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Associative Effects of the Il-8 and Il-17a Genes on Reproductive Function and the Occurrence of Lethal Mutations in Cows

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ABSTRACT

In this work, single-nucleotide polymorphisms (SNPs) in the II and III exon parts of the interleukin-8 (IL-8) gene and the II exon part of IL-17 (SNP c.126G>A) were studied. The effects of IL-17A gene alleles on the reproductive ability of Holstein cows (n=164, Medeu Commerce LLP) were determined. This study revealed the absence of genetic polymorphisms at the IL-8 gene locus, where carriers of only homozygous AA genotypes for both SNPs were identified. Concerning the IL-17A gene locus (SNP c.126G>A), an excessive occurrence of the heterozygous AG genotype (+15,622 specimens) was observed. Cows successfully inseminated within 45–60 and 61–90 days after calving most often had the IL-17A gene locus with homozygous GG genotypes (50.0 and 25.0%). In this group of animals, 8.06 and 19.5% had the homozygous AA genotype, and 7.77 and 25.55% had the heterozygous AG genotype. On the basis of these results, the authors believe that the G allele positively correlates with high reproductive ability in Holstein cows. The IL-17A polymorphism (SNP c.126G>A) can be used as a DNA marker of reproductive function. In cows of the Holstein breed at Mezhdurechensk AGRO LLP (n=150) and in stud bulls of Holstein and black-and-white breeds at Asyl Tulik JSC (n=37), the prevalence of the fertility haplotype was 4.67% and 5.40%, respectively, and the complex vertebral malformation hereditary anomalies occurred in 7.33% and 8.10%, respectively. The polymerase chain reaction-restriction fragment length polymorphism and real-time polymerase chain reaction methods confirmed the high diagnostic effectiveness during genetic screening. Thus, according to the existing international strategy for eliminating harmful mutations in breeding animals, examining 10–20% of livestock for carriers of genetic defects is recommended.

Key words: Reproductive function of cows, Fertility haplotype HH6, Complex vertebral malformation, Real-time polymerase chain reaction, Genetic monitoring

INTRODUCTION

Single-nucleotide polymorphisms (SNPs) in the promoter regions of exons II, III and IV of the interleukin-8 (IL-8) gene have been well studied. An associative relationship of IL-8 gene alleles with the immune system, the incidence of mastitis, and the content of somatic cells in milk in Holstein cows has been established (Narozhnykh et al. 2023). Cows with the homozygous CC genotype at the IL-8 gene locus have minimal somatic cell content in milk compared with animals with the CT and TT genotypes (Ridhowi et al. 2018; Beishova et al. 2024). Often, the etiological factor of endometritis in cows is high contamination of the uterus in the postpartum period with conditionally pathogenic microflora, as a result of which an inflammatory process develops, usually affecting the mucous membrane of the reproductive organs (Tanbayeva et al. 2024; Jakupov et al. 2024). The available lipopolysaccharides (LPS), as a result of pathogenic microflora entering the uterine cavity, stimulate an increase in the formation of IL-6 and IL-8 mRNAs, i.e., the expression of the corresponding genes increases. However, the mechanisms regulating this process remain unclear. We established the ability of LPS to increase the synthesis of the mRNAs of IL-6 and IL-8 (Wang et al. 2018). The main physiological role of IL-8 is to perform the function of a proinflammatory chemokine involved in the activation of neutrophils in response to infection and the suppression of

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the influence of a pathogenic agent. The alleles of the IL-8 gene significantly affect the number of somatic cells in the milk of Holstein cows. Thus, studying SNPs in the promoter, intron, and exon regions of the IL-8 gene is of practical importance (Stojkovic et al. 2017).

The reproductive ability of cows is one of the most important factors affecting the profitability of dairy farming. We have studied the relationships of IL-2 polymorphisms (exon 6, chromosome 17) and IL-10 polymorphisms (exon 5, chromosome 16) with parameters related to the reproductive function of cows, including the duration of opening, interval between calving, interval from calving to first estrus, stillbirth, and ease of calving (Taherian-Ghadi et al. 2022). Serum cytokines, such as IL-17, and the somatic cell content in milk may be potential indicators of mastitis in cows. The following SNPs were found in Holstein cows in China (n= 992): g-1578A>G, g-1835G>A, and g-398T>A in the 5' flanking region and g3164T>C and g3409G>C in the exon region of the IL-17 gene (Mohyuddin et al. 2022). Polymorphisms in the cytokines IL-17A and IL-17F can affect the activity and expression of inflammatory mediators, which can affect the activity of IL-17 (Straus 2013; Saraiva et al. 2013). The identification of alleles in the II exon of the IL-17A gene (SNP c.126G>A polymorphism) was carried out via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. We used the following primers for genotyping cows: F: 5'-TGTCCTGGACCATAGAATGTTCT-3' and R: 5'-TGGCTCTTCCAGGTTGACA-3', an amplicon length of 434bp, and a primer annealing temperature of 62°C. Depending on the genotype of the animal, fragments are formed after restriction by AciI endonuclease with the C-CGC recognition site in individuals with a homozygous AA genotype of 434bp, heterozygous AG genotypes of 434bp, 229bp, and 205bp, and homozygous GG genotypes of 229bp and 205bp (Shevchenko et al. 2023; Mukanova et al. 2024). However, the results of logistic regression analysis indicate that there is no significant relationship between the IL-17A genotype in Holstein cows and the occurrence of clinical mastitis; the number of somatic cells in milk has not been revealed. This finding suggests that the marker c.126G>A is irrelevant for genetic selection against mastitis in the analyzed breed (Dusza et al. 2018; Bessembayeva et al. 2024). Polymorphisms of the IL-17A and IL-17F genes (g.24345410A>G and g.24392436C>T) can be used to predict the risk of mastitis in cows. The relationships of the IL-17A and IL-17F genes with SCC/SCS and cytokine levels are presented. The results show that the 17A and IL-17F genes can be decisive modifiers of the inflammatory process and that SNP polymorphisms can be useful as DNA markers of genetic resistance to mastitis (Usman et al. 2017).

