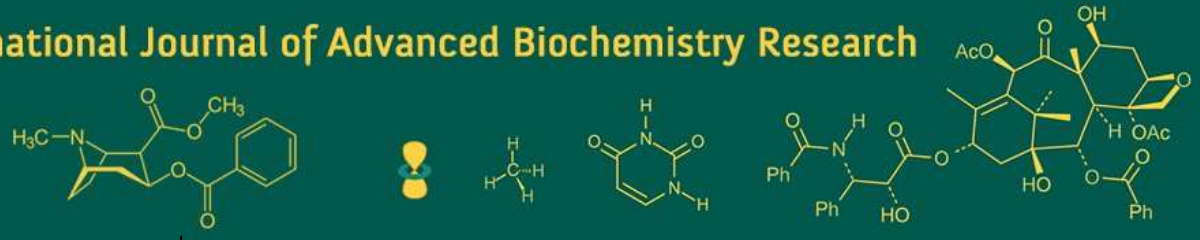
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(6): 343-349
www.biochemjournal.com
Received: 01-04-2024
Accepted: 06-05-2024

SB Khade
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

MP Sawane
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

SJ Komatwar
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

VD Pawar
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

MM Chopade
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

KN Pawankar
Department of Livestock Production Management, College of Veterinary and Animal Sciences, Udgir, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur, Maharashtra, India

Corresponding Author:
SB Khade
Department of Animal Genetics and Breeding, Bombay Veterinary College, Parel, Mumbai, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur Maharashtra, India

Analysis of deficiency of uridine monophosphate synthase syndrome and complex vertebral malformation in repeat breeding and anoestrous cattle using PCR-RFLP

SB Khade, MP Sawane, SJ Komatwar, VD Pawar, MM Chopade and KN Pawankar

DOI: https://doi.org/10.33545/26174693.2024.v8.i6e.1329

Abstract
In India the awareness about genetic disorders is too less among famers at field level, reproductive efficiency is a critical component of growing dairy, but reproductive insufficiency is highly economic problems today. Among that all reproductive problems, anoestrous and repeat breeding are major seen at field by many veterinarians, so it is important and needful to research and resolve the issues regarding to avoid farmers economic loss. There is possibility of mutant genes that are directly affect to livestock reproductive performance. With that concerned it is essential to screen the animals to avoid the increase disease prevalence. Present investigation undertaken, cows with anoestrous and repeat breeding to identify Complex Vertebral Malformation (CVM) and Uridine -5 Monophosphate Synthase (UMPS) molecular genetic disorders by Polymerase Chain Reaction (PCR) - Restriction Fragment Length Polymorphism (RFLP). For this study, Blood of 50 Anoestrous and 52 Repeat Breeding cows (102) were collected from Maharashtra state, India. The de-oxy ribose nucleic acid (DNA) has extracted, and PCR amplification was performed for SLC35A3 and UMPS genes at respective annealing temperature, shown 287 and 108 bp bands respectively. PCR product digested with restriction enzymes *PST* I and *Ava* I at 37 °C for 4 hours for CVM and DUMPS respectively. The results of CVM and DUMPS shown 264 & 23 bp bands and 53, 36 and 19 bp bands, respectively. But due to modest size, 19 bp has not visible in RFLP which is seen in wild animals. All selected animals had shown normal results for CVM and DUMPS.

Keywords: PCR-RFLP, DUMPS, repeat breeding

Introduction
As per 20th livestock census (2019) cattle population in India is 193.46 million (20th Livestock census- 2019, All India Report). Crossbred females account for 47 percent of the overall cattle population, while Indigenous or non-descript females account for 98 percent. Milking animals account for 20 million of the total crossbred or exotic females, while dry animals account for only 5 million. However, in indigenous females, milking animals are 31 million while dry animals are 16 million. The milk production produced by crossbred cattle is more than that of indigenous cattle. Its 27 and 21 percent in crossbred and indigenous cattle respectively (Basic Animal Husbandry Statistics, Government of India, 2017). In comparison to indigenous cattle breeds, Holstein Friesian crossbreds contribute more to milk production. The percentage of dry cows is only 2 percent in Crossbreed and 15 percent in Indigenous or nondescript cows. So, it is more important to keep the crossbred animals healthy and fit and it gives support for the rural economy (Ramesha *et al*., 2017) [41]. Milk is good source of Minerals, protein and Vitamin and fulfils the human health requirement. In Indian dairy sector today, reproductive efficiency is a critical component of a successful dairy operation, whereas reproductive insufficiency is one of the costliest concerns. Lactating animals are more susceptible to reproductive issues, which can have a substantial impact on the reproductive efficiency of a dairy herd (Ghavi, 2013) [42]. The most frequently occurring reproductive problems are Repeat breeding, anoestrous, extended calving intervals, early embryonic loss, ovarian cysts, and retained placenta.

