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Monitoring of recessive defects associated with low reproductive performance in dairy cattle in Uruguay

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Abstract

Background: Most dairy cattle breeds originate show an average generational inbreeding rate of 1%, which favors the occurrence of recessive defects associated with low reproductive performance.

Aim: The objective of this study was to monitor recessive defects associated with low reproductive performance in dairy cattle.

Methods: To monitor bulls carrying the Holstein Friesian haplotype (HH) 1, HH3, and HH4 haplotypes, we analyzed the records of 3,028 national and imported Holstein Friesian bulls from the 2021 updated sires' catalog published by "Evaluaciones Genéticas Lecheras"; and to determine the presence of these mentioned haplotypes, as well as Jersey haplotype (JH) 1 and complex vertebral malformation (CVM), were genotype with the GeneTitan® 2,500 single nucleotide polymorphism (SNP) bovine chip, estimate their frequencies and evaluate their impact on the fertility of 100 Holstein Friesian cows and 70 Holstein Friesian-Jersey crosses belonging to an experimental dairy.

Results: From a total of 1,468 (48.5%) bulls with genetic information from the sires' catalog for HH1 and 1,471 (48.6%) for HH3 and HH4, we found 90 (6.1%) carriers for HH1, 60 (4.1%) for HH3, and 6 (0.4%) for HH4, respectively. By genotyping with the chip, we calculated the herd frequency of the mutant alleles and herd prevalence of carriers for HH1 and CVM as q = 0.003 and 0.022; 0.59% and 4.3% (call rate >0.99), respectively. No mutant alleles were found for HH3, HH4, and JH1 in the analyzed population. We examined reproductive data by observing the presence of CVM and HH1 mutant alleles in repeat cows with an average of four services to achieve pregnancy.

Conclusion: This study demonstrated the presence of recessive defects associated with low reproductive performance in the analyzed population, which can affect the health and productivity of dairy cattle. Therefore, cows and bulls should be closely monitored through genetic testing to lower the incidence of recessive defects in dairy cattle.

Keywords: Artificial insemination, *Bos taurus*, Fertility, Hereditary diseases.

Introduction

Most dairy cattle breeds originate from a small number of founder animals through artificial insemination with a strong selection for a few productive traits (Fritz *et al*., 2013; Liebig *et al*., 2022). Consequently, these breeds show an average generational inbreeding rate of 1% that favors the appearance of recessive defects responsible for embryonic, fetal, and perinatal mortality because it increases homozygosity levels (Van Raden *et al*., 2011b; Fritz *et al*., 2013).

The implementation of routine genotyping of hundreds and thousands of animals within popular US dairy breeds using single nucleotide polymorphism (SNP) chips has provided comprehensive genomic data for all current breeding populations (Van Raden *et al*., 2011b; Howard *et al*., 2017). Van Raden *et al*. (2011b) were the first to propose examining this type of data to identify genomic regions in homozygosity-deficient animals. In their work, they identified and listed five haplotypes out of 11 candidates with detrimental effects on fetal development, which could lead to abortion and fetal death in three dairy cattle breeds: Holstein Friesian (HH1, HH3, and HH4), Jersey (JH1), and Brown Swiss (BH1). After two years, other new

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lethal haplotypes were reported in Holstein Friesian cattle, and in the meantime, some were solved at the molecular level, such as HH1 (*APAF1*, Adams *et al*., 2016), HH3 (*SMC2*, Daetwyler *et al*., 2014; McClure *et al*., 2014), HH4 (*GART*, Fritz *et al*., 2013) and HH5 (*TFB1M*, Schütz *et al*., 2016). In the Danish Holstein population, a haplotype associated with a missense variant in the *SLC35A3* gene that causes complex vertebral malformation (CVM) was identified (Thomsen *et al*., 2006). It was demonstrated that, in the homozygous state, the mutation typically results in intrauterine death. Sonstegard *et al*. (2013) identified the mutation in the *CWC15* gene that is associated with the JH1 haplotype and negatively impacts reproduction in Jersey cattle. Recently, Häfliger *et al*. (2022) updated the SNP positions from the new *Bos taurus* genome version *ARS-UCD1.2* (NCBI, *ARS-UCD1.2* assembly, 2018; Rosen *et al*., 2020) in the genomic regions of the haplotypes previously identified by other authors (Thomsen *et al*., 2006; Sonstegard *et al*., 2013; Daetwyler *et al*., 2014; McClure *et al*., 2014; Adams *et al*., 2016).