Increasing resistance to mastitis and pathologies of reproductive organs is becoming an important goal of dairy cow breeding programs. However, resistance to mastitis and endometritis is a complex trait, and identification of alleles associated with disease resistance is difficult (Brajnik and Ogorevc 2023). This study aimed to identify the polymorphism c.495C> T in the I exon of the osteopontin (OPN) gene and analyze its relationship with a predisposition to ketosis in Holstein–Frisian cows. Cows with ketosis most often had the CC genotype, whereas cows with the TT genotype had the lowest incidence of ketosis. The results show that the TT genotype at the c.495C>T locus of the OPN gene can be an effective DNA marker of predisposition to ketosis (Bauer et al. 2023). Scientists have carried out genotyping of cows, studied the C→T SNP polymorphism in exon III of the TLR4 gene, and established the effects of alleles of this gene on indicators of reproductive function, such as the number of inseminations per pregnancy, the age of the first calving, and the level of estrogen (Sutopo et al. 2023). According to the genotyping results at the CXCR 1c.735 gene locus, a genetic polymorphism was found in Holstein cows. The influence of alleles of the CXCR1c.735 gene on the parameters of milk productivity, the incidence of clinical and subclinical mastitis, the content of somatic cells in milk, and the reproductive function of cows (retention after birth, metritis, endometritis) was studied. However, the results did not reveal the influence of the alleles of this gene on the reproductive ability of the cows (Galvão et al. 2011).

The Holstein fertility haplotype HH6 is a new autosomal recessive defect in Holstein cattle (OMIA 002149-9913). Cattle embryos with a recessive homozygous genotype die in the first 35 days of pregnancy (Medrano and de Oliveira 2014; Fritz et al. 2018; Petrov et al. 2024). The ancestor of the genetic defect of the HH6 fertility haplotype was the stud bull MOUNTAIN USAM000002070579, which was born in 1987. This mutation was designated by the authors of the study as g.29773628 A>G mutation and registered in the National Center for Biotechnology Information (NCBI) database No. rs434666183. Carriers of the HH6 mutation were detected via classical PCR-RFLP analysis (Kamiński 2019). The following primers were used to detect carriers of the fertility haplotype HH6: G.29773628A>G; missense mutation in the coding part of the SED2 gene: WD: GTTCCGCGACTGGGTGAGAT; MT: GTTCCGCGACTGGGTGAGAC; and CR: GAACCACACCACACCGCCTT. Genotyping of DNA samples from stud bulls via competitive allele-specific PCR (KASP) analysis revealed that the average fluorescence contrast ((FAM-HEX)/(FAM+HEX)) was 0.673 and 0.086 for the wild-type and mutant-type alleles of stud bulls, respectively. Notably, these two genotypes can be distinguished by comparing their fluorescence signals. According to the results of genetic screening, the occurrence of heterozygous carriers of the HH6 fertility haplotype was 0.72% (Khan et al. 2021).

Common genetic defects in Holstein cattle include the fertility haplotypes HH1, HH2, HH3, HH4, HH5, HH6, HCD, BLAD, CVM, and BS. Scientists have developed a single-tube multiplex fluorescent amplification-resistant mutation (mf-ARMS) PCR method (single–tube multiplex fluorescent amplification-refractory mutation system (mf-ARMS)) for genotyping cows for harmful genetic defects and established the effectiveness of the developed method compared with the existing method with KASP. The genotyping of 484 Holstein cows revealed that 16.12% of the carriers presented at least one genetic defect. Compared with the KASP method, the new mf-ARMS PCR method has advantages in terms of latent mutation detection and economic and time efficiency. Thus, the use of mf-ARMS PCR for genotyping Holstein cattle is expected to reduce the frequency of lethal alleles and limit the transmission of

these genetic defects (Khan et al. 2024).

