# Main genetic defects of improving breeds in the population of Sychevsky cattle of the Smolensk region

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**Abstract.** The Sychevskaya breed belongs to local breeds of the combined direction of productivity. The use of the world's best gene pool of the Holstein and Simmental breeds allows to improve the productive qualities of the livestock, but at the same time it introduces a number of recessive genetic mutations into the gene pool of the Sychevskaya breed. 150 cows of the Vazuzsky type and 34 sires of the Sychevskaya breed were studied. The incidence of hidden carriers of thrombopathia was 1.4±0.009 % among cows and 5.9±0.04 % among breeding bulls. Animals in the cow group belonged to the Aromat 3433 line and other lines, and in the bull group - to the Toreador 3032 line. In the cows group, 12 carriers of subfertility and 6 carriers of cholesterol deficiency were identified, which is 8±0.022 % and 4±0.016 %, respectively. The linear affiliation of animals goes back to the lines of Redad 711620016730 (subfertility), Reflection Sovering 198998 and Klever 68 (HCD). The frequency of occurrence of undesirable alleles in the genotypes of Vazuzsky type cows according to the *TMEM95, RASGRP2*, and *APOB* genes was 0.040; 0.067; 0.020, and in the genotype of sires according to *RASGRP2* – 0.030.

## 1 Introduction

Local breeds were created and spread in many regions of Russia, taking into account their adaptability to natural and climatic conditions. One of these breeds is the Sychevskaya, which was bred in the Smolensk region by crossing local cattle with producers of the Simmental breed [1]. The best selected crossbreeds, raised in conditions of good feeding and maintenance, were bred "in their own". The Sychevskaya breed was approved by the Decree of the Council of Ministers of the USSR on September 8, 1950. Until 1985, the breed was improved according to the type of "closed" population, but to improve milk characteristics, they began to use red-and-white Holstein sires in breeding. As a result of this work, a selection achievement was registered - the Vazuzsky dairy type of the Sychevskaya breed (patent No. 4210 dated 06.11.2008) [2], [3]. Sychevskaya breed refers to breeds of combined direction of productivity and animals are characterized by dense strong body composition,

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harmonic body structure, evenly developed cup-shape udder and have a distinctive feature – good compatibility signs of milk and meat production. Breeding work with Sychevsky cattle is carried out under a single program of large-scale selection and is aimed at increasing milk production. For this purpose, it is widely practiced to cross breeding seed stock of Sychevsky cattle with sires of Simmental and Holstein red-mottled breeds. [4]. The use of the world's best gene pool makes it possible to improve the productive qualities of livestock and improve their adaptability to industrial milk production technologies. At the same time, recessive genetic mutations that cause morphological and functional disorders of organisms are widespread in Simmental and Holstein breeds. Most of the anomalies do not appear in a heterozygous state, that is, their carriers do not differ phenotypically from healthy animals, however, a quarter of the get obtained from such parents die at the embryonic stage or are born with defects incompatible with life, leading to a significant deterioration in reproduction indicators. According to the information contained in the OMIA database of the University of Sydney, 11 and 22 mutations were registered in the Simmental and Holstein breeds, respectively, on 20.10.2020 [5]. Genetic defects such as BMS, thrombopathia, and FN4, HH0, HCD, HH3, and HH5 fertility haplotypes have high frequencies and can occur in populations of Sychevsky cattle.

BMS (Bovine male subfertility) is an autosomal recessive defect related to nonsense mutations. Manifestation: homozygous sires are infertile (sterile), heterozygous sires and carrier cows have impaired reproductive ability. This mutation is associated with abnormal sperm development. Mutation on the BTA19 chromosome is localized. The transmembrane protein *TMEM95* is located on the surface of the sperm of fertile animals, while in subfertile animals it is absent, so the penetration of the sperm into the ovum is difficult [6].

Thrombopathia (TP) is a missence mutation in the *RASGRP2* gene inherited by an autosomal recessive type, which results in impaired blood clotting due to insufficient release of ADP from platelets. In the course of research conducted abroad in a sick calf, nucleotide variability was found, which led to the replacement of the proline amino acids with leucine. The general condition of these animals is normal, but they suffer after injuries, injections or surgical interventions from incessant bleeding from damaged skin, mucous membranes associated with blood clotting disorders. May cause death [7], [8].

