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Improvement of Diagnostic Methods for Carriers of HH2 and JH1 Fertility Haplotypes in Holstein and Jersey Cows

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ABSTRACT

This work aimed to improve the Holstein and Jersey cow HH2 and HJ1 fertility haplotype diagnostics, which indicate early embryonic mortality. Studies were conducted on 150 cows and 78 breeding bulls of the Holstein breed and 150 cows and 6 bulls of the Jersey breed. The Republic of Kazakhstan's cow population had 5.4 and 3.4% haplotypes associated with cow fertility, respectively. Due to its low cost, speed, and restrictions exclusion, the Tetra-primer ARMS-PCR reaction is recommended for detecting heterozygous carriers of the HH2 fertility haplotype. The proposed real-time polymerase chain reaction method accurately and quickly distinguishes homozygous healthy Jersey cattle and heterozygous carriers of the HJ1 haplotype of fertility. The DNA content in the reaction mixture should not be below 15ng/µL for a reliable reaction. Periodically screening breeding stock and bulls for genetic anomalies can help control the spread of harmful mutations.

Key words: Single nucleotide deletion, Point mutation, Polymerase chain reaction, Genetic monitoring, Cattle

INTRODUCTION

Currently, 669 hereditary anomalies have been identified in cattle, with over 60 genetic defects detectable through molecular genetic diagnostic techniques (Hozé et al. 2020). Annually, the incidence of diagnosed genetic disorders in cattle escalates, rendering research focused on identifying such anomalies pertinent and essential. Genetic defects are most frequently identified in Holstein, Ayrshire, Jersey, and Brown Swiss breeds, where artificial insemination utilising semen from superior bulls is extensively practised (Turysbayeva et al. 2023; Chelladurai et al. 2024). The primary manifestation of genetic disorders is embryonic mortality, resulting in diminished reproductive capacity in cows (Jakupov et al. 2021; Roman et al. 2024).

Ussenbekov et al. (2022) developed and successfully implemented modern molecular genetic diagnostic techniques, such as polymerase chain reaction-restriction fragment length polymorphism (PCR-PRDF) analysis and Real-PCR diagnostics, to identify hidden genetic defects in cattle. These included hereditary anomalies such as BLAD, CVM, DUMPS, BC, FXID, Brachyspina syndrome, fertility haplotypes in cows HH1, HH3, HH4, HH5 and HCD, as well as subfertility syndrome in breeding bulls. In Holstein cattle from other countries, the Republic of Kazakhstan found heterozygous carriers of Fertility Haplotype HCD, a mutation linked to low cholesterol. This happened 11.8% of the time in these animals.

According to Upperman et al. (2019), in nations with large breeding stock, it is not cost-effective to test every animal in the nation; instead, a study of at least 20% of the total cattle population is enough to determine the prevalence of hidden anomalies in the population. Holstein cattle have the most hidden genetic defects because they have a large population, are bred in 160 countries, and are covered by artificial insemination, which accelerates hereditary anomalies (Abutalip et al. 2024). Hozé et al. (2020) found that 1.6, 3.1, 4.2, 1.8%, HBY, 1.6, and 1.1% of Holsteins carry haplotypes that reduce fertility. The frequency of alleles associated with decreased reproductive potential in cows is the main reason for genetic monitoring for heterozygous carriers of fertility haplotypes, which is a necessary veterinary measure for the country's agroindustrial

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complex. Veterinary science must develop methods to detect hereditary anomalies. Brito et al. (2021) indicate that a lot of breeding aimed at improving genes caused an unwanted genetic load to build up in the population, which made dairy cattle less able to reproduce, stay healthy, live longer, and have other traits that are economically useful.

Shormanova et al. (2024) point out 9 haplotypes that are recommended for breeding organisations to control the spread of mutations associated with the fertility haplotypes HHC, HHB, HH0, HHCD, HHD, HH1, HH3, HH4, and HH5. Häfliger et al. (2022) identified 4 new haplotypes, HH13, HH21, HH25, and HH35, which also reduce the reproductive capacity of cows. In the work of Briano-Rodriguez et al. (2021), the frequency of recessive haplotypes was: HHM – 4.18%, HH0 – 3.39%, HCD – 2.61%, HHC – 2.09%, HHB-BLAD – 1.04%, HH1 – 4.44%, HH3 – 3.13%, HH4 – 1.04%, NN5 – 0.26%. The action of these mutations spreads to other breeds through interbreeding. Sudhakar et al. (2023) indicate the detection of genetic abnormalities in crossbred stock. According to Aubekerova et al. (2022), embryonic mortality ranged from 12.5 to 22.0%. Carriers of three fertility haplotypes, HH3, HH5, and HCD, were identified. Bimenova et al. (2022) indicate frequencies of HH3 – 3.23%, HH5 – 8.35%. A study by Kumar et al. (2021) used a tetra-amplification system, PCR primer-induced restriction analysis, and PCR restriction fragment length polymorphism to find haplotypes HH1, HH3, and HH4.