For all bovine breeds, 54 haplotypes with homozygous deficits have been reported; in 25 of them, the mutations associated with the responsible genes have been identified (OMIA, 2011). In the Holstein Friesian breed, 11 haplotypes have been identified; these mutations reduce the pregnancy rate and produce an increase in stillbirths and spontaneous abortion that aggravate the already critical situation of fertility loss (Van Raden *et al*., 2011b; Fritz *et al*., 2013; McClure *et al*., 2014; Adams *et al*., 2016; Schütz *et al*., 2016; Fritz *et al*., 2018; Hozé *et al*., 2020; Häfliger *et al*., 2022).

Given the economic importance of producing offspring for the selection of breeders and generating genetic influence of the resulting cows, the main objectives of this study were: (a) to monitor bulls with genetic information for the haplotypes HH1, HH3, and HH4, which are associated with low reproductive efficiency by analyzing the 2021 Holstein Friesian sires' catalog; and (b) to determine the presence of the mutant alleles of these haplotypes, as well as JH1 and CVM, with the GeneTitan® 2,500 SNP bovine chip of Affymetrix platform. We calculated their frequencies and evaluated their impact on fertility in Holstein Friesian cows and Holstein Friesian-Jersey crosses from a dairy farm of the experimental field of the Veterinary Faculty of the "Universidad de la República" (Libertad, San José, Uruguay).

Materials and Methods

Analysis of the 2021 Holstein Friesian sires' catalog

We analyzed the records of 3,028 national and imported Holstein Friesian bulls, born between 1964 and 2016, from the 2021 updated sire catalog published by "Evaluaciones Genéticas Lecheras," available at www.geneticalechera.com.uy. Each record was evaluated using the following national and international databases: (a) *ABS Global*: https://absbullsearch.absglobal.com/; (b) *DairyNZ*: https://www.dairynz.co.nz/animal/animal-evaluation/bull-team/; and (c) "Evaluaciones Genéticas Lecheras (Uruguay)."

Sampling and genetic material

DNA from 100 Holstein Friesian cows and 70 Holstein Friesian-Jersey crosses was extracted from blood using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's specifications. DNA concentration, quality, and purity were assessed with Quant-iT™ PicoGreen at 260 nm (Thermo Scientific, USA), considering an OD260/OD280 ratio between 1.8 and 2.0 and an OD260/OD230 ratio greater than 1.5.

Genetic analysis of genomic data with the GeneTitan® bovine chip.

These DNA samples were genotyped at "Genexa ADN Evolutivo" (Montevideo, Uruguay) with the GeneTitan® 2,500 SNP bovine chip, with Axiom Microarray Genotyping Technology platform from Affymetrix. With these DNA samples, Axiom technology was fine-tuned using the *B. taurus* reference genome sequence version, *ARS-UCD1.2* (NCBI, UCSC *bosTau9* version, *ARS-UCD1.2* assembly, 2018; Rosen *et al*., 2020; Häfliger *et al*., 2022), which is more accurate and has been unified for more effortless transfer and interpretation of results. The Affymetrix platform consists of small DNA molecules attached to fixed locations on the chip, which recognize the specific alleles of an SNP. The different alleles or nucleotides are detected by differential hybridization of the DNA sample. In order to identify those Holstein Friesian cows and Holstein Friesian-Jersey crosses carriers of the hereditary diseases: HH1 (rs448942533), HH3 (rs456206907), HH4 (rs465495560), JH1 (rs1115118696), and CVM (rs438228855), we classified as carriers those genotypes with a call rate greater than 99%, thus ensuring the high quality of the genomic data of the analyzed population. Allelic and genotypic frequencies were calculated by direct counting according to the method of Nei (1987).