Complex vertebral malformation (CVM) in cattle, the fertility haplotype HHC, a lethal autosomal recessive hereditary disease, was first described in 2001 (Agerholm et al. 2004).

A G/T mutation in the SLC35A3 gene at position 559 was studied. As a result of this mutation, the amino acid in the peptide at position 180 of valine was replaced by phenylalanine. On the basis of these studies, Japanese scientists selected primers to detect CVM mutations in cattle (Dennis et al. 1989; Agerholm et al. 2001). Among the 6,342 bulls surveyed, the percentages of heterozygous CVM carriers in the USA, Canada, the Netherlands, France, Germany, and Italy were 20.07, 6.42, 38.80, 42.85, 7.15, and 15.40%, respectively (Citek et al. 2006; Wang et al. 2012). The problem of controlling the spread of genetic defects in cattle in the context of globalization and the commercialization of breeding has become an important part of preventive veterinary medicine and corrective breeding. Polish scientists studied the prevalence of lethal CVM mutations in 2001--2005, and out of the 605 tested stud bulls, 150 heads were heterozygous carriers (Rusc and Kaminski 2007). The frequency of heterozygous CVM carriers among elite Holstein cows in the Czech Republic was 18.9% (Alaie et al. 2012). The results show that calves with birth defects are most often born from stud bulls that carry CVM (Rusc and Kaminski 2007). Chinese scientists amplified the desired DNA fragment of the SLC35A3 gene via real-time PCR (PCR with detection of accumulated amplification products in real-time) developed by Loche (Roche) via TaqMan probes (Zhang et al. 2012).

SNP 1117G>A, 1125A>C, and 1204T>C polymorphisms in the HSP70 gene have been identified in cattle. These genes are associated with thermal tolerance in seven breeds of Indonesia (Prihandini et al. 2022). An important criterion for the genetic diversity of any breed is the effective population size. Thus, the local Bali breed had the optimal population, although the effective population size of the Bali breeding center cattle was greater than that of commercial farmers because of the widespread use of artificial insemination. (effective population size, Ne) (Sudrajad et al. 2022).

Thus, the study of the associative effects of IL-8 and IL-17A gene alleles on the reproductive function of cows and the prevalence of hidden mutations accompanied by impaired embryonic development in cows is urgently needed for dairy cattle breeding in Kazakhstan. Recessive inheritance patterns of genetic defects allow carriers to function normally, but homozygous recessive genotypes cause embryo loss or neonatal death. Therefore, rapid identification of carriers is essential for controlling these genetic defects.

The purpose of this work was to study the effects of IL–8 and IL-17A gene alleles on the reproductive function of cows. The objective of this study was to evaluate the occurrence of carriers of HH6 and HHC fertility haplotypes in Holstein cows subjected to foreign selection and bred in Kazakhstan.

MATERIALS AND METHODS

The study was conducted in accordance with the recommendations for animal experiments outlined by the International Council for Laboratory Animal Science (ICLAS, 2024).

A total of 164 frozen blood samples from Holstein cattle from a breeding farm located in the Karasai district of the Almaty region, Kazakhstan, were used as material for genotyping the loci of the IL-8 and IL-17A genes. For PCR diagnostics of carriers of the HH6 and HHC fertility haplotypes, we used 150 blood samples of cows of the Holstein breed from the Mezhdurechensk AGRO LLP dairy farm and 37 sperm samples of stud bulls of the Holstein and black-and-white breeds from Asyl Tulik JSC. Blood for DNA extraction was taken from the jugular vein, sometimes from the caudal vein, in a volume of 2ml into vacuum tubes with ethylene diamine tetraacetate (EDTA). Genomic DNA was isolated from frozen blood from the Green Biotechnology and Cellular Engineering Laboratory of the Kazakh-Japanese Innovation Center of Kazakh National Agrarian Research University in two ways: via the classical phenolic method and via the PureLink™ Genomic DNA Mini Kit according to the manufacturer's instructions.

Frozen sperm from stud bulls were used as the other group of materials. DNA extraction from frozen sperm obtained from stud bulls was carried out via the Bahnak method (Bahnak 1993). The technique of DNA extraction from sperm. One milliliter of sperm was centrifuged for 5min at 4,000×g. The settled cells were washed with 0.15M NaCl solution and 2mm EDTA and centrifuged again for 5min at 4,000×g. This procedure was repeated two more times. After the last centrifugation, the upper layer was removed via a pipette, after which 5mL of buffer was added to the precipitate with the following composition: 6M guanidine thiocyanate, 25mM sodium citrate (pH 7.0), 0.5% saccharosyl, and 0.1 M2-mercaptoethanol. The mixture was subsequently incubated at 37°C for 30minutes. Before deproteinization, the lysed DNA mixture was diluted with 0.15M NaCl solution at a ratio of 1:4. Deproteinization was carried out according to the usual method by adding an equal volume of a mixture of phenol, chloroform, and isoamyl alcohol (24:24:1). After centrifugation, the top layer was carefully removed with a pipette and deposited in two volumes of 96% ethyl alcohol. The DNA was dried for 2–5min under a hood and dissolved in Tris/EDTA (TE) buffer. The quality of the isolated DNA was assessed by measuring the concentration via microspectrophotometric analysis (NanoDrop2000), and the degree of DNA purification was determined by the ratio of A260/A280. The sequences of the primers used and the conditions for amplification for genotyping DNA samples at the IL-8 II, IL-8 III, IL-8 III, and SDE2 gene loci are presented in Table 1 and 2.