There are certain monogenic disorders which affecting to reproductive efficiency in cattle. In exotic cattle breeds more than 50 genetic disorders have been identified (Debnath *et al*., 2016) [4]. Autosomal recessive genes, which impair bovine fertility, are responsible for most inherited problems in cattle. It involves Factor XI Deficiency syndrome (FXID), Complex Vertebral Malformation (CVM), Deficiency of Uridine Monophosphate Synthase syndrome (DUMPS).

CVM is a congenital autosomal recessive disorder that affects predominantly to the Holstein cattle. It is characterised by stillbirth, abortions, and preterm births. (Khade *et al*., 2014) [13]. Malformations of the spinal column's cervical and thoracic segments cause minor scoliosis, symmetric bilateral carpal joint contractions, neck, and shortening of the anterior limbs with medial rotation of the latter. Malformation of multiple vertebrae, mainly involving those at the cervico-thoracic junction, is a common feature (Agreholm *et al*., 2001) [37]. The causative mutation is on chromosome 3 in the bovine solute carrier family 35-member 3 (SLC35A3) gene (Thomson *et al*., 2006) [34]. There is G to T substitution at location 559 of the gene SLC35A3 on chromosome 3. CVM causes intrauterine mortality throughout pregnancy, resulting in repeated breeding and involuntary culling of cows, both of which result in economic losses (Berglund *et al*. 2004) [3].

DUMPS refers to deficiency of uridine 5 monophosphate enzyme in cattle. It is an inherited disease caused by single point mutation (C→T) at codon 405 within exon 5. DUMPS is a genetic disorder of cattle that causes early embryonic death upon uterine implantation. DUMPS is characterised by lowered blood activity of UMPS enzyme in the Holstein cattle (Schwenger *et al.*, 1993) [32]. DUMPS homozygous embryos do not sustain to term and die early in pregnancy. Approximately 40 days following conception, the embryos aborted or reabsorbed, resulting in recurring breeding issues. During the first trimester of gestation, the embryos are often reabsorbed which causes to form more services and longer calving intervals than normal animal and that makes additional problems in dairy herds. It is seen that the heterozygous carrier shows a decrease of almost 50% UMPS activity in kidney, spleen, muscle, and mammary glands. This leads to producing five to ten times higher concentrations of orotic acid in cow milk which is at high risk in human consumption (Robinson, 1980) [28] and might lead to threshold for fatty liver development. These, in analogy with a comparable human condition, would be expected to exhibit high perinatal morbidity and mortality (Robinson *et al.*, 1983) [27].

These genetic disorders primarily affect the Holstein Friesian breed. These conditions have been linked to embryonic deaths, abortions, and stillbirths, all of which have a negative impact on reproductive efficiency.

In India, breeding bulls are commonly used for breeding without being screened for monogenic autosomal disorders. With the widespread application of artificial insemination (AI), semen trading at National or International level and breeding bulls, genetic diseases can spread to a vast population in short time. The development of simple and fast procedures for accurately diagnosing mutations that cause genetic problems would help breeders in identifying carriers and carrying out breeding programmes to reduce genetic defects from the dairy population.

As a result, considering the financial losses and deadly effects of various illnesses in dairy cows, the carried investigation was undertaken to analyse animals with the reproductive disorders for these disorders. The objective is to investigate CVM and DUMPS monogenic disorders with repeat breeding and anoestrous cattle by using PCR-RFLP in cattle.

Materials and Methods

Animal selection

In the carried study, initially selected cows were not showing Oestrous or not conceiving even after proper nutrition and management practices despite all normal anatomical structure of animals. Total one hundred and two (102) animals were included with Holstein Friesian (HF) crossbred, Dangi, Gaolao and Nondescript breeds. Among all the animals selected, fifty-two (52) animals were under repeat breeding and fifty (50) animals were anoestrous. Almost of the animals were from well-established dairy farm, veterinary clinics, and farmers' fields from Maharashtra State.

Blood sample collection

The selected animals' blood was collected with proper hygiene and precautions to avoid any contamination. It was collected in the vacutainers (Make: BD Vacutainer®) containing ethylene diamine tetra acetate (EDTA) as blood anticoagulant. Firstly, gently shake the blood vacutainer to mix blood with EDTA properly and store in the cold chain-maintained transportation box. After collection, it carried out to the cytogenetic investigation laboratory then clean the vacutainers externally with ethanol (70%) and store the samples in refrigerator at 14 °C till the extraction of de-oxy ribose nucleic acid (DNA)

