and contributes to increasing the reliability of research and improving breeding work in dairy herds. The introduction of a control-assistant service remains open, although we have already discussed this issue at the national level (Vasilchenko, 2018). Approval of the method for assessing the breeding value of farm animals in the member states of the Eurasian Economic Union of the member countries of the EAEU, which is reflected in the new developed regulatory documents of the Eurasian Economic Commission for assessing the breeding value of dairy cattle, considering the ICAR method and as mentioned above, is also progressive character. Questions regarding the assessment of the daily dynamics of milk fat and protein in breeding cows, as reflected in the works of Dineen et al. (2020), Dynko et al. (2021) and Trukhachev et al. (2021).

Using foreign standards in the practice of breeding work (ICAR, Section 7 Bovine Functional Traits, 2020) for assessing the quality of milk, which establish more stringent requirements for the permissible level of somatic cells up to 200 thousand cells/cm<sup>3</sup>, in practice allow us to ensure the production of high-quality milk raw materials and the exploitation of a healthy milking contingent, free from mastitis diseases, which certainly makes it possible to more fully realize the genetically determined potential of milk productivity. Metlytska et al. (2018), Safina, (2018), and Ladyka et al. (2021) also pointed out the achievement of the desired milk productivity. Supplementing the practice of dairy herd management with mandatory monitoring of cattle for genetic abnormalities should also have to be as a progressive requirement. There had been studying that a few parental couples carriers of recessive monogenic diseases BLAD, CVM, BY, HCD and HH1, HH3, HH5 haplotypes. It is likely that some cows have reached the dominant form of these anomalies, which is reduced, including the fertility of animals. And although at the present stage, genetic studies are quite expensive and therefore cannot recommended everywhere for the entire livestock, it is necessary to cover stud bulls as much as possible with studies. Implementation of the ICAR method in breeding dairy cattle breeding in Russia will make it possible to assess the breeding value of dairy cattle using unified methods that have already tested in ICAR member countries. A wider implementation of this method will make it possible to form an export development vector for livestock breeders in the states of the Eurasian Economic Union. It should recognize that development of national coefficients for recalculating the daily dynamics of milk fat and protein content as promising areas. That will make it possible to unify national methods for assessing the breeding value of animals with the accepted methodological approaches of ICAR.

## CONCLUSION

Implementation of individual veterinary measures for two months allowed reducing the average somatic cell count by 1.85 times and reaching the level recommended by ICAR. The spread of individual indicators also significantly decreased. The standard deviation was 3.63% of the herd mean. According to the results of genetic studies of cows (65 heads), the number of animals with genetic abnormalities did not exceed 3% of the study group. But these genetic anomalies underline the need for widespread genetic monitoring to ensure the effective development of the dairy cattle industry. Therefore, introducing the ICAR method into the practice of national breeding work in dairy cattle breeding will make it possible to unify accounting practices with international principles and form the export potential. Using the individual monitoring of dairy raw materials quality assessment makes it possible to study the influence of para-typical (climatic) factors on the content of dairy components, as well as to stabilize the quality parameters in terms of the level of somatic cells not higher than 200 thousand cells/cm<sup>3</sup>.

## DECLARATIONS

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### Authors' contribution

Oleinik S. conceived of the presented idea; Skripkin V. developed the theory and performed the computations; Ershov F. analyzed and interpreted the data, and edited the manuscript for important intellectual contents; Shlykov S. and Omarov R. participated with data acquisition and critically revised the manuscript for improvements. All authors discussed the results and contributed to the final manuscript.

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## Conflict of interests

The authors have not declared any conflict of interests.

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