Haplotype HH2 mutation, also harmful to embryonic development, was found. Yang et al. (2022) say this mutation's main pathological factor is a change in the protein IFT80, which regulates blastula formation. Clark et al. (2021) found haplotype 2 (HH2) in 1.21% of US Holsteins. This study identified genetic abnormalities by amplifying, visualising, and sequencing target gRNA sequences on an agarose gel. According to Ortega et al. (2022), real-time PCR cannot detect mutant and original gene alleles, so it cannot always identify heterozygous carriers of this genotype. Zhang et al. (2020) indicate that the KASP reaction method can find almost all genetic anomalies in cattle by using specific primers for IFT80 genes that are either wild-type or mutant. Thus, the literature review shows that Holstein and Jersey cow HH2 and JH1 haplotype diagnostics and distribution are understudied.

The study's goal was to improve the ways of finding the HH2 and HJ1 fertility haplotypes in Holstein and Jersey cows by finding a single nucleotide deletion (g.107172616delT) in the 11th exon of the IFT8 gene using Tetra-Primer ARMS-PCR and the mutant allele type of the CWC15 gene using real-time polymerase chain reaction.

MATERIALS AND METHODS

The authors used frozen blood from 150 Holstein cows from Mezhdurechensk AGRO LLP and 150 Jersey cows from the breeding farm "Aidarbayev" in the Almaty region, along with frozen semen from bulls from producers Asyl Tulik JSC (60 samples of the Holstein breed), Taurus LLP (18 samples of the Holstein breed, and 6 samples of the Jersey breed). Nucleic acids were isolated from blood samples using a commercial PureLink™ Genomic DNA Mini Kit from Thermo Fisher Scientific (USA). The authors collected blood from the tail vein into vacuum tubes, using an ethylenediaminetetraacetic acid solution as an anticoagulant. The authors followed the instructions for the extraction protocol before using the commercial kit.

DNA was extracted from frozen breeding bull semen using the method described by Alarcón-Zúñiga et al. (2016). First, 1mL of semen was centrifuged at 4,000g for 5 minutes. After the initial centrifugation, the semen was washed with 0.15M sodium chloride and 2M EDTA to remove impurities. The settled cells were then subjected to a second centrifugation step at 4,000g for 5 minutes to further isolate the cellular material. This was done several times. After the last centrifugation, the top layer was pipetted off, and 5mL of cell lysis buffer was added to the precipitate. There was 0.2mg/mL proteinase K, 30 mM sodium citrate (pH 7), 0.3M β-mercaptoethanol, and 6M guanidine thiocyanate in the buffer. After washing the precipitate with 1mL of FCl solution (25 phenol: 24, chloroform: 1 isoamyl alcohol), it was centrifuged at 9,000rpm for 10min at 4°C. After repeating this with chloroform solution (24 chloroform: 1 isoamyl alcohol), RNase was incubated at 37°C for 30min. After precipitation with cold isopropanol and 0.1 volume of 3M sodium acetate, the mixture was incubated overnight at -20°C. The sample underwent centrifugation at 9,000rpm for 10min at 4°C. The top layer was pipetted off after centrifugation and precipitated in two volumes of 96% ethyl alcohol. DNA was dissolved in TE buffer after 2-5min of fume hood drying. NanoDrop™ 2000 microspectrophotometric analysis was used to find out how much isolated DNA there was, and the A260/A280 ratio showed how pure the DNA was.

The Tetra-Primer ARMS-PCR reaction detected HH2 fertility haplotype carriers. The Primer 1 program selected forward and reverse primer nucleotides. The desired gene fragment was amplified using external primers. A gene fragment was amplified using internal primers with an SNP polymorphism at the last nucleotide to detect wild-type or mutant alleles. Jersey cow DNA was tested using real-time polymerase chain reaction as an alternative diagnostic method. Forward external F-5'-GACAGACCACTCAGGATGCC-3' and reverse external R-5'-TCCTCCTCCAACTCTCTCCTCCTCCTGAAGTC-3' and internal forward labelled F-5'-FAM-ACGGTTTCAAACCTC-3' and reverse F-5'-VIC-ACGGGGTTTCGAACCTC-3 are recommended primer sequences.

The enhanced real-time PCR methodology for diagnosing HJ1 haplotype carriers in Jersey cattle involves preparing a reaction mixture in an Eppendorf tube. For one reaction, the components are as follows: 5.0µL of TaqMan Genotyping Master Mix, 0.25µL of the TaqMan test system (which includes the primer and probe mixture), and 3.75µL of bidistilled water. These components are combined to create a final reaction mixture that is ready for PCR amplification. The mixture is subsequently agitated using a vortex, and 9µL of the reaction mixture is allocated into strips, to which 1µL of each DNA sample, with a concentration of 20-40ng/µL, is incorporated. A StepOnePlus™ Real-Time PCR System amplifier from Thermo Fisher Scientific (USA) picks up the fluorescent signal and decides how much of it to amplify the DNA.