DNA extraction and Evaluation

Phenol Chloroform (PCI) method was applied and DNA extracted as described by Sambrook and Russel, 2001. DNA quality by spectrophotometry and quantity by agarose gel electrophoresis was checked for effective outcome. DNA was stored in the refrigerator for further requirements for Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP).

a) Spectrophotometry

The DNA were checked for its quantification and purity by ultraviolet (UV) spectrophotometry. For the PCR-RFLP analysis, used DNA samples with an (Optical density-OD) OD260/OD280 ratio of 1.7 to 2.0.

b) Agarose gel electrophoresis of extracted DNA

For gel electrophoresis Agarose (1%) and 1X TAE buffer (pH 8.0) was used. Boiled the Agarose and ethidium bromide (1 percent) @ 5 µl /100 ml of agarose gel was added. Subsequently, 5.0 µl DNA and 1.0 µl gel loading dye (6X) were filled in the wells. At 90 V Electrophoresis was carried out for 45 minutes. The quality and purity of the DNA (UVP, UK) was checked by observing the gel under UV light.

Table 1: Chemicals used for Agarose Gel Electrophoresis

Chemical	Composition
10X TAE, pH 8	48.4 gm Tris base
	11.4 ml Glacial acetic acid (17.4 M)
	20 ml EDTA (0.5 M)
Gel loading dye (6X)	10 Mm Tris -HCL (Ph 7.6)
	0.03 percent bromophenol blue
	60 percent glycerol
	60 Mm EDTA
Ethidium bromide (1 percent)	10 mg ethidium bromide
	1.0 ml distilled water

Assessment of annealing temperature: The OD ratio between 1.7 to 2.0 (OD 260: 280) DNA samples were used for PCR. The annealing temperature was tested in 55-65 °C range in the Master cycler gradient for the PCR amplification of SLC35A3 and UMPS genes with respective primer sequence (Table 2). For the SLC35A3 and UMPS genes, consistent findings were observed at 59.7 °C and 58.4 °C, respectively.

Table 2: Primers used for Polymerase Chain Reaction

Gene	Primer sequence (5'-3')	Reference
SLC35A3 Gene	Forward: 5'CACAATTTGTAGGTCTCACTGCA 3'	(Yathish *et al*., 2011) [35]
	Reverse: 5'CGATGAAAAAGGAACCAAAAGGG 3'	
UMP Gene	Forward: 5'GCA AAT GGC TGA AGA ACA TTC TG 3'	(Schwenger *et al*., 1993) [32]
	Reverse: 5'GCT TCT AAC TGA ACT CCT CGA GT 3'	

PCR amplification of SLC35A3 and UMPS: The PCR reactions were performed in 0.2 ml thin-walled PCR tubes with a final volume of 25 µl (Table 3). A composition for PCR were master mix constituted PCR super mix, forward primer, reverse primer, and distilled water and each PCR tube was then filled with ~100 ng/µl DNA, resulting to a final volume 25 µl. And the PCR tubes shifted in a pre-programmed thermal cycler (Master cycler Epigradient, Eppendorf, Germany) as per PCR protocol (Table 4) for different genetic disorders.

Table 3: PCR reaction component details

PCR Component	Volume	Concentration
PCR Super mix	22.5 µl	1X
Nuclease free water	0.5 µl	-
Forward primer	0.5 µl	10 pmole
Reverse primer	0.5 µl	10 pmole
Template DNA	1.0 µl	~100 ng
Total	25.0 µl	-

Table 4: PCR protocol for SLC35A3 and UMPS gene

Cycles	Step	SLC35A3 Gene		UMPS gene	
		Temperature	Time	Temperature	Time
1	Initial Denaturation	94 °C	02 min	95 °C	05 min
2	Denaturation	94 °C	45 sec	95 °C	01 min
	Annealing	59.7 °C	45 sec	58.4 °C	90 sec
	Extension	72 °C	45 sec	72 °C	90 sec
		Repeat cycle 2 for 40 times		Repeat cycle 2 for 35 times	
3	Final Extension	72 °C	10 min	72 °C	10 min

Gel electrophoresis of PCR product: After DNA amplification, 5 µl PCR product and 1 µl 6X gel loading dye from each amplified tube was mixed. Agarose gel (1.7%) and Ethidium bromide (1%) was incorporated in 1X TAE buffer. Then the mixed PCR sample was loaded properly in gel and electrophoresed at 90 V for 45 min in 1X TAE buffer. Then, the result was visualized under UV light and analysed it with molecular sized markers. The consistent findings were obtained at respective annealing temperature and the rest PCR samples were properly stored for further RFLP process.

Restriction digestion of amplified PCR product: The PCR products of the SLC35A3 gene were digested for 4 hours, UMPS gene for 6 hours in 37 °C water bath with concerned restriction enzymes. (Table 5).

