GENETIC VARIANTS IN FAM19A1, KCNB1 AND EDN3 GENE AND THEIR ASSOCIATION WITH UDDER TYPE TRAITS, CLINICAL MASTITIS AND MILK PRODUCTION IN KARAN FRIES AND SAHIWAL CATTLE

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL

(DEEMED UNIVERSITY)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ANIMAL GENETICS AND BREEDING

BY

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KARNAL-132001 (HARYANA), INDIA

2019

Regn. No. 15-P-AG-05

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This is to certify that the thesis entitled,"**GENETIC VARIANTS IN FAM19A1, KCNB1 AND EDN3 GENE AND THEIR ASSOCIATION WITH UDDER TYPE TRAITS, CLINICAL MASTITIS AND MILK PRODUCTION IN KARAN FRIES AND SAHIWAL CATTLE**" submitted by **RAGINI KUMARI** in partial fulfilment of the award of the degree of **Doctor Of Philosophy** in **Animal Genetics and Breeding** of the **ICAR-National Dairy Research Institute (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated: 06-03-2019

(ARCHANA VERMA)
MAJOR ADVISOR & CHAIRPERSON

DEDICATED TO
MY
BELOVED FAMILY
&
RESPECTED GUIDE

ACKNOWLEDGMENTS

Some glorifying moments come in this short eventful life that are to be kept in one corner of the heart for good, so that I can find out the significance of life recalling these sweet memories.

With all sense of ecstasy and profoundness, I wish to express my heartfelt gratitude to my respected guide Dr. Archana Verma, Principal Scientist, ICAR-NDRI, Karnal for herwholehearted cooperation, valuable guidance, unlimited patience, blessings, constant encouragement and care showered on me during experimentation. I will forever endure all her teachings, understanding and patientguidance to make me become what I am today. I feel greatly honoured to pursue my Ph.D degree under her guidance.

I wish to express my reverence to members of the advisory committee, Dr. I. D. Gupta, Principal Scientist, AGB division, Dr. A.K. Gupta, Principal Scientist, AGB division, Dr. S. S. Lathwal, Principal Scientist, LPM section, Dr. S. Jayakumar, senior Scientist, NBAGR and andDr. A. K. Mohanty, Principal Scientist, ABTC for planning of my experiment, for their timely suggestions, guidance and adviceduring the entire research work.

My profound reverence and gratitude to Dr. R.R.B Singh, Director and Vice-Chancellor, ICAR-NDRI (DU), Karnal, for providing me all the necessary facilities for successful completion of the work and financial support in the form of NDRI and UGC NFOBC fellowship during the entire period of my studies. Special thanks to Dr. A. K. Chakravarty (Former Head, AGB Division), Dr. A.K. Gupta (Former Head, AGB Division) and Dr. S.M. Deb (Head, AGB Division) for their valuable help and providing necessary facilities during my studies.

I wish to thank my respected teachers and divisional scientists, Dr. Avtar Singh, Dr. S.M.Deb, Dr. Anupma Mukherjee, Dr. Vikas Vohra and Dr. S. P. Dixit, for their strenuous efforts to impart good knowledge in the subject.

I express my sincere thanks to Suresh bhaiya, Raghubir uncle, Ruby, Panwar uncle, Baldev bhaiya, Mahendra bhaiya, ravinder bhaiya and all the staff members of AGB division for their cooperation during my research work.

I wish to record my sincere appreciation and thanks to my Seniors Ashwani sir, Mohsin sir, ArunPratap sir,Ramendra sir, Manvendra sir and Alok sir for their valuable suggestions and timely help.

Sweet memories shared with and nice ambience provided by Beena Ma'm, Diksha, Dimpee, Poonam, Rebeka, Prajwalita, Manoj, Vineeth, Anshuman, Suchit, Uday and Manjunatha sir are unforgettable. I wish to extend my heartfelt love and thanks to all my juniors, Saleem, Anand, shabha, Aneet, Ekta, Pooja.

I wish to express my heartiest thanks to my lab mates BeenaMa'm, Rebeka, Vineeth, Anshuman, Siddhu, Nisha, Sushil, Kaushalaya, Ravi, Anjali and Linda for their cooperation during my research work.

A formal line of appreciation would hardly meet the end of Justice in expressing my deep senseof heartiest regards and love to my revered papa and mummy their selfless love, blessings, affection, sacrifices, patience which always gave me the strength and encouragement. Their sacrifices and moral support made possible for me to attain this academic achievement in my life.

I exprees my heartiest love to Rashmi, Gautam, Saurabhfor their everlasting love and care. They always encouraged me and motivated me in every possible way.

Words would fail to express my ardent gratitude to my husband, Rakesh Kumar whose encouragement, dedication and unending love and care has always given me a new impetus to move forward. Without his constant support and understanding thecompletion of this thesis would not be possible. My thesis acknowledgement would be incomplete without thanking my baby-son Avyukt whose smiling face always made me happy and inspired me. Having him midway during my Ph.D. was certainly not easy for me but he has made my life wonderful. Words would never say how grateful I am to both of you.

My heart felt regard goes to my father in law, mother in law for their love and moral support.

Finally, I thank the Almighty God for always bestowing me with his grace and blessing without which I could not have achieved anything that I have today.

Not everyone mentioned but none is forgotten

Place: Karnal ***(RAGINI KUMARI)***

Date:

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ABBREVIATIONS

µg	:	Micro gram
µl	:	Micro litre
A	:	Adenine
Bp	:	Base pair
BTA	:	Bos taurus autosome
C	:	Cytosine
CDS	:	Coding sequence
CM	:	Centimorgan
CL		Central ligament
DFR		Distance between fore and rear teats
DLR		Distance between left and right teats
DMRT	:	Duncan's multiple range test
DNA	:	Deoyxribo nucleic acid
EDTA	:	Ethyelene Diamine Tetra acetic Acid
FTL	:	Front teat length
FTP	:	Front teat position
FTP	:	Front teat placement
FUA	:	Fore udder attachment
G	:	Guanine
Gm	:	Gram
LD	:	Linkage disequillibrium
LTCTEC	:	Long Term Changes in Teat-End Condition
M	:	Molar

Mb	:	Mega base pairs
Mg	:	Milli gram
Ml	:	Millilitre
NCBI	:	National Centre for Biotechnology Information
Ng	:	Nano gram
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
Pmol	:	Pico Mole
RFLP	:	Restriction Fragment Length Polymorphism
Rpm	:	Revolution Per Minute
RTP	:	Rear teat Position
RTL		Rear teat length
RTP	:	Rear teat Placement
RUH	:	Rear udder height
RUW	:	Rear udder width
SC	:	Skin Condition
SCC	:	Somatic cell count
SDF	:	Shortest distance from front teat end to floor
SDR	:	Shortest distance from rear teat end to floor
SE	:	Standard error
SNP	:	Single Nucleotide Polymorphism
SW	:	Sahiwal
T	:	Thymine
TBE	:	Tris Borate EDTA

TC	:	Teat circumference
TD	:	Teat diameter
TES	:	Teat end shape
TE	:	Tris EDTA
TS	:	Teat shape
TSI	:	Teat Size
UV	:	Ultra violet
UB	:	Udder Balance
UC	:	Udder circumference
UD	:	Udder depth
UL	:	Udder length
US	:	Udder shape
USUS	:	Udder suspension

ABSTRACT

The present investigation was carried out in lactating Sahiwal (*n*=87) and Karan Fries (*n*=166) cows to explore genetic polymorphism in FAM19A1, KCNB1 and EDN3 genes and to find out association of allelic variants with udder type traits, clinical mastitis and milk production. Total nine sets of primers were designed using Primer3 software. PCR products were subjected to custom sequencing and results for the respective region were analyzed using chromas software (version 2.6). Each sequence was aligned with corresponding *Bos taurus* reference sequence using ClustalW multiple sequence alignment program to identify SNPs. DNA sequencing revealed 9 SNPs in Karan Fries cows, 7 on FAM19A1 and 2 on KCNB1 gene and 10 SNPs, in Sahiwal cows 7 on FAM19A1, 2 on KCNB1 and 1 on EDN3 gene. PCR-RFLP analysis for genotyping was carried out using *BsrI, TaqI, AluI, HinfI, HindIII, MspI, BspHI* and *TspRI* enzymes for all 253 DNA samples. Association of each SNP locus with production and udder type traits was analyzed using GLM procedure of SPSS Version 22 while association of each SNP locus with incidence of mastitis and observational (visual) trait was analyzed using chi-square test. 'TT' genotype at locus T33069832C in FAM19A1 gene was associated with higher TC in both breeds. 'CC' genotype at C33464963A locus had minimum FTL and maximum no ring type skin in Karan Fries cows while in Sahiwal cows had minimum UB, DFR, DLR and maximum TC with minimum incidence of mastitis in both the breed. 'CT' genotype at locus C33471720T had maximum UW and CL with minimum UB in Karan Fries cows while, in Sahiwal cows had maximum RUH, RUW and minimum TC with highest production traits in both the breeds. CA' genotype at locus C33471980A had higher UL, UW, UC, TL, TDMY but with more number of rough ring than 'CC' genotype in Sahiwal cattle. However, in Karan Fries, 'CC' genotype had higher 305MY, TMY but minimum value of UD and DFR. 'GG' genotype of SNP at locus G33571161A recorded maximum UC, DFR, 305MY and TMY than 'GA' genotype in Karan Fries cows. Karan Fries cows with 'GG' genotype at locus G33589765A had significantly ($p<0.05$) highest MMY and UW than genotypes 'GA' and 'AA'. 'GG' genotype at locus G78216220A of KCNB1 gene had maximum UC, TD, TDMY but minimum DFR in Sahiwal cows while in Karan Fries cows 'AA' genotype at locus G78216220A was associated with highest UW, UL and lowest SDR. Sahiwal cows with 'AA' genotype at locus A78216335G of KCNB1 gene had significantly ($p<0.01$) lower RUH and RUW while in Karan Fries cows 'AA' genotype of this SNP had recorded higher RUH, RUW. 'CA' genotype at locus C57571910A of EDN3 gene had lower UL, UW and was also less susceptible to mastitis compared to 'CC' genotype. These findings suggest that CA' genotype at locus C33471980A and 'GG' genotype at locus G33571161A of FAM19A1 gene and 'AG' genotype at locus A78216335G of KCNB1 gene in Karan Fries cows and 'CA' genotype at C57571910A locus in EDN3 gene and 'CC' genotype at C33464963A locus of FAM19A1 gene in Sahiwal cows can be used as an aid to selection for higher milk production with desired udder conformation and lesser susceptibility to mastitis after validated in larger population.

सारांश

वर्तमान अध्ययन साहीवाल (८७) एवं करनफ्रीज (१६६) गायों का एफएएम19ए1, केसीएनबी1 और ईडीएन3 जीन में आनुवंशिक बहुरूपता की पहचान एवं इसका अयन गुणों, थनैला और दूध उत्पादन से सहसम्बन्धता का विश्लेषण करने के उद्देश्यों के साथ किया गया। कुल नौ प्राइमर सेटों को प्राइमर 3 सॉफ्टवेयर का उपयोग करके डिजाइन किया गया। पीसीआर उत्पादों का कस्टम अनुक्रम निर्धारण किया गया और संबंधित क्षेत्र को क्रोमस प्रारूप (संस्करण २.६) के द्वारा डीएनए अनुक्रमों का विश्लेषण किया गया। एकल न्यूक्लियोटाइड बहुरूपता को बॉश टॉरस के संदर्भ अनुक्रम के आधार पर कलस्टलडब्लू विभिन्न संरेखण प्रोग्राम द्वारा व वर्णलेख का दृश्य निरीक्षण करके पहचाना गया। डीएनए सीक्वेंसिंग से करनफ्रीज में ९ (एफएएम19ए1 में ७ और केसीएनबी1 जीन में २) और साहीवाल में १० (एफएएम19ए1 में ७, केसीएनबी1 में २ और ईडीएन3 जीन में १) एकल न्यूक्लियोटाइड बहुरूपता पाया गया। जीनोटाइपिंग के लिए पीसीआर आरएफएलपी विश्लेषण सभी २५३ डीएनए नमूनों पर *BsrI, TaqI, AluI, HinfI, HindIII, MspI, BspHI* और *TspRI* एंजाइमों का उपयोग कर के किया गया। प्रत्येक एकल न्यूक्लियोटाइड बहुरूपता अयन गुणों एवं दूध उत्पादन का सम्बन्ध विश्लेषण एसपीएसएस (संस्करण २२) GLM प्रक्रिया का उपयोग करके किया गया जबकि थनैला रोग और विसुअल गुणों का सम्बन्ध विश्लेषण काई-स्केयर का उपयोग करके किया गया। ‘TT 'जीनोटाइप का लोकस (T३३०६९८३२C) एफएएम19ए1 जीन में उच्च टीसी के साथ दोनों नस्लों में पाया गया। करनफ्रीज गायों में जीनोटाइप ‘CC’ (C३३४६४९६३A) में न्यूनतम एफटीएल, थनैला रोग की घटना और अधिकतम बिना रिंग की त्वचा जबकि साहीवाल गायों में न्यूनतम यूबी, डीएफआर, डीएलआर, थनैला रोग की घटना और अधिकतम टीसी पाया गया। करनफ्रीज में लोकस C३३४७१७२०T में CT 'जीनोटाइप में अधिकतम यूडब्लू, सिएल, दूध उत्पादन तथा न्यूनतम यूबी पाया गया जबकि साहीवाल गायों में अधिकतम आरयूएच, आरयूडब्लू, दूध उत्पादन और न्यूनतम टीसी मिला। साहीवाल गायों में जीनोटाइप ‘CA’ (C३३४७१९८०A) में उच्च यूएल, यूडब्लू, सिएल,टीएल, टीडीएमवाई और अधिक संख्या में खुरदरी रिंग की त्वचा पाया गया जबकि करनफ्रीज गायों में अधिकतम ३०५एमवाई, टीएमवाई और न्यूनतम यूडी, डीएफआर पाया गया। करनफ्रीज गायों में जीनोटाइप ‘GG’ (G३३५७११६१A) GA ’जीनोटाइप के मुकाबले अधिक यूसी, डीएफआर, ३०५एमवाई और टीएमवाई पाया गया। करनफ्रीज गायों में ‘GG’ जीनोटाइप (G३३५८९७६५A) ‘GA 'और' AA 'की तुलना में उच्चतम (p <०.०५) मासिक दुग्ध दर और यूडब्लू पाया गया। केसीएनबी1 जीन के लोकस G७८२१६२२०A में साहीवाल गायों में अधिकतम यूसी, टीडी, टीडीएमवाई और न्यूनतम डीएफआर पाया गया जबकि करनफ्रीज गायों में अधिक यूडब्लू, यूएल और निम्नतम एसडीआर पाया गया। केसीएनबी1 जीन के लोकस A७८२१६३३५G पर ‘AA ’जीनोटाइप वाली साहीवाल गायों में कम (p <०.०१) आरयूएच, आरयूडब्लू मिला जबकि AA जीनोटाइप वाली करनफ्रीज गायों में उच्च आरयूएच, आरयूडब्लू दर्ज किए गए। ईडीएन3 जीन के लोकेस C५७५७११०A में 'CA' जीनोटाइप में यूडब्लू, यूएल कम था और CC 'जीनोटाइप की तुलना में थनैला रोग के लिए भी कम संवेदनशील पाया गया। अतः करनफ्रीज गायों में ‘CA’ जीनोटाइप (C३३४७१९८०A) और ‘GG’ जीनोटाइप (G३३५७११६१A) एफएएम19ए1 जीन में और ‘AG’ जीनोटाइप (A७८२१६३३५G) केसीएनबी1 जीन के और साहिवाल गायों में ‘CA’ जीनोटाइप (C५७५७१९१०A) ईडीएन3 जीन तथा ‘CC’जीनोटाइप (C३३४६४९६३A) एफएएम19ए1 जीन में प्रयाप्त जानवरों की संख्या पर सिद्ध करके इसको वांछित अयन गुणों, थनैला रोग के लिए कम संवेदनशीलता के साथ उच्च दूध उत्पादन के लिए साहीवाल एवं करनफ्रीज गायों का चुनाव करने के लिए एक सहायता के रूप में किया जा सकता है।

CHAPTER –1

Introduction

1. INTRODUCTION

India is having an estimated number of 299.6 million bovines out of which cattle population is 190.9 million (Annual Report 2017-18, DAHD, GOI). Total crossbred population accounts for 39.73 million and Zebu/Non-descript cattle 151.17 million. India ranks first in milk production, achieving an annual output of 165.5 million tonnes with per capita availability of 355 gm/day (Annual Report 2017-18, DAHD, GOI). The total cow milk production in India is 76.41 million tonnes, the percentage share of milk production from indigenous/ non-descript cows in total cow milk production is 20.8 % while the crossbred cattle contributes 25.4 % to the total milk production. The main objective in dairy husbandry is to have a high yielding and well-functioning cow without any udder problems, which are major contributors to involuntary culling. Milk quantity, quality and production efficiency of cows is directly dependent on the udder health. The udder is the most important part of the body of the dairy cow. It was reported that both morphological and physiological mammary properties affect the milk yield of cattle (Tilki *et al.,* 2005). Higher selection pressure on production traits has been associated with occurrence of many production diseases in our cattle population. One of the most important diseases among them is Mastitis.

Udder health is generally considered as one of the most important traits in dairy cattle production (Miglior *et al*., 2005). Mastitis is one of the most important diseases of dairy animals which directly or indirectly affect the economy of the farmers (Sharma *et al.,* 2012). It adversely affects animal health, quality and quantity of milk, economics of milk production and shortens the productive life of the cow (Asaf *et al.,* 2014). Non-management factors such as season, parity, lactation stage, breed, udder conformation, milk production, milking speed and reproductive disorders are known to be associated with mastitis (Hagnestam *et al*., 2007; Nyman, 2007; Durr *et al*., 2008). In addition to the factors mentioned above, the genetic constitution and innate immune defense of a cow plays an important role in determining disease resistance in individual cows. Shook (1989) reported that there are several anatomical, physiological and immunological defense mechanisms in the cow against mastitis, and a large number of genes operate in these defenses.

The type traits are the body parts of dairy cow, which make her capable to produce milk and those traits which are directly or indirectly linked with each other are called as linear type traits. The linear type traits are the basis of modern day classification system of animal and are used to define the dairyness of a cow (ICAR, 2012). Moreover, improvement in these traits can improve the herd life of dairy cows besides improving their milk production level (Atkins and Shannon, 2002).

Udder is one of the most important physiological and conformational characteristics of all dairy animals. Udder type traits have always been an important issue in dairy cattle breeding as they are associated with lifetime production, and thus exert a major influence on the welfare and profitability of dairy cattle. Linear type traits describe biological extremes for a range of visual characteristics of an animal. They are relatively easy to measure and such information is generally available from a cow's first lactation. So, type traits have been used as indirect selection criteria for improving the herd life of dairy cow. Several researchers have shown a consistent relationship between udder conformation and udder health. It is known that the improper conformation of udder leads to the problem of udder deformities and cause udder disease like mastitis. Udder and teat conformation traits are highly heritable and have relation with milk SCC and hence mastitis (Klein *et al.,* 2005; Sharma *et al.,* 2011b). Deeper udder and long and thick teats increases the risk of mastitis (Singh *et al.*, 2014).

Sahiwal, one of the indigenous dairy breed of cattle is known to have good genetic potential to produce substantially large amount of milk in her productive life span. The unique characteristics which make her different from high producing exotic breeds such as Holstein-Friesian, Jerseys etc. is her resistant ability in extreme environmental and temperature fluctuations. Indigenous cows are considered less prone to mastitis but due to exploitation of these cows for more milk production the susceptibility of these animals to mastitis has increased. The Karan Fries crossbred dairy cattle was developed as a result of crossbreeding of Holstein Friesian with Tharparkar, followed by selection among interbreeds at NDRI, Karnal. This crossbred strain has good productivity but are also susceptible to mastitis. So far selection is being done mainly based on 305DMY and not much emphasis has been laid on the genes associated with udder type traits.

Use of molecular techniques in identification of QTLs and candidate genes affecting production traits, udder conformation and influencing mastitis resistance has opened new vistas for the genetic improvement in economic traits of dairy animals. Cole *et al.* (2011) reported that udder traits were affected by BTA 16, 22, X, 2, 10, 11, 20, 22 and 25, teat traits were affected by BTA 6, 7, 9, 16, 11, 26 and 17 by studying Genome-wide association analysis. Flury *et al.* (2014) found that Udder conformation traits are correlated with the incidence of clinical mastitis and the length of productive life. Most QTL for udder conformation traits were located in non-coding regions of the genome, which suggests that mutations in regulatory sequences are the major determinants of variation in mammary gland morphology in cattle (Pausch *et al.*, 2016).

Many genes have been identified which are associated with udder conformation and mastitis. FAM19A1 gene, related to MIP-1 alpha, a member of the CC-chemokine family, function as brain-specific chemokines or neurokines that acts as regulator of immune and nervous cells and is associated with somatic cell score and udder depth (Strillacci *et al.,* 2014). KCNB1 gene helps in maintenance and repair of udder epithelial tissue and development of mammary gland (Pal *et al*., 2006; Kim *et al*., 2012.) KCNB1 has association with milk yield, udder attachment, udder depth, height and width (Strillacci *et al.,* 2014). EDN3 is expressed in Heart, lung, liver, kidney, uterus and udder and helps in stimulation of udder cell growth and division (Duan *et al*., 2003). Wu *et al*. (2015) reported its association with mastitis. Sahana *et al*. (2014) reported the association of this gene with clinical mastitis and SCC. Data on nucleotide sequence polymorphism in the bovine for these genes are limited. Genetic variant study of these genes may be used as an aid to selection in Karan Fries and Sahiwal cows related to udder type traits, clinical mastitis and milk production. Keeping in view the importance of genes associated with linear type traits, mastitis and milk production together the present study is proposed to be conducted with the following objectives:

1. To explore genetic polymorphism in FAM19A1, KCNB1 and EDN3 genes in Karan Fries and Sahiwal Cattle
2. To find out the association of identified allelic variants with udder type traits, incidence of clinical mastitis and milk production

CHAPTER –2

Review of Literature

2. REVIEW OF LITERATURE

2.1 Linear type traits

International Committee for Animal Recording (ICAR), 2012 defines linear type traits for describing dairy cow as the basis of all modern type classification systems and foundation of all classification systems. Measurement of individual type traits form the basis of linear classification of traits instead of opinions. This system describes the degree and not the desirability of the trait. Moreover, the linear scores cover a biological range and variation within traits is identifiable.

2.2 Description of linear type traits

The precise description of each trait is well defined and it is essential to use the full range of linear scores to identify the intermediate and extremes of each trait within the population. The assessment parameters for the calculation should be based on the expected biological extremes. In the new linear type scoring systems, the methods are developed on the range of scale, *i.e.* 1 to 3, 1 to 9 or 1 to 50 points. The different scoring system for linear type trait which are being followed are:

- International Committee for Animal Recording (ICAR), 2012 World Holstein-Friesian Federation (WHFF), 2012 and Proceedings of 10th World Congress of Genetics Applied to Livestock Production (WCGALP), 2014 are 1 to 9 point scale scoring system whereas, Holstein Association USA, 2016 having 1 to 50 point scale scoring system (Table 2.1).

Saini and Gill (1989) recommended some important udder and teat measurements which are Udder length, Udder width, Udder depth, Udder circumference, Teat length and Teat diameter. Britt and Farnsworth (2011) also recommended some more important teat types/traits, which are Teat shape, Teat end classification score, Teat end shape and Skin condition.

The definition and description of different udder type traits as approved by ICAR (2012) are discussed below:

2.2.1 Fore udder attachment

It refers to the strength of attachment of fore udder to the abdominal wall. Udder attached over the whole width and has completely even transition with body, receives a score 9 whereas, udder with loose and narrow attachment with body receives score 1 (Figure 2.1). Fore udder attachment is not considered a true linear trait.

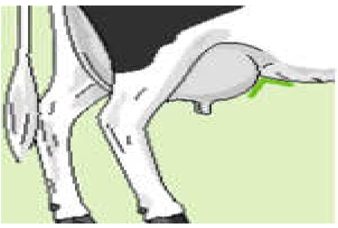
1-3: Weak and loose

4-6: Intermediate

7-9: Extremely strong and tight

Table 2.1 Different udder type traits included in different scoring system

ICAR & WHFF, 2012	WCGALP, 2014	H.A.U, 2016
Fore udder attachment	Fore udder attachment	Fore udder attachment
Rear udder height	Rear udder height	Rear udder height
Rear udder width	Rear udder width	Rear udder width
Udder depth	Udder depth	Udder depth
Udder balance	Udder balance	Udder balance
Teat length	Teat length	Teat length
Front teat placement	Front teat placement	Front teat placement
Rear teat placement	Rear teat placement	Rear teat placement
Central ligament	Teat thickness	Central ligament
Teat thickness	Fore udder length	
	Udder support	
	Teat diameter	
	Overall udder score	



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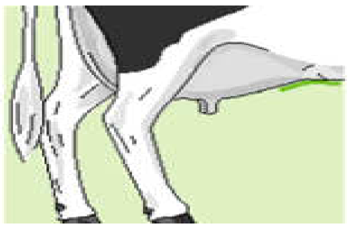


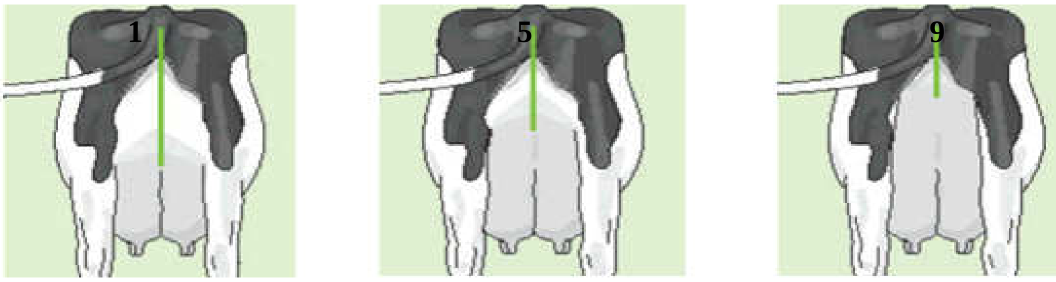
9

Figure 2.1 Diagrammatic representation of fore udder attachment under 9 point score system

2.2.2 Rear udder height

When assessing the rear udder height, the point of the milk secreting tissue has to be found. This point is assessed in relation to the distance between the pins and the hocks. For the point right in the middle, score 5 is given but if the point is much higher, then score 9 is given, while a very low receives score 1 (Figure 2.2).