The FN4 haplotype is localized on chromosome 12 (BTA12) in the region 10,859,759-12,805,107 (UMD3.1 genome assembly). It has been shown that the nucleotide substitution A→G at position 11102143 *SUGT1* (rs110793536, ARS-UCD1. 2) is associated with embryonic mortality and death of get in the first weeks of life, while the G allele is undesirable [9].

The HH0 haplotype is mapped in the 20-25 Mb region on chromosome 21 and is caused by a 3.3 kb deletion in the *FANCI* gene (Fanconi anemia complementation group I). *FANCI* is essential for maintaining chromosomal stability. A mutation in this gene in cattle causes disorders of embryonic development, manifested in a decrease in fetal weight, growth disorders, vertebral deformities in the form of shortening of the vertebral column and lengthening of the limbs. In addition, there are abnormalities in the development of internal organs, in particular heart, kidneys, and gonads [10].

Haplotype HCD is a lethal haplotype of Holstein cattle mapped on chromosome 11 in the *APOB* gene. It is characterized by a violation in the metabolism of cholesterol, which leads to weight loss, appetite loss, physical weakness, diarrhea, not amenable to medication. The result is the death of calves in the first weeks or months of life. Heterozygous animals have a low level of cholesterol in the blood, while homozygous animals have no cholesterol in the blood at all [11].

The HH3 haplotype is associated with fetal mortality in carrier calves before the 60th day of pregnancy. It is localized to BTA8 in the *SMC2* gene and occurs as a result of replacing

T→C at position 95410507 in exon 24 of the *SMC2* gene (UMD 3.1). This polymorphism causes the amino acid substitution of Phe→Ser at position 1135, localized within the HTPase domain of the encoded protein [12], [13].

The HH5 haplotype is a recessive inherited genetic defect which homozygous carriers die in the early stages of embryonic development. It is localized on BTA9 in the area of 45.8-47.6 Mb. The cause of HH5 is a deletion of the 138 BP long sequence in the position between 93233 BP and 93371 BP, which corresponds to the entire dimethyladenosine transferase 1 *TFB1M* gene. Since the dimethyladenosine transferase1 protein is necessary for the synthesis and functioning of ribosomes, a mutation in the *TFB1M* gene in the case of sires connubium (hidden carriers) with cows (hidden carriers) leads to various malformations of the embryo and early death before the 60th day of pregnancy [14].

The spread of such hereditary anomalies can cause huge economic damage to farms. In this regard, DNA diagnostics is an effective tool for controlling and managing risks caused by the manifestation of genetic defects in populations of breeding animals of the Sychevskaya breed.

The objective of the work was to analyze the frequency of occurrence of animals carrying genetic defects BMS, thrombopathia and fertility haplotypes FN4, HH0, HCD, HH3 and HH5 in the Smolensk population of animals of the Sychevskaya breed.

## 2 Materials and methods

DNA samples from 150 Vazuzsky type cows and 34 Sychevskaya breed sires from a breeding plant in the Smolensk region were used as research material. DNA was isolated from the tissue of cows (earmark) and spermodose (straws and paillettes) of sires with a set of DNA-Extran-2 (Sintol LLC, Russia) in accordance with the manufacturer's recommendations.

To diagnose BMS, thrombopathia, and fertility haplotypes FN4 and HH3, test systems based on real-time PCR (real-time PCR) were used. Primers and probes for identification of polymorphisms of the *SUGT1*, *TMEM95*, and *SMC2* genes were selected using the TaqMan technology [15], and for the *RASGRP2* gene - using the Snake technology [16].

The test systems included real-time amplification of a fragment of the *SUGT1*, *TMEM95, SMC2,* and *RASGRP2* genes with lengths of 76, 79, 218, and 93 BP containing the mutation region using two specific primers and two allele-specific probes stained with FAM and R6G dyes.