Table 5: Restriction enzyme

Gene	Restriction enzyme	Recognition Site
SLC35A3 gene	Pst I	5'-CTGCA^G---3' 3'--G^GACGTC--5'
UMPS	Ava I	5' - C^YCGRG-3' 3' - GRGCY^C-5'

Gel electrophoresis of digested PCR product: The digested PCR products was visualized in 2.5% and 5% agarose for SLC35A3 and UMPS genes respectively. In agarose gel, ethidium bromide @ 5 µl/100 ml was added. The 6X gel loading dye was used @ 1.5 ml/15 ml of RFLP product and electrophoresed at 90V for 60 minutes in 1X

TAE buffer. For SLC35A3 Step DNA Ladder (Range 50-3000 bp) and Ultra Low range (ULR) for UMPS was used as a molecular marker and visualized bands under UV light. By comparing the band size to a molecular size marker, the band size was determined.

Table 6: Components used in digestion of PCR product

Reagent	Quantity (ml)
Restriction enzyme	1.0
Restriction enzyme buffer	1.5
PCR product	10.0
Nuclease free water	2.5
Total Reaction volume	15.0

Results and discussion:

The aim of this study was to observe for monogenic disorders such as CVM and DUMPS in animals with reproductive disorders such as repeat breeding and anoestrous, which obstruct production and reproduction and result in financial losses. It has already been observed that a repeat breeder cow results in a $324 economic loss over a fertile cow. The occurrence of factor XI deficiency (FXID) carriers in generally fertile and repeat breeder cows, as well as possible economic losses from 'longer calving intervals' and 'additional service (Akyuz *et al*., 2012) [2]. CVM and DUMPS were created primarily to investigate the link between these disorders and reproductive problems with the help of molecular techniques and results were discussed in detail as under.

Genomic DNA extraction and Analysis:

Gel electrophoresis was carried out for genomic DNA examination (Plate 1). Nanodrop was used to check the DNA quantity. The DNA with (Optical density-OD) OD260/OD280 ratio ranging between 1.7 to 2.0 were subjected for further use.

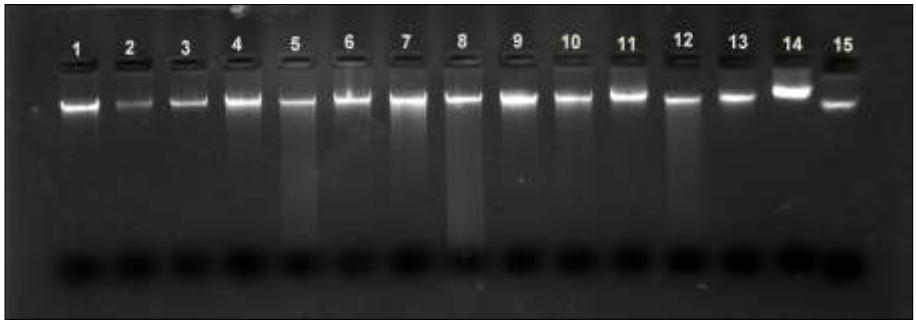


Plate 1: Extracted DNA samples

Amplification of SLC35A3 gene:

The PCR products were analysed in 1.7 percent agarose gel and revealed 287 bp single band (Plate 2) band size in UV Spectrophotometer. The same results were observed by Yathish *et al*. (2011) [35] and Khade *et al*. (2014) [13].

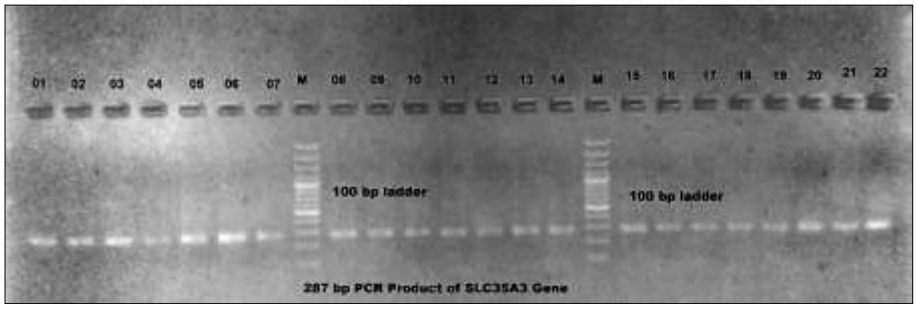


Plate 2: PCR Amplification of SLC35A3 Gene (100 bp ladder)

RFLP analysis of SLC35A3 gene

The PCR products were digested with *Pst I* restriction enzyme (RE) for 4 hours at 37 °C and visualised under UV spectrophotometer light. It revealed 264 bp and 23 bp sizes two bands (Plate 3). But the size of 23 bp was too small so it is not apparent. The result shown that selected animals were found to be normal, with no carriers or affected for SLC35A3 gene.