Evaluation of reproductive data

We evaluated reproductive data for Holstein Friesian and Holstein Friesian-Jersey crossbred cows carrying the allelic variants previously identified by genotyping with the GeneTitan® bovine chip. These cows have had conventional reproductive management with predominant artificial insemination, gestation diagnosis no later than 70 days post-insemination, and no hormonal treatments during the experimental period. Data were provided through the diary of the experimental field of the Veterinary Faculty.

Ethical approval

All procedures were carried out following the rules and standards of the Ethics Committee on the Use of Animals and approved by the "Comisión Honoraria de Experimentación Animal de la Universidad de la República," Uruguay (protocol from CEUA n° 1526).

Results

Analysis of the 2021 Holstein Friesian sires' catalog

We analyzed the Holstein Friesian sires' catalog of the genetic evaluations updated to 2021, observing that 87.12% (n = 2,638) of the genetic contribution came mainly from the US, Canada, and Uruguay for haplotypes HH1 (Table 1), HH3 (Table 2), and HH4 (Table 3). Of the 3,028 bulls analyzed, only 48.5% (n = 1,468) had a recorded genetic test for the HH1 haplotype (Table 1), and another 48.6% (n = 1,471) for the HH3 (Table 2) and HH4 (Table 3) haplotypes. Of these, 6.1% (n = 90) were carriers for haplotype HH1 (HH1C) from the US, Canada, Netherlands, and Italy; 4.1% (n = 60) for HH3 (HH3C) from the US, Canada, the Netherlands, Italy, and other countries; and 0.4% (n = 6) for HH4 (HH4C) from the Netherlands and other countries. When evaluating the progeny of HH1C bulls for dairy production in Uruguay, we observed there were 10,788 daughters included in the national genetic evaluation system (Table 1). We also observed them for HH3C and HH4C bulls, with 6,317 (Table 2) and 573 daughters (Table 3), respectively.

Genotyping with the GeneTitan® bovine chip

Genotyping detected the following variants: HH1 in a single Holstein Friesian-Jersey cross cow, and CVM in seven Holstein Friesian cows in a total of 170 genotyped cows (call rate >0.99) (Table 4). The frequency of the mutant allele for HH1 in the population analyzed was low, q = 0.003; and the prevalence of carriers was 0.59% (call rate >0.99). For CVM, it was q = 0.022 and 4.3% (call rate >0.99). No mutant alleles were found for HH3, HH4, and JH1 in the analyzed population.

Analysis of reproductive data of cows carrying the associated mutations

We analyzed the reproductive data using the number of inseminations in cows carrying the mutations that had been genotyped with the GeneTitan® bovine chip. Two cows, CVM and HH1 carriers, gave birth to dead calves; these cows had an average of four service repeats to achieve pregnancy (Table 5).

Table 1. Genetic status for the HH1 haplotype of bulls used as sires in Uruguayan dairy genetic evaluation systems until 2021 according to country of origin.

Country	Total bulls (n)	N/D bulls** (n)	Genetic test (%, n)	HH1T[a] (n)	HH1C[b] (n)	Offspring of HH1C bulls for milk production (n)
United States	1,603	581	63.8 (1,022)	960	62	6,971
Canada	375	65	82.7 (310)	288	22	3,632
The Netherlands	93	55	40.9 (38)	33	5	169
Uruguay	660	657	0.5 (3)	3	0	0
Italy	29	6	79.3 (23)	22	1	16
New Zealand	126	122	3.8 (4)	4	0	0
Other*	142	74	47.9 (68)	68	0	0
Total	3,028	1,560	48.5 (1,468)	1,378	90	10,788

*Other: Argentina, Australia, Belgium, Denmark, France, Germany, Italy, Poland, Spain, Sweden, United Kingdom, and United States. **N/D: No data. [a]Disease-free individuals. [b]Carrier individuals.