The DNA concentration in 150 samples from Jersey cows ranged from 159.4ng/µL to 1793.2ng/µL; the mean concentration was 444.7ng/µL, and the DNA purification grade ranged from 1.77 to 2. To optimize the DNA concentration for real-time PCR, DNA was diluted in a ratio of 10µL of tested DNA and added to 50µL of distilled water. In the first step, after diluting the DNA samples, the DNA concentration was remeasured, which ranged from 11.2ng/µL to 56.8ng/µL. The first 48 DNA samples were tested by real-time PCR using this technique by diluting the DNA samples and the remaining 102 DNA samples were tested without first diluting the DNA samples (Fig. 1 and 2).

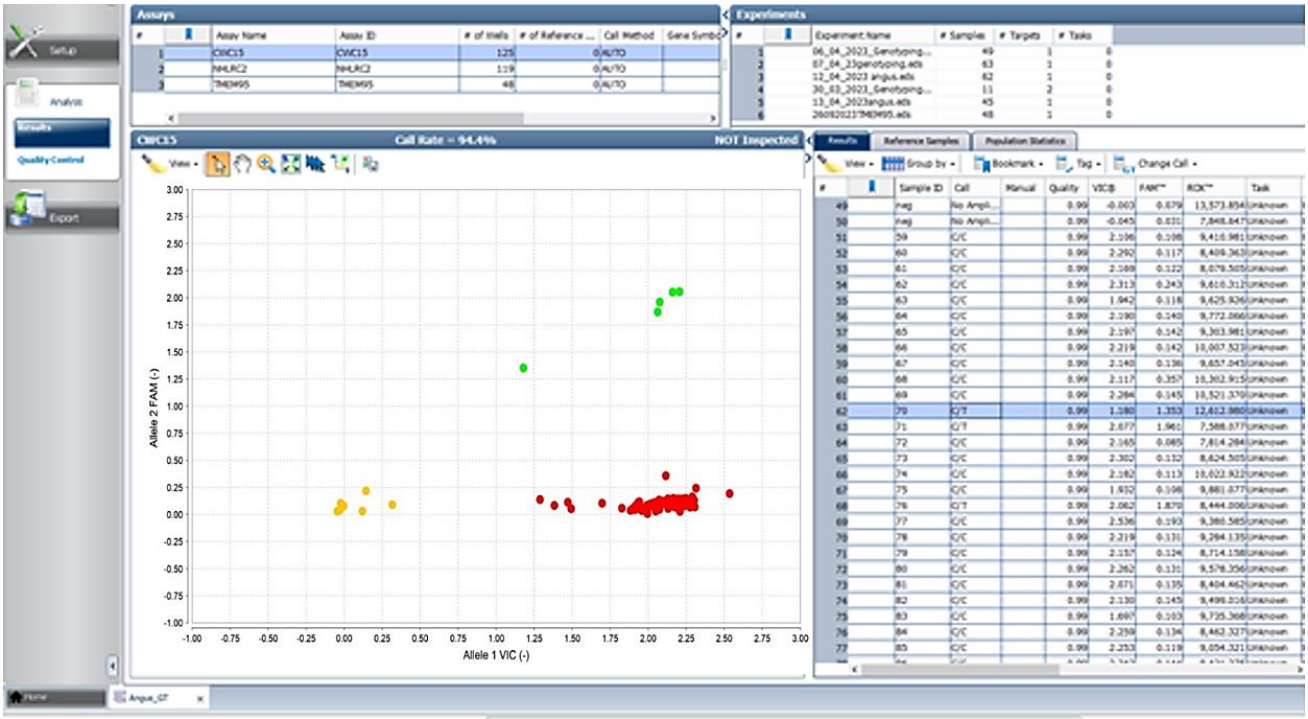

Fig. 1: Allelic discrimination plot, CT heterozygous carrier of CWC15 mutation. Note: samples, cow and numbers 2957, 65510, 6073, 11487, 2160 (amplification with VIC probe, wild type, allele C, amplification with FAM probe, mutant type, allele T). Sample No. 70, highlighted in blue (amplification with VIC probe, wild type, allele C, amplification with FAM probe, mutant type, allele T).