1-3:	Very low
4-6:	Intermediate
7-9:	High



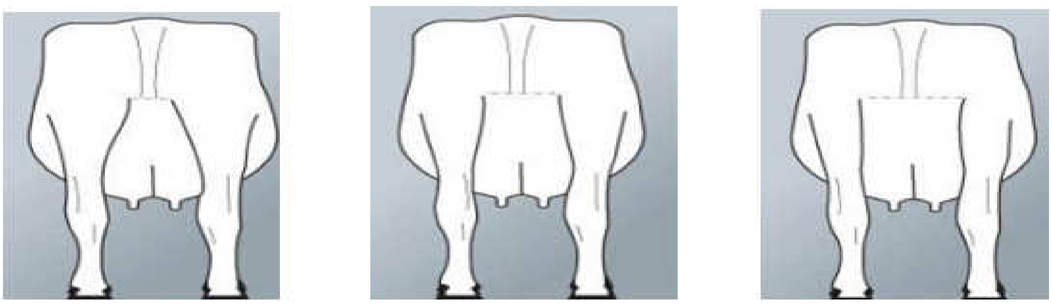
1 5 9

Figure 2. 2 Diagrammatic representation of rear udder height under 9 point score system

2.2.3 Rear udder width

It refers to the width of the udder at the point where the milk secretion tissue is attached to the body (Figure 2.3).

1:	Narrow
5:	Intermediate
9:	Wide



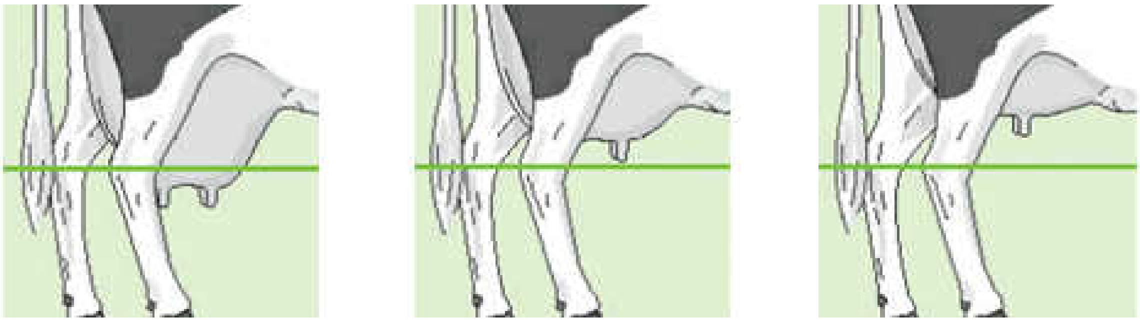
1 5 9

Figure 2.3 Diagrammatic representation of rear udder width under 9 point score system

2.2.4 Udder depth

The udder depth is assessed by the distance between the bottom of the udder and the hock (Figure 2.4). Potential point of reference is measure from the level of the hock.

1: Deep
5: Intermediate
9: Shallow



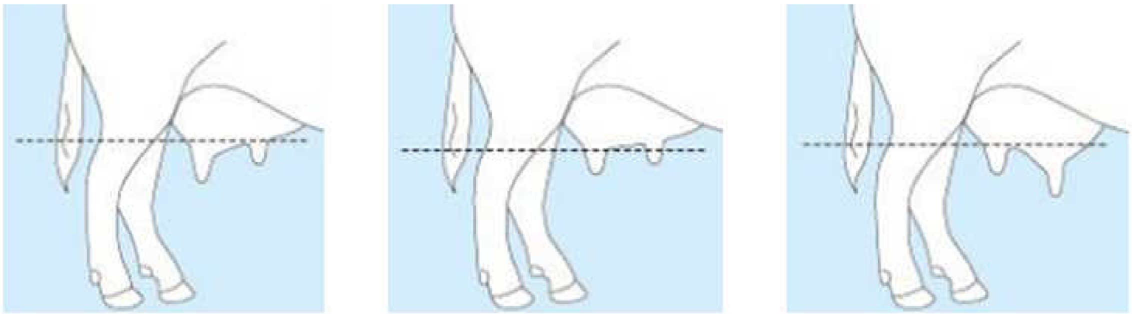
1 **5** **9**

Figure 2.4 Diagrammatic representation of udder depth under 9 point score system

2.2.5 Udder balance

The balance of the udder floor between front and rear udder (Figure 2.5).

1: Front quarter higher than rear quarter
5: Intermediate
9: Front quarter lower than rear quarter



1 **5** **9**

Figure 2.5 Diagrammatic representation of udder balance under 9 point score system

2.2.6 Teat length

It is the length of the front teat. The distance between the root and the tip of the teat (Figure 2.6).

1-3: Short
4-6: Intermediate
7-9: Long

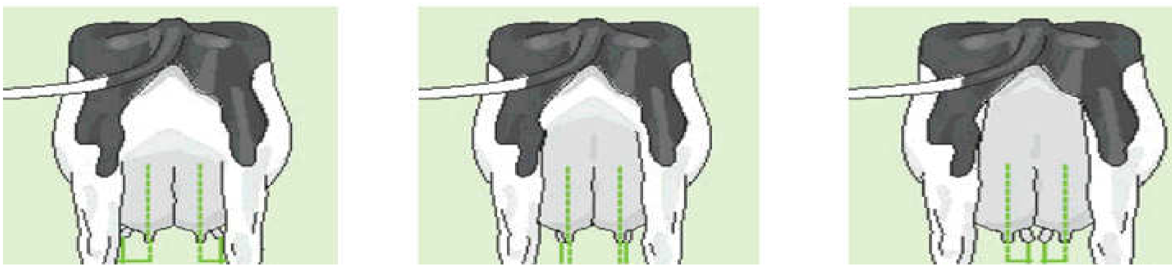
1 5 9

Figure 2.6 Diagrammatic representation of teat length under 9 point score system

2.2.7 Front teat placement

It refers to the position of front teat from centre of quarter as viewed from the rear. Front teats which are placed at the inner side of the quarter and pointing inwards receives score 9 whereas when placed on the outside of the quarter and pointing outwards obtain score 1 (Figure 2.7).

1-3: Outside of quarter
4-6: Middle of quarter
7-9: Inside of quarter



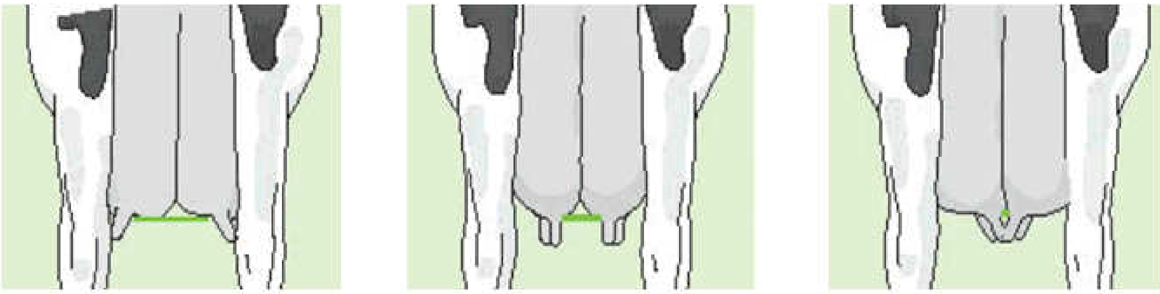
1 5 9

Figure 2.7 Diagrammatic representation of front teat placement under 9 point score system

2.2.8 Rear teat placement

The rear teat placement is assessed from the centre of quarter. Rear teats which are very close and are placed inside the quarter close to the ligament, obtain score 9 whereas when placed a far from each other and on the outside of the quarter, receives score 1 (Figure 2.8).

1-3: Outside
4-6: Mid point
7-9: Inside of quarter



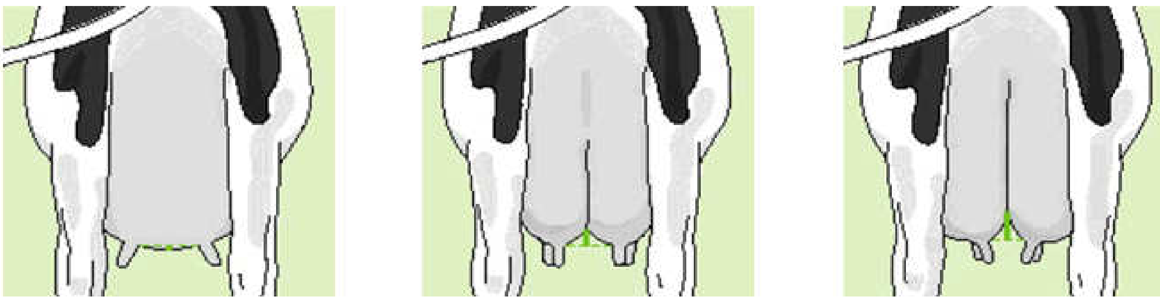
1 5 9

Figure 2.8 Diagrammatic representation of rear teat placement under 9 point score system

2.2.9 Central ligament

The depth of cleft at the base of the rear udder (Figure 2.9).

1-3: Convex to flat floor (flat), broken ligament
4-6: Intermediate
7-9: Deep cleft/strong ligament



1 5 9

Figure 2.9 Diagrammatic representation of central ligament under 9 point score system

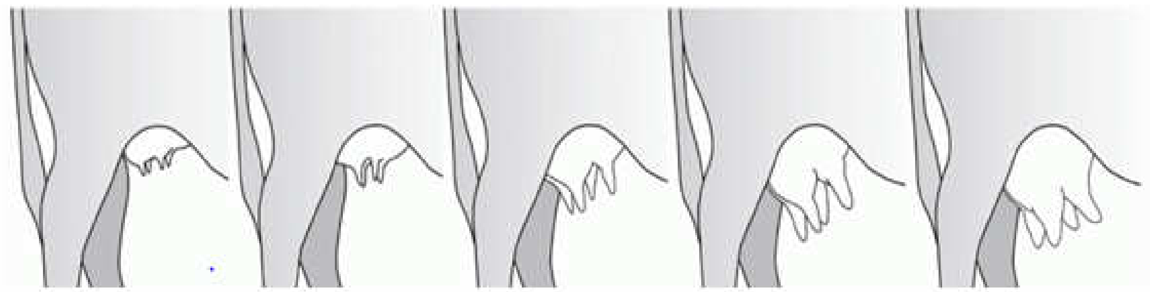
2.2.10 Teat thickness

Thickness of the teat in the middle of the front teat.

1-3: Thin
4-6: Intermediate
7-9: Thick

2.2.11 Teat Size

It is based on the subjective assessments of teat length and circumference of the animal (Figure 2.10).

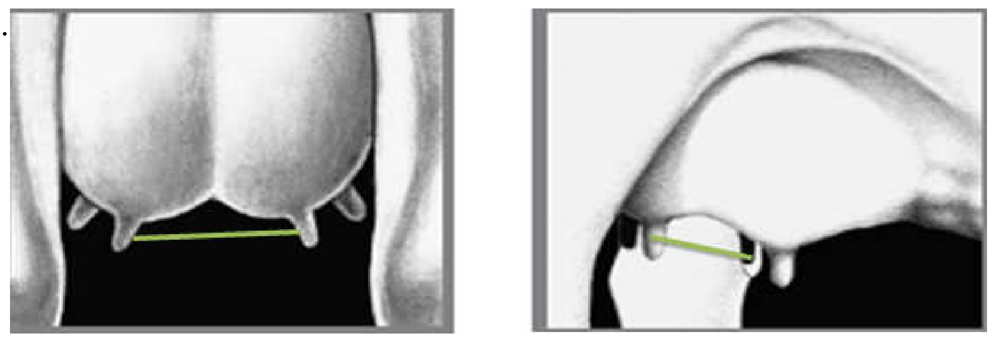


Very small symmetrical | Small symmetrical | Intermediate symmetrical | Large asymmetrical | Very large asymmetrical

Figure 2.10 Diagrammatic representation of teat size

2.2.12 Distance between teats

It describes the distance between left and right teats and between fore and rear teats at the midpoint of teat length (Figure 2.11).



Distance between left and right teats **Distance between fore and rear teats**

Figure 2.11 Diagrammatic representation of distance between teats score system

2.2.13 Teat end shape /Teat tip shape

Figure 2.12 Diagrammatic representation of teat end shape /teat tip shape

2.2.14 Udder suspension

It describes subjective assessments of udder support. Udder suspension with very tight and very pronounced median suspensory ligament receives score 9, with tight attachment and pronounced median suspensory ligament receives a score of 7. Udder suspension with intermediate attachment has a score of 5. Weak Suspensory ligament with loose attachment gives a score of 3 while very weak, very loose and pendulous attachment receives a score of 1 (Figure 2.13).

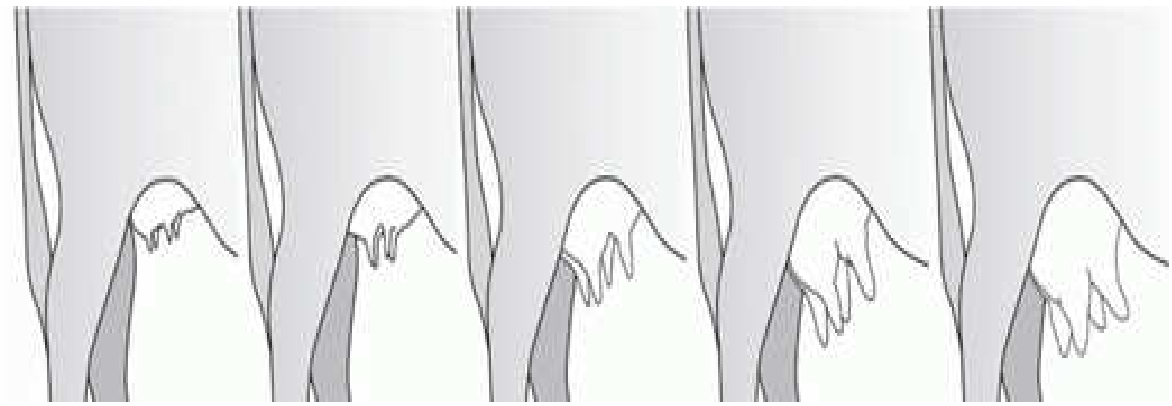


Figure 2.13 Diagrammatic representation of udder suspension score system Britt and Farnsworth (2011); Mein *et al*. (2001)

2.2.15 Longer-term changes in teat-end condition

It refers to the amount and hardness of the keratin in the teat ends. Mein *et al*. (2001) proposed a scoring system that consists of four descriptive characteristics to define Longer-Term Changes in Teat-End Condition (Figure 2.14).

1. **No ring**

   Teat end is smooth with small, even orifice
2. **Smooth/slightly rough ring**

   A raised ring is available. The ring is smooth/slightly rough with no keratin
3. **Rough ring**

   A raised, roughened ring with isolated mounds of old keratin extending 1-3 mm from the orifice
4. **Very Rough ring**

   A raised ring with rough mounds of old keratin extending 4 mm or more from the orifice - flowered appearance

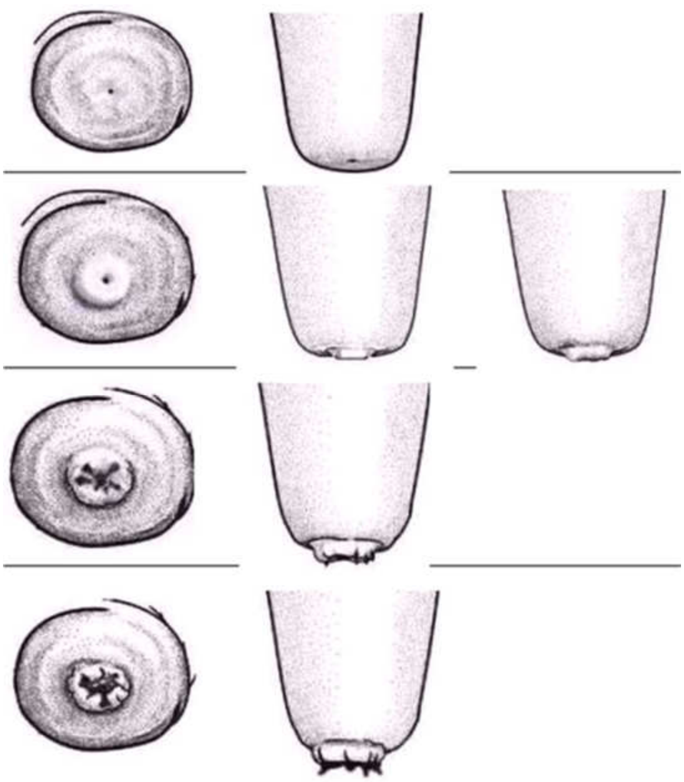


Figure 2.14 Diagrammatic representation of long term changes in teat end condition

2.2.16 Skin condition

Cold, wet or muddy conditions induce hardening or thickening of teat skin. Machine milking further exacerbates problems of chapping or cracking. Chemical irritation associated with disinfectant type or concentration, inappropriate type or concentration of emollients promote teat chapping. Skin conditioners reduce evaporation from the skin and act as humectants to maintain or improve the teat skin condition (Rasmussen and Larsen, 1998). The dryness of black teats tends to be over-estimated by observation alone. Evaluation is improved by lightly rubbing the teat skin with a finger. In the absence of cracks and sores, differences in skin condition classified as smooth or rough do not seem to influence new mastitis infection rates (Rasmussen and Larsen, 1998).

They proposed the following simple classification system:

1. Normal :Smooth sheen, soft, healthy skin
2. Dry :Scaly, flaky or rough skin but with no cracking)
3. Open lesions (allow space for specific description) :Chapped, cracked, black spot

2.3 Udder type scores

Different linear scales for scoring udder type have been reported in literature as 1 to 3 points (Smith *et al*., 1985), 1 to 9 points (Meyer and Burnside, 1987; Brotherstone *et al*., 2010), 1 to 50 points (Foster *et al*., 1989; Misztal *et al*., 1992) and 50 to 99 points (Norman *et al*., 1988; Dahiya *et al*., 2006). The score of a cow depends on five classification score. Mammary system (udder & teat), Dairy Strength, feet and legs, front end & body capacity and rump. These account for 40%, 20%, 20%, 15% and 5% respectively of the score **(**WHFF, 2005**)**. So, the mammary system constitute the major part of a dairy cow. Mitra *et al.* (1998) studied 13 linear type traits using 50 point (50-99) scale in Karan Fries cattle and found that most of the phenotypic correlations between different type traits were low to moderate while genetic correlations among traits were either beyond range or associated with large standard errors. They concluded that linear type traits could serve as indicators for selection of superior cows in the absence of milk production records especially under field condition. Dahiya *et al.* (2006) to study 15 linear traits on 50 point scale to study the importance of type traits for milk production potential. Dairy character and most of the udder traits had high association with the milk production. They concluded that linear type traits could serve as indicators for selection of superior cows in the absence of milk production records especially in field condition. Various works had been reported by different researchers on the type traits. The frequencies of teat shape and udder shape in different breed of cattle are presented in Table 2.2 and 2.3 respectively. Mean and standard error of different udder and teat type traits in different breeds reported by different authors are presented in Table 2.4.

Table 2.2 Various teat shapes and their percentage in different breeds of cattle

Breed	Shape of Teat (in %)					Reference
	Cylindrical	Conical/ Funnel	Pear	Bottle	Long and thick teats	
Crossbred	51.61	12.9	35.48	-	-	Sonwane *et al.*, 2002
Holstein	73.3	18.8	3.2	-	4.7	Uzmay *et al.*, 2003
Carora cows	32.84	48.56	-	15.34	-	Riera-Nieves *et al.*, 2005
Karan Fries	11.9	78.1	-	10	-	Singh, 2005
Karan Fries	75	25	-	-	-	Rao, 2006
Sahiwal	21.5	78.5	-	-	-	Singh, 2006
Sahiwal	38.89	44.4	-	16.67	-	Ahlawat, 2007
Karan Fries	37.42	48.27	-	14.31	-	Kamboj *et al.*, 2008
Crossbred	24.75	48.63	4.5	22.13	-	Patel and Trivedi, 2018
Gir	36.67	32.17	12.16	19	-	Modh *et al.*, 2017

2.4 Relationship of linear type traits with production traits

Udder conformation traits are known to correlate with the incidence of clinical mastitis and the length of productive life (Flury *et al*., 2014). Most phenotypic correlation between type traits and milk yield ranges from 0.18 to 0.38 (Norman *et al.*, 1988). Meyer and Burnside (1987) and Foster *et al*. (1988) found dairy character and udder depth having highest association among all linear type traits with lactation milk yield of Holstein cows. In Sahiwal cattle dairy character and most of udder traits had high association with milk production (Dahiya *et al.*, 2006). Bermingham *et al*. (2007) found taller, wider, deeper and more angular cows with deep udders tended to produce more milk in cows. Similar results were reported in Sahiwal cattle by Dubey, (2010). In Crossbred cows udder length, udder width, udder depth and fore teat diameter were positively associated with milk yield (Patel *et al.*, 2016). They found that the correlations between milk yield and various udder measurements viz., udder length (0.499), udder width (0.413) and udder depth (0.178) were found positive and significant ($P<0.05$) to highly significant ($P<0.01$) which reflected that all the three udder measurements should be the important criteria for selection of dairy cows as the udder length, width and depth decides the capacity of udder which reflects the milk yield. Similar results were found in Sahiwal (Ahlawat *et al*., 2008), Holdeo crossbred cows (Waghmore and Siddiqui, 2000), Kankrej & Crossbred cows (Kshatriya *et al.*, 2009) Vrindavani cattle (Singh *et al*., 2010) and Gir (Singhai *et al.*, 2013). Ranjeet *et al.* (2010) reported a positive and significant correlation ($P<0.05$) between total milk yield with udder length and width in Vrindavani cattle. Liu *et al.* (2014) found more milk production from wider rear udder in Holstein cows. Modh *et al.* (2017) in Gir cows revealed that the correlation between milk yield and udder width (0.194) was found significant ($P<0.05$) while, correlation between milk yield and udder length (0.128) and depth (0.157) was non-significant. Correlation between milk yield and length of fore teats (0.143) and length of rear teats (0.102) were non-significant. Genetic and phenotypic correlations between the linear type traits and the production traits in Holstein-Friesian dairy cattle was studied by Brotherstone (1994) and he reported that all phenotypic correlations between the linear type traits and the yield traits were small, but moderate genetic correlations were found between the yield traits and udder depth. Gibson (2015) in Brown Swiss cattle found positive correlation of yield traits with rear udder height and rear udder width while negative correlation with udder depth and fore udder attachment. In Holstein Cows genetic correlations between type and production traits were found

Table 2.3 Various udder shapes and their percentage in different breeds of cattle

Breed	Shape of Udder (in %)								Reference
	Bowl	Round	Goaty	Globular	Pendulous	Trough	ball	Rear-heavy/ unbalanced	
Crossbred	35.48	16.12	-	-	41.43	6.45	-	-	Sonwane *et al.*, 2002
Holstein	-	-	-	-	6.7	55.9	25.9	11.5	Uzmay *et al.*, 2003
Dairy cows	16.67	54.17	29.16	-	-	-	-	-	Bhuiyan *et al.*, 2004
Karan Fries	-	11.94	15.09	-	19.47	45.94	-	7.51	Singh, 2005
Karan Fries	-	50	-	-	6.25	43.75	-	-	Rao, 2006
Sahiwal	-	42.8	-	-	7.14	50	-	-	Singh, 2006
Sahiwal	-	33.3	-	-	22.2	44.4	-	-	Ahlawat, 2007
Crossbred	-	-	-	53.64	2.58	37.69	-	-	Gajbhiye *et al.*, 2007
Karan Fries	-	11.94	15.09	-	19.47	45.94	-	7.51	Kamboj *et al.*, 2007
Gaolao	60-90	10-30	10-20	-	-	-	-	-	Chachare and Walkunde, 2011
Friesian	20.4	75.51	2.04	-	2.04	-	-	-	Tag *et al.*, 2011
Crossbred	-	19.5	7.5		16.5	56.5	-	-	Patel and Trivedi, 2018
Gir	-	22	38	-	6	34	-	-	Modh *et al.*, 2017

Table 2.4 Mean±SE of different udder and teat type traits in different breeds of cattle

Breed	UL	TL	TD	SD	UD	UW	RUH	UB	CL	FUA	TP	Reference
Crossbred	49.87 ±0.56	F-7.32 ± 0.09 R-6.72 ± 0.09	F-7.32 ± 0.09; R-1.47 ± 0.03	-	14.65 ±0.20	51.08 ±0.58	-	-	-	-	-	Waghmore and Siddiqui, 2000
Red and White, Poland	43.92 ±3.87	F- 5.72 ±0.82 R-4.72 ±0.74	F-2.88 ±0.29; R-2.86 ± 0.32	F-53.90 ±5.01 R-53.45 ±5.46	F-26.92±2.57	-	-	-	-	-	-	Kuczaj, 2003
Dairy Cow	33.20 ±0.71	-	-	-	12.57 ±0.32	32.12 ±0.68	-	-	-	-	-	Bhuiyan *et al.* 2004
Crossbred	52.65 ±2.26	-	-	-	20.30 ±0.86	59.21 ±2.16	-	-	-	-	-	Kshatriya *et al.*,2009
Vrindavani	49.2 ±0.8 to 62.9 ± 1.2	5.0±0.1 to 6.4±0.2	2.1± 0.05 to 2.7±0.1	-	19.4±0.4 to 28.5 ±0.7	55.6±0.9-73.5±1.3	-	-	-	-	-	Singh *et al.*, 2009
Sahiwal	-	6.92 ±0.17	2.78 ±0.06	-	12.39 ±0.42	-	12.65 ±0.23	0.54 ±0.19	1.76 ±0.07	127.38 ±1.56	F-5.34 ±0.14 R-6.37 ±0.11	Dubey, 2010
Cows	-	F-4.77 ±0.51 R-4.66 ± 0.54	F-1.68 ± 0.13 R-1.70 ± 0.08	-	-	-	-	-	-	-	-	Hussain *et al.*, 2012
Gir	45.99 ±2.07	7.07 ±0.42	3.49 ±0.21	-	20.29 ±1.04	20.51 ±0.99	-	-	-	-	-	Singhai *et al.*, 2013
Dairy cow	-	-	-	F-51.26 ±5.98; R-51.13 ±6.3	-	-	-	-	-	-	-	Nakov *et al.*, 2014
Zebu cows, Camroon	24.02 ±2.85	-	-	-	13.11±2.36	-	-	-	-	-	-	Meingoas *et al.*, 2017
Gir	61.95 ±1.20	3.95 ±0.64	1.98 ±0.30	-	25.62±0.43	62.99±1.17	-	-	-	-	-	Modh *et al.*, 2017
Crossbred	58.24 ±0.68	5.73 ±0.06	2.68 ±0.03	-	23.06 ±0.34	65.45 ±0.70	-	-	-	-	-	Patel and Trivedi, 2018

UL-Udder Length, **TL**- Teat length, **TD**-Teat diameter, **SD**-Shortest distance from floor, **UD**-Udder Depth, **UW**- Udder width, **RUH**-Rear udder height, **UB**-Udder Balance, **CL**-Central ligament, **FUA**-Fore udder attachment, **TP**-Teat placement, **F**-Fore, **R**-Rear low in which Udder depth had the highest negative genetic correlation with production traits (Campos *et al*., 2015). Ghosh and Prasad (1998) found Positive significant association of test day milk yield with udder length, udder width and udder depth in Jersey and Red Sindhi crossbred cattle. Khan and Khan (2016) also reported as udder length, width, circumference and depth increases as test day milk yield increases in Sahiwal cattle.