Table 2. Genetic status for the HH3 haplotype of bulls used as sires in Uruguayan dairy genetic evaluation systems until 2021 according to country of origin.

Country	Total bulls (n)	N/D bulls** (n)	Genetic test (%, n)	HH3T[a] (n)	HH3C[b] (n)	Offspring of HH3C bulls for milk production (n)
United States	1,601	580	63.8 (1,021)	967	54	5,935
Canada	377	64	83 (313)	311	2	146
The Netherlands	93	55	40.9 (38)	37	1	38
Italy	29	6	79.3 (23)	22	1	48
Uruguay	660	657	0.5 (3)	3	0	0
New Zealand	126	122	3.2 (4)	4	0	0
Other*	142	73	12 (69)	67	2	150
Total	3,028	1,557	48.6 (1,471)	1,411	60	6,317

*Other: Argentina, Australia, Belgium, Denmark, France, Germany, Italy, Poland, Spain, Sweden, United Kingdom, and United States. **N/D: No data. [a]Disease-free individuals. [b]Carrier individuals.

Table 3. Genetic status for the HH4 haplotype of bulls used as sires in Uruguayan dairy genetic evaluation systems until 2021 according to country of origin.

Country	Total bulls (*n*)	N/D bulls** (*n*)	Genetic test (%, *n*)	HH4T[a] (*n*)	HH4C[b] (*n*)	Offspring of HH4C bulls for milk production (*n*)
United States	1,601	580	63.8 (1,021)	1,021	0	0
Canada	377	64	83 (313)	313	0	0
The Netherlands	93	55	40.9 (38)	37	1	86
Italy	29	6	79.3 (23)	23	0	0
Uruguay	660	657	0.5 (3)	3	0	0
New Zealand	126	122	3.2 (4)	4	0	0
Other*	142	73	12 (69)	64	5	487
Total	3,028	1,557	48.6 (1,471)	1,465	6	573

*Other: Argentina, Australia, Belgium, Denmark, France, Germany, Italy, Poland, Spain, Sweden, United Kingdom, and United States. **N/D: No data. [a]Disease-free individuals. [b]Carrier individuals.

Table 4. Number of individuals genotyped free and carriers of lethal hereditary diseases using the GeneTitan® bovine chip; the genes associated with their identification in NCBI, and their location in the genome.

Hereditary lethal disease (OMIA ID and variant)[1]	Gene (dbSNP)[2]	Chromosome: Genomic location[3]	Number of genotyped individuals (call rate > 0.99)	Number of individuals for each genotype	
				Non-carriers	Carriers
HH1 (000001-9913; 286)	APAF1 (rs448942533)	5: g.62810245C>T	170	169	1
HH3 (001824-9913; 211)	SMC2 (rs456206907)	8: g.93753358T>C	169	169	0
HH4 (001826-9913; 182)	GART (rs465495560)	1: g.1997582A>C	170	170	0
JH1 (001697-9913; 287)	CWC15 (rs1115118696)	15: g.15449431C>T	170	170	0
CVM (001340-9913; 187)	SLC35A3 (rs438228855)	3: g.43261945C>A	170	163	7

[1]OMIA: Online Mendelian Inheritance in Animals, https://www.omia.org/home/, HH1: Holstein Friesian Haplotype 1; HH3: Holstein Friesian Haplotype 3; HH4: Holstein Friesian Haplotype 4; JH 1; CVM: Complex vertebral malformation. [2]dbSNP: National Center for Biotechnology Information, NCBI, https://www.ncbi.nlm.nih.gov/gene. [3]National Center for Biotechnology Information, NCBI, ARS-UCD1.2 assembly, 2018, https://www.ncbi.nlm.nih.gov/assembly.

Table 5. Number of service repeats and calving in cows carrying the associated mutations previously identified with the GeneTitan® bovine chip.