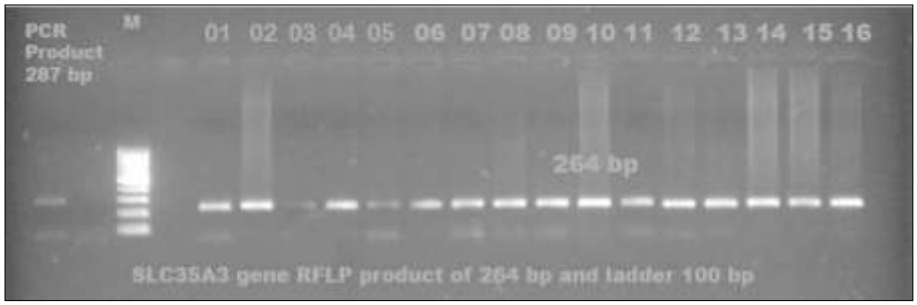


Plate 3: RFLP of SLC35A3 Gene (100 bp ladder)

The digested PCR products of SLC35A3 gene produces 264 bp and 23 bp size bands in wild animal, 287, 264 and 23 bp size in carrier animals, and only one fragment of size 287 bp in homozygous recessive animals. The findings for CVM were wild type genotype and that agreed with Yathish *et al.* (2011) [35] with no CVM carriers in 55 Karan Fries bull calves. The same results have reported Khade *et al*. (2014) [13] in 50 HF crossbred cattle maintained at Maharashtra, India as well as Eydivandi *et al.* (2011) [5].

Mahdi (2008) [15] found two CVM carrier bulls with 20.35 percent frequency. Kumar (2009) [14] reported 76.36 percent animal's carrier as well as Mahdipour *et al.* (2010) [16] found 12 animal's carriers in 52 Karan fries bull for CVM. CVM carriers were found in 17.76% of top sires in the United States, according to various studies (Holstein Association USA 2006), Germany 13.20%, Japan 32.50% (Nagahata *et al*. 2002) [19], Sweden 23.00% (Berglund *et al*. 2004) [3], and Poland 25.00 percent (Rusc and Kaminski, 2007) [30].

In Slovakia, Gabor *et al*. (2012) [6] discovered four CVM carriers among 47 Holstein bulls. Zhang *et al*. (2012) [36] discovered 56 CVM carrier bulls, equating to a heterozygote carrier frequency of 9.54 percent. Paiva *et al*. (2013) [22] discovered that out of 783 Girolando bull samples, 1.53% were CVM carrier animals. According to Meydan *et al*. (2013) [18], observed that selected animals carried normal genes. According to Hemati *et al*. (2015) [10], observed some dominant homozygote (AA) and heterozygote (Aa) for CVM in Holstein cows. Abraham *et al*. (2019) [38] identified 42 carrier cows for CVM, Neziha and Hasret (2019) [39] found all animals with wild type of gene for CVM.

Amplification of UMPS gene

The PCR findings of UMPS gene revealed 108 bp size (Plate 4). These findings were observed by Grzybowski *et al*. (1998) [9], Ghanem *et al*. (2006) [8], Patel *et al*. (2006) [23], Meydan *et al*. (2010) [17] and Khade *et al*. (2016) [12].

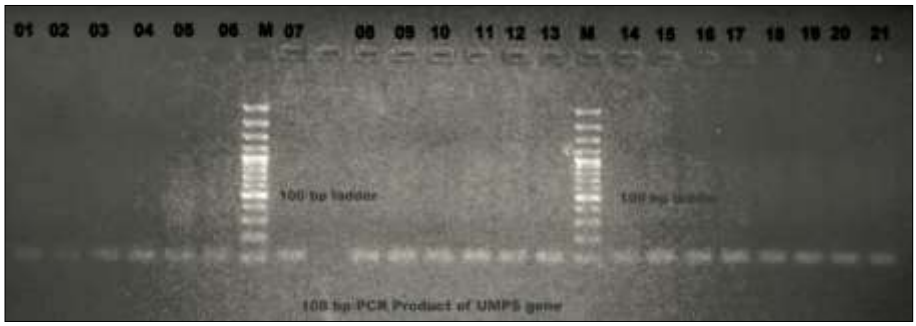


Plate 4: PCR Amplification of UMPS Gene (100 bp ladder)

RFLP analysis of UMPS gene

Following that, the PCR products were digested with the *Ava* I restriction enzyme at 37 °C for 6 hours, revealing the normal genotype with three bands of 53, 36, and 19 bp for all the animals. (Plate 5). But 19 bp band is too short so it is not visible in RFLP. In carrier animal, four bands *viz*., 89, 53, 36 and 19 bp will show in RFLP product. First time DUMPS was recorded in North America and Europe by Shanks and Robinson, (1990) [33]. The results shown that all animals were wild type allele for DUMPS.