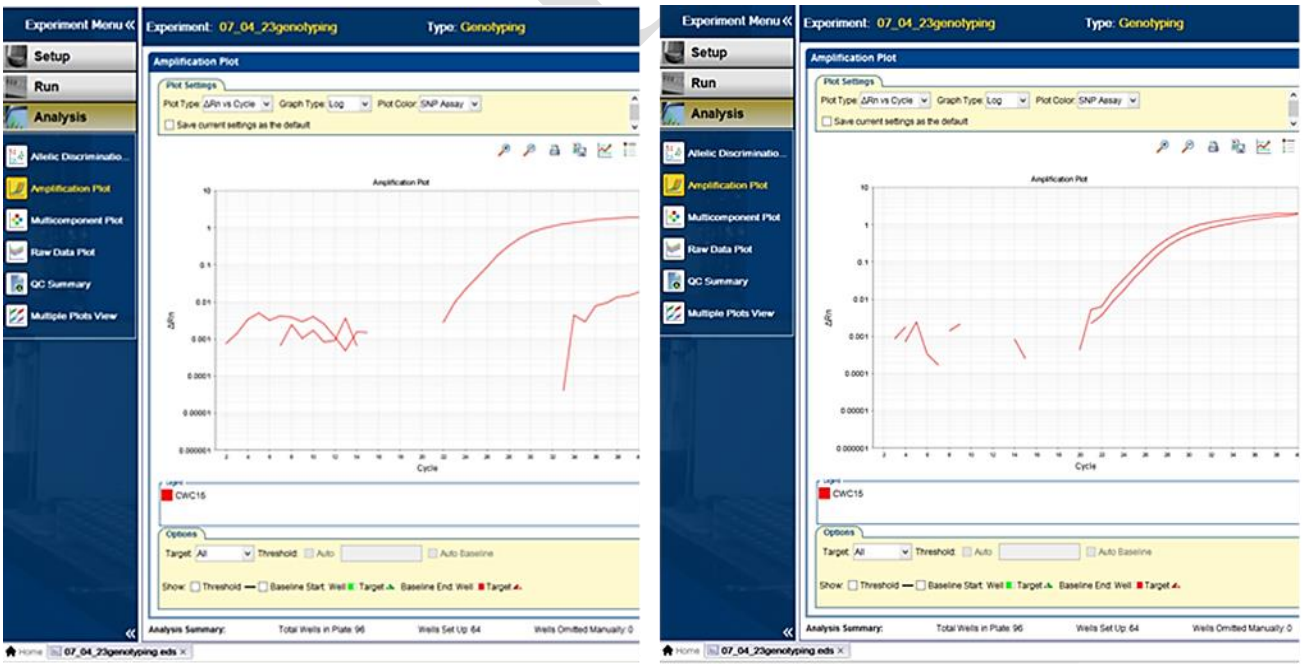

Fig. 2: Graphical representation of Real-Time PCR diagnostic results: a) homozygous genotype at the CWC15 gene locus (amplification with VIC probe, wild type, allele C, amplification with FAM probe, mutant type, allele T); b) heterozygous genotype at the CWC15 gene locus (amplification with VIC probe, wild type, allele C, amplification with FAM probe, mutant type, allele T), Amplification Plot format.

RESULTS

Allele affiliation to fertility haplotypes HH2 and JH1 was assessed in Holstein cows (n=150) and bulls (n=78), as well as Jersey cows (n=150) and bulls (n=6), using Tetra-Primer ARMS-PCR, PCR-PDRF analysis, and real-time polymerase chain reaction. Upon detecting deletions and point mutations in the exon region of the relevant genes, the localisation of SNP substitutions was ascertained. The nucleotide sequences of the IFT80 and CWC15 genes were analysed using polymerase chain reaction (PCR). Table 1 shows the location of a single nucleotide deletion (T) in exon 11 of the IFT80 gene and a point mutation [C→T] in exon 3 of the CWC15 gene.

Considering the position of the single-nucleotide deletion for the Tetra-Primer ARMS-PCR reaction, tetra primer sequences were chosen utilising Primer 1 software. The subsequent primers were employed for the genotyping of DNA samples at the IFT80 gene locus: 5'-CATCTTTTTATTCTGTATTTTTTTTTTAGGCT-3' – forward primer for the T allele, 5'-CCACACCATCTACAAGAAGAAGAAGAT-3' – primer for the A allele, with corresponding reverse primers (5'-3'): TTTCAGGTTGTTGTTTTTTTTTATATATTTCG-3' and (5'-3'): CAGAGAGACAGACAGTCTGTGTGCATT-3. Using PCR, the following products were made: the primer for allele T had a unique chain length of 145 nucleotide bases, and the amplificon for allele A had 184 bases. The primers were annealed at 55°C. The temperature was suggested by Primer 1 software for amplification using the specified primers. Fig. 3 and 4 illustrate a segment of the IFT80 gene acquired through PCR employing the Tetra-Primer ARMS-PCR technique.

To detect JH1 fertility haplotype carriers in Jersey cows, PCR was performed using the forward primer (5'-GGGACTGAGGAGGATGAAGTTGC-3') and reverse primer (5'-GGTTGGGGGAATACGGAAAGGT-3'). The amplification profile included an initial denaturation at 95°C for 8 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 10min. PCR products were analyzed via electropherogram, and TaqI restriction enzyme digestion (30min at 65°C) differentiated mutant from normal alleles. The 1246 bp PCR product contains two TaqI restriction sites: one common and one informative for identifying JH1 mutation carriers. In homozygous healthy animals, TaqI digestion produces three fragments (715, 402 and 129bp), while heterozygous carriers show four fragments (844, 715, 402, and 129 bp) (Sudhakar et al. 2023). Fig. 5-7 depict the CWC15 gene fragment of the JH1 haplotype in Jersey cattle.