Fluorescence detection for these genes occurred at temperatures of 61, 63, 61, and 66°C, respectively. Alleles were determined by the nature of kinetic fluorescence curves using Real-Time PC.

Test systems developed at the Center for Biotechnology and Molecular Diagnostics of the L.K. Ernst Federal Research Center for Animal Husbandry were used to diagnose the HH0, HCD, and HH5 fertility haplotypes [17], [18], [19], [20].

The test systems are based on the AS-PCR method, which involves the use of two reverse primers specific to the healthy and defective allele and one common direct primer. In this case, fragments of different lengths are amplified, which makes it possible to differentiate mutant and non-mutant alleles of the *FANCI, APOB*, and *TFB1M* genes by agarose gel electrophoresis. The results were detected by electrophoresis of AS-PCR products in 2% agarose gel with the addition of dimidium bromide and visualization under ultraviolet light. To identify the fragment lengths, a molecular marker with a length of 100 BP (500×2) was used (Biosan LLC, Russia).

The work used the data of zootechnical and breeding registration of the farm, grading of livestock and catalogs of sires of the breeding enterprise. Digital data obtained in the course

of research were processed biometrically on a personal computer using Microsoft Excel programs according to the methods of Zhivotovsky L.A. [21].

The frequency of occurrence of genotypes was calculated using the formula (1):

$$pi = ni / N \quad (1)$$

where ni - number of animals with the i-th genotype, and N - sample size.
Allele frequencies were calculated using the following formula (2):

$$pi = (2*Nii+Niy) / (2*N) \quad (2)$$

where pi - frequency of occurrence of the i-th allele, Nii - number of animals homozygous for the i-th allele, Niy - number of animals heterozygous for the i-th allele (y - any other allele), and N - sample size.
The genotype frequency error was calculated using the formula (3):

$$Sp=\sqrt{(1-p)} /N \quad (3)$$

where p - genotype frequency and N - sample size

## 3 Results and discussion

The study of 150 cows and 34 sires using the developed test systems showed that among the animals of the Vazuzsky type of the Sychevskaya breed bred in the Smolensk region, there are animals that carry genetic defects of the Simmental and Holstein breeds. The results of the screening are shown in table 1.

**Table 1**. Results of genotyping of Sychevsky cattle of the Smolensk region for carriers of genetic mutations.

| Mutations under study | Group | | | |
|---|---|---|---|---|
| | Cows (n=150) | | Sires (n=34) | |
| | Healthy | Carriers | Healthy | Carriers |
| FH4 | 150 | 0 | 34 | 0 |
| BMS | 138 (92%) | 12 (8%)±0.022 | 34 | 0 |
| TP | 148 (98.6%) | 2 (1.4%)±0.009 | 32 (94.1%) | 2 (5.9%)±0.040 |
| HH0 | 150 | 0 | 34 | 0 |
| HCD | 144 (96%) | 6 (4%)±0.016 | 34 | 0 |
| HH3 | 150 | 0 | 34 | 0 |
| HH5 | 150 | 0 | 34 | 0 |

The genetic defect thrombopathia was detected in both groups of animals studied. The frequency of occurrence of hidden carriers was 1.4% among cows and 5.9% among sires. Animals in the cow group belonged to the Aromat 3433 line and other lines, and in the bull group - to the Toreador 3032 line.

Animals carrying genetic defects subfertility and cholesterol deficiency were found only among cows. We identified 12 carriers of subfertility and 6 carriers of cholesterol deficiency, which is 8% and 4%, respectively. The linear affiliation of these animals goes back to the lines of Redad 711620016730 (subfertility), Reflection Sovering 198998 and Klever 68 (HCD).

The frequency of occurrence of undesirable alleles in the genotypes of Vazuzsky type cows according to the *TMEM95, RASGRP2*, and *APOB* genes was 0.040; 0.067; 0.020, and in the genotype of sires according to *RASGRP2* – 0.030.

Figures 1 and 2 show possible genotypes of the studied animals based on the *TMEM95* (BMS) and *RASGRP2* (TP) genes:

CC and AA (BMS/ TP) - normal genotype (there are two normal copies of the gene, homozygote). The animal is healthy, does not carry a lethal mutation.