Simple correlations between teat length, total milking yield and 305-day milk were positive and significant (Moore *et al*., 1981). Correlation coefficient of teat length and diameter with milk production were not encouraging in Karan Fries Cattle (Gupta *et al.,* 1991) whereas Patel *et al.* (2016) found a positive and significant ($P<0.05$) association of fore teat diameter with milk yield. Rao (2006) found effect of teat shape on milk yield and composition to be non-significant in Karan Fries cattle. They found significant and positive correlation of teat length with milk yield and a negative significant correlation between rear teat diameter and milk yield. Tilki *et al.* (2005) revealed that teat length, udder height, distance between front teats, between rear teats and between front and rear teats significantly affected milk yield in Brown Swiss. Milk yield increases with decreasing udder height and increasing distance between teats. Yakubu (2011) reported that the milk yield had positive and significant association with fore right teat length, fore left teat length, rear right teat length, rear left teat length and udder circumference ($r=0.538$-0.766; $P<0.01$) in Bunaji (White Fulani) cows. However, milk yield negatively correlated with udder height ($r=-0.420$; $P<0.01$).

The udder shape reported to influence the milk production (Pawlina *et al.,* 2000; Bhuiyan *et al.,* 2004). Tag *et al.* (2011) noted that the cows with bowl shaped udder would release more milk yield in milking, higher milking rate and average milk flow rate followed by the cows with round and pendulous shaped udder, while the less milk yield was detected in the cows with goat shaped udder. Ahmed *et al.* (2005) observed the relationship of udders and teat morphology with mastitis and milk yield and found that, trough-shaped udders were common in primiparous crossbred dairy cows. Bhuiyan *et al.* (2004) showed that bowl shaped udder yielded higher milk yield. Gajbhiye *et al.* (2007) concluded that cows with a trough shaped udder had higher milk yield as compared with globular and pendulous shaped udder . It was also noted that longer length and width of the udder were directly proportional to milk yield in crossbred cattle. It can be concluded that udder measurements can be a reliable criteria for selecting cows for milk production.

2.5 Relationship of linear type traits with mastitis

Consistent relationship between udder conformation and udder health had been reported by many researchers. The improper conformation of udder leads to the problem of udder deformities and cause udder disease like mastitis. Van Dorp *et al*. (1998) showed the genetic correlations between udder conformation traits and mastitis. Height of the udder, length, diameter and shape of the teat had been found to be correlated to udder injury and incidence of udder disease. The udder shape, location and strength of udder attachments are hereditary and the genetic selection has the ability to alter the anatomical structure of cow's udder (Atkins, 2007). Udder depth, fore-udder attachment and udder balance were positively associated with Somatic cell score and clinical mastitis in Holstein (Rupp and Boichard, 1999). Amin *et al*. (2002) also found deeper udder and low udder height from floor to be associated with clinical mastitis in Holstein cows. Wattiaux (2005) reported that the Cows with deeper udder are more prone to mastitis. Singh *et al.* (2014) also reported the association of udder rear longer, deeper udder and less distance of udder to floor as a cause to clinical mastitis. Weak fore udder attachment had the high chance of clinical mastitis (Sorensen *et al*., 2010). Nash *et al.* (2002) found reduce in the total number of clinical mastitis episodes with stronger fore udder attachment, shallow udders and deeper udder cleft in Holstein cows. On the basis of shapes of udder and teats, the highest incidences of subclinical mastitis was observed in pendulous shaped udder followed by unbalanced udder , goat type, round and trough shaped udder (Kamboj *et al.,* 2008). Higher incidences of subclinical mastitis in pendulous udders and lowest with trough-shaped udders has also been reported in Holstein and Sahiwal cows by Uzmay *et al.* (2003) and Ahlawat *et al*. (2008). Sabin George *et al.* (2007) found higher occurrence of subclinical mastitis in hind quarters (53.07 %) than the fore quarters (46.93 %) and also higher incidence in left quarters (53.50 %) than the right quarters (46.50 %) in crossbred cattle. Khan and Muhammad (2015) also found the occurrence of subclinical mastitis more in hind quarters than fore quarters in Crossbred cows while Shahid *et al.* (2011) observed higher incidence of sub clinical mastitis in fore quarters (30 %) than the hind quarters (20 %) in crossbred cows. Kuczaj (2003) reported a significant relationship between cow udder size (distance from the floor, suspension and udder and teat shape) and incidence of mastitis. Nakov *et al*. (2014) reported higher risk of Clinical mastitis incidence in the rear udder quarters compared to the front udder quarters and found the relative risk of Clinical mastitis was lower for primiparous cows which increases with further parity. The odds ratio of Clinical mastitis increased significantly as udder morphology worsened, distance from teat ends to floor decreased and the teat ends were flat (Nakov *et al*., 2014). They further concluded conformation udder traits could be used for the genetic selection of dairy cows for mastitis resistance.

Velazquez (2000) reported that teat traits have an influence on Mastitis. Uzmay *et al.* (2003) reported that the risk of sub-clinical mastitis was highest for cows with long and thick teats and cylindrical teats. Bottle shaped teats and less distance of anterior and posterior teat from ground are associated with Somatic cell score (SCC) in Sahiwal cattle (Ahlawat, 2007). SCC were significantly associated with teat length, teat end shape, teat diameter in Jersey cross bred (Bharti *et al*., 2015). Guarin *et al.* (2017) reported positive association of clinical mastitis with Pre milking diameter of the teat apex in dairy cattle. Sabin George *et al.* (2007) reported that the incidence of subclinical mastitis increased with increase in teat length. Higher incidence of subclinical mastitis in the teat with larger diameter was found by Bardakcioglu *et al.* (2011). Among various shapes of teat, quarters with cylindrical teats had significantly ($P<0.05$) higher (41.59 %) incidence of subclinical mastitis than conical teats (15.63 %) and bottle shaped teats (13.18 %) (Kamboj *et al.,* 2008). Patel 2014 also reported highest incidence of subclinical mastitis in cows having cylindrical teats followed by funnel, bottle and pear shape teat.

2.6 Effects of non-genetic factors on production traits, udder conformation and incidence of clinical mastitis

Various non-genetic factors viz. parity, season of calving, stage of lactation found to effect milk production traits, udder conformation and incidence of mastitis.

2.6.1 Effect of non-genetic factors on production traits

2.6.1.1 Effect of season of calving

Significant effect ($P<0.05$) of season of calving on 305DMY has been reported by Katok and Yanar (2012).Kumar *et al.* (2016) and Kumar *et al.* (2017) observed non-significant effect of season of calving on milk and its constituents in dairy animals. Ratwan *et al*. (2016) observed significant effect of period and season of calving in Jersey crossbred cattle while Kumar *et al.* (2012) found non-significant effect of season of calving on 305 MY in Sahiwal cattle. All the monthly test day (MTDY) milk yields were highly significant in Karan Fries cattle for the season of calving.

2.6.1.2 Effect of parity

Significant effect of parity on 305DMY has been reported by Rehman and Khan (2012), Khan and Khan (2016) and Kakati *et al.* (2017). Significant effect of parity on TMY has been observed by Bajwa *et al.* (2004) and Rehman and Khan (2012). While non-significant effect was reported by Rehman *et al.* (2008). Fahim *et al.* (2017) reported that the highest milk yield was produced by the crossbred cows in 4th parity, whereas, the lowest milk yield was produced by the cows in their 1st parity. There has been a significant difference ($P<0.01$) in milk yield among the different parity. Jinger *et al.* (2014) and Japheth *et al.* (2015) also reported significant ($P<0.01$) influence of parity on milk yield in Karan Fries cows. Khan and Khan (2016) also reported the significant ($P<0.001$) effect of parity on 305 day milk yield and test day yield in Sahiwal cattle.

2.6.1.3 Effect of stage of lactation

Total milk yield was significantly ($P<0.05$) higher during the early stages of milk production than in mid and late stages in Holstein dairy cows (Strapák *et al.*, 2011). Fahim *et al.* (2017) reported that the total milk/day/animal was significantly ($P<0.01$) higher during the early stages of milk production than in mid and late stages in crossbred cows. Highly significant ($p<0.01$) effect of stage of lactation on TDMY in Sahiwal, Karan Fries, Tharparkar and Murrah was reported by Sarkar *et al*. (2006) and in Holstein dairy cows by Cobanglu *et al*. (2017). Khan and Khan (2016) reported the effect of stage of lactation on TDMY in Sahiwal cattle.

2.6.2 Effect of non-genetic factors on udder conformation traits

Dairy character and rear udder width was found to be significantly influenced ($p<0.01$) with stage of lactation whereas fore udder attachment, rear udder width and udder depth has been affected by Parity (Mitra *et al.*, 1998). Value of scores for fore udder attachment, rear udder height and width and teat position reduced with the increase in stage of lactation (Marinov *et al*. (2015). Deng *et al.* (2012) reported that all udder measurements increased with parity order but stage of lactation did not affect the udder measurements. Khatri *et al.* (2017) found that all udder and teat measurements were found to be increasing with the increment in lactation number in buffaloes.

Khan and Khan (2016) found stage of lactation, parity and herd as a significant source of variation for udder length, width, depth, circumference, 305 day milk yield and test day yield (P<0.001). The udder length had the highest phenotypic correlation with test day yield at first stage of lactation followed by udder width, udder circumference and udder depth.

Zwertvaegher *et al*. (2012) and Mingoas *et al*. (2017) reported that udder height and udder depth significantly increased (p<0.05) with parity while both the traits decreased (p<0.05) at the 3rd stage of lactation. They also reported that there was no significant variation of teat size according to parity and stage of lactation.

2.6.3 Effect of non-genetic factors on incidence of clinical mastitis

Frequencies of mastitis differ between parities and the risk of clinical mastitis increases with increasing parity (Sargeant *et al.*, 1998; Heikkila *et al.*, 2012; Breen *et al.*, 2009; Boujenane *et al*., 2015) while Lescourret *et al.* (1993) did not find any significant effect of parity on the number of clinical mastitis cases per lactation. Boujenane *et al*. (2015) found that Calving season had a significant effect on the number of mastitis cases (P < 0.05). Cows affected in summer had a higher risk of mastitis during the 305 days lactation period than cows that calved during the other seasons. Steeneveld *et al.* (2008) reported that the incidence rate of clinical mastitis in the first 10 days of lactation was higher in heifers than in multiparous cows. However, after 10 days of lactation, it was higher in multiparous cows than in heifers. Nakov *et al.* (2014) in logistic regression study reported that the relative risk of clinical mastitis was lower for primiparous cows, and increased with increased parity.

In the multivariable logistic regression model, the herd-level factors significantly associated ($p < 0.05$) with the presence of mastitis were herd size, bedding material, and milking the mastitic cows, whereas at the cow-level, breed, stage of lactation, parity, udder and leg hygiene, and teat end shape have a significant effect on the occurrence of mastitis. (Abebe *et al*., 2016).

2.7 Genes having association with udder conformation traits, mastitis and milk production

Many researchers have found the different genes present on various chromosomes to be associated with type traits, production traits and mastitis in different breeds of cattle (Table 2.5).

Hiendleder *et al*. (2003) identified a QTL having significant F-values for udder conformation traits in the Holstein breed on BTA6 at 88 cM based on a granddaughter-design (16 half sib families with 872 sons) and 264 microsatellite markers.

Cole *et al.* (2011) studied a Genome-wide association analysis and identified a number of candidate genes and chromosome regions associated with 31 dairy traits in contemporary U.S. Holstein cows. They reported that highly significant genes and chromosome regions include BTA13's guanine nucleotide binding protein, alpha stimulating (GNAS) region for milk, fat and protein yields. They further found that udder traits were affected by BTA16, BTA22, BTAX, BTA2, BTA10, BTA11, BTA20, BTA22 and BTA25, teat traits were affected by BTA6, BTA7, BTA9, BTA16, BTA11, BTA26 and BTA17, and feet/legs traits were affected by BTA11, BTA13, BTA18, BTA20, and BTA26.

Flury *et al*. (2014) found that Udder conformation traits are correlated with the incidence of clinical mastitis and the length of productive life. The results of a genome-wide association study based on imputed high-density genotypes of 1,637 -Brown Swiss sires and de-regressed breeding values for 13 udder traits. For seven of the totally 13 investigated traits significant SNPs could be observed. For seven traits significant signals could be observed in five regions on chromosome 3 (117.31-118.36 Mb); chromosome 5 (31.50-31.52 Mb), chromosome 6 (88.74-90.80 Mb), chromosome 17 (63.12-65.39), and chromosome 25 (10.89-10.93 Mb). For fore udder length and teats diameter significant SNPs were found in a known region around 90 Mb on BTA6. For the trait rear udder height significant SNPs are positioned in the coding region of the SNX29 gene. Several significant SNPs around 62 Mb on BTA17 are associated with rear udder width, front teat placement and rear teat placement.

Pausch *et al.* (2016) carried out whole-genome sequence data from 157 key ancestors of the German Fleckvieh cattle population. They found seven QTL were associated with multiple phenotypes. Phenotypic data on seven udder conformation traits (teat thickness, teat length, teat position, udder depth, central ligament, fore udder attachment and fore udder length) were taken.

Table 2.5 Candidate genes for udder conformation, mastitis and milk production

Genes	BTA	Associated with	Reference
ETS2	1	Mastitis and milk production	Ogorevc *et al*., 2009
ROBO1		Udder cleft, milk production	Strillacci *et al*., 2014
SP5	2	Udder conformation	Pausch *et al*., 2016
LRRFIP1	3	Fore udder attachment	Flury *et al*., 2014
LOC104970680		Udder conformation	Flury *et al.*, 2014
MLPH		Fore udder attachment	Flury *et al*.,2014
RAB17		Udder conformation	Flury *et al*., 2014
COL6A3		Udder conformation	Flury *et al.*, 2014
PLXNA4	4	Clinical mastitis, Udder depth	Strillaci *et al*., 2014
PD1A6		Teat length	Wu *et al*., 2015
PTHLH	5	Milk traits and mastitis	Ogorevc *et al*.,2009
HMGA2		Adipose tissue development, regulation of growth hormone secretion	Tolleson, 2016
SPCS3		Udder support	Tolleson, 2016
LOC100848544		Udder support	Tolleson, 2016
VDR		Calcium related metabolism and calcium homeostasis	Gao *et al.*, 2013
LOC785294		Udder support	Tolleson, 2016
LOC100139418		Udder support	Tolleson, 2016
PTPRR		mammary epithelial cell proliferation, differentiation and apoptosis	Gallo-Hendrikx *et al.*, 2001
LOC786074		Udder support	Tolleson, 2016
C5H12orf28		Udder support	Tolleson, 2016
IL22		Cell growth, differentiation and motility. Antimicrobial immunity, inflammation, tissue repair - mastitis prevention and resistance	Tolleson, 2016
DYRK2		Proper mammary gland development	Gallo-Hendrikx *et al.*, 2001
LOC100848387		Udder support, Average teat diameter	Tolleson, 2016
DCK	6	Clinical mastitis, SCC	Wu *et al*., 2015
GC		Clinical mastitis, SCC, Udder conformation	Pausch *et al*., 2016, Wu *et al*., 2015
NPFFR2		Clinical Mastitis, SCC, udder conformation	Pausch *et al.*, 2016

…

ABCG2		Mastitis and milk production	Ogorevc *et al.*, 2009
LETM1		Fore udder attachment, Rear Udder Height, Udder depth	Cole *et al.*, 2011
WHSC2		Udder Depth , Udder Cleft	Cole *et al.*, 2011
WDFY3		Fore Teat Placement, Rear Teat Placement, Teat Length	Cole *et al.*, 2011
RASSF6		Udder conformation	Pausch *et al.*, 2016
SFXN1		Udder depth and milk production	Gonzalez *et al.*, 2017
ANKRD31	10	Udder depth and milk production	
WDR41		Udder depth and milk production	
CRIM1	11	Udder conformation	Pausch *et al.*, 2016
RXFP2	12	Udder conformation	Strillaci *et al.*, 2014
KCNB1	13	Milk yield, Udder attachment , Udder Depth	Strillaci *et al.*, 2014
EDN3		Clinical Mastitis, SCC	Sahana *et al.*, 2014
FGF2		Clinical Mastitis, milk production	Wang *et al.*, 2008
RBM19	17	Udder conformation	Flury *et al.*, 2014
TBX5		Rear Udder Width , Rear Teat Placement, Fore teat Placement	Flury *et al.*, 2014
ABHD2	21	Teat length	Cole *et al.*, 2011
FAM19A1	22	Protein yield, Udder Depth, SCC	Strillaci *et al.*, 2014
SUCLG2		Udder conformation,	Cole *et al.*, 2011
SNX29	25	Rear Udder Height	Flury *et al.*, 2014

Most QTL were located in non-coding regions of the genome but in close proximity of candidate genes that could be involved in mammary gland morphology (SP5, GC, NPFFR2, CRIM1, RXFP2, TBX5, RBM19 and ADAM12). Most QTL for udder conformation traits were located in non-coding regions of the genome, which suggests that mutations in regulatory sequences are the major determinants of variation in mammary gland morphology in cattle.

Tolleson (2016) detected a total of 15 significant SNP for udder support exclusively within a 26.4 Mb region on BTA 5 (21.6 to 48.1 Mb) while average teat diameter had one associated marker located on BTA 5 in *Bos indicus* and *Bos taurus* cross cows. No SNP associations were detected for average teat length or any individual teat traits. Out of these SNPs, three SNPs were located within genes; VDR, IL22, and PTPRR. The other 12 SNPs were located at a distance of 9,882 to 192,136 bp from the nearest known genes. These results identified associated markers within genes that have an involvement in the development and regulation of the mammary system.

Gonzalez *et al.* (2017) studied a genome-wide association analysis for a set of sampled and imputed SNPs with 16 conformation traits in a population of Holstein cows from a desert area of North western Mexico. Eight SNPs were found to be significant in two phenotypes (udder depth and body depth). No significant SNPs were detected for any of the other traits. Seven SNPs were significant for the udder depth trait, all located on chromosome 10 (BTA10). Out of these 7 SNPs, 4 SNPs were located inside genes (SFXN1, ANKRD31 and WDR41) whereas the rest were located within an average distance of 155 kb from the annotated genes. The udder depth trait resulted in seven associated SNPs, related to marbling score, milk yield, fat yield, protein yield, and protein percentage.

Merete *et al.* (2018) used the AWM-PCIT (Association Weight Matrix with a Partial Correlation in Information) algorithm as a post-GWAS analysis tool to identify candidate genes associated with cattle morphology traits. They used data from 78,440 cows belonging to three French dairy cattle breeds for five udder morphology traits, five production traits, somatic cell score and clinical mastitis. The AWM-PCIT detected ten potential candidate genes for udder related traits: ESR1, FGF2, FGFR2, GLI2, IQGAP3, PGR, PRLR, RREB1, BTRC, and TGFBR2.

2.8 Organization and location of FAM19A1 gene

It is a member of the TAFA family which is composed of five highly homologous genes that encode small secreted proteins. This gene is located on bovine chromosome number 22 (ENSBTAG00000019041.5) conferring a total length of 520.04 kb. FAM19A1 gene comprises 4 exons and 3 introns. It has a translation length of 133 residues (Figure 2. 15 and Table 2.6).

Table 2.6 Nomenclature of FAM19A1 gene

Synonym	**Family with sequence similarity 19 member A1 (chemokine (C-C motif-like)**
Exons	04
Coding exons	04
Introns	03
Gene size	520040 bp
Transcription length	402 bp
Translation length	133 residues
Gene type	Protein coding

2.8.1 Biological role of FAM19A1 gene

This gene is a member of the TAFA family which is composed of five highly homologous genes that encode small secreted proteins. These proteins contain conserved cysteine residues at fixed positions, and are distantly related to MIP-1alpha (Macrophage inflammatory protein), a member of the CC-chemokine family. The TAFA proteins are predominantly expressed in specific regions of the brain, and are postulated to function as brain-specific chemokines or neurokines that act as regulators of immune and nervous cells. Chemokine receptors expressed on neutrophil surfaces play role in neutrophil activation, phagocytosis of pathogens and chemotaxis.

Gene ID **782913**	• **Family with sequence similarity 19 member A1, C-C motif chemokine like** • **TAFA chemokine like family member 1**	**Ensemble Version** **ENSBTAG00000019041.5**

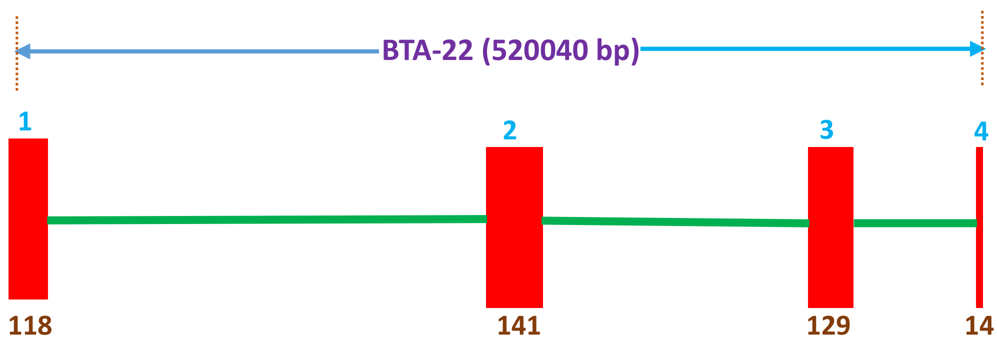


Figure 2.15 Organization of FAM19A1 gene in *Bos taurus*

2.9 Organization and location of KCNB1 gene

KCNB1 is a protein coding gene located on bovine chromosome 13 (ENSBTAG00000027320). It has a length of 110.35 kb and has 2 exons and 1 intron having a translation length of 858 residues (Figure 2. 16 and Table 2.7).

Table 2.7 Nomenclature of KCNB1 gene

Synonym	**Potassium voltage-gated channel, subfamily B member 1**
Exons	02
Coding exons	02
Introns	01
Gene size	110350 bp
Transcription length	3,747 bp
Translation length	858 residues
Gene type	Protein coding

2.9.1 Biological role of KCNB1 gene

Potassium channels are membrane proteins that allow rapid and selective flow of K^+ ions across the cell membrane and thus generate electrical signals in cells. Voltage gated K^+ channels (kv channels) present in all animal cells open and close upon changes in the transmembrane potential. Voltage-gated potassium ion channels (Kv) play an important role in a variety of cellular processes, regulation of apoptosis, cell growth and differentiation, the release of neurotransmitters and hormones, maintenance of cardiac and udder epithelial tissue (Bosma and Hille, 1992; Pal *et al*., 2006; Kim *et al*., 2012). Potassium channels in mammary tissue become more susceptible to β-CN (1-28) activity under heat stress.

Regulations of the mammary epithelial potassium channel sensitivity to the inhibitory effect of β-CN (1-28) is part of the regulatory system. This negative feedback system was shown to comprise an endogenous milk enzymatic system, the plasminogen activator (PA)-plasminogen (PG)-plasmin (PL) that specifically forms a β-casein (CN) fragment (1-28) from β-CN which acts as the negative control signal by closing potassium channels on the apical membrane of the epithelial cells of the mammary gland (Silanikove *et al*., 2006).

Interestingly, a further activation of the PA-PG-PL system which was coupled with more extensive degradation of casein induced involution of the mammary gland in lactating cows and forcefully activated the innate immune system ((Silanikove *et al*., 2005). Based on these findings a casein hydrolyzate preparation was developed to reduce the suffering from mammary gland engorgement associated with abrupt cessation of milking (Leitner *et al*., 2007) and to treat and prevent common clinical and subclinical infections of the udder in dairy cows (Silanikove *et al*., 2012).

The concept that PA-PG-PL- β-CN f (1-28) is involved in milk-born negative feedback regulation of milk secretion was supported experimentally under conditions that simulated stress and by exposure of cows to dehydration (Shamay *et al*., 2000).

2.10 Organization and location of EDN3 gene

The protein encoded by this gene is a member of the endothelin family. Endothelins are endothelium-derived vasoactive peptides involved in a variety of biological functions. The active form of this protein is a 21 amino acid peptide processed from the precursor protein. The active peptide is a ligand for endothelin receptor type B (EDNRB). This gene is located on bovine chromosome 13 (ENSBTAG00000012109), having 6 exons, 5 introns and a translation length of 270 residues (Figure 2.17 and Table 2.8).