The drawback of the current diagnostic method for the HJ2 fertility haplotype is the substantial size of the resultant amplicon, coupled with the existence of two TaqI endonuclease restriction sites within the amplified region of the CWC15 gene; the second restriction site is non-informative, being shared by both individuals with mutant and wild-type alleles of the CWC15 gene. In homozygous individuals, the electropherogram reveals fragments of 715bp, 402bp, and 129bp, whereas heterozygous individuals exhibit fragments of 844bp, 715bp, 402bp, and 129bp. The latter are poorly visualised on the electropherogram due to the significant disparity in fragment lengths, complicating the selection of the optimal agarose concentration.

Table 1: Name of genetic defects and their genetic nature, localization site of deletion and point mutation

Characteristics	Name of genetic defects	
	Fertility haplotype HH2	Fertility haplotype JH1
Gene name	IFT80	CWC15
The gene is located on a chromosome	1	15
Gene length	131,808bp.	8,939bp.
The defect was caused by	Deletions in exon 11	A point mutation in exon 3.
Single nucleotide deletion in part of the IFT80 gene and point mutation of the exon 3 part of the CWC15 gene	Deletion TTTTTTTAGACA[T]TTTCTTCTTCTTTCTTGTAGT TTTTTTTTAGACATTTTCTTCTTCTTGTAG del [T]	Point mutation [C→T] CTGAAGAGAGGTTC→TGAA ACCGTGAC
Position of localization of the deletion or point mutation	Deletion (g.107172616delt) in exon 11	Mutation (C→T) at position 15,707,169 in exon 3 of the gene
Detection method and PCR product size	Tetra-Primer ARMS-PCR 281bp, 184bp, 145bp.	PCR-PDRF, 1246bp. (2020) PCR-PDRF, 313bp (authors' results).
The restrictions used and its restriction site	Not used	Taqi, TCG/A
Fragments after restriction and horizontal electrophoresis	The size of the amplificon of the T allele: 145n. The size of the amplificon of the A allele: 184n. Amplificon size of both primers: 281n.	844bp, 715bp, 402bp, 129p.n. 313bp, 192bp, 121p.n. (design of the authors2024)

*TTTCAGGTTGTTTTTATATTTCG*TTCTTTATATATCTATGTCTTATTATTGTTTTAATTGTAA
GATTTAGATATTTCCAAAGCATAAAATATTTTCTAATATGAAAATGAAATTATAGTAAA
TCCTACTTGAGAATGCATCTTTATTCTGTATTTTTTAGACATTTTCTTCTTGTAGATGGT
GGTGGTATCTATTTATATTCTTATGAAGGGCGCTTCCTTTCCTCTCCAAAATTTCCTGGA
ATGAGAACAGATATTCTA*AATGCACAGACTGTCTCTCTG*.

Fig. 3: IFT80 gene fragment. Note: red italic font indicates IFT80 gene regions complementary to external forward and reverse primers, PCR product size 281bp; red underlined regions of the gene complementary to internal forward primers; yellow underlined regions corresponding to internal reverse primers. A fragment of 145bp, representing the allele's mutant type, holds diagnostic value.

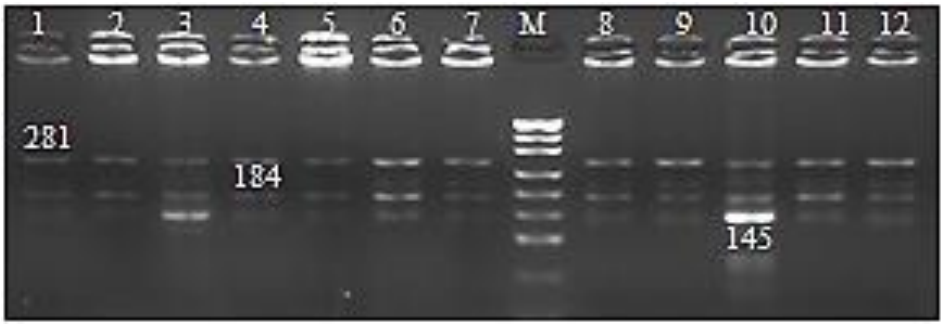

Fig. 4: Electropherogram of IFT80 gene amplificated Tetra-Primer ARMS-PCR reaction. Note: 4.0% agarose, wells 1-2, 4-7, 8-9, 11-12 PCR product with external primer pairs, fragments 281bp, 184bp homozygous healthy animals, wells 3, 10 – heterozygous carrier of HH2 fertility haplotype, fragments 281bp, 184bp, 145bp, M-DNA marker pUC19/MspI.