Our primers F-CCATGGCATTTAATATGGGACT 59.94
R-TCAGCAGCAGCAGAAAATGTA 59.77

Experimental simulations on the identification of IL-8 gene alleles in *Bos taurus* samples were carried out with the following sequence: search for the IL-8 gene sequence on the NCBI website, analysis of the gene sequence in GenBank and FASTA formats, and determination of SNPs in the exon of the IL-8 gene (Sonali 2014).

For genetic monitoring of the HH6 haplotype in cows, we used the following primers: direct F-5'-GACGGAAGCCCTCACTATCA-3' and reverse R-5'-CTTCTCTTAGCAACGCCTCG-3', as described in our work. The identification of the wild-type and mutant allele

Table 1: Primer sequences for cow genotyping by the IL-8 II, IL-8 III, IL-17A/AciI, and SDE2 gene loci

Gene name	Primer sequences 5'→3'	Fraction GC %	References
IL-8 II	F-GCCAAGCTGTGCTTATGGAT-3'	50.00	Sonali (2014)
IL-8 II	R-GCTGGATTCTTCCACGTCTC-3'	55.00	
IL-8 III	F-CCATGGCATTTAATATGGGACT-3'	40.90	Our design
IL-8 III	R-TCAGCAGCAGCAGAAAATGTA-3'	40.90	
IL-17A	F-CATCTCATCAGCTTTTCACTTAACA-3'	36.00	Ziyabek et al. (2021)
IL-17A	R-AGAGGTTTTATCTGGGGTGCTTA-3'	40.00	
SDE2	F-GACGGAAGCCCTCACTATCA-3'	55.00	Fritz et al. (2018)
SDE2	R-CTTCTCTTAGCAACGCCTCG-3'	55.00	

Table 2: PCR conditions for genotyping DNA samples from Holstein cows by the IL-8 II, IL-8 III, IL-17A, and SDE2 gene loci

Amplification conditions	IL-8 II	IL-8 III	IL-17A	SDE2
Initial denaturation	95°C; 3 min	95°C; 3 min	95°C; 5 min	95°C; 3 min
Denaturation	95°C; 30 s	95°C; 30 s	95°C; 45 s	94°C; 30 s
Annealing	56°C; 45 s	60°C; 45 s	60°C; 30 s	61°C; 30 s
Elongation	72°C; 45 s	72°C; 45 s	72°C; 50 s	72°C; 30 s
Number of cycles	35	35	34	35
Final synthesis	72°C; 10 min	72°C; 10 min	72°C; 7 min	72°C; 5 min

types of the SDE2 gene was carried out via PCR restriction of the product by the BccI endonuclease with the CCATC recognition site (Khan et al. 2021). Below, one can see a fragment of the SDE2 gene and a sequence of the gene complementary to the reverse primer: CR: 5'-GAACCACACCACACCGCCTT-3', described previously (Fritz et al. 2018).

5'-GCGACTGGGTGAGATGGCGGAGACGGCGGCGC TGGTGTGGCTTCGGGGCCCTGGCT

TCGGGTGC*AAGGCGGTGTGGTGTGGTTC*-3'. The size of the amplified fragment of the SDE2 gene was 524 base pairs (bp). However, using information from the NCBI website in FASTA format, we determined the sequence of the SDE2 gene section with a length of 84 bp. The rest of the sequence of this gene appears to be located in the 5'-flanking or promoter region of the specified gene.

The genotyping of the DNA samples from the Stud bulls from Asyl-Tulik JSC was carried out via real-time PCR. In our work, the following primers with the following sequences were used for real-time PCR SNP diagnosis of CVM point mutations: F-5'-AGCTGGCACAATTTGTAGGT-3' and R-5'-CTCAAAGTAAACCCCAGCAAAGC-3' and labeled primers F-VIC-5'-TCATGGCAGTTCTCA-3' and R-FAM-5'-TCATGGCATTTCTCA-3'. The amplification conditions were as follows: I, denaturation at 95°C for 10min; I, denaturation at 95°C for 15sec; and II, annealing of the primers and elongation at 60°C for 1min; the number of cycles was 40. The real-time PCR components used were as follows: TaqMan Genotyping Master Mix (40× genotyping kit), 12.5μL; direct and reverse primers, 0.625μL; DNA, 1.0μL; and distilled water, 10.8μL. After this, the mixture was mixed on a vortex, 24μL of the reaction mixture was transferred to the strips, and 1μL of DNA was added at a concentration of 20–40ng/μL. We used latex gloves to avoid contamination of the strips. Allelic recognition was performed by analyzing amplification graphs in real time. Theoretically, VIC-type probes are complementary only to the wild type and form a standard amplification graph, whereas FAM probes are complementary only to mutant alleles and form an amplification graph. Thus, the genotype can be accurately determined by comparing the amplification graphs.