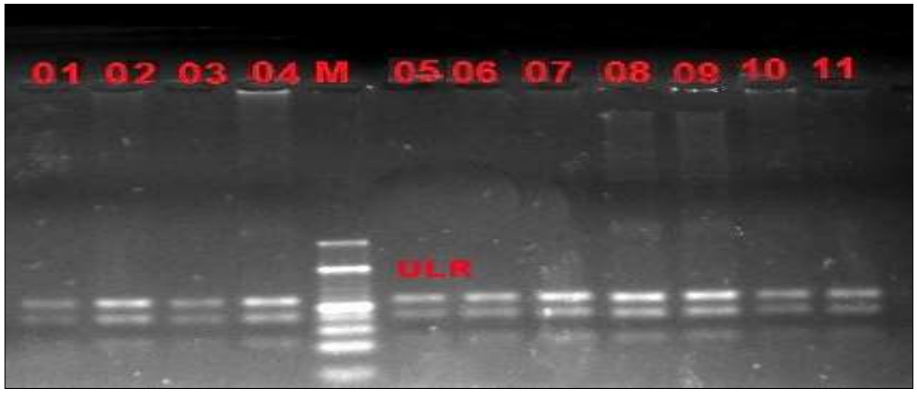


Plate 5: RFLP of UMPS Gene (Ultra low range ladder) (53 and 36 bp size and 19 bp is not visible)

The findings are in accordance with Kaminski *et al*. (2005) [11], with no DUMPS occurrence. In a study of 642 HF animals, Patel *et al*. (2006) [23] found no DUMPS. In Iranian HF bulls, Rahimi *et al*. (2006) [25], Rezaee *et al*. (2009) [26], and Eydivandi *et al*. (2011) [5] found no carriers for DUMPS. In Turkish HF cattle, Meydan *et al*. (2010) [17] and Oner *et al*. (2010) [20] found no carriers for DUMPS. Karsli *et al*. (2011) found no carriers for DUMPS. Gaur *et al*. (2012) [7], reported one HF carrier bull for DUMPS in India. Paiva *et al*. (2013) [22], Meydan *et al*. (2013) [18], Khade *et al*. (2016) [12], and Debnath *et al*. (2016) [4], Magotra *et al*. (2020) [40] all have screened the animals for the UMPS gene and found no carriers.

Conclusion

Genetic disorders are one of the most important part and it play a significant role in the dairy industry. Now as days there is vast use of assisted reproductive techniques such as artificial insemination, embryo transfer, and *in-vitro* fertilisation for livestock development through selective breeding. Genetic diseases in cattle are having a significant influence on the national livestock economy by affecting to

the production and reproduction. There are a variety of monogenic recessive disorders that are peculiar to the Holstein Friesian cattle breed and have been documented to cause significant economic losses. The primary cause of these problems in the population is the breeding bulls utilised. Increased numbers of undiscovered carriers of cytogenetic abnormalities and recessive disorders might result in significant financial losses for the livestock business. Other potential sources of disease are superior germplasm bull mothers and other breeding cows. Hence, the study carried out in 102 cows with reproductive disorders to screen out animals for genetic disorders, if any. The carried work can be concluded as under.

- All animals found normal CVM and DUMPS monogenic disorders. As a result, it's possible that these diseases aren't directly linked to reproductive issues in the population investigated.
- These genetic diseases in the affected animal were directly or indirectly linked to reproductive disorders in cattle, resulting in diminished or subfertility in cattle.
- Screening of animals is important to avoid the transfer of disease to normal animals, reduce risk to human health and to avoid loss to the farmers.

Acknowledgement: The Bombay Veterinary College, Mumbai, Maharashtra, India, given all essential facilities as well as guidance to carry out the research work. So, the authors are thankful to the Associate Dean of the college.

Author's contribution

S.B.K and M.P.S: Conceptualization, validation, and original draft preparation. V.D.P, M.M.C and R.D.D: Review and editing. All authors read and approved the manuscript.