AC and AG (BMS / TP) - there is one mutant and one normal copy of the genes (heterozygote). The animal is a carrier of the disease, does not suffer from the disease, but can transmit a mutant copy of the gene to the get.

AA and GG - there are two copies of the mutant gene (homozygote for the mutant allele). Animals with this genotype are culled from the herd due to sterility (sires) and death

The identified animal genotypes for the *APOB* gene associated with the HCD fertility haplotype are shown in figure 3. Hidden carriers of fertility haplotypes FH4, HH0, HH3, and HH5 were not found among the animals studied.

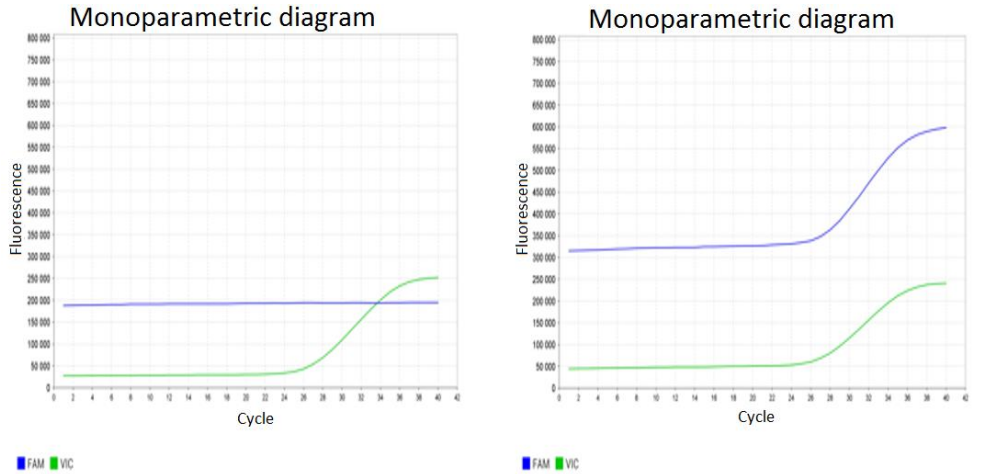


**Fig. 1**. Detection of animal genotyping results in real time by the nature of subfertility fluorescence curves (BMS) (on the left – CC genotype (homozygote); on the right – AC genotype (carrier heterozygote).

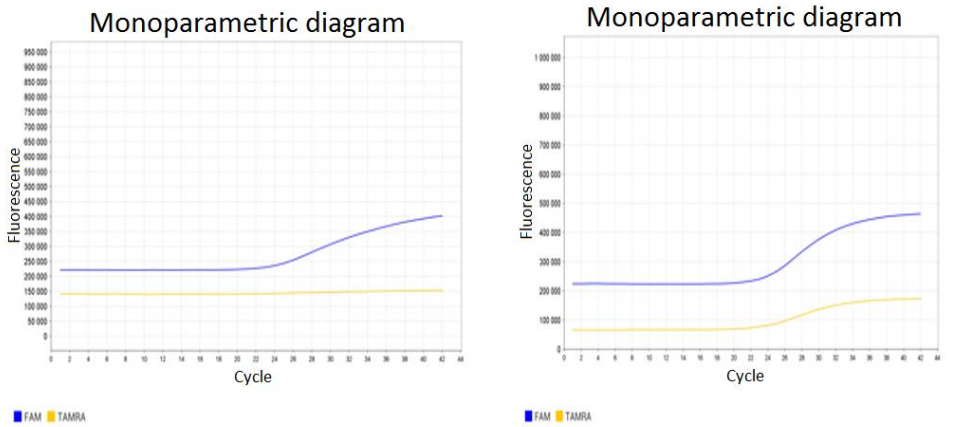


**Fig. 2.** Detection of the results of animal genotyping in real time by the nature of the fluorescence

curves for thrombopathia (TP) (on the left – the AA genotype (homozygote); on the right – the AG genotype (carrier heterozygote).