RESULTS

We studied SNPs located in exons II and III of the gene via the locus of the IL-8 gene and performed genotyping of 164 DNA samples from Holstein cows from the Medeu Commerce LLP breeding farm via PCR-RFLP analysis (Fig. 1 and 3). The absence of genetic polymorphisms in the studied animals was found for both SNPs. All the animals were carriers of the homozygous genotypes AA and AA for both SNPs (Fig. 2 and 4).

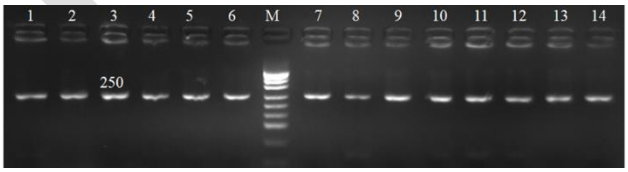

Fig. 1: Electrophoregram of the PCR product of the IL-8 exon 2 gene; amplicon length: 250 bp; M-DNA marker pUC19/MspI

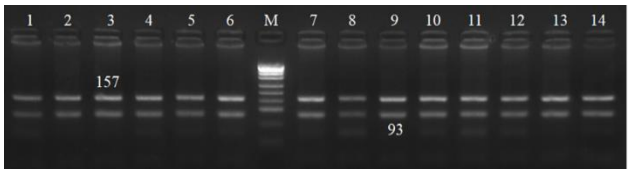

Fig. 2: Electrophoregram of the PCR product of the IL-8 gene, exon 2, after restriction by endonuclease HaeIII, wells 1--6, 7--14 homozygous AA genotype, fragments: 157 bp, 93 bp. M-DNA marker pUC19/MspI

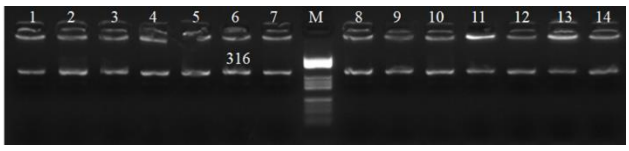

Fig. 3: PCR electropherogram of the IL-8 gene product, exon 3, amplicon length: 316 bp, M-DNA marker pUC19/MspI

To identify alleles of the IL-17A gene (c.126G>A), 3 primer sequences were selected via the Primer 3 program: F 5'-CATCTCATCAGCTTTTCACTTAACA-3' and R 5'-AGAGGTTTTATCTGGGGTGCTTA-3'. The PCR size of the IL-17A gene product was 380 bp; after restriction by AciI endonuclease, the following fragments were formed:

380bp, 253bp, and 127bp in heterozygous AG genotype animals; 380bp in the homozygous AA genotype; and 253bp and 127bp in the homozygous GG genotype specimens (Fig. 5 and 6) (Ziyabek et al. 2021).

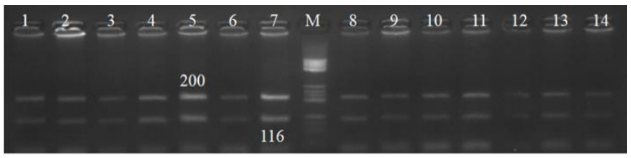

Fig. 4: Electrophoregram of the PCR product of the IL-8 gene, exon 3, after restriction by SspI endonuclease, wells 1--7, 8--14 homozygous genotype AA, fragments: 200 bp, 116 bp, M-DNA marker pUC19/MspI

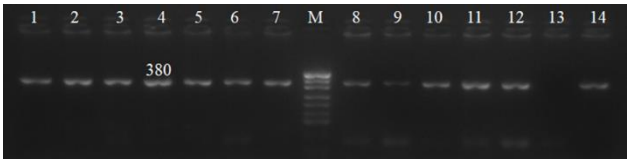

Fig. 5: Electrophoregram of the PCR product of the IL-17A gene. Wells 1--7 and 8--14 were amplified with a size of 380 bp, and the M-DNA marker pUC19/MspI

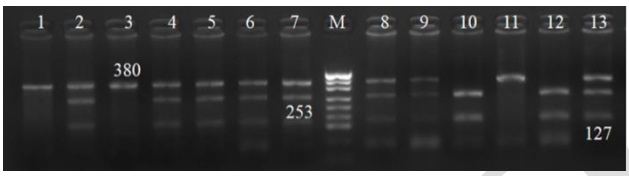

Fig. 6: Electrophoregram of the IL-17A gene amplicon after restriction by AciI endonuclease; wells 1, 3, and 11, homozygous AA genotype: 380 bp; wells 2, 4--7, 8--9, and 13, heterozygous AG genotype: 380 bp, 253 bp and 127 bp; wells 10,12, homozygous GG genotype: 253 bp and 127 bp; M-DNA marker pUC19/MspI