References

1. 20th Livestock Census-2019. All India Report. Government of India. Ministry of Fisheries, Animal Husbandry & Dairying. Department of Animal Husbandry & Dairying, Animal Husbandry Statistics Division, Krishi Bhawan, New Delhi.
2. Akyuz B, Sacriozkan S, Bayram D. Factor XI mutation in normally fertile and repeat breeding Holstein cows in the Middle Anatolin region of Turkey: A financial approach. Animal Production Scienc. 2012;52:1042-1045.
3. Berguland B, Person A, Stalhammer H. Effects of complex vertebral malformation of fertility in Swedish Holstein cattle. Acta Veterinaria Scandinavica. 2004;45:161-165.
4. Debnath A, Kumar A, Maan S, Kumar V, Joshi VG, Nanda T, *et al*. Molecular Screening Of Crossbred Cow Bulls For Important Genetic Disorders. Haryana Vet. 2016;55(1):93-96.
5. Eydivandi C, Seyedabadi HR, Amirinia C. Identification of BLAD, DUMPS and CVM deficiency in Khuzestan Holstein cattle population of Iran. Global Veterinaria. 2011;6(6):519-524.
6. Gabor M, Miluchova A, Tracovika A, Riecka Z, Candrack J, Vavirhinova K, *et al*. Detection of Complex vertebral malformation Slovak Holstein cattle by high resolution melting analysis. Acta Veterinaria (Beogard). 2012;62(2-3):239-248.
7. Gaur U, Sathe TG, Roy A, Patel RK, Sunkara PSS. First case of genetic polymorphism in Uridine Monophosphate Synthase gene in an Indian Holstein bull. Inter. J Vet. Sci. 2012;2(1):32-34.
8. Ghanem MET, Nakao M, Nishibori M. Deficiency of uridine monophosphate synthase (DUMPS) and X-chromosome deletion in fetal mummification in cattle. Anim. Reprod. Sci. 2006;91(1-2):45-54.
9. Grzybowski G, Grzybowski T, Wozniak M, Chacinska BI, Smuda E, Ubieniecki K, *et al*. Badania przesiewowe na obecnosc genu wczesnej obumieralnooeci zarodków DUMPS u bydla w Polsce. Med Wet. 1998;54:189-193.
10. Hemati B, Gharaie-Fathabad S, Fazeli MH, Namvar Z, Ranji M. Investigation of Bovine Leukocyte Adhesion Deficiency (BLAD) and Complex Vertebral Malformation (CVM) in a Population of Iranian Holstein Cows. Iranian Journal of Applied Animal Science. 2015;5(1):69-72.
11. Kaminski S, Grzybowski G, Prusak B, Rusc A. No incidence of DUMPS carriers in polish dairy cattle. Appl. Genet. 2005;46:395-397.
12. Khade SB, Pawar VD, Chopade MM, Komatwar SJ, Pathade SS. Genotyping of The HF Crossbred Cattle for Umps Gene Using PCR-RFLP. International Journal of Science, Environment and Technology. 2016;5(5):3245-3248.
13. Khade SB, Pawar VD, Doiphode AY, Sawane MP, Umrikar UD. Genotyping of Holstein Friesian Crossbred Cattle for CVM and FXI Deficiency Loci. Indian Journal of Veterinary and Animal Science Research. 2014;43(5):340-345.
14. Kumar V, Sharma. Genetic screening of K F animals for bovine leukocyte adhesion deficiency syndrome (BLAD) and complex vertebral malformation (CVM). M.V.Sc. Thesis, NDRI, Karnal; c2009.
15. Mahdi M. Genetic screening of Karan Fries bulls for bovine leukocyte adhesion deficiency and complex vertebral malformation. M. Sc. Thesis, NDRI, Karnal; c2008.
16. Mahdipour M, Sharma A, Dubey P, Kumar V, Misra B, Singh A, *et al*. Identification of complex vertebral malformation using Plolymerase Chain Reaction-Primer Introduced Restrication Analysis in Karan Fries Bulls. Current Trends in Biotechnology and Pharmacy. 2010;4(4):850-854.
17. Meydan H, Yildiz MA, Agerholm J. Screening for bovine leukocyte adhesion eficiency, deficiency of uridine monophosphate synthase, complex vertebral malformation, bovine citrullinaemia, and factor XI deficiency in Holstein cows reared in Turkey. Acta Veterinaria Scandinavica; c2010. p. 52-56.
18. Meydan H, Ugurlu M, Yildiza MA. Monitoring of BLAD, DUMPS, CVM, BC and FXID in Turkish Native Cattle Breeds. Journal of Agricultural Sciences. 2013;18:239-245.
19. Nagahata H, Oota H, Nitanai A, Oikawa S, Higuchi H, Nakade T, *et al*. Complex vertebral malformation in a stillborn Holstein Calf in Japan. J Vet. Med. Sci. 2002;64(12):1107-1112.
20. Oner Y, Keskin A, Elmaci C. Identification of BLAO, DUMPS, Citrullinemia and Factor FXI deficiency in Holstein Cattle in Turkey. Asia. J Anim. and vet. Sci.; c2010. p. s1-6.