GGGACTGAGGATGAAGTTGCGTTTCTTGAATTGTCTTCCATTACTACAGCTGTGTGTTT
TTTAGAAGTGACTTTGCCTTGTCTTGTTTCTACTTTAGACAGACCACTCAGGATGCCCCT
GAAGAGGTTCGAAACCGTGACTTCAGGAGAGAGTTGGAGGAGAGAGAGAGAGCTGCT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GATTTGATTCAGTGAGTTAACTAATGTTTGTTTTCTCCCAGAACATACAACCTCCTCTTC
AGTGTCAAAGAAGCCTCGACTGGACCAGATTCCTGCTGCCAACCTTGATGCAGATGAT
CCACTAACAGATGTATGTTTATTTCTTCCTCTTATCTCCATGTAGTAATTTACTTACCAT
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
CTTCCCTAAAAAATAATTCCTGGTATTAAACCATGGAAATTGGCTAGCTTTACCTCTTC
AGACTGGGCTCTTCATCAATAGCATCCCCTCCAGTGAACGTAGGGACCTTTCCGTATTC
CCAACC

Fig. 5: CWC15 gene fragment.

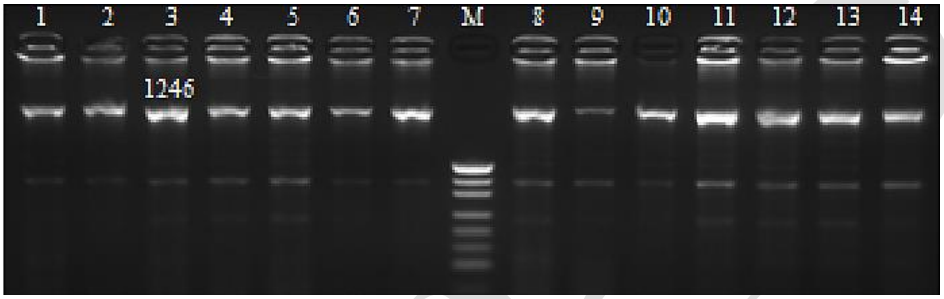

Fig. 6: Electropherogram of CWC15 gene amplificated. Note: 2.5% agarose, wells 1-7, 8-14 PCR product, 1246bp fragment, M-DNA marker pUC19/MspI.

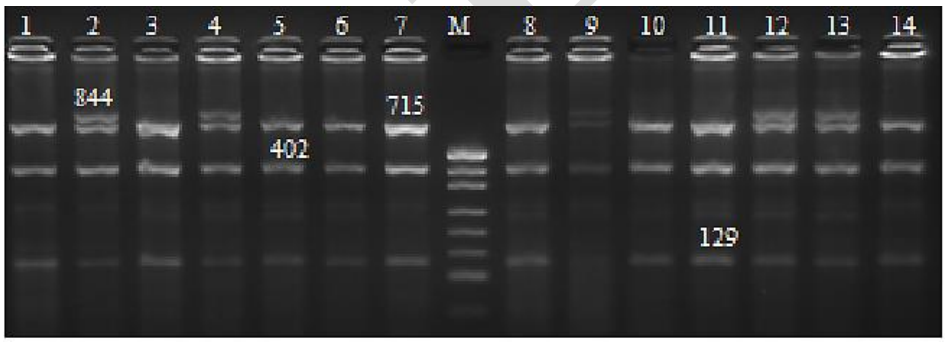

Fig. 7: Electropherogram of CWC15 gene amplificated after restriction with TaqI endonuclease. Note: 2.5% agarose, wells 1, 3, 5-7, 8-11, 14 homozygous healthy individuals, fragments 715bp, 402bp, 129bp, wells 2, 4, 12, 13 are heterozygous carriers of the JH1 fertility haplotype, fragments 844bp, 715bp, 402bp, 129bp, M-DNA marker pUC19/MspI.

To optimize the diagnostic method of HH2 haplotype fertility using the Primer 3 program, the design of forward and reverse primers, forward F-5'-TGCCTCTCTTAGTAACCTTCGGA-3' and reverse R-5'-ACACACAATGTTACGAGAGAGAGAGAGATGT-3', was carried out. The optimal temperature of primer annealing was determined at 58°C with the PCR product size of 313bp. The fact that 313, 192, and 91bp fragments were found in the electropherogram shows that the sample has a heterozygous CT genotype (Fig. 8-10).

Two formats were used to interpret real-time PCR diagnostic results: allelic discrimination and detection of heterozygous carriers in Amplification Plot format, where homozygous individuals at the CWC15 gene locus show a characteristic graphic image, the amplifier displays the curve from VIC probe amplification (C wild-type allele) starting from cycle 22, and the curve from the mutant type allele (T) starting from cycle 34. In the 22nd cycle of real-time PCR in heterozygous individuals, the visual curve from amplification with the VIC probe (C wild-type allele) and the FAM probe (T mutant-type allele) can be seen and stays the same strength. TaqMan@Genotyper Software Version 1.7.1 was used to analyze real-time PCR results to determine homozygous and heterozygous individuals. The amplification with labeled probes (VIC and FAM) failed in samples No. 45 and No. 56 due to low DNA concentrations (8.87ng/μL and 12.55ng/μL, respectively).