Table 2.8 Nomenclature of EDN3 gene

Synonym	**Endothelin 3**
Exons	6
Coding exons	6
Introns	5
Gene size	250007bp
Transcription length	1469 bp
Translation length	270 residues
Gene type	Protein coding

Gene ID 539528	• Potassium voltage-gated channel subfamily B member 1	Ensemble Version ENSBTAG00000027320

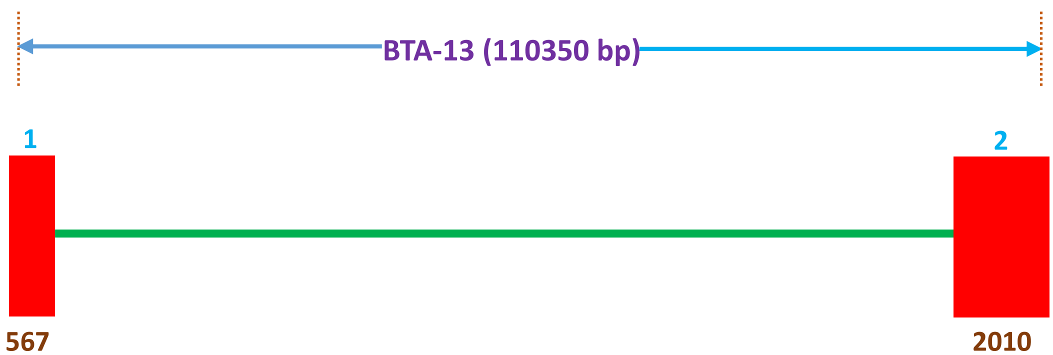


Figure 2.16 Organization of KCNB1 gene in *Bos taurus*

Gene ID		Ensemble Version
513753	• Endothelin 3	ENSBTAG00000012109

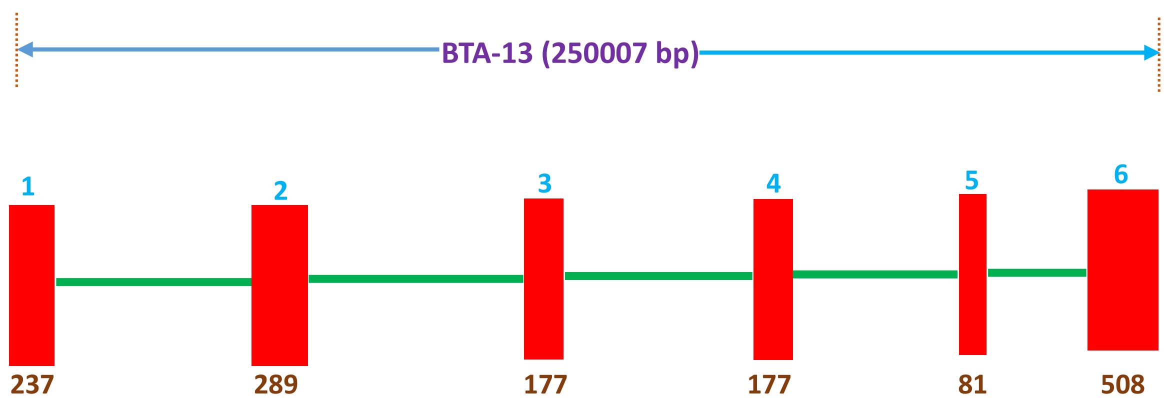


Figure 2.17 Organization of EDN3 gene in *Bos taurus*

2.10.1 Biological role of EDN3 gene

EDN3 (Endothelin 3) is a protein coding gene. Protein in the endothelin family are produced in various cells and tissues, where they are involved in the development and function of blood vessels, the production of certain hormones and the stimulation of cell growth and division. Endothelins are endothelium derived vasoactive peptides involved in variety of biological functions. The active peptide is a ligand for endothelin receptor type B (EDNRB). The interaction of this endothelin with EDNRB is essential for development of neural crest derived cell lineages such as melanocytes and enteric neurons. Three endogenous isoforms are known to exist i.e. ET-1, ET-2 and ET-3. Due to their physiological role as vasoactive peptides, endothelins are linked to certain cardiac, vascular and renal diseases. Two distinct endothelin receptors have so far been cloned in mammals as ETA and ETB receptor (Baynash *et al*., 1994; Duan *et al*., 2003). Expression of EDN3 was detected throughout the organs examined which included heart, lung, liver, kidney, spleen, stomach, uterus and udder (Fujimori *et al*., 2004). EDN3 enhances release of nitric oxide and prostaglandins which induced vasodilation in mammary gland (Yamashita *et al*., 1991).

2.11 Polymorphism and association studies in FAM19A1, KCNB1 and EDN3 Genes

Strillacci *et al.* (2014) studied quantitative Trait Loci (QTL) for SCS in Valdostana Red Pied cattle using the Illumina Bovine HD BeadChip using a selective DNA pooling (SDP) approach. A total of 171 SNPs reached the genome-wide significance for association with SCS. Fifty-two SNPs were annotated within genes, some of which are involved in the immune response to mastitis. On BTAs 1, 2, 3, 4, 9, 13, 15, 17, 21 and 22 the largest number of markers in association to the trait was found. They further reported that the FAM19A1 (family with sequence similarity 19 (chemokine (C-C motif)-like), member A1) on BTA22 associated with SCS. They found rs110064285, rs135018045 and rs133223316 to be associated with the trait. They also reported that these SNPs also to be associated with udder depth. They also reported that SNP rs41710487 of KCNB1 (potassium voltage-gated channel, Shab-related subfamily, member) having association with milk yield, udder attachment, udder depth, udder height, udder width.

Sahana *et al.* (2014) studied a genome-wide association in Nordic Holstein cattle to confirm and fine-map quantitative trait loci (QTL) for mastitis traits identified earlier using linkage analysis with sparse microsatellite markers in the same population. Strong associations of SNPs with mastitis traits were observed on bovine autosomes 6, 13, 14 and 20. They reported a QTL region between 57509023 and 57581780 bp on BTA13 which overlapped with the QTL at 57.54 Mb. This region overlaps with the endothelin 3 (EDN3) gene that influences neutrophils activation which are the major leukocytes that respond to the inflammatory stimuli.

Wu *et al*. (2015) deliberate the association analysis for udder health based on SNP-panel and sequence data in Danish Holsteins. They reported that the comparison between candidate QTL regions and known genes (NPFFR2, SLC4A4, DCK, LIFR, and EDN3) may be considered as candidate genes for mastitis susceptibility.

CHAPTER –3

Materials & Methods

3. MATERIALS AND METHODS

3.1 Experimental animals

The present study was conducted in a total of 253 cattle comprising 87 Sahiwal cattle and 166 Karan Fries cattle maintained at Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana.

3.2 Geographical and Climatic description

Karnal a city of Haryana State in North of India is situated between 29.68°N latitude and 76.98°E longitude with altitude ranging from 235 to 252 meters above from mean sea level. The climate of Karnal is sub tropical with temperature in summer months i.e. April to June, ranging between 24°C- 46°C. Karnal experiences moderate rainfall in the months of July and lasts till September with annual rainfall of 744 mm. The winter months (October, November, December and January) are extremely cold with the temperature ranging from 4°C to 32°C.

3.3 Source of Data

Data for udder type traits, milk yield and incidence of clinical mastitis were recorded for all the animals taken in study. The data for udder type traits were collected from all lactating cows of within the period of research work from the herd of Sahiwal and Karan Fries cattle maintained at Livestock Research Centre of ICAR-NDRI, Karnal. The milk production data was collected from daily milk yield register kept in the livestock record unit, Animal Genetics and Breeding division of ICAR-NDRI, Karnal. Incidence of mastitis was taken from Animal Health Complex of ICAR-NDRI, Karnal. All the udder conformation traits were taken from the recommendation made by ICAR, 2012; WHFF; WCGALP, 2014; H.A.U., 2016 and few others were taken according to the importance of traits in relation to milk production and mastitis.

3.4 Information of Karan Fries and Sahiwal cattle

3.4.1 General information

The data on Karan Fries and Sahiwal cows were collected from the information available on history-cum-pedigree sheets maintained in the record room of Animal Genetics & Breeding Division.

The following information was collected for analysis purpose:

- Animal Number
- Sire Number
- Dam Number
- Age at scoring in months
- Last date of calving
- Stage of lactation
- Parity
- Season of calving

3.4.2 Traits recorded

The following production traits were recorded from the history sheet and data of incidence of clinical mastitis were recorded from clinical register of animal health complex.

- Monthly milk yield (kg)
- Test day milk yield (kg)
- 305- Days or less milk yield (kg)
- Total milk yield (kg)
- Incidence of clinical mastitis

3.4.3 Traits generated

The following udder and teat measurements were scored linearly on possible score scale from one biological extreme to other in the present study (Table 3.1).

Table 3.1 Description of the udder and teat type traits

S.No	Type traits	Class & Description	Measurement
ICAR & WHFF (2012)			
1.	Fore udder attachment (Degree)	Weak and loose	Angle between the abdominal wall and udder
		Intermediate acceptable	
		Strong and tight	
2.	Rear udder height (cm)	Very low	Distance between the bottom of the vulva and the milk secreting tissue
		Intermediate	
		High	
3.	Udder depth (cm)	Below hocks	Distance from lowest part of udder floor to hock
		Level with hock	
		Intermediate	
		Shallow	
4.	Udder balance (cm)	Deep rear udder	Depth of the rear udder was assessed in relation to the depth of the front udder
		Front and rear udder on same level	
		Deep front udder	
5.	Rear udder width (at attachment) (cm)	Wide	Width of the udder at the point where the milk secretion tissue is attached to the body
		Intermediate wide	
		Narrow	
6.	Central Ligament (udder cleft) (cm)	Convex to flat floor (flat), broken ligament	The depth of cleft at the base of the rear udder
		Slight definition (Intermediate)	
		Deep cleft/strong ligament	
7.	Fore teat placement (Visual)	Wide	The position of the front teat from centre of quarter
		Intermediate	
		Close groups	
8.	Rear teat Placement (Visual)	Wide	The position of the rear teat from centre of quarter
		Intermediate	

		Close groups	
9.	Teat length (cm)	Short	Distance between bases of the teat to the tip of the teat
		Intermediate	
		Long	
Saini and Gill (1989); Rao (2006)			
10.	Udder shape	Pendulous	Visual Observation
		Trough	
		Round	
11.	Udder suspension (Visual Observation)	Very tight	Subjective assessments of udder support
		Tight	
		Intermediate/medium	
		Pendulous	
		Very Pendulous/Broken floor	
12.	Udder length (cm)	Long	Distance between rear & fore attachment of udder along the median line
		Intermediate	
		Short	
13.	Udder circumference (cm)	Large/long	Measuring around the base of udder
		Intermediate	
		Short	
WCGALP (2014)			
14.	Teat Size (Visual Observation)	Very small	Subjective assessments of teat length and circumference
		Small	
		Intermediate/medium	
		Large	
		Very large/ballon shaped	
15.	Teat circumference (cm)	Thick	Measured at the midpoint of the teat length
		Intermediate	
		Thin	
16.	Distance between teats	Long	Distance between teats at the

	(cm)	Intermediate	midpoint of teat length
		Short	
17.	Teat end shape /Teat tip shape	Flat	Visual observation
		Round	
		Funnel shape (inverted)	
		Disk	
		Pointed (hyperkeratosis)	
Britt and Farnsworth (2011) ; Mein *et al*.(2001) ; WCGALP (2014)			
18.	Teat shape	Funnel/Conical	Visual appraisal
		Cylinder	
		Wide/Flat	
		Bottle	
19.	Longer-Term Changes In Teat-End Condition	Smooth bottom, no or smooth callous, no lesions	Visual appraisal
		Raised callous ring with slight roughness	
		Rough callous with hyperkeratosis	
		Very rough callous with hyperkeratosis and radial cracking	
		Open skin, haemorrhage, trauma or any abnormal condition	
20.	Skin Condition	Normal – Smooth and healthy	Visual appraisal
		Dry – No cracking	
		Rough-Dryness + cracking	

3.5 Procedure of measurements and scoring of type traits

3.5.1 Procedure of measurement of type traits

The measurements were done on each animal by securing the animal in a standing position on a levelled floor for accuracy. The data were recorded one to two hours before the evening milking. Teat and udder measurement were done by vernier calliper and measuring tape respectively. The measurements were taken in centimeter (cm).

3.5.2 Scoring of type traits

The range for each trait was calculated by subtracting the minimum value from the maximum value. As the number of classes in which the animals were evaluated is 9, thus range will be divided by 9 so as to get the unit score point. This unit score point will be added to that of minimum value so as to get the range for score 1. The subsequent score classes were obtained by adding unit score point to the highest unit of pervious classes.

The scoring of various traits in Sahiwal cattle are as follows:

3.5.2.1 Fore udder attachment (FUA)

In Sahiwal cattle, FUA ranged from 33 degree to 150 degree. The difference of 117degree was obtained which was divided by 9, to get the unit score of 13 degree. The minimum and maximum value of this was 82 degree and 174 degree respectively in Karan Fries cattle.Thus range of 92 degree was divided by 9, to get the unit score of 10.22 degree.

Table 3.2 Sub classes of score points and their interpretation for fore udder attachment

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	loose	2	24
4-6	Intermediate	48	115
7-9	Strong	37	27

3.5.2.2 Rear udder height (RUH)

The minimum and maximum value of RUH was 8.89 cm and 36.87 cm respectively in Sahiwal cattle. Thus range of 27.98 cm was divided by 9, to get the unit score of 3.31 cm. In Karan Fries cattle, RUH ranged from 7.62 cm to 41.91 cm and difference of 34.29 cm was obtained. This was divided by 9, to get the unit score of 3.81 cm.

Table 3.3 Sub classes of score points and their interpretation for rear udder height

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Low	14	48
4-6	Intermediate	55	108
7-9	High	18	10

3.5.2.3 Rear udder width (RUW)

The RUW in Sahiwal cattle ranged from 4.82 cm to 15.25 cm and difference of 10.43 cm was obtained which was divided by 9, to get the unit score of 1.15 cm. RUW ranged from 5.59 cm to 20.32 cm in Karan Fries cattle. The difference of 14.73 cm was divided by 9, to get the unit score of 1.64 cm.

Table 3.4 Sub classes of score points and their interpretation for rear udder width

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Narrow	37	38
4-6	Intermediate	41	103
7-9	Wide	16	22

3.5.2.4 Udder depth (UD)

The minimum and maximum value of udder depth was 9.69 cm and 23.61 cm respectively in Sahiwal cattle. The range of 13.92 cm was divided by 9, to get the unit score of 1.54 cm. In Karan Fries this ranged from 14.24 cm to 29.00 cm. Thus difference of 14.76 cm was obtained which was divided by 9, to get the unit score of 1.64 cm.

Table 3.5 Sub classes of score points and their interpretation for udder depth

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Shallow	5	16
4-6	Intermediate	25	110
7-9	deep	57	40

3.5.2.5 Udder balance (UB)

In Sahiwal cattle maximum and minimum value of UB viz. 11.43 cm and 5.12 cm respectively and dividing the range of 16.51 cm by 9, a unit score of 1.83 cm was obtained. UB ranged between viz. 12.95cm to 23.11 cm in Karan Fries cattle the difference of 36.06 cm was obtained which was divided by 9, to get the unit score of 4.00 cm.

Table 3.6 Sub classes of score points and their interpretation for udder balance

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Deep rear udder	10	78
4-6	Same level	59	72
7-9	Deep front udder	18	16

3.5.2.6 Udder length (UL)

In Sahiwal cattle minimum and maximum value of UL was 39.37 cm and 68.61 cm respectively .Thus range of 29.24 cm was divided by 9, to get the unit score of 3.24 cm. In Karan Fries cattle UL ranged from 40.64 cm to 93.98 cm and a difference of 53.34cm was obtained which was divided by 9, to get the unit score of 5.93 cm.

Table 3.7 Sub classes of score points and their interpretation for udder length

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	33	80
4-6	Intermediate	41	72
7-9	long	13	14

3.5.2.7 Udder width (UW)

The UW ranged from 48.76 to 85.65 cm in Sahiwal cattle. The difference of 36.89 cm was obtained which was divided by 9, to get the unit score of 4.09 cm. In Karan Fries cattle the minimum value of Udder width was 36.07 cm and maximum value was 99.06 cm. Thus range of 62.99 cm was divided by 9, to get the unit score of 7 cm.

Table 3.8 Sub classes of score points and their interpretation for udder width

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Narrow	36	18
4-6	Intermediate	38	122
7-9	Wide	13	26

3.5.2.8 Udder circumference (UC)

The udder circumference ranged from 110.24 cm to 168.91 cm in Sahiwal cattle.The difference of 58.67 cm was obtained which was divided by 9, to get the unit score of 6.51cm. The maximum and minimum value of this trait was 74.16 cm and 225.29 cm respectively. Thus range of 151.13 cm was divided by 9, to get the unit score of 16.79 cm.

Table 3.9 Sub classes of score points and their interpretation for udder circumference

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Small	15	38
4-6	Intermediate	46	115
7-9	Large	26	13

3.5.2.9 Central ligament (CL)

In Sahiwal cattle minimum and maximum value of this trait was 0.0 cm and 6.35 cm respectively. Thus range of 6.35 cm was divided by 9, to get the unit score of 0.71 cm. In Karan Fries cattle central ligament ranged from 0.0 cm to 8.12 cm. Thus difference of 8.12cm was obtained which was divided by 9, to get the unit score of 0.9 cm.

Table 3.10 Sub classes of score points and their interpretation for central ligament

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Weak	8	20
4-6	Intermediate	49	115
7-9	Strong	20	31

3.5.2.10 Teat circumference (TC)

Teat circumference ranged from 6.32 cm to 14.78 cm in Sahiwal cattle. The difference of 8.46 cm was obtained which was divided by 9, to get the unit score of 0.94 cm. In Karan Fries cattle this ranged from 5.54 cm to 11.68 cm and difference of 6.14 cm was obtained which was divided by 9, to get the unit score of 0.68 cm.

Table 3.11 Sub classes of score points and their interpretation for teat circumference

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Thin	44	95
4-6	Intermediate	31	57
7-9	Thick	11	14

3.5.2.11 Rear teat length (RTL)

In Sahiwal cattle RTL ranged from 2.79cm to 11.04 cm .Thus difference of 8.25cm was divided by 9, to get the unit score of 0.92 cm. in Karan Fries cattle minimum and maximum value of this trait was 2.41 cm and 7.49 cm respectively. Thus range of 5.08 cm was divided by 9, to get the unit score of 0.56 cm.

Table 3.12 Sub classes of score points and their interpretation for rear teat length

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	46	64
4-6	Intermediate	31	80
7-9	Long	10	22

3.5.2.12 Fore teat length (FTL)

The FTL ranged from 3.17 cm to 11.68 cm in Sahiwal cattle. Thus difference of 8.51 cm was obtained which was divided by 9, to get the unit score of 0.95 cm. In Karan Fries cattle FTL ranged from 2.03 cm to 11.68 cm. thus difference of 9.65 cm divided by 9, to get the unit score of 1.07 cm.

Table 3.13 Sub classes of score points and their interpretation for fore teat length

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	48	87
4-6	Intermediate	33	66
7-9	Long	6	13

3.5.2.13 Distance between front and rear teats (DFR)

In Sahiwal cattle DFR ranged from 1.39 cm to 11.38 cm.Thus differences of 9.99 cm was obtained which was divided by 9, to get the unit score of 1.11 cm. In Karan Fries cattle minimum and maximum value of DFR was 1.78 cm and 13.97 cm respectively. Thus range of 12.19 cm was divided by 9, to get the unit score of 1.35 cm.

Table 3.14 Sub classes of score points and their interpretation for distance between front and rear teats

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	34	66
4-6	Intermediate	44	87
7-9	Long	9	13

3.5.2.14 Distance between left and right teats (DLR)

The minimum value for DLR was 2.29 cm and maximum value was 15.42 cm in Sahiwal cattle. Thus difference of 13.13 cm was obtained which was divided by 9, to get the unit score of 1.45 cm. In Karan Fries cattle minimum and maximum value for this trait was 0.88 cm to 20.32 cm. Thus range of 19.44 cm was divided by 9, to get the unit score of 2.16 cm.

Table 3.15 Sub classes of score points and their interpretation for distance between left and right teats

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	43	49
4-6	Intermediate	34	103
7-9	Long	13	14

3.5.2.15 Shortest distance from front teat ends to floor (SDF)

In Sahiwal cattle minimum and maximum value for SDF was 28.44 cm and 55.88 cm respectively. Thus range of 27.44 cm was divided by 9, to get the unit score of 3.04 cm. In Karan Fries cattle value of this trait ranged between 35.43 cm and 64.00 cm. The difference of 28.57 cm was obtained which was divided by 9, to get the unit score of 3.17 cm.

Table 3.16 Sub classes of score points and their interpretation for shortest distance from front teat ends to floor

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	17	47
4-6	Intermediate	55	81
7-9	Long	15	38

3.5.2.16 Shortest distance from rear teat ends to floor (SDR)

In Sahiwal cattle the SDR ranged from 25.40 cm to 53.34 cm. Thus range of 27.94 cm was obtained which was divided by 9, to get the unit score of 3.30 cm. Minimum and maximum value of this trait in Karan Fries cattle was 27.56 cm and 62.48 cm respectively. Thus the range of 34.92 cm was divided by 9, to get the unit score of 3.88 cm.

Table 3.17 Sub classes of score points and their interpretation for shortest distance from rear teat ends to floor

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Short	9	23
4-6	Intermediate	39	77
7-9	Long	39	66

3.5.2.17 Teat diameter

The minimum value for teat diameter was 1.80 cm and maximum value was 4.49 cm in Sahiwal cattle. Thus, the range of 2.69 cm was divided by 9 to get the unit score of 0.29 cm. In Karan Fries cattle the minimum and maximum value for this trait was 1.50 cm and 3.71 cm respectively. The difference of 2.21 cm was obtained which was divided by 9, to get the unit score of 0.24 cm.

Table 3.18 Sub classes of score points and their interpretation for teat diameter

Range of score point	Interpretation	Frequencies	
		Sahiwal	Karan Fries
1-3	Thin	55	94
4-6	Intermediate	23	67
7-9	Thick	9	5

3.6 Standardization of Data

The records of the animals with known pedigree and normal lactation were considered in the study. The lactation records of animals producing less than 500 kg milk in a lactation and a lactation length less than 100 days were considered as abnormal and were not included in the present study.

3.7 Classification of data

The data on udder and teat measurements, production traits and incidence of mastitis were classified for determining the effect of parity, season and stage of lactation of animal as in the following.

3.7.1 Classification of season

The climatic conditions throughout the year shows wide variation, difference being more between the seasons and less within the seasons. Hence evaluation and correction of records for seasons was important for standardizing the variations on trait. Based on the meteorological factors such as maximum and minimum temperature and relative humidity a year was classified into four seasons based on prevalent climatic conditions in the region as recorded in CSSRI, Karnal. The different seasons are as follows:

Table 3.19 Classification of cows according to different season of calving

Season	Duration	Code
Winter	December-March	1
Summer	April-June	2
Rainy	July-September	3
Autumn	October- November	4

3.7.2 Classification of order of parity

To estimate the effect of parity, the parity was classified as follows:

Table 3.20 Classification of cows according to different parity

Parity Group	Code
1st Parity	1
2nd Parity	2
3rd Parity	3
4th Parity	4
5th and above Parity	5

3.7.3 Classification of stage of lactation

Stage of lactation was calculated on the basis of days in milk in the animal. So accordingly was divided into three stages:

Table 3.21 Classification of cows according to different stages of lactation

Stage of lactation	Duration	Code
1st stage	<3 months	1
2nd stage	3 - 6 months	2
3rd stage	> 6 months	3

3.7.4 Level of milk production

Table 3.22 Classification of cows according to different level of milk production

Parity Group	Code	Sahiwal	Karan Fries
High Yielder	1	>1800kg	>4500kg
Medium Yielder	2	1000-1800kg	1900-4500kg
Low yielder	3	<1000kg	<1900kg

3.8 Isolation of Genomic DNA

3.8.1 Blood collection

About 10 ml of whole blood was collected aseptically from Jugular Vein of the animal into a sterile vacutainer tube containing 0.5 per cent anticoagulant Ethylene Diamine Tetra Acetic Acid (EDTA). The tube was shaken gently to facilitate thorough mixing of blood with the anti-coagulant. The samples were transported to the laboratory in an icebox and stored at -20°C till further processing for DNA isolation.

3.8.2 DNA extraction

DNA was isolated from blood using Phenol-chloroform method, as described by Sambrook and Russell (2001) with minor modifications. The steps involved in this method were as follows:

Cell lysis

1. Ten ml Blood was taken in an Oakridge tube with two volumes of ice-cold RBC lysis buffer, mixed well by vortexing and the tubes were kept on ice for 10 minutes.
2. Centrifuged at 8500 rpm for 10 minutes at 4^{o}C and the supernatant were decanted.
3. WBC pellet was resuspended in one volume of RBC lysis buffer and kept on ice for 10 minutes and centrifuged as given in above step.
4. 2nd and 3rd steps were repeated for complete lysis red blood cells until a clear pellet of white blood cells was obtained.
5. WBC pellet was resuspended in DNA extraction buffer (equal to the volume of blood sample collected) by vortexing.
6. SDS (20%) was added @ 200 μl per 10ml of whole blood. The mixture was gently mixed, and Proteinase-K was added @ 40 μl (20 mg/ml stock solution) per 10 ml of blood and incubated at 56^{o}C overnight.

Phenol extraction

1. Equal volume of Tris (pH 8.0) saturated phenol was added to the above mixture and mixed gently by inversion to form a uniform suspension (5-10 minutes).
2. The mixture was centrifuged at 10,000 rpm for 15 minutes at 20^{o}C.
3. Upper aqueous phase was gently aspirated using a wide bore sterile Pasteur pipette, without disturbing the interphase of protein, and transferred to a fresh Oakridge tube.
4. A mixture of saturated phenol, chloroform and isoamyl alcohol in a ratio of 25:24:1 was added in equal volume and mixed gently by inverting the tube until a uniform suspension was formed.
5. The mixture was centrifuged at 10,000 rpm for 15 minutes at 20^{o}C and the upper aqueous phase was gently aspirated using a wide bore sterile Pasteur pipette without disturbing the interphase of protein and transferred into a fresh Oakridge tube.

6. A mixture of chloroform and isoamyl alcohol at a ratio of 24:1 was added and mixed properly by inverting the tube. The mixture was centrifuged again at 10,000 rpm for 15 minutes and the aqueous phase was aspirated into a fresh sterile glass tube.