The genetic defect fertility haplotype HH6 in cattle arose as a result of a point mutation at position g.29773628 A>G in the coding region of the SDE2 gene. Therefore, the PCR-PDRF method was chosen to diagnose heterozygous carriers of this genetic defect, and the PCR product was hydrolyzed by BccI restrictase to identify wild-type and mutant alleles of the SDE2 gene (Fig. 7 and 8).

To identify carriers of the mutation in the SLC35A3 gene, real-time PCR was used with a real-time StepOnePlus device. The selection of sequences for real-time PCR was carried out by Applied Biosystems on the basis of the results to determine the location of the point mutation in the coding part of the SLC35A3 gene (Zhang et al. 2012). The design of the test system for real-time PCR diagnostics was carried out via special Primer Express software (Applied Biosystems 2002). Allelic discrimination of the wild-type and mutant types of the SLC35A3 gene was carried out according to the following principle: in carriers of the wild-type allele, amplification was successfully performed with a VIC probe, and one curve was detected on the display (Fig. 9). If the test sample belongs to a heterozygous carrier, amplification occurs simultaneously with two types, wild-type and mutant types of alleles with VIC and FAM probes. Accordingly, two curves are visible on the display, which are characteristic of heterozygous mutation carriers (Fig. 10).

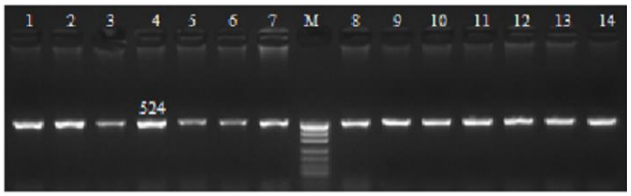

Fig. 7: Electrophoregram of the amplicon of the SDE2 gene; fragment size, 524 bp; M-DNA marker, pUC19/MspI

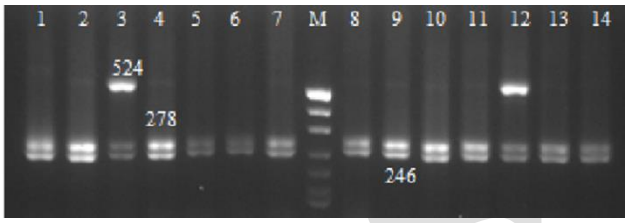

Fig. 8: Electrophoregram of the amplicon of the SDE2 gene; after restriction by BccI endonuclease, wells 1--2, 4--7, 8--11, and 13--14 homozygous healthy animals; fragments: 278 bp and 246 bp; wells: 3.12 heterozygous individuals; fragments: 524 bp, 278 bp, and 246 bp; M-DNA marker pUC19/MspI

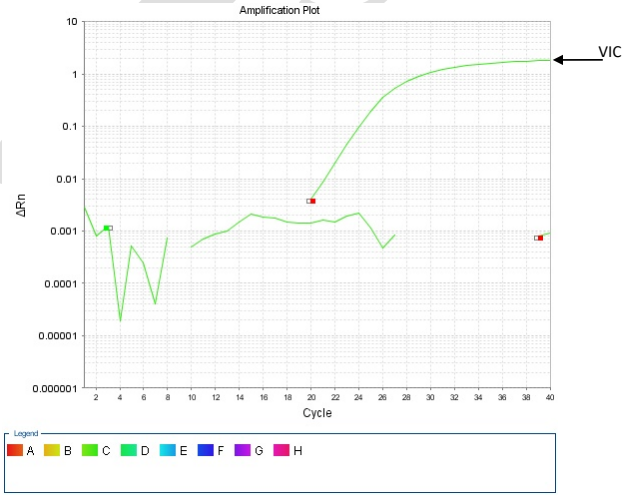

Fig. 9: Results of real-time PCR diagnosis of a point mutation in the SLC35A3 gene in a homozygous healthy animal at the CVM locus (amplification with a VIC probe, wild type).

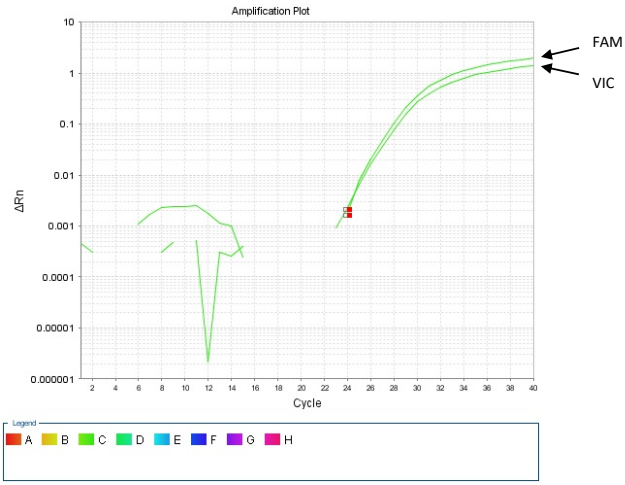

Fig. 10: Graphical representation of the results of real-time PCR diagnostics of a point mutation in the SLC35A3 gene and a heterozygous CVM carrier (amplification with a FAM probe, mutant type, and with a VIC probe, wild type).