Cow ID	Breed	No. of calvings		Service repeat	Lethal hereditary disease (associated gene)
97	Holstein Friesian	Multiparous (2015-2022)	6 calvings (2 stillbirths)	In 2019 (4 services to get pregnant) In 2020 (4 services to get pregnant)	CVM (SLC35A3)
636	Holstein Friesian	Multiparous (2019-2022)	4 calvings	No service repeat	CVM (SLC35A3)
531	Holstein Friesian	Multiparous (2017--2022)	4 calvings	No service repeat	CVM (SLC35A3)
724	Holstein Friesian	Multiparous (2019--2020)	4 calvings	No service repeat	CVM (SLC35A3)
830	Holstein Friesian	Multiparous (2020--2022)	2 calvings	No service repeat	CVM (SLC35A3)
32	Holstein Friesian	Multiparous (2018--2022)	3 calvings	No service repeat	CVM (SLC35A3)
311	Holstein Friesian	Multiparous (2015--2022)	8 calvings	No service repeat	CVM (SLC35A3)
605	Holstein Friesian-Jersey cross	Multiparous (2018--2022)	4 calvings (2 stillbirths)	In 2019 (4 services to get pregnant)	HH1 (APAF1)

Discussion

The sires' catalog of the dairy genetic evaluations includes bulls with at least one daughter included in the genetic evaluation system for the Holstein Friesian breed in Uruguay. After analysis of the catalog, it appears a strong component of genetic material mainly from the US and Canada; these had been already found in other investigations (Artigas *et al*., 2020) that showed a predominance of these countries, where presumably the founding mutations of the HH1 and HH3 haplotypes originated (Van Raden *et al*., 2011b; Van Raden *et al*., 2012; Fritz *et al*., 2013; Fritz *et al*., 2018; Häfliger *et al*., 2022). It has been noted that the HH4 mutation came from Europe (Fritz *et al*., 2013; Cole *et al*., 2016), as it was not found in the bulls from the United States and Canada from the dairy genetic evaluation.

After analyzing all the bulls with genetic information in the Holstein Friesian breed gene pool, we detected 90 animals carrying the HH1 haplotype (HH1C = 6.1%), 60 carrying HH3 (HH3C = 4.1%), and six carrying HH4 (HH4C = 0.4%). The number of bulls carrying these haplotypes could be higher, given the low number of animals with genetic information available in public databases regarding their genetic status for the disease, including 99.5% of Uruguayan Holstein Friesian bulls. A similar pattern has been found for brachyspina inherited disease (Artigas *et al*., 2020). The high proportion of bulls without molecular diagnostic tests in the sire catalog can be explained by the fact that this analysis includes bulls before diagnostic tests for mutations in the *APAF1* (HH1), *SCM2* (HH3), and *GART* (HH4) genes were published (Fritz *et al*., 2013; McClure *et al*., 2014; Adams *et al*., 2016; Cole *et al*., 2016).

Bulls' carriers of the HH1C mutation from the 2021 sires´ catalog contributed genetically to Holstein Friesian breed improvement programs, having many daughters (n = 10,788) in national dairy genetic evaluation systems. The same happened with the daughters of HH3C (n = 6,317) and HH4C (n = 573) bulls. Since the inheritance mechanism is autosomal recessive, we assume that, on average, 50% of the daughters (n = 5,394) were HH1C carriers, just as for HH3C (n = 3,158) and HH4C (n = 286), thus indicating the presence of the mutant alleles in the Uruguayan selection nucleus. Since all daughters (n = 10,788 for HH1C; n = 6,317 for HH3C; n = 573 for HH4C) were evaluated for milk production traits, they left offspring at the national level.

Among the 26 HH1C bulls used in Uruguay, stand out O-Bee Manfred Justice (HOLUSAM000122358313), Tesk Holm Valiant Rockie (HOLUSAM000001841366), Lemax Pawnee Memorial (HOLUSAM000001765326), End-Road PVF Boliver (HOLUSAM000123586443) and MJR Blackstar Emory (HOLUSAM000002114601), direct descendants of Walkway Chief Mark (HOLUSAM000001773417), Milu Betty Ivanhoe Chief (HOLUSAM000001578139), SWD Valiant (HOLUSAM000001650414), and Pawnee Farm Arlinda Chief (HOLUSAM000001427381), which are the main disseminators of the mutation in the *APAF1* (apoptotic peptidase activating factor) gene that blocks approximately one-third of the encoded protein (Larkin *et al*., 2012; Van Raden *et al*., 2012; Adams *et al*., 2016; Cole *et al*., 2016). A substitution of a thymine for a cytosine (c.1741C>T) characterizes this mutation (Adams *et al*., 2016). This mutation causes fetal and embryonic loss between 60 and 200 days of gestation and a reduced conception rate in cows; consequently, fertility is reduced in carrier bulls (Adams *et al*., 2016).