Table 2 shows that out of the 150 DNA samples from Holstein cows that were tested, 8 (or 5.4%) had a harmful single nucleotide deletion (g.107172616delT) in the 11-exon part of the IFT80 gene. However, the 78 Holstein bulls tested did not carry the HH2 fertility haplotype.

A total of 5 heterozygous carriers of the mutation (C→T) at position 15,707,169 in exon 3 of the CWC15 gene were identified in Jersey cows; the producer bulls were found to have a homozygous genotype with the wild-type C allele.

Table 2: Prevalence of heterozygous carriers of fertility haplotypes HH2 in Holstein cows and JH1 in Jersey cows

Breed and number of animals	Name of fertility haplotypes, genes, and diagnostic methods					
	Fertility haplotype HH2, (IFT80), Tetra-Primer ARMS-PCR			Fertility haplotype JH1, (CWC15), PCR-PDRF, Real-Time PCR		
	wt/wt AA	wt/mt AT	mt/mt TT	wt/wt CC	wt/mt CT	mt/mt TT
Holstein (cows, n=150)	142/94.6%	8/5.4%	0	-	-	-
Holstein (bulls, n=78)	78/100%	0	0	-	-	-
Jerseys I (cows, n=150)	-	-	-	145/96.6%	5/3.4%	0
Jerseys I (bulls, n=6)	-	-		6/100%	0	0
Total	220/96.4%	8/3.6%	0	151/96.7%	5/3.3%	0

TGCCTCTTAGTAACCTTCGGATAATGATTTTGTTGATTCCCCTAGTGGAGTTTTCTGCTT
TAGGGACTGAGGATGAAGTTGCGTTTCTTGAATTGTCTTCCATTACTACAGCTGTGTGT
TTTTTAGAAGTGACTTTGCCTTGTCTTGTTTCTACTTTAGACAGACCACTCAGGATGCCC
CTGAAGAGGTTCGAAACCGTGACTTCAGGAGAGAGTTGGAGGAGAGAGAGAGAGCTG
CTGCAAGAGAAAAAAACAGAGATCGGCCAACCCGAGGTACCAATACTATCTTAGACAT
CTCTCTCGTAACATTGTGT

Fig. 8: The CWC15 gene region of Jersey cattle, fertility haplotype JH1 and localization of a point mutation at position 15,707,169 at BTA15 in exon 3 of the gene.

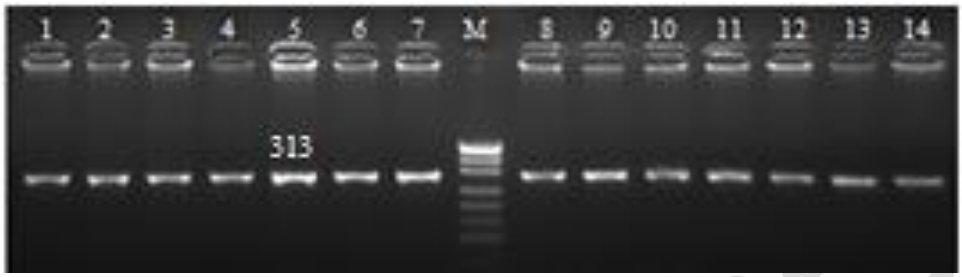

Fig. 9: PCR electropherogram of the CWC15 gene product. Note: 4% agarose, wells 1-7, 8-14 DNA samples, amplification length 313bp, M-DNA marker pUC19/MspI.

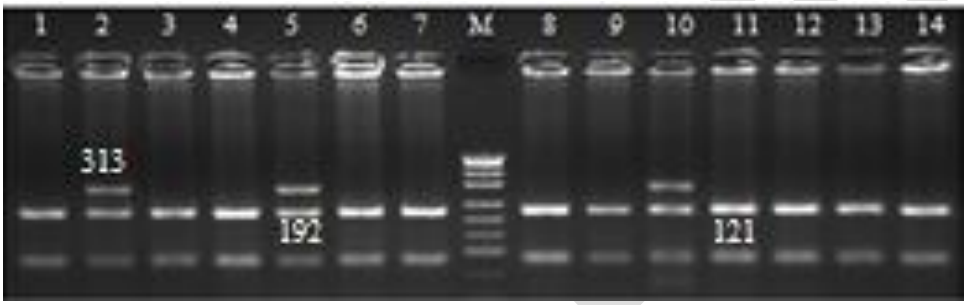

Fig. 10: Electropherogram of CWC15 gene amplificated after restriction with TaqI endonuclease. Note: 4% agarose gel, wells 1, 3, 4, 6-9, 11-14 homozygous healthy animals, fragments 192bp, 121bp wells 2, 5, 10, heterozygous carriers, fragments: 313bp, 192bp and 121bp, M-DNA marker pUC19/MspI.