DNA Precipitation

1. Sodium acetate (3 M, pH 5.2) was added to the aqueous layer to a final concentration of 0.33 M, mixed gently, and two volumes of ice-cold ethanol was added and tilted gently for DNA precipitation.
2. The precipitated DNA was spooled out with the help of bent Pasteur pipette and transferred to eppendorf tube with 1.00 ml of 70% ethanol, mixed well and centrifuged at 10,000 rpm for 5 minutes at 4^{o}C. This step was repeated three times.
3. The DNA was dried by keeping the eppendorf tubes open in a sterile incubator at 37^{o}C. The DNA was dissolved in 500 µl TE buffer (pH 8.0), covered with parafilm, kept in a water bath maintained at 65^{o}C for 30 minutes, after DNAse treatment, the tubes were kept at 4^{o}C for 4-5 days and finally stored at -20^{o}C.

3.9 Quality and Quantity Checking of DNA

The quality of DNA was checked by agarose gel electrophoresis. Two µl of DNA was loaded in 0.8 per cent agarose gel (0.80 gm Agarose + 10 ml TBE (10 X) + 90 ml double distilled water) in horizontal mini electrophoresis unit and electrophoresed using 1x TBE buffer as running buffer at 150 volts for about two hours. Gel was stained with 1% Ethidium Bromide solution (@ 1µl/100 ml of gel), and viewed under Gel Documentation system (MiniBisLabnet Pvt. Ltd) and photographed. Sharp bands were considered as correct concentration of the DNA. Quality and quantity of DNA was also estimated by UV- Spectrophotometer. About 2 µl DNA was dissolved in 98 µl of double distilled water and loaded into a 100 cuvette. An optical density (OD) value was determined at wavelengths of 260 nm and 280 nm in Spectrophotometer with reference to blank sample. DNA samples having the OD_{260} /OD_{280} ratio between 1.7 and 1.9 were considered pure and used for further analysis. The concentration of DNA was estimated using the formula:

$$\textbf{Quantity of DNA in µg/ml} = OD_{260/280} \text{ value} \times 50 \times \text{dilution factor}$$

3.10 Dilution of samples (preparation of template DNA) for PCR

The stock DNA was diluted with autoclaved double distilled water to a final concentration of 50 ng/µl and used as the working solution. Formula used for calculation of dilution is given below:

$$\textbf{Dilution of DNA} = 50\text{ng} \times 100\ \mu\text{l} / \text{O. D. of sample}$$

The DNA stock in µl equivalent to the resultant value from the above formula is taken and made up to 100 µl with help of Millipore water.

3.11 Polymerase Chain Reaction (PCR) Amplification of Target Region of Bovine FAM19A1, KCNB1 and EDN3 genes

3.11.1 Primers

In silico primer designing for target regions of Bovine FAM19A1 gene (Accessionno.ENSBTAG00000019041), KCNB1 gene (Accession no. ENSBTAG00000027320) and EDN3 gene (Accession no. ENSBTAG00000012109) was carried out using Primer3 software (http://www.primer3.ut.ee). Seven sets of forward and reverse region-specific oligonucleotide primers for FAM19A1 and one set for each KCNB1 and EDN3 gene were designed. While designing the primers, oligonucleotide melting temperature, oligonucleotide length, GC content, primer-dimer possibilities, PCR product size, positional constrains within the source sequence and miscellaneous constrains were considered. The primers designed were checked for specificity by BLAST programme. Primers were procured from Eurofins Genomics India Pvt. Ltd, Bengaluru. A simple formula for calculation of the melting temperature (Tm) of primer is:

$$Tm = 4(G+C) + 2(A+T)^{o}C$$

The sequence of primers and their respective nucleotide numbers are given in Table 3.23.

Table 3.23 Sequence of primers and their respective number of bases of bovine FAM19A1, KCNB1 and EDN3 gene

Genes	Primer Sets		Sequence 5'-3'	No. of Bases
FAM19A1	1.	F	GTAGCAAAGGGACATGACGT	20
		R	GGCCAAGGTATCCACAAACA	20
	2.	F	AGAGTGAGGGAAGAGGGATT	23
		R	AGCACATAGAGAATTAGCGTGA	21
	3.	F	CTCATGGGTTCTTGCTGCTC	20
		R	GTTGTGGAGGAAACGAGCAT	20
	4.	F	TGCCTGTCTTCTCCCAGATG	20
		R	GGGCTGGCATTTGTCTAACT	20
	5.	F	AGCCCCTTCTTAGCCTTCTA	20
		R	GGTCATCTTGTCTGTCCCCT	20
	6.	F	AGGTTCAAAAGGACCTAATGCA	22
		R	ATCCAAATACCCGCCTCACT	20
	7.	F	ACCACTTACCATTACTTAGGGCT	23
		R	GGATCACTGGGCGAGAGTAT	20
KCNB1	8.	F	TTCAAATCCCGACTCCACCA	20
		R	TAACACACAAAAGTCGCCCC	20
EDN3	9.	F	GAAAAGCCCTAACCCACAGC	20
		R	AGGCTTTCTGAACACTTTGCA	21

3.11.2 Reaction mixture

The working solutions of both forward and reverse primers were prepared from stock solutions by adding primer and autoclaved double distilled water to obtain final concentration of 10 pmol for each primer.

- Template DNA stock solution was diluted to working concentration of 50 to 100 ng/µl using autoclaved double distilled water.
- Each PCR reaction cocktail was prepared using PCR Master Mix (2X) (Fermentas)
- Final reaction cocktail (25 µl) was as follows:

Forward primer:	0.5 µl
Reverse primer:	0.5 µl
PCR Master Mix (2X):	13.0 µl
Milli Q Water:	9.0 µl
Template DNA:	2.0 µl

- The above contents of PCR reaction cocktail were mixed thoroughly by vortexing followed by brief spin.
- All the tubes were marked with respective DNA sample number.

3.11.3 PCR programme

The PCR amplification was performed using Thermal cycler (BioRad T100). Each 0.2 ml tube containing PCR reaction cocktail was kept in Thermal cycler for amplification of targeted region of Bovine FAM19A1, KCNB1 and EDN3 gene. Detailed PCR programme and annealing temperatures are given in Table 3.24.

Table 3.24 PCR programme for Primer set of FAM19A1, KCNB1 and EDN3 Gene

S. No	Step	Temperature				Time
1	Initial denaturation	95°C				3 min
2	Denaturation	94°C				1 min
3	Annealing	Primer 1		Primer 6		1 min
		Primer 2		Primer 7		
		Primer 3		Primer 8		
		Primer 4		Primer 9		
		Primer 5				
4	Extension	72°C				1 min
Go to step 2, (34 X)						
5	Final Extension	72°C				10 min
6	Hold	4°C				-

3.12 Checking PCR amplification

3.12.1 Gel assembly

The gel plates and combs were cleaned with 70% ethanol and rinsed with distilled water and air-dried. The edges of the plate were sealed with cellophane tape to avoid leakage of the gel and then the combs were fixed. The gel and combs were handled with gloved hands to avoid any fingerprints and the entire assembly was kept on a level stage.

3.12.2 Agarose gel electrophoresis

After PCR amplification, the PCR product was checked on 1.5% agarose gel to verify the amplification of target region. The agarose gel was prepared using 1.8gm of agarose powder dissolved in 120 ml 1x TBE solution .This mixture was then heated in microwave for about 2 minutes till it became colourless and allowed to cool to 50^{o}C and finally 1% ethidium bromide was added @ 2 µl/100 ml of gel. This mixture was then poured in gel plate for solidifying and two combs were placed for making wells. Care was taken to avoid bubble formation while pouring the gel. The gel was allowed to solidify for about half an hour. After solidification the combs are removed gently without damaging the wells. The solidified gel was placed in the electrophoresis chamber containing 650 ml of 1X TBE buffer. 5 µl of PCR product were loaded slowly in separate wells and the 100 bp marker was loaded in one of the well of the gel. The chamber was closed with the lid and power supply turned on to 100 volts for 30 minutes.

3.12.3 Visualization of gel

At the end of run, power supply was turned off and the gel was carefully removed from the chamber and placed on trans illuminator, viewed under UV light and photographs were taken with the help of gel documentation system. A single sharp band indicated proper amplification of the target DNA.

3.13 Restriction fragment length polymorphism (RFLP)

The PCR amplified products were subjected to restriction digestion with suitable restriction enzymes. The PCR products (10 µl) were digested with restriction enzymes as per the manufacturer's protocol. The reaction mixture was centrifuged for few seconds for uniform mixing and then incubated at the specific temperature of respective restriction enzymes for 12-16 hours. The restriction digested fragments was separated on 2.5% agarose gels and resolved by ethidium bromide. Photographs were taken using gel documentation system to screen for restriction fragment length polymorphism in the population to be studied.

3.14 DNA sequencing

The amplified PCR products from all sets of primers were sent to 1st base sequencing INT (Malaysia) for purification and custom sequencing from both ends (5′ and 3′ ends).

3.14.1 Sequence data analysis for SNP detection and genotyping

DNA sequencing results for the respective region of bovine FAM19A1, KCNB1 and EDN3 gene were visualized using Chromas software (version 2.6.6). Each edited sequence was aligned with corresponding reference sequence using ClustalW multiple sequence alignment program for DNA (www.ebi.ac.uk/tools/msa/clustalw2) to identify SNPs. The coding sequence were translated into amino acid sequence using online ExPasy translate tools.

3.15 Genotypic and allelic frequencies

Allele and genotype frequencies were calculated using POPGENE software package. Effective allele number (ne) which estimates the reciprocal of and Shannon Index (I) which estimates measure of gene diversity to find out that Hardy-Weinberg equilibrium exists within population or not. Polymorphism Information Content (PIC) value was calculated with the help of POPGENE software.

PIC (Polymorphism Information Content): According to the classification of PIC

PIC value less than 0.25	**:** Low polymorphism
PIC value between 0.25 to 0.5	**:** Intermediate polymorphism
PIC value above from 0.5	**:** High polymorphism

3.16 Statistical analysis

The statistical analysis was carried out using SPSS 22.0 Version under the following headings:

3.16.1 Descriptive analysis

The descriptive analysis for mean, standard error and coefficients of variation of all the traits were carried out using standard statistical procedures (Snedecor and Cochran, 1994)**.**

3.16.2 Assessing the effect of non-genetic factors

To overcome the non-orthogonality of effects due to unequal and disproportionate sub-class frequencies least-squares analysis was applied to identify the significance of important non-genetic factors as described by Harvey (1990). The following model was considered with the assumptions that the different components being fitted into the model are linear, independent and additive.

$$Y_{ijkl} = \mu + L_i + P_j + S_k + e_{ijkl}$$

Where,

Y_{ijkl}	=	The observation (udder type trait score)
μ	=	Overall mean
L_i	=	Fixed effect of i^{th} stage of lactation (i = 1 to 3)
P_j	=	Fixed effect of j^{th} parity (j = 1 to 5)
S_k	=	Effect of k^{th} season of calving (k = 1 to 4)
e_{ijkl}	=	Random error associated with Y_{ijkl} observation and assumed to be NID (0, σ^2e)

3.16.3 Association of type traits with genotype

The association of genotypes with the corresponding udder type scores/ traits and milk production of the animals will be assessed using the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,

Y_{ij}	=	Adjusted value of type traits of j^{th} animal of i^{th} genotype
μ	=	Overall mean
G_i	=	Effect of i^{th} Genotypes
e_{ij}	=	Random error associated with Y_{ij} observation and assumed to be NID (0, σ^2e)

3.16.4 Effect of non genetic factors on incidence of clinical mastitis

It is very difficult to identify the non-genetic factors influencing threshold traits in dairy animals using conventional analysis wherein the normality of residual error is assumed. Under such conditions of binary or discontinuous nature of response variable, logistic regression can facilitate efficiency in exploring the relationship between the dependent andindependent variables as well as for predicting the response. Logistic regressions work with odds rather than proportions. The odds are the ratio of the proportions for the two possible mutually exclusive outcomes. If p is the proportion for one outcome, then (1-p) is the proportion for the alternate outcome: ODDS = p / (1-p). It was carried out using the maximum likelihood method of LOGISTIC procedure of SPSS Version 22.

Dichotomous Logistic Regression Model:

$$\ln\left[\frac{p}{1-p}\right] = \beta_0 + \sum_{j=1}^{c} \beta_j X_j$$

Here marginal one unit increase in X_j brings about an increase in Log [pi/1 – pi] by β_1

P = Probability of Mastitis resistance animals

B_o = Intercept

B_jX_j = Partial regression coefficients

3.16.5 Estimation of phenotypic correlations (r_p) between milk production and udder type traits

Phenotypic correlation (r_p) between 2 traits was computed by standard statistical procedure using Pearson's correlation coefficient (Snedecor and Cochran, 1994).

3.16.6 Association of genotypes with incidence of clinical mastitis

Association of genotypes with incidence of mastitis animals was calculated using χ^2 test (Snedecor and Cochron, 1994) as follows:

$$\chi^2 = \sum(\text{Observed - Expected})^2 / \text{Expected}$$

3.16.7 Association of udder type traits (visual observations) with incidence of clinical mastitis

Association of udder type traits with mastitis affected animals and non affected animals were calculated using χ^2test (Snedecor and Cochron, 1994) as follows:

$$\chi^2 = \sum(\text{Observed - Expected})^2 / \text{Expected}$$

CHAPTER –4

Results and Discussion

4. RESULTS AND DISCUSSION

The present investigation was carried out to identify polymorphism in genomic sequences of FAM19A1, KCNB1 and EDN3 genes and to explore association of identified allelic variants with udder type traits, incidence of clinical mastitis and milk production in Sahiwal and Karan Fries cows. The results obtained from the experiments are presented below along with the discussion under the following sections:

4.1 Genomic DNA isolation

Blood samples of Sahiwal (*n*=87) and Karan Fries (*n*=166) cattle were collected from Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana. Genomic DNA was extracted from blood samples by using a standard Phenol Chloroform extraction method (Sambrook and Russell, 2001). The yield and purity of DNA samples were estimated by UV Spectrophotometer. The purity of DNA samples (determined as O.D ratio at 260 nm / 280 nm) ranged from 1.70 to 1.92, with a mean of 1.79 ± 0.02 indicating high purity of the extracted DNA. Quality of DNA was checked with 0.8% agarose gel electrophoresis. Plate 4.1 and Plate 4.2 shows the genomic DNA of Sahiwal and Karan Fries cattle, respectively. The overall yield ranged from 112 to 3756 μg in Sahiwal and 110 to 4114 μg in Karan Fries samples. The results of purity and mean yield of DNA samples are presented in Table 4.1

Table 4.1 Mean yield and purity of DNA

Parameter		Sahiwal	Karan Fries
Yield (μg)	Range	112-3756	110-4114
	Mean±SE	1248±122	1240.7±162
Purity (OD_{260}/OD_{280})	Range	1.70-1.92	
	Mean±SE	1.79±0.02	

4.2 Standardization of Polymerase Chain Reaction (PCR)

DNA template concentrations varied between 100 to125 ng per 25 μl of reaction mixture with increments of 25 ng. There was no apparent difference in the yield and specificity of the PCR product observable by visual appraisal in agarose gel in case of different samples. Hence, a concentration of 100 to 125 ng per 25 μl reaction volume were used for PCR amplification of DNA.

4.2.1 Primer concentration

The concentration of forward and reverse primer was optimized by setting up PCR trials. The optimum level of primer used was 10 pmol/µl. When primers were used at less than optimum level, lower yield of the desired product was obtained. This might be due to limited amount of the primers (Sambrook and Russell, 2001).

4.2.2 Annealing temperature (T_a)

One of the most precarious factors is the annealing temperature (T_a) which affects both yield and specificity of the PCR product. Seven primer sets of FAM19A1, one primer set of KCNB1 and one primer set of EDN3 gene were used to amplify targeted region in both the breeds. At lower annealing temperatures, nonspecific products were formed while at higher than optimal temperature yield of products were reduced.

4.2.3 Polymerase Chain Reaction (PCR)

Nine sets of primers were used to amplify targeted regions of FAM19A1, KCNB1 and EDN3 genes in 253 (166 Karan Fries and 87 Sahiwal) DNA samples. PCR reactions were set up for a total volume of 25 µl for each sample. The primer sequences, annealing temperatures, targeted regions and amplicon sizes of all primer sets are given in Table 4.2.

4.2.4 Quality of PCR product

Targeted genomic regions of all primer sets were successfully amplified in all the animals considered in the study. The quality and sizes of PCR products for targeted regions of primers were checked on 1.5 % agarose gel electrophoresis (Plate 4.3 to 4.20).

4.3 DNA sequencing and analysis

PCR products of all nine primer sets of both the breeds were sent for purification and custom sequencing to 1^{st} Base sequencing INT (Malaysia) for all the amplicons. Sequencing data was analyzed using Chromas software and the low quality bases were trimmed. For identifying SNPs in FAM19A1 gene, reference sequence (Accession no. ENSBTAG00000019041.5), in KCNB1 gene, reference sequence (Accession no. ENSBTAG00000027320) and in EDN3 gene reference sequence (Accession no. ENSBTAG00000012109) of *Bos taurus* available at ENSEMBL genome browser (http: //www. www.ensembl.org) were aligned with sequencing results of each targeted region using ClustalW multiple sequence alignment software.

The multiple sequence alignment using ClustalW was performed for all seven targeted regions of FAM19A1 gene between *Bos taurus* sequence (Accession no. ENSBTAG00000019041.5), and observed sequences of Karan Fries and Sahiwal cattle respectively (Figure 4.1 to 4.7). Similarly, multiple sequence alignment was performed for

Plate 4.1 Quality checking of working DNA isolated from whole blood of Sahiwal cows

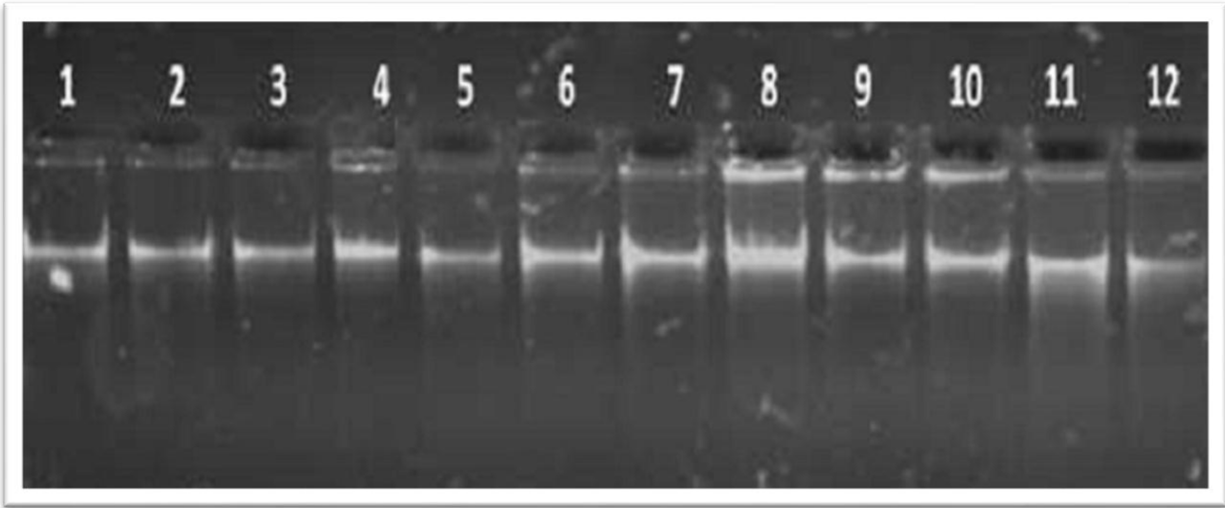


Plate 4.2 Quality checking of working DNA isolated from whole blood of Karan Fries cows

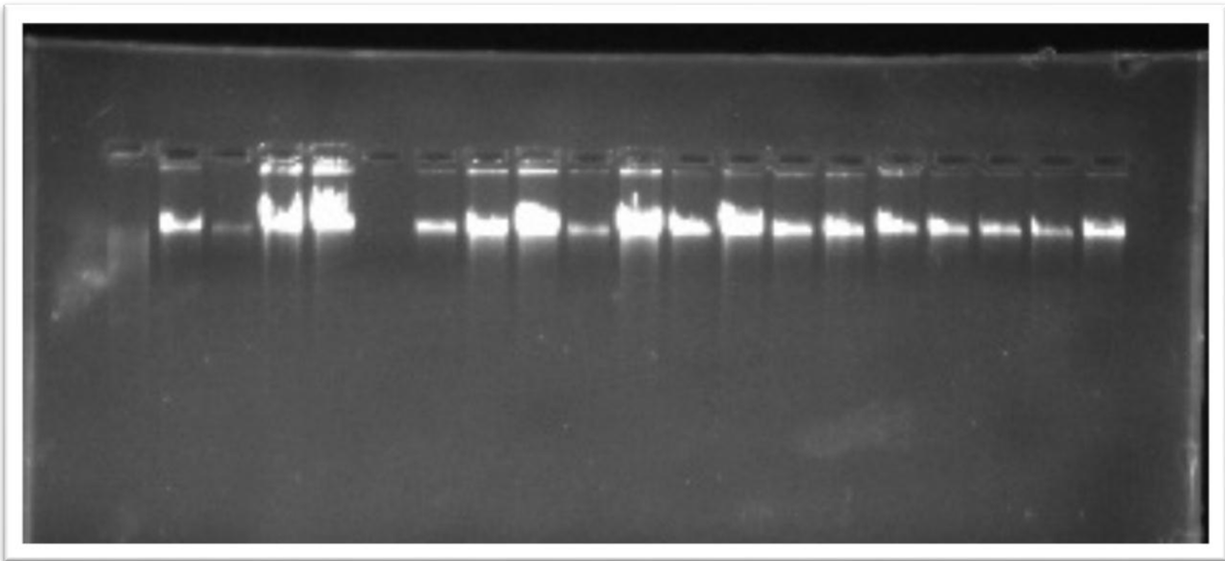
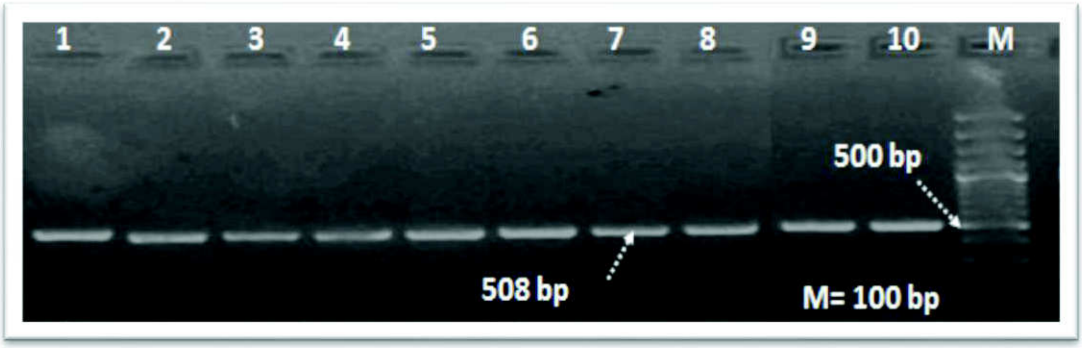
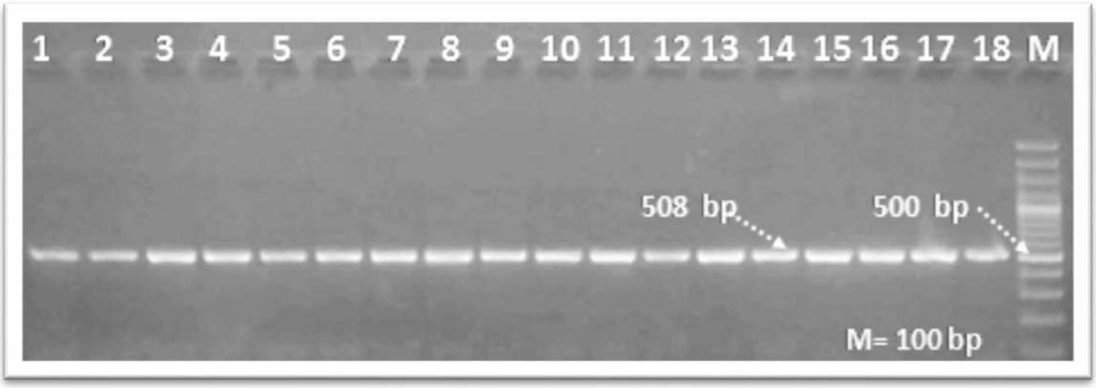


Plate 4.3 Resolution of PCR product of primer 1 of FAM19A1 gene in Sahiwal cows



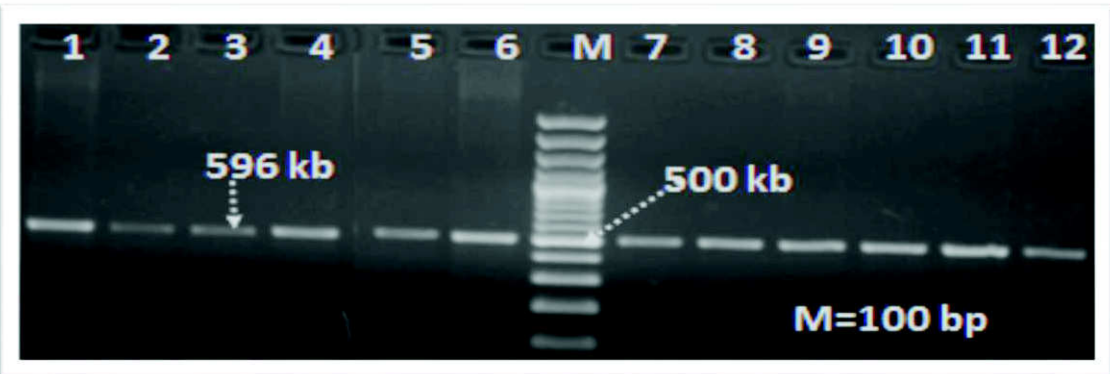
Lane 1-10 :PCR product (508 bp) of Sahiwal cow samples
Lane M :100 bp DNA ladder

Plate 4.4 Resolution of PCR product of primer 1 of FAM19A1 gene in Karan Fries cows