Of the HH3C bulls used in Uruguay, stands out Arlinda Melwood (HOLUSAM000001879149), a direct descendant of Glendell Arlinda Chief (HOLUSAM000001556373), another major disseminator of the *SMC2* mutation (McClure *et al*., 2014; Cole *et al*., 2016). This mutation in the *SMC2* (structural maintenance of chromosomes) gene is characterized by a thymine-to-cytosine substitution (*c.3404T>C*) in exon 24 (Daetwyler *et al*., 2014; McClure *et al*., 2014). Häfliger *et al*. (2022) updated the position of the SNP responsible for this mutation (rs45206907) on chromosome 8 (93,753,358) according to the new version of the reference genomic sequence assembly (NCBI, *ARS-UCD1.2* assembly, 2018). The *SMC2* gene plays an essential role in DNA repair and chromosome condensation and segregation during cell division. This point mutation changes amino acid 1135 from phenylalanine to serine (F1135S) and alters the function of the NTP*ase* domain of the encoded protein. Daetwyler *et al*. (2014) analyzed 1,000 bull genomes to identify the *SMC2* mutation, to which embryonic loss was ascribed as a negative effect.

Of the HH4C used in Uruguay, Jocko Besne (HOLFRAM005694028588) is a son of Besne Buck (HOLFRAM004486041658) from Europe, from which he inherited the *GART* mutation (Fritz *et al*., 2013; Cole *et al*., 2016). The *GART* enzyme phosphoribosylglycinamide synthetase is required for purine nucleotide synthesis. The *GART* mutation is associated with decreased fertility and embryonic mortality in the first month of gestation (Fritz *et al*., 2013).

Bulls carrying haplotypes HH1 (*APAF1*), HH3 (*SMC2*), and HH4 (*GART*) have been used to produce offspring for the selection of sires to be used in artificial insemination, thus having a substantial genetic influence on the resulting cow population in the different dairy farms in Uruguay.

Although the mutations associated with haplotypes HH1 (*APAF1*), HH3 (SMC2), HH4 (*GART*), JH1 (*CWC15*), and CVM (SLC*35A3*) were identified 10 years ago, this study was the first report in Uruguay that evaluates reproductive data genomic data from genotyping with the GeneTitan® bovine chip, which allowed a much more accurate imputation compared

to that of other commercial chips. This technology allowed the detection of two mutations associated with the *APAF1* (HH1) and *SLC35A3* (CVM) genes, which affect fetal development and may cause abortion and fetal death in Holstein Friesian cows and Holstein Friesian-Jersey crosses. In the case of the *APAF1* (HH1) mutation, we obtained a herd prevalence of 0.59% with a call rate close to 100%. This value was low compared to that reported by Briano-Rodríguez *et al*. (2021) (4.4%), who performed genotyping with the commercial GeneSeek Genomic Profiler -GGP-Bovine 50K chip, with which they detected 16 Holstein Friesian calves carrying the APAF1 (HH1) mutation in eight dairy farms in Uruguay. Therefore, the *APAF1* (HH1) mutation was still dispersed in Uruguay's Holstein Friesian dairy herd. The prevalence of *APAF1* (HH1) carrier cows in Germany was similar to that of this study (1.8%, Schütz *et al*., 2016). In the USA, Holstein Friesian cows carrying the *APAF1* (HH1) mutation were found at a higher frequency of 4.5% and 2.25% (Van Raden *et al*., 2011b; Adams *et al*., 2016, respectively). However, it had been observed that between 1980 and 1990, the frequency of carrier cows was 0.08, with a subsequent decrease to approximately 0.03 in 2010 (Van Raden *et al*. 2011b). VanRaden *et al*. (2011a) determined the negative effect of this haplotype on reproduction, estimating an impact on conception rate of -0.35%, and evaluated the 60-day non-return rate (NR60), which was -1.1. It was estimated that this mutation caused more than 5,00,000 abortions and a loss of $ 420 million to the dairy industry (Adams *et al*., 2016).