DISCUSSION

The expression of genes of the cytokine family is important in the pathogenesis of inflammatory processes, including those in reproductive organs and the mammary gland. Most studies on the influence of IL-6, IL-8, and IL-17A gene alleles consider the associative effect of alleles of these genes with resistance to mastitis and the content of somatic cells in milk (Usman et al. 2017; Ridhowi et al. 2018; Dusza et al. 2018; Taherian-Ghadi et al. 2022). The associative effects of the IL-6, IL-8, and IL-17A alleles on the reproductive function of cows have been poorly studied. Currently, researchers are conducting functional studies on the influence of gene alleles viewed as candidates for various economically useful traits in dairy cattle breeding, searching for DNA markers of disease resistance and ensuring high reproductive functions. Thus, scientific research has established that cows with homozygous GG genotypes have better results than animals with other AA and AG genotypes in terms of the TNFa gene locus (Kawasaki et al. 2014; Bimenova et al. 2019). No genetic polymorphisms were detected in the studied population via the IL-8 gene locus in exons II and III, and a single homozygous AA genotype was detected in both studied loci, which indicates disruptions of the gene balance and the absence of genetic diversity. By the IL-17A gene locus, the genotypes of cows with the SNP c.126G>A were determined, and genetic variants with frequencies of the AA (0.38), AG (0.55), and GG (0.07) genotypes were identified. Here, the animals with the heterozygous AG genotype prevailed (55.0%), and excessive occurrence of the A allele (0.65) was observed (Table 3). The studied cows presented the same level of deficiency in the frequency of genetic variants AA and GG, which was -7,811 individuals, and the digital value of χ^2 was 7,2348.

In the Commonwealth of Independent States (CIS), the optimal timing of cow insemination after calving is controversial. Most researchers believe that cows should be inseminated within 30–45 days after calving. However, other researchers consider the optimal period of insemination for cows with the current technology of feeding and keeping, considering high milk productivity, to be from 60-90 days after calving (Crowe et al. 2018). The parameters of the reproductive function of cows are influenced by multiple factors, such as pathologies of the reproductive organs, endocrine disorders, feeding, keeping, metabolic disorders, and genetic factors. In our study, the effects of an IL-17A gene allele (SNP c.126G>A) on the reproductive function of cows were analyzed. As a criterion for assessing reproductive function, the level of effective fertilization of cows within 45–60, 61–90, 91–120, or more than 121 days after calving was determined. A total of 164 heads were genotyped (cows of the Holstein breed with 1st, 2nd, or 3rd lactation with a productivity of 8,500–9,000kg of milk).

According to the IL-17A gene locus (SNP c.126G>A), 62 heads with the AA genotype, 90 heads with the heterozygous AG genotype, and 12 heads with the homozygous GG genotype were identified. The distribution of genetic variants in the group of cows with high reproductive function, successfully inseminated from 45-60 days after calving, was 8.06% with the genetic variant AA, 7.77% with the genotype AG, and 50.0% with the genotype GG. In the second group of animals, where the indicators of reproductive ability were optimal, in cows successfully inseminated from 61-90 days after calving, the frequency of genetic variants was as follows: AA: 19.5%, AG: 25.55%, and GG: 25.0%. In cows with sufficiently low indicators of reproductive function that were successfully inseminated within 91–120 days and more than 121 days after calving, the distribution of genetic variants of the IL-17A gene (SNP c.126G>A) was as follows: AA: 33.8%, 38.7%, AG: 41.11%, 25.55%, and GG: 25.0%, 8.33%, respectively. Thus, the analysis of the effectiveness of artificial insemination of cows with different genotypes by the IL-17A gene locus (SNP c.126G>A) indicated that in the first group with high fertility, most animals (50.0%) had the homozygous GG genotype. The percentages of cows with the AA and AG genotypes were low, at 8.06% and 7.77%, respectively. In the third and fourth groups of animals, where fertilization was low, most animals had the AA (33.8 and 38.7%) and AG (41.11 and 25.55%) genotypes, whereas the proportion of cows with the GG genotype was minimal and reached 16.6 and 8.33%, respectively. In the studied cows, reproductive ability indicators were high in cows with the homozygous GG genotype. Thus, this genetic variant is a desirable genotype for the IL-17A gene locus (SNP c.126G>A).