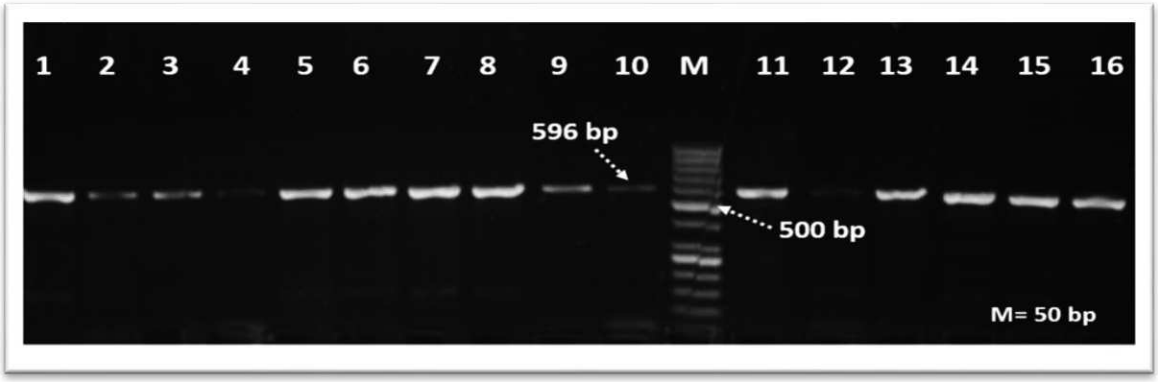
Lane 1-18 : PCR product (508 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.5 Resolution of PCR product of primer 2 of FAM19A1 gene in Sahiwal cows



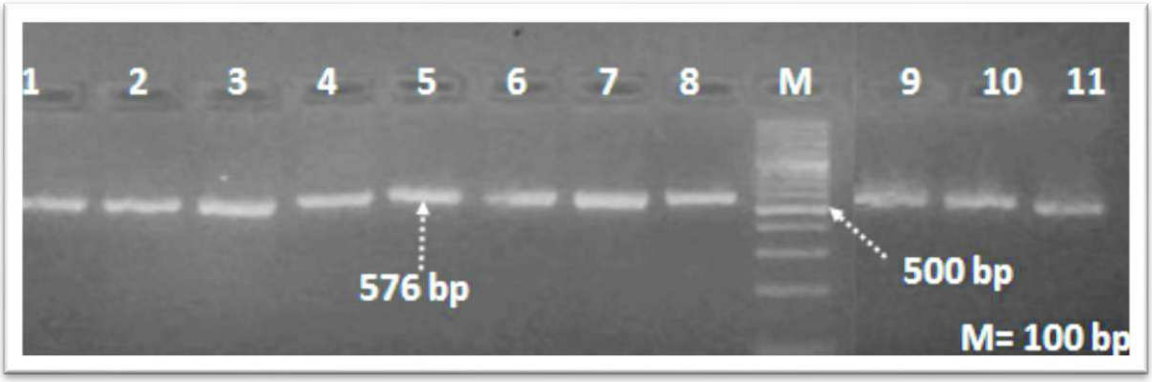
Lane 1-18 : PCR product (596 bp) of Sahiwal cow samples
Lane M : 100 bp DNA ladder

Plate 4.6 Resolution of PCR product of primer 2 of FAM19A1 gene in Karan Fries cows



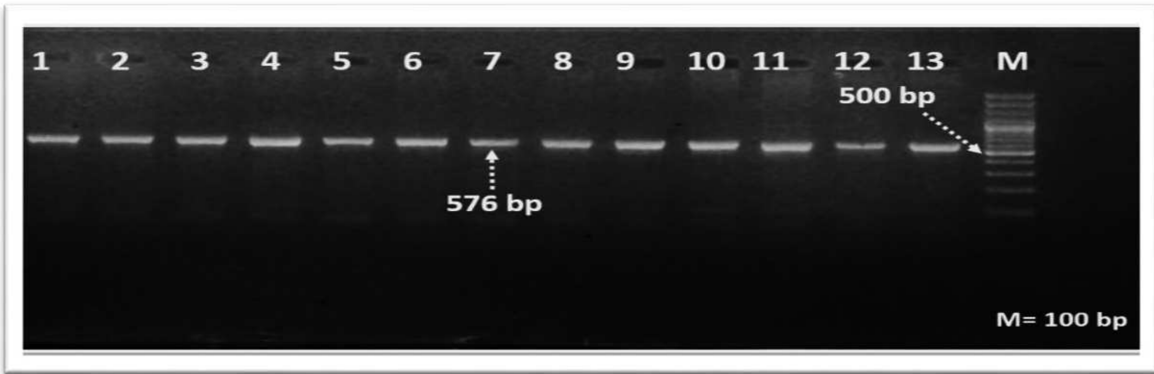
Lane 1-16 : PCR product (596 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.7 Resolution of PCR product of primer 3 of FAM19A1 gene in Sahiwal cows



Lane 1-11 : PCR product (576 bp) of Sahiwal cow samples
Lane M : 100 bp DNA ladder

Plate 4.8 Resolution of PCR product of primer 3 of FAM19A1 gene in Karan Fries cows

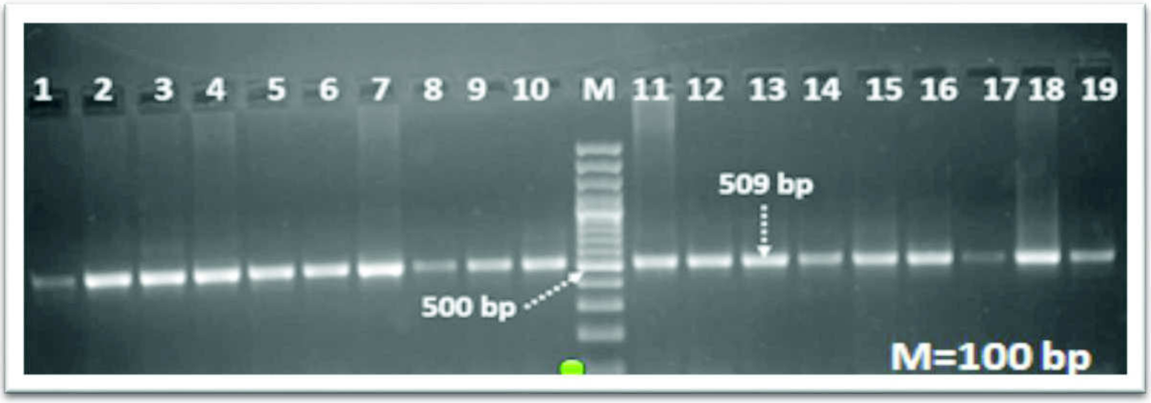


Lane 1-13 : PCR product (576 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.9 Resolution of PCR product of primer 4 of FAM19A1 gene in Sahiwal cows

Lane 1-14 : PCR product (509 bp) of Sahiwal cow samples
Lane M : 100 bp DNA ladder

Plate 4.10 Resolution of PCR product of primer 4 of FAM19A1 gene in Karan Fries cows

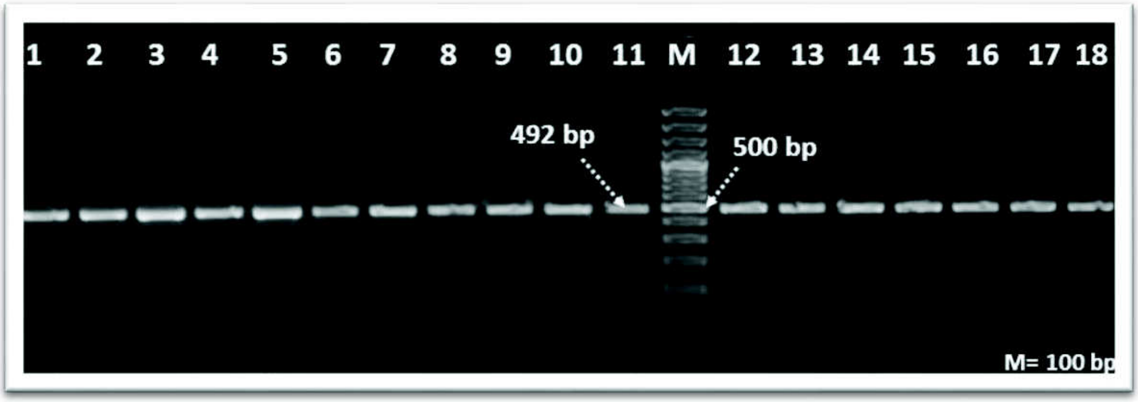


Lane 1-19 : PCR product (509 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.11 Resolution of PCR product of primer 5 of FAM19A1 gene in Sahiwal cows

Lane 1-19 : PCR product (492 bp) of Sahiwal cow samples
Lane M : 100 bp DNA ladder

Plate 4.12 Resolution of PCR product of primer 5 of FAM19A1 gene in Karan Fries cows

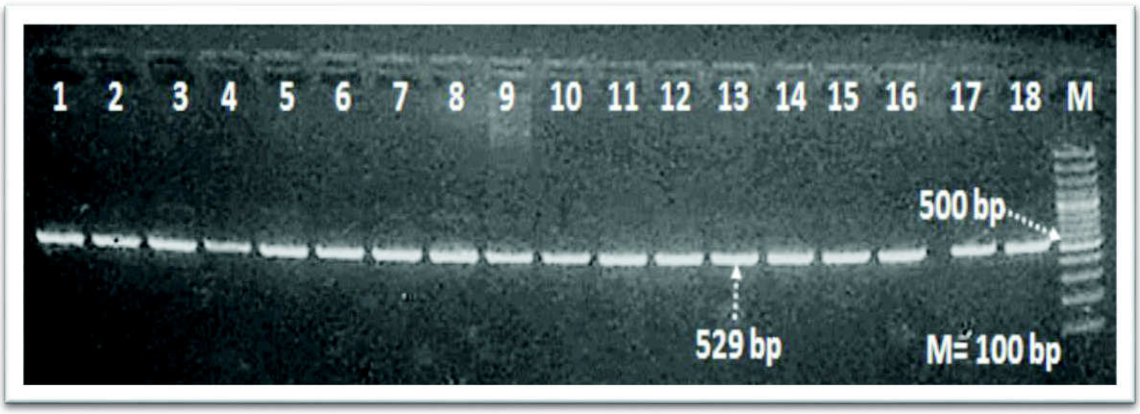


Lane 1-18 : PCR product (492 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.13 Resolution of PCR product of primer 6 of FAM19A1 gene in Sahiwal cows

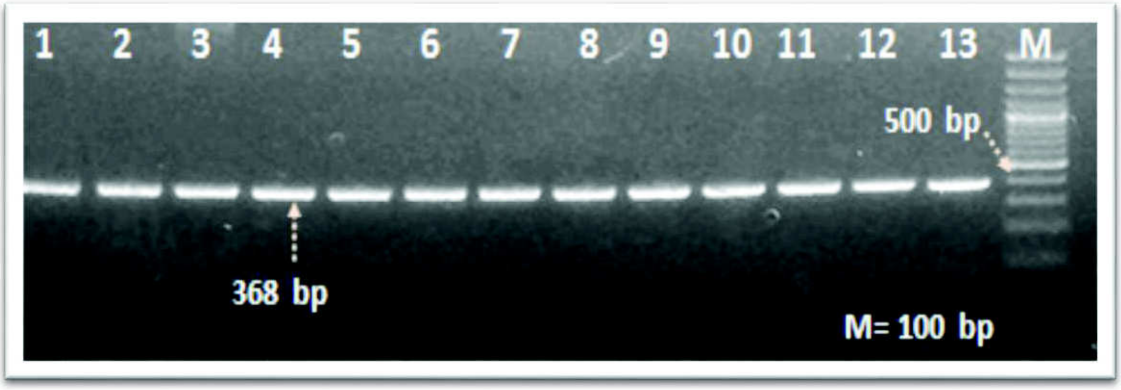
Lane 1-19 : PCR product (529 bp) of Sahiwal cow samples
Lane M : 100 bp DNA ladder

Plate 4.14 Resolution of PCR product of primer 6 of FAM19A1 gene in Karan Fries cows



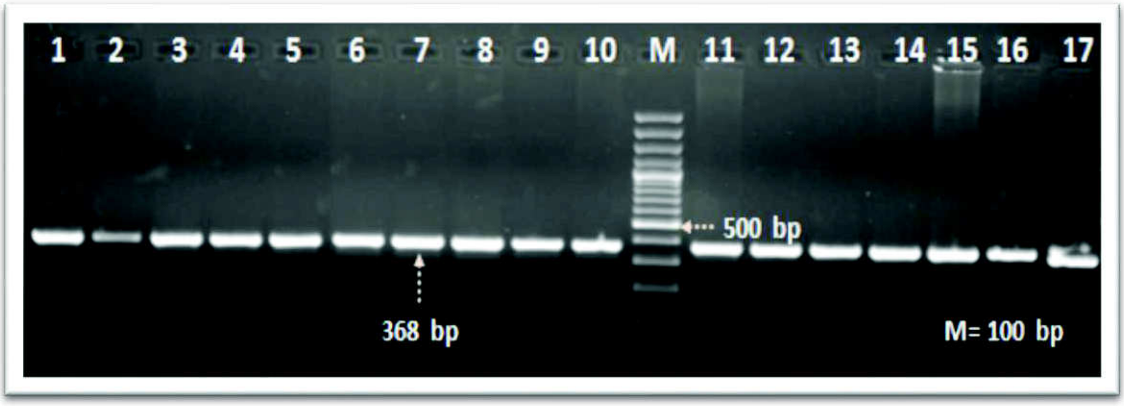
Lane 1-18 : PCR product (529 bp) of Karan Fries cow samples
Lane M : 100 bp DNA ladder

Plate 4.15 Resolution of PCR product of primer 7 of FAM19A1 gene in Sahiwal cows



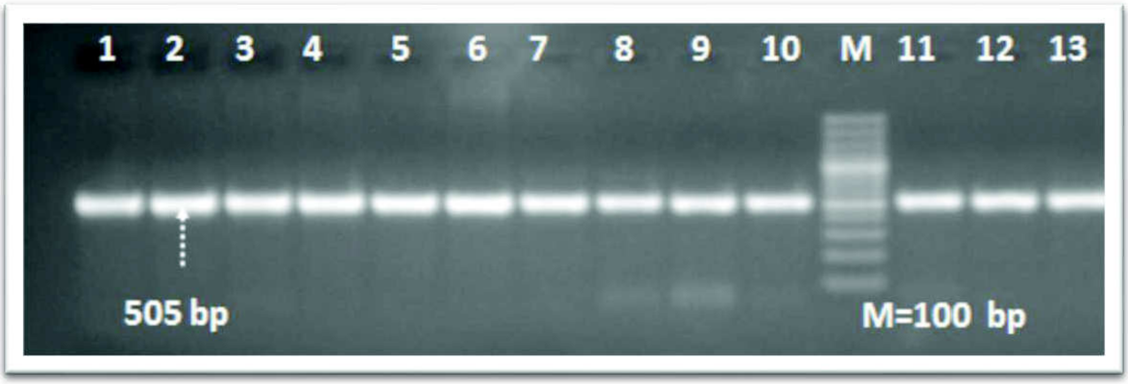
Lane 1-13	: PCR product (368 bp) of Sahiwal cow samples
Lane M	: 100 bp DNA ladder

Plate 4.16 Resolution of PCR product of primer 7 of FAM19A1 gene in Karan Fries cows



Lane 1-17	: PCR product (368 bp) of Karan Fries cow samples
Lane M	: 100 bp DNA ladder

Plate 4.17 Resolution of PCR product of primer 1 of KCNB1 gene in Sahiwal cows

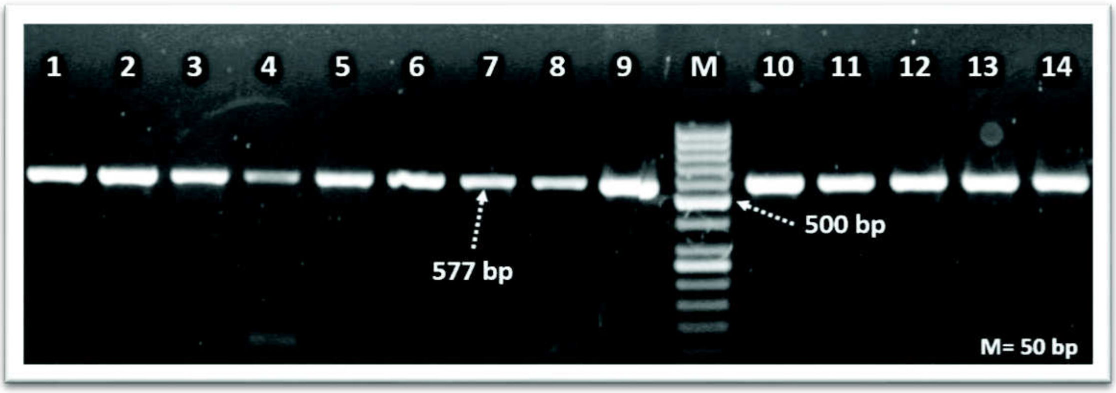


Lane 1-13	: PCR product (505 bp) of Sahiwal cow samples
Lane M	: 100 bp DNA ladder

Plate 4.18 Resolution of PCR product of primer 1 of KCNB1 gene in Karan Fries cows

Lane 1-10	: PCR product (505bp) of Karan Fries cow samples
Lane M	: 100 bp DNA ladder

Plate 4.19 Resolution of PCR product of primer 1 of EDN3 gene in Sahiwal cows



Lane 1-14	: PCR product (577 bp) of Sahiwal cow samples
Lane M	: 50 bp DNA ladder

Plate 4.20 Resolution of PCR product of primer 1 of EDN3 gene in Karan Fries cows

Lane 1-14	: PCR product (577 bp) of Karan Fries cow samples
Lane M	: 50 bp DNA ladder

one targeted region between each of KCNB1 *Bos taurus* sequence (Accession no. ENSBTAG00000027320) and EDN3 gene *Bos taurus* sequence (Accession no. ENSBTAG00000012109) with observed sequences of Karan Fries and Sahiwal cattle, respectively (Figure 4.8 and 4.9).

The nucleotide changes of FAM19A1 gene (Figure 4.10 to 4.17), KCNB1 gene (Figure 4.18 and 4.19) and EDN3 gene (Figure 4.20 and 4.21) were identified and shown in respective chromatographs of Karan Fries and Sahiwal cattle.

Table 4.2 Sequence, annealing temperatures, target region and amplicon sizes of all Primer sets

Gene	Primer Sets		Sequence 5'-3'	Region Covered	Amplicon Size (bp)	*Ta (°C)
FAM19A1	1	F R	GTAGCAAAGGACATGACGT GGCCAAGGTATCCACAAACA	Exon 1 and flanking regions	508	60.5
	2	F R	AGAGTGAGGGAAGAGGGATT AGCACATAGAGAATTAGCGTGA	Intron 1	596	55.8
	3	F R	CTCATGGGTTCTTGCTG CTC GTTGTGGAGGAAACGAGCAT	Intron 1	576	59.1
	4	F R	TGCCTGTCTTCTCCCAGATG GGGCTGGCATTTGTCTAACT	Exon 2 and flanking regions	509	60.5
	5	F R	AGCCCCTTCTTAGCCTTCTA GGTCATCTTGTCTGTCCCCT	Intron 2	492	62.0
	6	F R	AGGTTCAAAAGGACCTAATGCA ATCCAAATACCCGCCTCACT	Exon 3 and flanking regions	529	56.9
	7	F R	ACCACTTACCATTACTTAGGT GGATCACTGGGCGAGAGTAT	Exon 4 and flanking regions	368	56.9
KCNB1	1	F R	TTCAAATCCCGACTCCACCA TAACACACAAAAGTCGCCCC	Intron 1	505	62.2
EDN3	1	F R	GAAAAGCCCTAACCCACAGC AGGCTTTCTGAACACTTTGCA	Exon 1 and flanking regions	577	62.2

* Annealing temperature same in Karan Fries and Sahiwal cows

4.4 SNP identification in FAM19A1, KCNB1 and EDN3 gene in Sahiwal and Karan Fries cows

Total 10 nucleotide changes in Sahiwal cows (T33069832C, C33219027T, C33464963A, C33471720T, C33471980A, G33571161A, G33589765A, G78216220A, A78216335G, C57571910A) and 9 nucleotide changes in Karan Fries cows (T33069832C, C33219027T, C33464963A, C33471720T, C33471980A, G33571161A, G33589765A, G78216220A, A78216335G) of FAM19A1, KCNB1 and EDN3 genes were found in the present study (Table 4.3).

The PCR product of 508 bp harbouring SNP at T33069832C locus in intron 1 of FAM19A1 gene was digested with *BsrI* enzyme. Three genotypes were identified in both Sahiwal and Karan Fries cattle. These genotypes were defined by three distinct band patterns i.e. TT (508 bp), TC (508, 260 and 248 bp) and CC (260 and 248 bp) as shown in Plate 4.21 and 4.22 for Sahiwal and Karan Fries cattle, respectively. Their chromatograph showing nucleotide change is depicted in Figure 4.10.

The PCR product of 576 bp size revealed SNP at locus C33219027T in intron 1 of FAM19A1 gene was digested with *TaqI* enzyme. Three genotypes defined by three distinct band patterns were identified in both Sahiwal and Karan Fries cattle. These genotypes were CC (438 and 138 bp), CT (576, 438 and 138 bp) and TT (576 bp) as shown in plate 4.23 and 4.24 for Sahiwal and Karan Fries cattle, respectively. Their chromatograph showing nucleotide change is represented in Figure 4.11.

The 509 bp PCR product delineated SNP at C33464963A locus in intron 2 of FAM19A1 gene. This was digested with *AluI* enzyme unveiled two genotypes in Sahiwal and Karan Fries cattle. These genotypes were CC (369 and 140 bp) and CA (509, 369 and 140 bp) as shown in Plate 4.25 and 4.26 for Sahiwal and Karan Fries cattle, respectively and their chromatograph showing nucleotide change is shown in Figure 4.12.

Two SNPs were found for 492 bp PCR product in intron 2 of FAM19A1 gene in both the breeds. First SNP at locus C33471720T was digested with *HinfI* enzyme and resulted in three genotypes with three distinct patterns. These genotypes were CC (322 and 170 bp), CT (492, 322 and 170 bp) and TT (492 bp) as shown for Sahiwal and Karan Fries cattle in plate 4.27 and 4.28, respectively and their chromatograph showing nucleotide change is shown in Figure 4.13.

Other SNP was found at locus C33471980A in 492 bp PCR product. This was digested with *HindIII* enzyme and revealed two genotypes with two banding pattern in both the breeds. These genotypes were CC (431,61 bp) and CA (431, 61 and 492 bp), shown for

Figure 4.1 ClustalW Alignment of exon 1 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos Taurus*

```
Bos_taurus      GTAGCAAAGGGACATGACGTCTGAGACCCCCCTTCTTTCATCAGTGGGACTGACAGAGCT  60
SW_F1           ------------------------------GGTATTCTTCTCAGTGGGACTGACAGAGCT  30
SW_F2           ---------------------------TGGTTCTTCTTTATCAGTGGGACTGACAGAGCT  33
KF_F1           --------------------------GGGTTAGTTCTTTCTCAGTGGGACTGACAGAGCT  34
KF_F2           -----------------------------GTTGTACTTTCTCAGTGGGACTGACAGAGCT  31
                                                     *  ********************

Bos_taurus      GGGGGCTCAAAGCCGGGGGTAATTCTTCCTTTCTAATGTTCTCTTTAGAGAATGGCAATG  120
SW_F1           GGGGGCTCAAAGCCGGGGGTAATTCTTCCTTTCTAATGTTCTCTTTAGAGAATGGCAATG  90
SW_F2           GGGGGCTCAAAGCCGGGGGTAATTCTTCCTTTCTAATGTTCTCTTTAGAGAATGGCAATG  93
KF_F1           GGGGGCTCAAAGCCGGGGGTAATTCTTCCTTTCTAATGTTCTCTTTAGAGAATGGCAATG  94
KF_F2           GGGGGCTCAAAGCCGGGGGTAATTCTTCCTTTCTAATGTTCTCTTTAGAGAATGGCAATG  91
                ************************************************************

Bos_taurus      GTCTCTGCGATGTCCTGGGTCCTGTATTTGTGGATAAGTGCTTGCGCGGTGCTGCTCTGC  180
SW_F1           GTCTCTGCGATGTCCTGGGTCCTGTATTTGTGGATAAGTGCTTGCGCGGTGCTGCTCTGC  150
SW_F2           GTCTCTGCGATGTCCTGGGTCCTGTATTTGTGGATAAGTGCTTGCGCGGTGCTGCTCTGC  153
KF_F1           GTCTCTGCGATGTCCTGGGTCCTGTATTTGTGGATAAGTGCTTGCGCGGTGCTGCTCTGC  154
KF_F2           GTCTCTGCGATGTCCTGGGTCCTGTATTTGTGGATAAGTGCTTGCGCGGTGCTGCTCTGC  151
                ************************************************************

Bos_taurus      CATGGATCCCTTCAGCACACTTTCCAGCAGCATCACCTGCACCGACCAGGTAAGTCGGAG  240
SW_F1           CATGGATCCCTTCAGCACACTTTCCAGCAGCATCACCTGCACCGACCAGGTAAGTCGGAG  210
SW_F2           CATGGATCCCTTCAGCACACTTTCCAGCAGCATCACCTGCACCGACCAGGTAAGTCGGAG  213
KF_F1           CATGGATCCCTTCAGCACACTTTCCAGCAGCATCACCTGCACCGACCAGGTAAGTCGGAG  214
KF_F2           CATGGATCCCTTCAGCACACTTTCCAGCAGCATCACCTGCACCGACCAGGTAAGTCGGAG  211
                ************************************************************

Bos_taurus      GCTGGCTCCACAGTAGCCTTCAGTCTCCAGCCTCACTGTGGTCTTCTAACAGATGGTTGA  300
SW_F1           GCTGGCTCCACAGTAGCCTTCAGTCTCCAGCCTCACTGTGGTCTTCTAACAGATGGTTGA  270
SW_F2           GCTGGCTCCACAGTAGCCTCCAGTCTCCAGCCTCACTGTGGTCTTCTAACAGATGGTTGA  273
KF_F1           GCTGGCTCCACAGTAGCCTTCAGTCTCCAGCCTCACTGTGGTCTTCTAACAGATGGTTGA  274
KF_F2           GCTGGCTCCACAGTAGCCTCCAGTCTCCAGCCTCACTGTGGTCTTCTAACAGATGGTTGA  271
                ******************* ****************************************

Bos_taurus      AGGCAAGTGACAGAAAGTGCTACAGTGTGGGCAGCTTGCCTCGGAATTCCAAGACTTACT  360
SW_F1           AGGCAAGTGACAGAAAGTGCTACAGTGTGGGCAGCTTGCCTCGGAATTCCAAGACTTACT  330
SW_F2           AGGCAAGTGACAGAAAGTGCTACAGTGTGGGCAGCTTGCCTCGGAATTCCAAGACTTACT  333
KF_F1           AGGCAAGTGACAGAAAGTGCTACAGTGTGGGCAGCTTGCCTCGGAATTCCAAGACTTACT  334
KF_F2           AGGCAAGTGACAGAAAGTGCTACAGTGTGGGCAGCTTGCCTCGGAATTCCAAGACTTACT  331
                ************************************************************

Bos_taurus      GTTGGCTGGATGCTACGGGGAGTGTGGGGGATACTAAAATGCCATGGATGTATTTCCAAT  420
SW_F1           GTTGGCTGGATGCTACGGGGAGTGTGGGGGATACTAAAATGCCATGGATGTATTTCCAAT  390
SW_F2           GTTGGCTGGATGCTACGGGGAGTGTGGGGGATACTAAAATGCCATGGATGTATTTCCAAT  393
KF_F1           GTTGGCTGGATGCTACGGGGAGTGTGGGGGATACTAAAATGCCATGGATGTATTTCCAAT  394
KF_F2           GTTGGCTGGATGCTACGGGGAGTGTGGGGGATACTAAAATGCCATGGATGTATTTCCAAT  391
                ************************************************************

Bos_taurus      AAATGTTTAGAAAATGTGATTCTGTCTAGGAGTCCCACCATCCCTTGGTGCATAAAGTAG  480
SW_F1           AAATGTTTAGAAAATGTGATTCTGTCTAGGAGTCCCACCATCCCTTGGTGCATAAAGTAG  450
SW_F2           AAATGTTTAGAAAATGTGATTCTGTCTAGGAGTCCCACCATCCCTTGGTGCATAAAGTAG  453
KF_F1           AAATGTTTAGAAAATGTGATTCTGTCTAGGAGTCCCACCATCCCTTGGTGCATAAAGTAG  454
KF_F2           AAATGTTTAGAAAATGTGATTCTGTCTAGGAGTCCCACCATCCCTTGGTGCATAAAGTAG  451
                ************************************************************

Bos_taurus      AAGTCTTTTGTTTGTGGATACCTTGGCC------ 508
SW_F1           AAGTCTTTTGTTTGTGGAACCCTTGGCCACAA-- 482
SW_F2           AAGTCTTTTGTTTGTGGAACCCTTGGCCACAA-- 485
KF_F1           AAGTCTTTTGTTTGTGGAACCCTTGGCCAAGG-- 486
KF_F2           AAGTCTTTTGTTTGTGGATACCTTGGCCAAGCAG 485
                ******************  ********
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Figure 4.2 ClustalW Alignment of intron 1 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos Taurus*