The *SLC35A3* (CVM) mutation was reported in the OMIA catalog (001340-9913) and has been studied in cattle of the Holstein Friesian breed since 2006 (Thomsen *et al*., 2006; Ruść and Kamiński, 2007; Meydan *et al*., 2010) and traced back to the elite North American sire Carlin-M Ivanhoe Bell (HOUSA000001667366), who received the lethal recessive mutation from his sire Penstate Ivanhoe Star (HOLUSAM000001441440) (Chu *et al*., 2008). CVM disease was primarily caused by a defining mutation in the membrane transporter protein UDP-N-acetyl glucosamine of the *SLC35A3* gene (Thomsen *et al*., 2006) and results in the substitution of valine for phenylalanine (V180F) at position 180 (Ruść and Kamiński, 2007). The genotyping of the SNP responsible for this disease (rs438228855, located at position 43,261,945 of bovine chromosome 3) was performed with the GeneTitan® bovine chip. The genotyping was highly accurate and efficient, with a herd prevalence of carrier cows of 4.3% (with a call rate of 100%). This prevalence was intermediate compared to other reports made in Uruguay using other genotyping PCR-HRM/melting methodologies (6.45%, Branda-Sica *et al*., 2019) and GeneSeek Genomic Profiler -GGP- 50K bovine chip (2.09%, Briano-Rodriguez *et al*., 2021). This study found that the herd prevalence of CVM carriers was similar to that of cows in Turkey (3.4%, Meydan *et al*., 2010). Nevertheless, the prevalence was low when compared with other countries, such as Denmark (31.0%, Thomsen *et al*., 2006), Poland (24.8%, Rusc, and Kaminski, 2007), and Japan (13.0%, Ghanem *et al*., 2008). The reason for these differences was differences in the regions of origin of the sampled population, although it might also be influenced using semen from bulls' carriers of the mutation over time. Most countries have developed improvement programs to decrease the frequency of CVM carriers in the cattle population (Ruść and Kamiński, 2007). Hence, in some Holstein Friesian populations in Uruguay, the frequency of CVM carriers still seems to be high regarding the other autosomal recessive mutations.

Acquiring repeat breeder cows that are carriers of CVM and HH1 may be attributed to ovarian dysfunction (Ghanem *et al*., 2017). In a previous study, Ghanem *et al*. (2010) found that progesterone concentration tended to decrease during the estrous cycle in CVM carrier cows due to luteal dysfunctions, which could reduce the conception rate and increase the frequency of repeat cow syndrome. Due to the above reasons, Ghanem *et al*. (2017) studied fertility in *APAF1* mutation (HH1) carrier cows by measuring progesterone concentration in skim milk as a tool to indicate an ovarian function in cows, finding that progesterone concentrations did not differ significantly ($p = 0.8$) between HH1 carrier and non-carrier cows at the first 45 days after artificial insemination.