DISCUSSION

Cows' low reproductive capacity has been Kazakhstan's biggest dairy farming issue recently. The length of the interbreeding period and the number of early embryonic deaths decrease milk productivity and litter size. Most genetic disorders that reduce cow reproductive capacity are recessive, making diagnosis difficult (Busol et al. 2023; Nyzhnyk et al. 2024). They do not affect the animal's phenotype in heterozygotes, but homozygous animals die early in the embryo stage. This method of spreading genetic mutations among breeding stock in all countries is most likely caused by the intensive use of improved bull semen products for artificial insemination in regional cattle improvement programs (Guo et al. 2021). Thus, bulls whose semen is used to inseminate cows in Kazakhstan must be genetically controlled. Testing has become standard in developed cattle breeding countries, including Canada (Wang et al. 2020), the USA (Nani and Peñagaricano 2020) and China (Khan et al. 2021). Mass genetic monitoring of breeding stock (cows) is ineffective because the costs outweigh the benefits (Abutalip et al. 2024; Hermadi et al. 2024). Thus, selection-genetic and genealogical analysis of cows with elongated sexual cycles or early embryonic mortality is more appropriate.

Shormanova et al. (2024) reports Kazakh animal mutant haplotype frequencies for HH1, HH3, HH4, HH5, and HCD. These studies lack information on another genotype anomaly associated with embryo death, so they cannot fully predict reproductive problems of genetic character in Kazakhstani Holstein and dairy cattle. The primary bias in the detection of HH2 mutant alleles stemmed from the enhancement of the Tetra-Primer ARMS-PCR deletion detection technique, which utilised Primer 1 primer sets. Gel electrophoresis can distinguish the amplification products because they differ in nucleotide chain size (Mussayeva et al. 2021; Melnikova and Gilsanz 2023). The studies found 3.6% recessive haplotype HH2 in suckling cows, which is slightly higher than in other countries (Dechow et al. 2020). According to Mohshina and Gedik (2022), 1.7-2.6% of mutant allele T, which causes embryonic mortality, was found in the US, France, Canada, and Italy. The higher prevalence of this haplotype in Kazakhstani Holstein cows may be due to the lack of diagnostic studies in previous years and breeding work to remove mutant alleles from the herd. This may have contributed to Kazakhstan's dairy cattle population. Thus, studying the prevalence of HH2 genotypes in Kazakhstani cattle requires further testing and genealogical comparison.

Kazakhstan's Jersey cattle population has a similar JH1 mutation load. The replacement of one nucleotide in the

CWC15 gene in Jersey cattle disrupts placentation and results in embryo death. No JH1 abnormal allele frequency diagnostics in Kazakhstan were found. An improved real-time PCR method was developed to identify HJ1 fertility haplotype carriers. Then, the improved method was compared to PCR-PDRF analysis. Both methods efficiently detected the mutant allele in animals and yielded similar results. Real-time PCR results depend on DNA concentration in the reaction mixture (Hryshchuk et al. 2023). When DNA concentration is below 15 ng/µL, the reaction does not occur, highlighting the need to control this indicator in diagnostic studies. This study found 96.6% dominant homozygotes and 3.4% mutant heterozygotes for the CWC15 gene. The mutant haplotype was 9.6% in the USA (Cole et al. 2021), 14.7% in Canada (Van Doormaal 2021) and 23.3% in India among breeding bulls. Uruguay had no HJ1 haplotype of reduced fertility (Bimenova et al. 2022). The lower frequency of the mutant haplotype in Jersey cattle in Kazakhstan may be due to the purchase of breeding cattle from Europe, but there is no recent data on this genetic anomaly.

Kazakhstani breeding bulls used for artificial insemination had no mutant or abnormal genes. This is because commercial companies Asyl Tulik JSC and Taurus LLP formed their herd with foreign selection producers who passed control for genetic and chromosomal anomalies. Therefore, the frequency of mutant genotypes HH2 and HJ1 in Holstein and Jersey cattle, along with diagnostic methods, indicate that the Republic of Kazakhstan has developed and tested a comprehensive range of methods for identifying genetic anomalies in dairy cattle that can reduce fertility.

Conclusion

The authors researched to find better ways to find people who carry the mutant haplotypes HH2 and JH1, which make cattle less fertile and cause early embryonic death, and to find out how common recessive haplotypes are in Kazakhstani Holstein and Jersey breeds. Using improved Tetra-Primer ARMS-PCR techniques allows the identification of animals carrying the heterozygous HH2 genotype. Real-time PCR for detecting heterozygous genotypes is limited by DNA concentration; amplification does not occur below 15ng/µL, while high concentrations do not affect results, simplifying the reaction. The frequency of the mutant HH2 allele in Holstein cattle was 5.4%, slightly higher than in other countries, and the prevalence of the JH1 fertility haplotype in Jersey cows was 3.4%. Genetic analyses of breeding bulls used in artificial insemination programs did not reveal mutant genotypes. The authors recommend further research that involves more animals from different farms and countries and compares the results with genealogical studies and artificial insemination efficiency.

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