```
Bos_taurus      AGAGTGAGGGAAGAGGGATTAACTACAAAGGACCACAAAGAAATTTTAGGGTGATGAGAA 60
SW_F            ----------------------------GCCAGGGAAAAAAGATTTTAGGGTGATGAGAA 32
KF_F            ------------------------------ACCATGACAAAGATTTTAGGGTGATGAGAA 30
                                                    * * * ******************

Bos_taurus      TATACTATGTATACCTCTGTTGAAATGTATAGAACGTTACACTTCAAGCTGTTGCAGTTT 120
SW_F            TATACTATGTATACCTCTGTTGAAATGTATAGAACGTTACACTTCAAGCTGTTGCAGTTT 92
KF_F            TATACTATGTATACCTCTGTTGAAATGTATAGAACGTTACACTTCAAGCTGTTGCAGTTT 90
                ************************************************************

Bos_taurus      TTATTGTATCTAAATTATATCTCACCAAGTCTCACTTTGAGGGAAAAAACAGATTTCCTC 180
SW_F            TTATTGTATCTAAATTATATCTCACCAAGTCTCACTTTGAGGGAAAAAACAGATTTCCTC 152
KF_F            TTATTGTATCTAAATTATATCTCACCAAGTCTCACTTTGAGGGAAAAAACAGATTTCCTC 150
                ************************************************************

Bos_taurus      TGGAAAAAATACCTGTCTTGTACTTTTTGGTTCTCTTGCAAAGAAACTTTTCTGAAAACGT 240
SW_F            TGGAAAAAATACCTGTCTTGTACTTTTTGGTTCTCTTGCAAAGAAACTTTTCTGAAAACGT 212
KF_F            TGGAAAAAATACCTGTCTTGTACTTTTTGGTTCTCTTGCAAAGAAACTTTTCTGAAAACGT 210
                ************************************************************

Bos_taurus      GTATGAAAGCAGTCACAATGTATAAGAGAAGTTGAGAGTTTGGTGAGGGAATCTCTTTCT 300
SW_F            GTATGAAAGCAGTCACAATGTATAAGAGAAGTTGAGAGTTTGGTGAGGGAATCTCTTTCT 272
KF_F            GTATGAAAGCAGTCACAATGTATAAGAGAAGTTGAGAGTTTGGTGAGGGAATCTCTTTCT 270
                ************************************************************

Bos_taurus      GTCTCTATCTCTGTCTGTCTCTTTATCGCTCTCTCATTTAAAAATAAAACTGCATAAACC 360
SW_F            GTCTCTATCTCTGTCTGTCTCTTTATCGCTCTCTCATTTAAAAATAAAACTGCATAAACC 332
KF_F            GTCTCTATCTCTGTCTGTCTCTTTATCGCTCTCTCATTTAAAAATAAAACTGCATAAACC 330
                ************************************************************

Bos_taurus      CAGGAATCGAACCCAGGTATCCCGCATTGCGGGCAGACGCTTTGCCATCTGAGCCACCAG 420
SW_F            CAGGAATCGAACCCAGGTATCCCGCATTGCGGGCAGACGCTTTGCCATCTGAGCCACCAG 392
KF_F            CAGGAATCGAACCCAGGTATCCCGCATTGCGGGCAGACGCTTTGCCATCTGAGCCACCAG 390
                ************************************************************

Bos_taurus      GGAAGTGGGTATTAAAGACAATAAAATAAGACATTTTGAGTGTAATAATTACAGTTTCAT 480
SW_F            GGAAGTGGGTATTAAAGACAATAAAATAAGACATTTTGAGTGTAATAATTACAGTTTCAT 452
KF_F            GGAAGTGGGTATTAAAGACAATAAAATAAGACATTTTGAGTGTAATAATTACAGTTTCAT 450
                ************************************************************

Bos_taurus      CATTGTCATTATTTTAAGGAAATTATAATGAGGAAAACAAAAGTGCATTTTTTATTTGTT 540
SW_F            CATTGTCATTATTTTAAGGAAATTATAATGAGGAAAACAAAAGTGCATTTTTTATTTGTT 512
KF_F            CATTGTCATTATTTTAAGGAAATTATAATGAGGAAAACAAAAGTGCATTTTTTATTTGTT 510
                ************************************************************

Bos_taurus      ACTGAACACAATTAATTTTCAAAGAAGTTTTTGTTCACGCTAATTCTCTATGTGCT---- 596
SW_F            ACTGAACACAATTAATTTTCAAAGAAGTTTTTGTTCACGCTAATTTCCTATTGTGCTAGA 572
KF_F            ACTGAACACAATTAATTTTCAAAGAAGTTTTTGTTCACGCTAATTCCCTATGTGCTAGAA 570
                *********************************************  ****
```

Figure 4.3 ClustalW Alignment of intron 1 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos Taurus*

```
Bos_taurus      CTCATGGGTTCTTGCTGCTCCACCATGTGTACCTGCAAAGCCAGTGAAGGGAATGAAGGC  60
SW_F1           ------------------TCACATAAATGCAGCAGTGAGGGATGAGGCGGGAATGAAGGC  42
SW_F2           ---------------GGGGCAATGCTAAACAGTCCGAATGAAGGCATGCAGCACTGGACC  45
KF_F1           -----------------GTGAAGCATGCTAACAGGGAATGAAGGCATGCAGCACTGGACC  43
KF_F2           --GCGGGAATGCAGCAGTATCAGGGTATGATGCGGGAATGAAGGCATGCAGCACTGGACC  58
                                                     * *   *      * *      *

Bos_taurus      ATGCAGCACTGGACCGGCAGGAAGTACTTTCTTAAGCCTGCCTCTTCTTTATTTCTTTCA  120
SW_F1           ATGCAGCACTGGACCGGCAGGAAGTACTTTCTTAAGCCTGCCTCTTCTTTATTTCTTTCA  102
SW_F2           ATGCAGCACTGGACCGGCAGGAAGTACTTTCTTAAGCCTGCCTCTTCTTTATTTCTTTCA  105
KF_F1           ATGCAGCACTGGACCGGCAGGAAGTACTTTCTTAAGCCTGCCTCTTCTTTATTTCTTTCG  103
KF_F2           ATGCAGCACTGGACCGGCAGGAAGTACTTTCTTAAGCCTGCCTCTTCTTTATTTCTTTCA  118
                ***********************************************************

Bos_taurus      CCACTTGTTGAAATTAATCGAAATTAATGCCTTGGGTTCTTTCTGATTGTTTCTTCTCCC  180
SW_F1           CCACTTGTTGAAATTAATCGAAATTAATGCCTTGGGTTCTTTCTGATTGTTTCTTCTCCC  162
SW_F2           CCACTTGTTGAAATTAATTGAAATTAATGCCTTGGGTTCTTTCTGATTGTTTCTTCTCCC  165
KF_F1           CCACTTGTTGAAATTAATTGAAATTAATGCCTTGGGTTCTTTCTGATTGTTTCTTCTCCC  163
KF_F2           CCACTTGTTGAAATTAATCGAAATTAATGCCTTGGGTTCTTTCTGATTGTTTCTTCTCCC  178
                ****************** *****************************************

Bos_taurus      TACTCTCATGTCTATAGAAATATGAAATATTTTGTATGTTTTTTCCTATCCTCATCTTGC  240
SW_F1           TACTCTCATGTCTATAGAAATATGAAATATTTTGTATGTTTTTTCCTATCCTCATCTTGC  222
SW_F2           TACTCTCATGTCTATAGAAATATGAAATATTTTGTATGTTTTTTCCTATCCTCATCTTGC  225
KF_F1           TACTCTCATGTCTATAGAAATATGAAATATTTTGTATGTTTTTTCCTATCCTCATCTTGC  223
KF_F2           TACTCTCATGTCTATAGAAATATGAAATATTTTGTATGTTTTTTCCTATCCTCATCTTGC  238
                ************************************************************

Bos_taurus      TAATTATTTTTAAAAGTAGTATCTCCATGGTGAAACCACAGTAATAAGAATAATAGTGCA  300
SW_F1           TAATTATTTTTAAAAGTAGTATCTCCATGGTGAAACCACAGTAATAAGAATAATAGTGCA  282
SW_F2           TAATTATTTTTAAAAGTAGTATCTCCATGGTGAAACCACAGTAATAAGAATAATAGTGCA  285
KF_F1           TAATTATTTTTAAAAGTAGTATCTCCATGGTGAAACCACAGTAATAAGAATAATAGTGCA  283
KF_F2           TAATTATTTTTAAAAGTAGTATCTCCATGGTGAAACCACAGTAATAAGAATAATAGTGCA  298
                ************************************************************

Bos_taurus      CTCATCCTATGTTAACCATTCTATTAAATATTTTACATCAGATATTTCATTGAGTCCTGA  360
SW_F1           CTCATCCTATGTTAACCATTCTATTAAATATTTTACATCAGATATTTCATTGAGTCCTGA  342
SW_F2           CTCATCCTATGTTAACCATTCTATTAAATATTTTACATCAGATATTTCATTGAGTCCTGA  345
KF_F1           CTCATCCTATGTTAACCATTCTATTAAATATTTTACATCAGATATTTCATTGAGTCCTGA  343
KF_F2           CTCATCCTATGTTAACCATTCTATTAAATATTTTACATCAGATATTTCATTGAGTCCTGA  358
                ************************************************************

Bos_taurus      ACACAACCTAATGAGATTGGCTTCATTTTCCTCTGGAAACCAGAAGCTAAGTGACTCATC  420
SW_F1           ACACAACCTAATGAGATTGGCTTCATTTTCCTCTGGAAACCAGAAGCTAAGTGACTCATC  402
SW_F2           ACACAACCTAATGAGATTGGCTTCATTTTCCTCTGGAAACCAGAAGCTAAGTGACTCATC  405
KF_F1           ACACAACCTAATGAGATTGGCTTCATTTTCCTCTGGAAACCAGAAGCTAAGTGACTCATC  403
KF_F2           ACACAACCTAATGAGATTGGCTTCATTTTCCTCTGGAAACCAGAAGCTAAGTGACTCATC  418
                ************************************************************

Bos_taurus      AATGTCGTGGCTCTTCTAGTAAATGGGACCTTGGTAATTTAAACTTGGAACAACCAGTCC  480
SW_F1           AATGTCGTGGCTCTTCTAGTAAATGGGACCTTGGTAATTTAAACTTGGAACAACCAGTCC  462
SW_F2           AATGTCGTGGCTCTTCTAGTAAATGGGACCTTGGTAATTTAAACTTGGAACAACCAGTCC  465
KF_F1           AATGTCGTGGCTCTTCTAGTAAATGGGACCTTGGTAATTTAAACTTGGAACAACCAGTCC  463
KF_F2           AATGTCGTGGCTCTTCTAGTAAATGGGACCTTGGTAATTTAAACTTGGAACAACCAGTCC  478
                ************************************************************

Bos_taurus      TTCTTCAAAGCCTGTCCTCCATGTCAGGATGACTTGGAGGTATGCTTATTTCAGGTATAT  540
SW_F1           TTCTTCAAAGCCTGTCCTCCATGTCAGGATGACTTGGAGGTATGCTTATTTCAGGTATAT  522
SW_F2           TTCTTCAAAGCCTGTCCTCCATGTCAGGATGACTTGGAGGTATGCTTATTTCAGGTATAT  525
KF_F1           TTCTTCAAAGCCTGTCCTCCATGTCAGGATGACTTGGAGGTATGCTTATTTCAGGTATAT  523
KF_F2           TTCTTCAAAGCCTGTCCTCCATGTCAGGATGACTTGGAGGTATGCTTATTTCAGGTATAT  538
                ************************************************************

Bos_taurus      ACAGATATATACAGGTATGCTCGTTTCCTCCACAAC---      576
SW_F1           ACAGATATATACAGGTATGCTCGTTTCCTCCACAACAGT      561
SW_F2           ACAGATATATACAGGTATGCTCGTTTCCTCCACAACAGT      564
KF_F1           ACAGATATATACAGGTATGCTCGTTTCCTCCACAACAGA      562
KF_F2           ACAGATATATACAGGTATGCTCGTTTCCTCCACAACATT      577
                ************************************
```

Figure 4.4 ClustalW Alignment of exon 2 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos Taurus*

```
Bos_taurus      TGCCTGTCTTCTCCCAGATGACATGACACATCCCTTGTATGCAATCTTGTATACATGTAT 60
SW_F            --------------------------CGGATTTACTTGCATGCATCTTGTATACATGTAT 34
KF_F            ----------------------------GGCATACTTGCATGCATCTTGTATACATGTAT 32
                                                   *       *****************

Bos_taurus      AGATACATGTTGTAGGAGAGAATCCCATAGTAACTCTACTTTATGGAAACCCTTCCCTTT 120
SW_F            AGATACATGTTGTAGGAGAGAATCCCATAGTAACTCTACTTTATGGAAACCCTTCCCTTT 94
KF_F            AGATACATGTTGTAGGAGAGAATCCCATAGTAACTCTACTTTATGGAAACCCTTCCCTTT 92
                ************************************************************

Bos_taurus      ACGTTTTTCACTTTCCGAACCCCTCCACTCACACGTGCACATACCTGAATGGCCACTGAA 180
SW_F            ACGTTTTTCACTTTCCGAACCCCTCCACTCACACGTGCACATACCTGAATGGCCACTGAA 154
KF_F            ACGTTTTTCACTTTCCGAACCCCTCCACTCACACGTGCACATACCTGAATGGCCACTGAA 152
                ************************************************************

Bos_taurus      TTATCTCTAATCAGCATCCCGCTTCTTTCTCTCTTGCCAGAAGGAGGGACGTGTGAAGTG 240
SW_F            TTATCTCTAATCAGCATCCCGCTTCTTTCTCTCTTGCCAGAAGGAGGGACGTGTGAAGTG 214
KF_F            TTATCTCTAATCAGCATCCCGCTTCTTTCTCTCTTGCCAGAAGGAGGGACGTGTGAAGTG 212
                ************************************************************

Bos_taurus      ATTGCTGCGCACAGATGTTGTAATAAAAACCGCATTGAGGAGCGGTCGCAAACAGTCAAG 300
SW_F            ATTGCTGCGCACAGATGTTGTAATAAAAACCGCATTGAGGAGCGGTCGCAAACAGTCAAG 274
KF_F            ATTGCTGCGCACAGATGTTGTAATAAAAACCGCATTGAGGAGCGGTCGCAAACAGTCAAG 272
                ************************************************************

Bos_taurus      TGTTCCTGTCTACCTGGGAAAGTGGCTGGAACAACAAGAAACCGACCTTCCTGTGTCGAT 360
SW_F            TGTTCCTGTCTACCTGGGAAAGTGGCTGGAACAACAAGAAACCGACCTTCCTGTGTCGAT 334
KF_F            TGTTCCTGTCTACCTGGGAAAGTGGCTGGAACAACAAGAAACCGACCTTCCTGTGTCGAT 332
                ************************************************************

Bos_taurus      GGTAAGTAGCTGGTTTCACTCCTCTCTCTATGGGTTCACCTGTGTTGTTATGGTGTAAAA 420
SW_F            GGTAAGTAGATGGTTTCACTCCTCTCTCTATGGGTTCACCTGTGTTGTTATGGTGTAAAA 394
KF_F            GGTAAGTAGATGGTTTCACTCCTCTCTCTATGGGTTCACCTGTGTTGTTATGGTGTAAAA 392
                ********* **************************************************

Bos_taurus      TATATATATATTTTGTTTCATCTAAATATGGTTCAGGATAATAAAGAAGGTGTCTCTACA 480
SW_F            TATATATATATTTTGTTTCATCTAAATATGGTTCAGGATAATAAAGAAGGTGTCTCTACA 454
KF_F            TATATATATATTTTGTTTCATCTAAATATGGTTCAGGATAATAAAGAAGGTGTCTCTACA 452
                ************************************************************

Bos_taurus      ATGCTGCAAAGTTAGACAAATGCCAGCCC----  509
SW_F            ATGCTGCAAAGTTAGACAATGGCCAGCCCATAG  487
KF_F            ATGCTGCAAAGTTAGACAATGGCCAGCCCAAGT  485
                *******************  ********
```

Figure 4.5 ClustalW Alignment of intron 2 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos taurus*

```
Bos_taurus      AGCCCCTTCTTAGCCTTCTAAAACATGATAGCGTATTAAAGGCTGAGAAGCTCTGCAGAC 60
SW_F1           -----------------------GGATCGTTCGGTTTTAAAGGCTGAGAGCTCTGCAGAC 37
SW_F2           ----------------------------AAGCTATCGATTATGCTGAGAGCTCTGCAGAC 32
KF_F1           ---------------------------CGGTCTGTCGATTAGGCTGAGAGCTCTGCAGAC 33
KF_F2           ---------------------------CGGCTTTCGAATTACGCTGAGAGCTCTGCAGAC 33
                                                                ************

Bos_taurus      CAGGAACCTGTAGAACTTTAGGCCAGTATTCCCTAAACATATTTGACCATAGAACCCTCT 120
SW_F1           CAGGAACCTGTAGAACTTTAGGCCAGTATTCCCTAAACATATTTGACCATAGAACCCTCT 97
SW_F2           CAGGAACCTGTAGAACTTTAGGCCAGTATTCCCTAAACATATTTGACCATAGAACCCTCT 92
KF_F1           CAGGAACCTGTAGAACTTTAGGCCAGTATTCCCTAAACATATTTGACCATAGAACCCTCT 93
KF_F2           CAGGAACCTGTAGAACTTTAGGCCAGTATTCCCTAAACATATTTGACCATAGAACCCTCT 93
                ************************************************************

Bos_taurus      CCTGTGTAACACTTGTTTATATCCTGCAGACCCAATGTTCCATGAAATGGACTCGGGACC 180
SW_F1           CCTGTGTAACACTTGTTTATATCCTGCAGACCCAATGTTCCATGAAATGGACTCGGGACC 157
SW_F2           CCTGTGTAACACTTGTTTATATCCTGCAGACCCAATGTTCCATGAAATGGACTTGGGACC 152
KF_F1           CCTGTGTAACACTTGTTTATATCCTGCAGACCCAATGTTCCATGAAATGGACTCGGGACC 153
KF_F2           CCTGTGTAACACTTGTTTATATCCTGCAGACCCAATGTTCCATGAAATGGACTTGGGACC 153
                ***************************************************** ******

Bos_taurus      ATGTTGGTGTTAGACTGTTTAGACTGTGTTTTTGTTTTTGTTTCCAGGAGAGTGCTTTAG 240
SW_F1           ATGTTGGTGTTAGACTGTTTAGACTGTGTTTTTGTTTTTGTTTCCAGGAGAGTGCTTTAG 217
SW_F2           ATGTTGGTGTTAGACTGTTTAGACTGTGTTTTTGTTTTTGTTTCCAGGAGAGTGCTTTAG 212
KF_F1           ATGTTGGTGTTAGACTGTTTAGACTGTGTTTTTGTTTTTGTTTCCAGGAGAGTGCTTTAG 213
KF_F2           ATGTTGGTGTTAGACTGTTTAGACTGTGTTTTTGTTTTTGTTTCCAGGAGAGTGCTTTAG 213
                ************************************************************

Bos_taurus      GTATTCCAAGACCAGCTGATAATTCCTTTGCCCATAACTCAGTGTTTTGCCAAAGACTGG 300
SW_F1           GTATTCCAAGACCAGCTGATAATTCCTTTGCCCATAACTCAGTGTTTTGCCAAAGACTGG 277
SW_F2           GTATTCCAAGACCAGCTGATAATTCCTTTGCCCATAACTCAGTGTTTTGCCAAAGACTGG 272
KF_F1           GTATTCCAAGACCAGCTGATAATTCCTTTGCCCATAACTCAGTGTTTTGCCAAAGACTGG 273
KF_F2           GTATTCCAAGACCAGCTGATAATTCCTTTGCCCATAACTCAGTGTTTTGCCAAAGACTGG 273
                ************************************************************

Bos_taurus      CTTTTCCAAAGACCATGCAGTGCACAACAGGTCTTGGCAGTAATGTGGTTTATCACATTT 360
SW_F1           CTTTTCCAAAGACCATGCAGTGCACAACAGGTCTTGGCAGTAATGTGGTTTATCACATTT 337
SW_F2           CTTTTCCAAAGACCATGCAGTGCACAACAGGTCTTGGCAGTAATGTGGTTTATCACATTT 332
KF_F1           CTTTTCCAAAGACCATGCAGTGCACAACAGGTCTTGGCAGTAATGTGGTTTATCACATTT 333
KF_F2           CTTTTCCAAAGACCATGCAGTGCACAACAGGTCTTGGCAGTAATGTGGTTTATCACATTT 333
                ************************************************************

Bos_taurus      CAGTGATATGTCAGGCTCAACTTAGGTTTGGAAAGCATCTAGCAAACTTCCAGGGAAATG 420
SW_F1           CAGTGATATGTCAGGCTCAACTTAGGTTTGGAAAGCATCTAGCAAACTTCCAGGGAAATG 397
SW_F2           CAGTGATATGTCAGGCTCAACTTAGGTTTGGAAAGCATCTAGCAAACTTCCAGGGAAATG 392
KF_F1           CAGTGATATGTCAGGCTCAACTTAGGTTTGGAAAGCATCTAGCAAACTTCCAGGGAAATG 393
KF_F2           CAGTGATATGTCAGGCTCAACTTAGGTTTGGAAAGCATCTAGCAAACTTCCAGGGAAATG 393
                ************************************************************

Bos_taurus      TACTATGAAAAAGCTTAAGCCCTTATTTTTATTCACACATATTAAATATTTTAGGGGACA 480
SW_F1           TACTATGAAAAAGATTAAGCCCTTATTTTTATTCACACATATTAAATATTTTAGGGGACA 457
SW_F2           TACTATGAAAAAGCTTAAGCCCTTATTTTTATTCACACATATTAAATATTTTAGGGGACA 452
KF_F1           TACTATGAAAAAGCTTAAGCCCTTATTTTTATTCACACATATTAAATATTTTAGGGGACA 453
KF_F2           TACTATGAAAAAGATTAAGCCCTTATTTTTATTCACACATATTAAATATTTTAGGGGACA 453
                ************* **********************************************

Bos_taurus      GACAAGATGACC----  492
KF_F2           GAAAAGATGCCCAGT-  468
SW_F1           GAAAAGATGACCAGGG  473
SW_F2           GACAAGATGACCATA-  467
KF_F1           GAAAAGATGACCATTA  469
                ** ****** **
```

Figure 4.6 ClustalW Alignment of exon 3 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos taurus*

```
Bos_taurus      AGGTTCAAAAGGACCTAATGCATCTCATAGAAAATTAACATCTATAGAAGGACACAGTCT 60
SW_F1           -----------------------------CCAGGCATACATCTATAGAAGGACACAGTCT 31
SW_F2           ----------------------------CCTGGACTTACATCTATAGAAGGACACAGTCT 32
KF_F1           ---------------------------CCAAGCACTTACATCTATAGAAGGACACAGTCT 33
                                                    ************************

Bos_taurus      CACGCCCAGATTGGATGAATTGCAAACAAACTTGTTTCCTGTCTTTGCACAGCCTCCATT 120
SW_F1           CACGCCCAGATTGGATGAATTGCAAACAAACTTGTTTCCTGTCTTTGCACAGCCTCCATT 91
SW_F2           CACGCCCAGATTGGATGAATTGCAAACAAACTTGTTTCCTGTCTTTGCACAGCCTCCATT 92
KF_F1           CACGCCCAGATTGGATGAATTGCAAACAAACTTGTTTCCTGTCTTTGCACAGCCTCCATT 93
                ************************************************************