21. Online Mendelian inheritance in animals; http://omia.angis.org.au (accessed 10 April 2021).
22. Paiva DS, Fonseca I, Pinto ISB, Ianella P, Campos TA, Caetano AR, *et al*. Incidence of bovine leukocyte adhesion deficiency, complex vertebral malformation, and deficiency of uridine-5monophosphate synthase carriers in Brazilian Girolando cattle. Genetics and Molecular Research. 2013;12(3):3186-3192.
23. Patel RK, Krishna MS, Kalpesh JS, Jenabhai BC, Krothaalli RS, Sambasiya R, *et al*. Lack of carriers of citrullinemia and DUMPS in Indian Holstein cattle. J Appl. Genet. 2006;47:239-242.
24. Poli MA, Dewey R, Semorile L, Luzano ME, Albarino CG, Grau O, *et al*. PCR screening for carriers of BLAD, DUMPS in Argentine Holstein cattle. Zentralbl Veterinary. 1996;43:163-168.
25. Rahimi G, Javaremi N, Olek K. Genotyping BLAD, DUMPS and K- CSN loci in Holstein young bulls of the national animal breeding centre of Iran. Pakistan Journal Biological Sciences. 2006;9(7):1389-1392.
26. Rezaee AR, Nassiry MR, Sadeghi B, Shafagh Motlagh A, Tahmoorespour M, Valizadeh R, *et al*. Implication of complex vertebral malformation and deficiency of uridine monophosphate synthase on molecular based testing in the Iranian Holstein bull's population. African Journal of Biotechnology. 2009;8(22):6077-6081.
27. Robinson JL, Drabik MR, Dombrowski DB, Clark JH. Consequence of UMP synthase deficiency in cattle. Proc. Natl Sci. USA. 1983;80(2):321-323.
28. Robinson JL. Bovine Milk Orotic Acid Variability and Significance for Human Nutrition. Symposium Milk Synthesis. 1980:865-871.
29. Ghavi HA. Effects of main reproductive and health problems on the performance of dairy cows: A review. Span J Agric. Res. 2013;11(3):718-735.
30. Rusc A, Kaminski S. Prevalence of complex vertebral malformation carriers among Polish Holstein-Friesian Bulls. J Appl. Genet. 2007;48(3):247-252.
31. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press; c2001. p. 3.17-3.32.
32. Schwenger B, Schober S, Simon D. DUMPS cattle carry a point mutation in the uridine monophosphate synthase gene. Genomics. 1993;161:241-244.
33. Shanks RD, Robinson J. Deficiency of uridine monophosphate synthase among Holstein cattle. C. Vet. 1990;80:119-122.
34. Thomson B, Horn P, Panitz F, Bendixen E, Petersen AH, Holm L, *et al*. A missense mutation in the bovine SLC35A3 gene, encoding a UDP-N- acetylglucosamine transporter, causes complex vertebral malformation. Genome Res. 2006;16:97-105.
35. Yathish HM, Sharma A, Kumar V, Jain A, Chakraborty D, Singh A, *et al*. Genetic polymorphism of SLC35A3 gene in Karan Fries bull calves. Indian Journal of Animal Sciences. 2011;81(9):951-953.
36. Zhang Y, Fan X, Sun D, Wang Y, Yan Xie YY, Zhang S, *et al*. A novel method for rapid and reliable detection of complex vertebral malformation and bovine leucocyte adhesion deficiency in Holstein cattle. J Anim. Sci. Biotechnol. 2012;3:24.
37. Agerholm JS, Bendixen C, Andersen O, Arnbjerg J. Complex vertebral malformation in Holstein calves. J Vet Diagn. Invest. 2001;13:283-289.
38. Virgen-Mendez A, Ayala-Valdovinos MA, Galindo-Garcia J, Sanchez DR, Lemus-Flores C, Duifhuis-Rivera T, *et al*. Carrier frequency of autosomal recessive disorders (BC, BLAD, FXID and CVM) in Holstein cows in Jalisco, Mexico. Pesquisa Veterinaria Brasileira, Brazilian Journal of Veterinary Research. 2019;39(7):481-484.
39. Neziha, Hasret. Detection of allele and genotype frequencies of bovine leukocyte adhesion deficiency, factor XI deficiency and complex vertebral malformation disease genes in Holstein cattle. Ankara Univ. Vet Fak Derg. 2019;66:311-315.
40. Mogotra A, Patil CS, Malik BS, Garg AR. Genetic screening of deficiency of uridine monophosphate synth (dumps), bovine citrullinaemia (bc) and factor xi deficiency (fxid) Hardhenu, Sahiwal and Hariana Bull. Int. J Bio-resour Stress Mgt., 2020, 11(1).
41. Ramesha KP, Rao A, Alex R, Geetha GR, Basavaraju M, Kataktalware MA, *et al*. Screening for Genetic Disorders in Indian Murrah and Surti Buffalo (*Bubalus bubalis*) Bulls. Buffalo Bulletin. 2017;36(1):133-142.