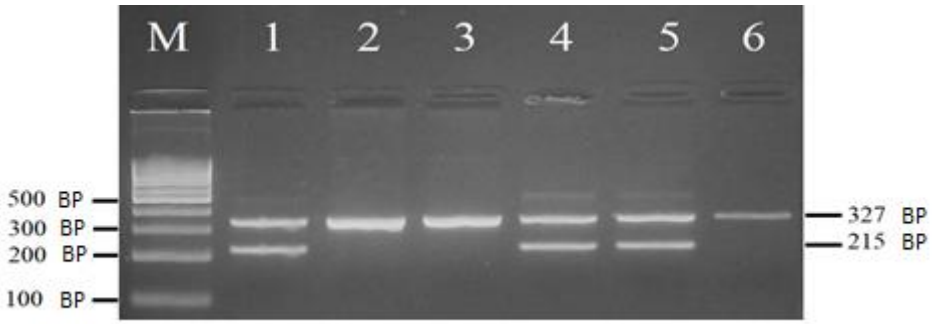


**Fig. 3**. Gel electrophoresis of PCR analysis products of the *APOB* gene associated with hereditary cholesterol deficiency (HCD). *Note:* path M - marker of "steps" length of the marker in base pairs (BP) indicated to the left of the photograph; 2, 3, 6, - not HCD carrier; 1, 4, 5 - hidden HCD carrier (length of amplified fragments in base pairs is shown on the right from the pictures: 327 BP - fragment corresponding to the normal allele, 215 BP - fragment corresponding to the mutant allele).

As can be seen from the results of the study, the most widespread in both groups of animals were hereditary anomalies characteristic of the Simmental breed of cattle, which had a great influence on the formation of the gene pool of animals in the Sychevskaya population. Analyzing the ways of distribution of genetic defects, we can assume that the high frequency of BMS and TP occurrence is not only a consequence of the participation of animals of the Simmental breed in the creation of the Sychevskaya livestock population of the Smolensk region, but also the lack of mandatory molecular genetic control of incoming breeding material from abroad. According to German scientists, the frequency of the haplotype associated with subfertility is 7.2% [22]. Earlier studies in the Russian Federation also showed high rates of subfertility in cows and sires of the Simmental breed of the Central and the Siberian Federal Districts - 5.7% [23]. The incidence of thrombopathia in the Sychevskaya livestock population of the Smolensk region is consistent with the data obtained by Swiss authors (10%) [24]. Since this mutation was detected in the genotype of both sires and cows, it is advisable and economically justified to screen both the paternal and maternal sides of the Sychevsky and Simmental livestock.

The spread of cholesterol deficiency in the Sychevskaya livestock population is associated with the blood transfusion of Holstein animals during the creation of the Vazuzsky type. Data from domestic studies show that the frequency of HCD occurrence among breeding sires is quite high (about 10%) [25], [26].

## 4 Conclusions

Thus, the analysis of the distribution of hereditary anomalies BMS, thrombopathia and fertility haplotypes FH4, HH0, HCD, HH3 and HH5 in the Smolensk population of animals of the Sychevskaya breed was carried out:

1. The results obtained give an idea of the frequency of occurrence of animals carrying genetic defects of Simmental and Holstein breeds in the population of Sychevsky cattle. According to the *RASGRP2* gene polymorphism, the frequency of occurrence in the group of cows and sires was 1.4%±0.009 and 5.9%±0.04. The frequency of subfertility and cholesterol deficiency in the group of cows was 8%±0.022 and 4%±0.016.

2. The frequencies of undesirable alleles for the genes *TMEM95, RASGRP2*, *ABOP* in the group of cows were equal to 0.040; 0.067; 0.020, in the group of sires for the *RASGRP2* gene - 0.030.

3. Obtained data on the frequency of occurrence of genetic defects indicate the need for molecular genetic control of both Sychevsky cattle and breeding material of improving breeds.

4. Test systems developed at the Center for Biotechnology and Molecular Diagnostics of Animals are a reliable and inexpensive tool for detecting animals that carry hereditary defects. This will allow to control the process of their distribution in the breed and avoid possible economic losses and problems with the gene pool in the future.

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