Table 3: The theoretical and actual distributions of genetic variants associated with the IL-17A/AciI gene locus in cows (n=164) from the Medeu Commerce LLP breeding farm

Groups	Allele frequency		In the II exon part of IL-17A/AciI (SNP c.126G>A)			χ^2
	A	G	AA	AG	GG	
			Theoretical distribution of genotypes			
Cows			69.811	74.378	19.811	7.2348
			Actual distribution of genotypes			
Cows	0.65	0.35	62	90	12	
Deviation			-7.811	+15.622	-7.811	

Table 4: Results of genotyping cows by the IL-17A gene locus and the associative effect of alleles of this gene on the reproductive function of cows

Holstein cows with different indicators of reproductive function	Genotype distribution by IL-17A locus (SNP c.126G>A)					
	AA		AG		GG	
	n	%	n	%	n	%
Insemination within 45-60 days (n=30)	5	8.06	7	7.77	6	50.0
Insemination within 61-90 days (n=30)	12	19.5	23	25.55	3	25.0
Insemination within 91-120 days (n=30)	21	33.8	37	41.11	2	16.6
Insemination after more than 121 days (n=30)	24	38.7	23	25.55	1	8.33
Total	62		90		12	

Table 5: The prevalence of heterozygous carriers of the HH6 fertility haplotype in Holstein cows and the occurrence of HHC carriers in stud bulls of the Asyl Tulik JSC breeding center

Genetic defect, gene, diagnosis method	Breed, sex, and number of animals	
	Holstein, cows (n=150)	Holstein, black and white, bulls (n=37)
Fertility haplotype HH6, SDE2, PCR-PDRF		
wt/wt (homozygous genotype)	143 (95.33)	35 (94.60)
wt/mt (heterozygous genotype)	7 (4.67)	2 (5.40)
mt/mt (homozygous genotype)	0	0
CVM, SLC35A3, real-time PCR		
wt/wt (homozygous TT genotype)	139 (92.67)	34 (91.89)
wt/mt (heterozygous TC genotype)	11 (7.33)	3 (8.10)
mt/mt (homozygous CC genotype)	0	0

The analysis of the data in Table 5 shows that the prevalence of heterozygous carriers of the HH6 fertility haplotype was 4.67% in cows (n=150) and 5.40% in stud bulls (n=37) from Asyl Tulik JSC. A higher incidence rate was observed for the CVM genetic defect, with values of 7.33% in cows and 8.10% in stud bulls. The use of PCR-RFLP to detect carriers of the CVM genetic defect is a complex and time-consuming process since there is no appropriate restriction to detect a point mutation. Various variants of the PCR method are typically used to detect CVM carriers, such as amplification of a gene site via allele-specific primers (AS-PCR), a restriction site created during amplification to detect a point mutation (CRS-PCR), and introduction of a single nucleotide replacement in the primer sequence to create a restriction site for an endonuclease (PCR-PIRA (primer-introduced restriction analysis)) (Nagahata et al. 2002; Reshetnikova et al. 2024). Therefore, we developed a real-time PCR diagnostic method for detecting carriers of CVM genetic defects. Heterozygous mutation carriers undergo amplification with two VIC and FAM probes, and two curves are formed. Thus, of the 150 Holstein cows tested, seven were carriers of the fertility haplotype (HH6), and 11 were CVM carriers, of which two were carriers of two genetic anomalies (HH6, CVM) (1.33%).

Conclusion

According to our results, the studied cows (n=164) of the Holstein breed from the Medeu Commerce LLP breeding farm presented genetic monomorphism in exons II and III of the IL-8 gene; therefore, the effects of IL-8 gene alleles on the reproductive function of cows have not been established. By another locus of the IL-17A gene (SNP c.126G>A), a well-expressed genetic polymorphism was found in the tested cows (n=164), where the occurrence of the genetic variants AA, AG, and GG was 0.38, 0.55, and 0.07, respectively. To assess the effect of IL-17A gene alleles, we used the following criterion: the time of successful insemination of cows after calving. Cows fertilized within 45–60 and 61–90 days after calving mostly had homozygous GG genotypes (50.0 and 25.0%, respectively), which indirectly indicates a positive effect of this genotype on cow fertility. DNA certification of 150 Holstein breed cows from the Mezhdurechensk AGRO LLP dairy farm and 37 Holstein bulls and black-and-white breeds from Asyl Tulik JSC was carried out. Both genetic defects were detected in the studied animal population (HH6, CVM). The frequency of the fertility haplotype was 4.67% in cows and 5.40% in stud bulls, whereas the frequency of the CVM haplotype was 7.33% in cows and 8.10% in stud bulls. The detection of heterozygous carriers of the HH6 fertility haplotype via PCR-RFLP analysis and complex spinal deformity (CVM) via real-time PCR proved to be an effective, fast, and accurate diagnostic method. To control the spread of hereditary abnormalities in breeding animals, genetic screening should be carried out, especially for stud bulls.

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