All cows validated in this study had a normal homozygous genotype for the diseases associated with haplotypes HH3 (*SMC2*), HH4 (*GART*), and JH1 (*CWC15*), with no mutant alleles identified in the population analyzed. However, some studies associated the lethal effects of HH3 (*SMC2*), HH4 (*GART*), and JH1 (*CWC15*) haplotypes with embryonic losses immediately after conception, which are not phenotypically observable; nevertheless, the reduction of conception rate associated with these mutations occurs before 60 days, while embryonic losses associated to HH1 (*APAF1*) and CVM occur throughout the entire gestation (Van Raden *et al*., 2011a; Van Raden *et al*., 2012; Norman *et al*., 2012; Fritz *et al*., 2013). Van Raden *et al*. (2012) reported an HH3 heterozygous cows (*SMC2*) frequency of 4.7% and a 60-day non-return rate (NR60) of -3.1% for this haplotype. Häfliger *et al*. (2022) detected a purely recessive embryonic lethal effect of *SMC2* mutation that allows indirect detection in the non-return rate in Canadian Holstein Friesian heifers. In Germany, Schütz *et al*. (2016) detected HH3 and HH4 carriers with frequencies of 5.1% and 4%, respectively. Fritz *et al*. (2013) reported frequencies of 3.6% for HH4 haplotype, which was associated with a reduction in births in French Holstein Friesian cows, where they observed a reduction in calving rate of -5.8% in heifers and -1.74% in carrier cows when mated to carrier males and daughters of HH4 carrier males. In Uruguay, Briano-

Rodriguez *et al*. (2021) found mutant alleles of HH3 (3.13%) and HH4 (1.04%) haplotypes in a population of Holstein Friesian calves born in 2016. Today, these calves would have become breeders with genetic information and should have been registered in the catalog of parents of the dairy genetic evaluation of the Holstein Friesian breed. In any case, the female calves could have remained as replacements for the farm. For the Jersey breed, there are no reports on the presence of JH1 (*CWC15*) disease carriers in Uruguay. This disease was reported by US ARS-USDA scientists in 2013 (Sonstegard *et al*., 2013), and its origin was traced to the elite North American bull Observer Chocolate Soldier (JERUSAM000000596832), born in 1962 (Van Raden *et al*., 2011a). This bull sired 1,454 daughters for milk production, and consequently, its impact was further magnified with 107 sons and 715 grandsires used in artificial insemination to generate >50,000 granddaughters and >2,00,000 great-granddaughters. As a result, the frequency of JH1 heterozygotes experienced an initial rapid increase, remaining stable at 20%–25% since 1980. Van Raden *et al*. (2004) examined 52,449 fertility records of JH1 heterozygous bulls mated to daughters of JH1 heterozygous bulls and found a conception success rate of 33.3% versus 37.0% for 2,90,373 recent mating of normal bulls to daughters of normal bulls. The reduction in conception rate of 3.7% confirmed JH1 as a recessive lethal disease, in rough agreement with the expected reduction of 4.6%. The conception success rate was 36.3% in 57,523 mating of heterozygous bulls to daughters of normal bulls, with a slight reduction of 0.7% potentially caused by heterozygous dams with JH1 maternal inheritance (Sonstegard *et al*., 2013).

Conclusion

This study demonstrated the presence of recessive defects associated with low reproductive performance in the analyzed population, which can have a negative impact on fertility, calf viability, and overall herd productivity. Therefore, cows and bulls should be closely monitored through genetic testing to lower the incidence of recessive defects in dairy cattle. Furthermore, it is essential to implement preventive measures such as genetic counseling for producers, education on the consequences of these defects, and promoting responsible use of assisted reproduction to minimize the risk of transmitting these undesirable traits.

It is recommended to conduct studies aimed at quantifying the economic losses in dairy farms with the presence of Holstein Friesian haplotypes. In addition, pedigree and genotyping studies of animals should be carried out as a tool to prevent mating between carriers of certain haplotypes.

To prevent the spread of JH1 haplotype, it is advisable to analyze the limited set of 90 records from domestic and imported Jersey bulls born between 1978 and 2012. This data can be sourced from the dairy genetic evaluation available at *www.geneticalechera.com.uy*. The analysis should aim to identify the presence of the mutant allele within the Jersey 2022 sires' catalog.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors contributions

A.B.S. and S.L. designed the experimental procedures. A.B.S., R.A., E.T., E.K., and S.L. performed the experimental work. A.B.S., R.A., P.N., and S.L. conceptualized and designed the study. All authors read and approved the final manuscript.

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Data availability

The data supporting the findings of this study are available within the manuscript. Any other data are available from the corresponding author upon reasonable request.

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