Bos_taurus      GTGATCGGGAAATGGTGGTGTGAGATGGAGCCTTGTCTAGAAGGAGAAGAGTGTAAGACA 180
SW_F1           GTGATCGGGAAATGGTGGTGTGAGATGGAGCCTTGTCTAGAAGGAGAAGAGTGTAAGACA 151
SW_F2           GTGATCGGGAAATGGTGGTGTGAGATGGAGCCTTGTCTAGAAGGAGAAGAGTGTAAGACA 152
KF_F1           GTGATCGGGAAATGGTGGTGTGAGATGGAGCCTTGTCTAGAAGGAGAAGAGTGTAAGACA 153
                ************************************************************

Bos_taurus      CTCCCTGATAATTCTGGATGGATGTGTGCTACAGGCAATAAAATCAAGACCACAAGAGTA 240
SW_F1           CTCCCTGATAATTCTGGATGGATGTGTGCTACAGGCAATAAAATCAAGACCACAAGAGTA 211
SW_F2           CTCCCTGATAATTCTGGATGGATGTGTGCTACAGGCAATAAAATCAAGACCACAAGAGTA 212
KF_F1           CTCCCTGATAATTCTGGATGGATGTGTGCTACAGGCAATAAAATCAAGACCACAAGAGTA 213
                ************************************************************

Bos_taurus      AGTTGTTTTCTTTTTTTGGAAATGTCTCATGCATGTTACAGACTGTACTGTTTGCCTTTG 300
SW_F1           AGTTGTTTTCTTTTTTTGGAAATGTCTCATGCATGTTACAGACTGTACTGTTTGCCTTTG 271
SW_F2           AGTTGTTTTCTTTTTTTGGAAATGTCTCATGCATGTTACAGACTGTACTGTTTGCCTTTG 272
KF_F1           AGTTGTTTTCTTTTTTTGGAAATGTCTCATGCATGTTACAGACTGTACTGTTTGCCTTTG 273
                ************************************************************

Bos_taurus      TGTTGTATAAAGAACACCCAGAGAAGCTTCGAACAAGAGAGAAAGAAGCCTTTTCCCCAG 360
SW_F1           TGTTGTATAAAGAACACCCAGAGAAGCTTCGAACAAGAGAGAAAGAAGCCTTTTCCCCAG 331
SW_F2           TGTTGTATAAAGAACACCCAGAGAAGCTTCAAACAAGAGAGAAAGAAGCCTTTTCCCCAG 332
KF_F1           TGTTGTATAAAGAACACCCAGAGAAGCTTCGAACAAGAGAGAAAGAAGCCTTTTCCCCAG 333
                ****************************** *****************************

Bos_taurus      CAGAAACTATTTTTGACGGTTCAGATCAGTGTCCATCCATCAAAGAGAAGTTAGAGATTG 420
SW_F1           CAGAAACTATTTTTGACGGTTCAGATCAGTGTCCATCCATCAAAGAGAAGTTAGAGATTG 391
SW_F2           CAGAAACTATTTTTGACGGTTCAGATCAGTGTCCATCCATCAAAGAGAAGTTAGAGATTG 392
KF_F1           CAGAAACTATTTTTGACGGTTCAGATCAGTGTCCATCCATCAAAGAGAAGTTAGAGATTG 393
                ************************************************************

Bos_taurus      AACTTGACTTCTCCATCCTGAGCAGTTTAGTGTTTCTGTTATGGACTCATAAACAAAGCT 480
SW_F1           AACTTGACTTCTCCATCCTGAGCAGTTTAGTGTTTCTGTTATGGACTCATAAACAAAGCT 451
SW_F2           AACTTGACTTCTCCATCCTGAGCAGTTTAGTGTTTCTGTTATGGACTCATAAACAAAGCT 452
KF_F1           AACTTGACTTCTCCATCCTGAGCAGTTTAGTGTTTCTGTTATGGACTCATAAACAAAGCT 453
                ************************************************************

Bos_taurus      AGGCTGCCATCACCAAAGGGAAGAGGGGCAGTGAGGCGGGTATTTGGAT----        529
SW_F1           AGGCTGCCATCACCAAAGGGAAGAGGGGCAGTGAGGCGGTATTTTGGATACAT        504
SW_F2           AGGCTGCCATCACCAAAGGGAAGAGGGGCAGTGAGGCGGTAATTTGGATACAA        505
KF_F1           AGGCTGCCATCACCAAAGGGAAGAGGGGCAGTGAGGCGGGAATTTGGATATAC        506
                *****************************************************
```

Figure 4.7 ClustalW Alignment of exon 4 sequence of FAM19A1 gene in Sahiwal and Karan Fries cows with *Bos taurus*

```
Bos_taurus      ACCACTTACCATTACTTAGGGCTTCCCAGGTGGCCCTAGTGGTAAAGAACCCGCCTGCCA 60
SW_F1           -----------------------------CCATGCTACTAGTGGTAAGACCCGCCTGCCA 31
SW_F2           -----------------------------ACATGAGACTAGTGGTAAGACCCGCCTGCCA 31
KF_F1           -----------------------------GCATGCTGGCTAGTGGTAGACCCGCCTGCCA 31
KF_F2           ----------------------------ACAATGCGGACTAGTGGTAGACCCGCCTGCCA 32
                                                                ************

Bos_taurus      AGGCAGGAGATGTAAGACACCCAGGTTCAATCCCTGGGTTGGGAAGATCCCCTGGAGGAG 120
SW_F1           AGGCAGGAGATGTAAGACACCCAGGTTCAATCCCTGGGTTGGGAAGATCCCCTGGAGGAG 91
SW_F2           AGGCAGGAGATGTAAGACACCCAGGTTCAATCCCTGGGTTGGGAAGATCCCCTGGAGGAG 91
KF_F1           AGGCAGGAGATGTAAGACACCCAGGTTCAATCCCTGGGTTGGGAAGATCCCCTGGAGGAG 91
KF_F2           AGGCAGGAGATGTAAGACACCCAGGTTCAATCCCTGGGTTGGGAAGATCCCCTGGAGGAG 92
                ************************************************************

Bos_taurus      GGTATGGCAACCCACTCCAGTATTCTTGCCCGGAGAACCCCGTGGATAGAAGAGCCTGGT 180
SW_F1           GGTATGGCAACCCACTCCAGTATTCTTGCCCGGAGAACCCCGTGGATAGAAGAGCCTGGT 151
SW_F2           GGTATGGCAACCCACTCCAGTATTCTTGCCCGGAGAACCCCGTGGATAGAAGAGCCTGGT 151
KF_F1           GGTATGGCAACCCACTCCAGTATTCTTGCCCGGAGAACCCCGTGGATAGAAGAGCCTGGT 151
KF_F2           GGTATGGCAACCCACTCCAGTATTCTTGCCCGGAGAACCCCGTGGATAGAAGAGCCTGGT 152
                ************************************************************

Bos_taurus      GGGCTATAGTTCATAGGGTCACAAAGAATCGAACATGACTGAAGTGACTCTGCACACATC 240
SW_F1           GGGCTATAGTTCATAGGGTCACAAAGAATCGAACATGACTGAAGTGACTCTGCACACATC 211
SW_F2           GGGCTATAGTTCATAGGGTCACAAAGAATCAAACATGACTGAAGTGACTCTGCACACATC 211
KF_F1           GGGCTATAGTTCATAGGGTCACAAAGAATCGAACATGACTGAAGTGACTCTGCACACATC 211
KF_F2           GGGCTATAGTTCATAGGGTCACAAAGAATCAAACATGACTGAAGTGACTCTGCACACATC 212
                ****************************** *****************************

Bos_taurus      CACCATCACTTAGAGACTACTTCAAGGACTACTGAAGCTGTATTTGTTGGTGATTTCACC 300
SW_F1           CACCATCACTTAGAGACTACTTCAAGGACTACTGAAGCTGTATTTGTTGGTGATTTCACC 271
SW_F2           CACCATCACTTAGAGACTACTTCAAGGACTACTGAAGCTGTATTTGTTGGTGATTTCACC 271
KF_F1           CACCATCACTTAGAGACTACTTCAAGGACTACTGAAGCTGTATTTGTTGGTGATTTCACC 271
KF_F2           CACCATCACTTAGAGACTACTTCAAGGACTACTGAAGCTGTATTTGTTGGTGATTTCACC 272
                ************************************************************

Bos_taurus      TGCGTCTCCTTCCCATTGCATGTGATTTGTAAAACAGCCATGCAGAATATACTCTCGCCC 360
SW_F1           TGCGTCTCCTTCCCATTGCATGTGATTTGTAAAACAGCCATGCAGAATATACTCTCGCCC 331
SW_F2           TGCGTCTCCTTCCCATTGCATGTGATTTGTAAAACAGCCATGCAGAATATACTCTCGCCC 331
KF_F1           TGCGTCTCCTTCCCATTGCATGTGATTTGTAAAACAGCCATGCAGAATATACTCTCGCCC 331
KF_F2           TGCGTCTCCTTCCCATTGCATGTGATTTGTAAAACAGCCATGCAGAATATACTCTCGCCC 332
                ************************************************************

Bos_taurus      AGTGATCC-----     368
SW_F1           AGTGATCCACAAG     344
SW_F2           AGTGATCCAAA--     342
KF_F1           AGTGATCCATAA-     343
KF_F2           AGTGATCCAAA--     343
                ********
```

Figure 4.8 ClustalW Alignment of Primer 1 sequence of KCNB1 gene in Sahiwal and Karan Fries cows with *Bos taurus*

```
Bos_taurus      TTCAAATCCCGACTCCACCACCAGTACCTGGAGCCTCCAGCAGGTCACTTCCTCTCTCTC 60
SW_F1           -------------------------AACAATTCGAACTCAGCAGTCACTTCCTCTCTCTC 35
SW_F2           ---------------------------GAACGGGGGCTCAGCAGTCACTTCCTCTCTCTC 33
KF_F1           ---------------------------GAACGGGGGCTCAGCAGTCACTTCCTCTCTCTC 33
KF_F2           ---------------------------AACGTGAGACTCAGCAGTCACTTCCTCTCTCTC 33
                                                    *      *****************

Bos_taurus      TGAGCTGACGTTTTCTCTGAACCTCATCTTCAGAGACCAGTGCCAGGAGAGAGACTCCCT 120
SW_F1           TGAGCTGACGTTTTCTCTGAACCTCATCTTCAGAGACCAGTGCCAGGAGAGAGACTCCCT 95
SW_F2           TGAGCTGACGTTTTCTCTGAACCTCATCTTCAGAGACCAGTGCCAGGAGAGAGACTCCCT 93
KF_F1           TGAGCTGACGTTTTCTCTGAACCTCATCTTCAGAGACCAGTGCCAGGAGAGAGACTCCCT 93
KF_F2           TGAGCTGACGTTTTCTCTGAACCTCATCTTCAGAGACCAGTGCCAGGAGAGAGACTCCCT 93
                ************************************************************

Bos_taurus      TCCTCTTCCCTGGCTGTTTCCGCCAAGTCCCAAGGTTGGCTCTGATTGGCCACACTTGGA 180
SW_F1           TCCTCTTCCCTGGCTGTTTCCGCCAAGTCCCAAGGTTGGCTCTGATTGGCCACACTTGGA 155
SW_F2           TCCTCTTCCCTGGCTGTTTCCGCCAAGTCCCAAGGTTGGCTCTGATTGGCCACACTTGGA 153
KF_F1           TCCTCTTCCCTGGCTGTTTCCGCCAAGTCCCAAGGTTGGCTCTGATTGGCCACACTTGGA 153
KF_F2           TCCTCTTCCCTGGCTGTTTCCGCCAAGTCCCAAGGTTGGCTCTGATTGGCCACACTTGGA 153
                ************************************************************

Bos_taurus      TCACGTGCTCATCTCTGAACCAATCCCTGTGGCCAGAGGAGGACATCACTGAGGGGACTC 240
SW_F1           TCACGTGCTCATCTCTGAACCAATCCCTGTGGCCAGAGGAGGACATCACTGAGGGGACTC 215
SW_F2           TCACGTGCTCATCTCTGAACCAATCCCTGTGGCCAGAGGAGGACATCACTGAGGGGACTC 213
KF_F1           TCACGTGCTCATCTCTGAACCAATCCCTGTGGCCAGAGGAGGACATCACTGAGGGGACTC 213
KF_F2           TCACGTGCTCATCTCTGAACCAATCCCTGTGGCCAGAGGAGGACATCACTGAGGGGACTC 213
                ************************************************************

Bos_taurus      ACTTAGGTCAGAAACCACCACTGAGTGCAGGGGAAATACCCGGAATGTATTTACTTAATA 300
SW_F1           ACTTAGGTCAGAAACCACCACTGAGTGCAGGGGAAATACCCGGAATGTATTTACTTAATA 275
SW_F2           ACTTAGGTCAGAAACCACCACTGAGTGCAGGGGAAATACCCGAAATGTATTTACTTAATA 273
KF_F1           ACTTAGGTCAGAAACCACCACTGAGTGCAGGGGAAATACCCGAAATGTATTTACTTAATA 273
KF_F2           ACTTAGGTCAGAAACCACCACTGAGTGCAGGGGAAATACCCGGAATGTATTTACTTAATA 273
                ****************************************** *****************

Bos_taurus      TTTGCTTAATACTCTTATTTAAAAATATTTACTTAATATTCTTCTTTAAATTCATTAATT 360
SW_F1           TTTGCTTAATACTCTTATTTAAAAATATTTACTTAATATTCTTCTTTAAATTCATTAATT 335
SW_F2           TTTGCTTAATACTCTTATTTAAAAATATTTACTTAATATTCTTCTTTAAATTCATTAATT 333
KF_F1           TTTGCTTAATACTCTTATTTAAAAATATTTACTTAATATTCTTCTTTAAATTCATTAATT 333
KF_F2           TTTGCTTAATACTCTTATTTAAAAATATTTACTTAATATTCTTCTTTAAATTCATTAATT 333
                ************************************************************

Bos_taurus      TATTTTACTTCAATCAAATTATTTTAAAAGAATTATCATGAAGAATTGGCACAATTCGCC 420
SW_F1           TATTTTACTTCAATCAAATTATTTTAAAAGAATTATCGTGAAGAATTGGCACAATTCGCC 395
SW_F2           TATTTTACTTCAATCAAATTATTTTAAAAGAATTATCATGAAGAATTGGCACAATTCGCC 393
KF_F1           TATTTTACTTCAATCAAATTATTTTAAAAGAATTATCGTGAAGAATTGGCACAATTCGCC 393
KF_F2           TATTTTACTTCAATCAAATTATTTTAAAAGAATTATCATGAAGAATTGGCACAATTCGCC 393
                ************************************* **********************

Bos_taurus      ATAAATCATGTTGTGATATTCTAGCCAGATGCTGGAGCCTGACCCTTGCTTTCTTTGGGT 480
SW_F1           ATAAATCATGTTGTGATATTCTAGCCAGATGCTGGAGCCTGACCCTTGCTTTCTTTGGGT 455
SW_F2           ATAAATCATGTTGTGATATTCTAGCCAGATGCTGGAGCCTGACCCTTGCTTTCTTTGGGT 453
KF_F1           ATAAATCATGTTGTGATATTCTAGCCAGATGCTGGAGCCTGACCCTTGCTTTCTTTGGGT 453
KF_F2           ATAAATCATGTTGTGATATTCTAGCCAGATGCTGGAGCCTGACCCTTGCTTTCTTTGGGT 453
                ************************************************************

Bos_taurus      TCAAAGGGGCGACTTTTGTGTGTTA----------        505
SW_F1           TCAAAGGGGCGACTTTTTGTGTGTTAATA------        484
SW_F2           TCAAAGGGGCGACTTTTGTGTGTTAAAAAGGGGGA        488
KF_F1           TCAAAGGGGCGACTTTTGTGTGTTAAAAAGGGGGA        488
KF_F2           TCAAAGGGGCGACTTTTGTGTGTTAAAA-------        481
                *****************      *
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Figure 4.9 ClustalW Alignment of Primer 1 sequence of EDN3 gene in Sahiwal and Karan Fries cows with *Bos taurus*

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Bos_taurus      GAAAAGCCCTAACCCACAGCCGGCAGCCCTGAGCCGGGATGGGCAGCGCGCTCTGAAGTT 60
SW_F1           -------------------------TGGAACTTCACGGGAGGGCAGCGCGCTCTGATGTT 35
SW_F2           ------------------------GGGACGGTAACGGGGAGGGC-AGCGCGCTCTGAGTT 35
KF_F            ---------------------------TGGCGGTACGGGAGGGC-AGCGCGCTCTGAGTT 32
                                                    **  ****             ***

Bos_taurus      TGTGACGGTCGCAGACGACTCCTGGCCGCAGCCCGGGACGCGGCGAGCCAGCGGCCGGCC 120
SW_F1           TGTGACGGTCGCAGACGACTCCTGGCCGCAGCCCGGGACGCGGCGAGCCAGCGGCCGGCC 95
SW_F2           TGTGACGGTCGCAGACGACTCCTGGCCGCAGCCCGGGACGCGGCGAGCCAGCGGCCGGCC 95
KF_F            TGTGACGGTCGCAGACGACTCCTGGCCGCAGCCCGGGACGCGGCGAGCCAGCGGCCGGCC 92
                ************************************************************

Bos_taurus      TCGAAGCATCTGCAGTTGGAGGGTGAGGCCCACTGTGCCCGGTCCGAGCGCCCTTTGGCG 180
SW_F1           TCGAAGCATCTGCAGTTGGAGGGTGAGGCCCAATGTGCCCGGTCCGAGCGCCCTTTGGCG 155
SW_F2           TCGAAGCATCTGCAGTTGGAGGGTGAGGCCCACTGTGCCCGGTCCGAGCGCCCTTTGGCG 155
KF_F            TCGAAGCATCTGCAGTTGGAGGGTGAGGCCCACTGTGCCCGGTCCGAGCGCCCTTTGGCG 152
                ******************************** ***************************

Bos_taurus      GCCACAGGCGCCCGTCCTCCGGCGCGGTGCGCCCGCGCCCGATCGGGGTTCATGGAGCTG 240
SW_F1           GCCACAGGCGCCCGTCCTCCGGCGCGGTGCGCCCGCGCCCGATCGGGGTTCATGGAGCTG 215
SW_F2           GCCACAGGCGCCCGTCCTCCGGCGCGGTGCGCCCGCGCCCGATCGGGGTTCATGGAGCTG 215
KF_F            GCCACAGGCGCCCGTCCTCCGGCGCGGTGCGCCCGCGCCCGATCGGGGTTCATGGAGCTG 212
                ************************************************************

Bos_taurus      GGGCTGTGCTTCCTTTTCGGGCTCGCTGTGACCTCCGCCGCAGGTAAGCGGGCCGGGAGG 300
SW_F1           GGGCTGTGCTTCCTTTTCGGGCTCGCTGTGACCTCCGCCGCAGGTAAGCGGGCCGGGAGG 275
SW_F2           GGGCTGTGCTTCCTTTTCGGGCTCGCTGTGACCTCCGCCGCAGGTAAGCGGGCCGGGAGG 275
KF_F            GGGCTGTGCTTCCTTTTCGGGCTCGCTGTGACCTCCGCCGCAGGTAAGCGGGCCGGGAGG 272
                ************************************************************

Bos_taurus      CGCGCGTCTCCTGGCGCGAGCGCACACACAAAAGGACCCCGGATCGGGGGCGCGGGGAGG 360
SW_F1           CGCGCGTCTCCTGGCGCGAGCGCACACACAAAAGGACCCCGGATCGGGGGCGCGGGGAGG 335
SW_F2           CGCGCGTCTCCTGGCGCGAGCGCACACACAAAAGGACCCCGGATCGGGGGCGCGGGGAGG 335
KF_F            CGCGCGTCTCCTGGCGCGAGCGCACACACAAAAGGACCCCGGATCGGGGGCGCGGGGAGG 332
                ************************************************************

Bos_taurus      GTGCCTCGCCGGGGGAGCGGCCTGGAGGCCGGCTAGAGGGCAGAAGCTGAGGGCACGGAC 420
SW_F1           GTGCCTCGCCGGGGGAGCGGCCTGGAGGCCGGCTAGAGGGCAGAAGCTGAGGGCACGGAC 395
SW_F2           GTGCCTCGCCGGGGGAGCGGCCTGGAGGCCGGCTAGAGGGCAGAAGCTGAGGGCACGGAC 395
KF_F            GTGCCTCGCCGGGGGAGCGGCCTGGAGGCCGGCTAGAGGGCAGAAGCTGAGGGCACGGAC 392
                ************************************************************

Bos_taurus      CACCCCGCGGGCCCGGGAGGTTGCAGCCCACACGCAGGCAGGAGACCTGGGGCCCGGGCC 480
SW_F1           CACCCCGCGGGCCCGGGAGGTTGCAGCCCACACGCAGGCAGGAGACCTGGGGCCCGGGCC 455
SW_F2           CACCCCGCGGGCCCGGGAGGTTGCAGCCCACACGCAGGCAGGAGACCTGGGGCCCGGGCC 455
KF_F            CACCCCGCGGGCCCGGGAGGTTGCAGCCCACACGCAGGCAGGAGACCTGGGGCCCGGGCC 452
                ************************************************************

Bos_taurus      CGAGTTGGTCAATTTTTCATGAAGTCGGGAACTTTTCCAAAAGTTTGCAAACGATTCAGA 540
SW_F1           CGAGTTGGTCAATTTTTCATGAAGTCGGGAACTTTTCCAAAAGTTTGCAAACGATTCAGA 515
SW_F2           CGAGTTGGTCAATTTTTCATGAAGTCGGGAACTTTTCCAAAAGTTTGCAAACGATTCAGA 515
KF_F            CGAGTTGGTCAATTTTTCATGAAGTCGGGAACTTTTCCAAAAGTTTGCAAACGATTCAGA 512
                ************************************************************

Bos_taurus      AACTGTGCAAAATGACTGCAAAGTGTTCAGAAAGCCT----- 577
SW_F1           AACTGTGCAAAATGACTGCAAAGTGTTAAGAAAAGCCTAAGG 557
SW_F2           AACTGTGCAAAATGACTGCAAAGTGTTCAAAAAAGCCTAA-- 555
KF_F            AACTGTGCAAAATGACTGCAAAGTGTTCAAGAAAAGCCTAA- 553
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Figure 4.10 Chromatograph showing the SNP locus [T33069832C] in FAM19A1 gene in Sahiwal and Karan Fries cows

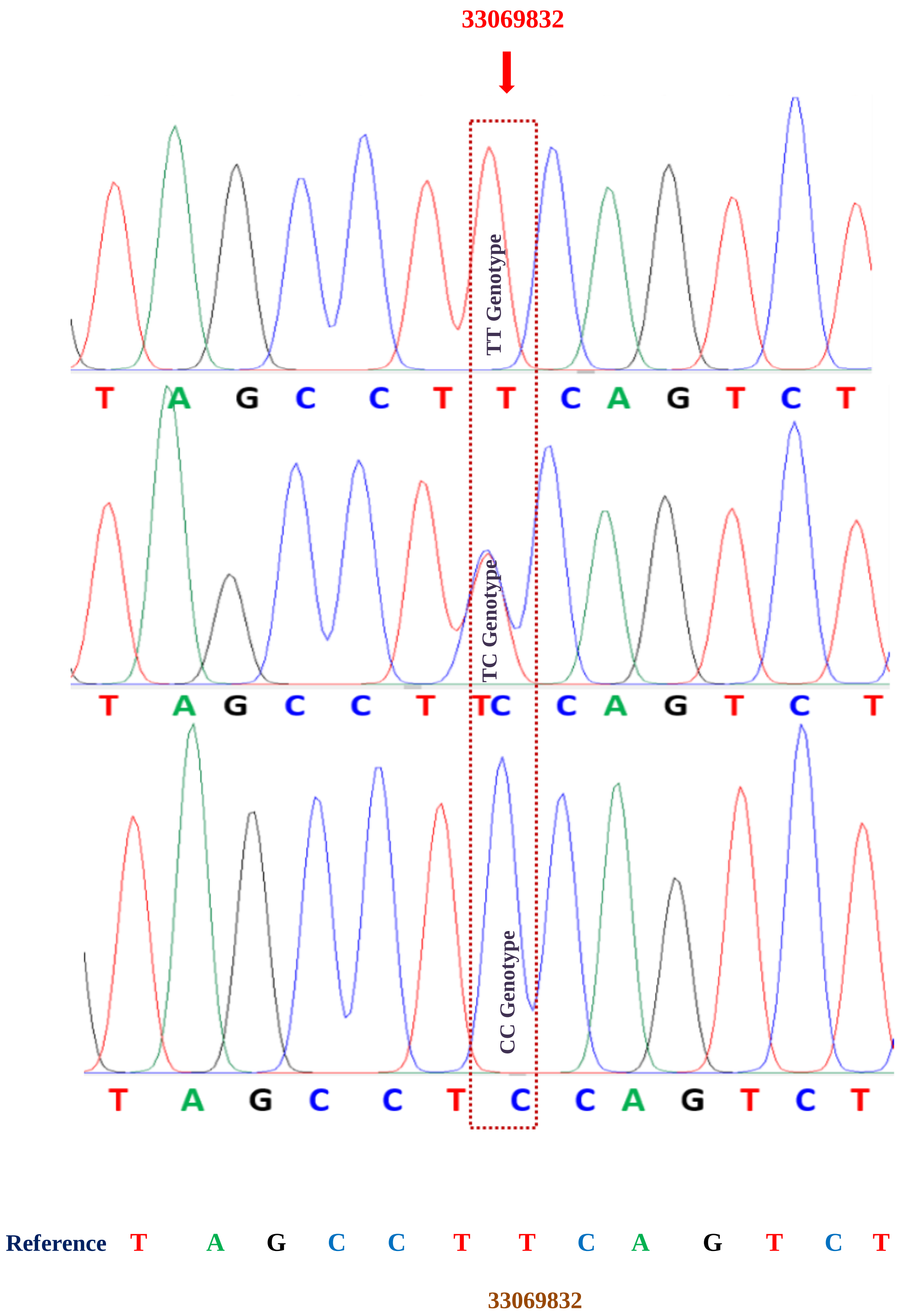


Figure 4.11 Chromatograph showing the SNP locus [C33219027T] in FAM19A1 gene in Sahiwal and Karan Fries cows

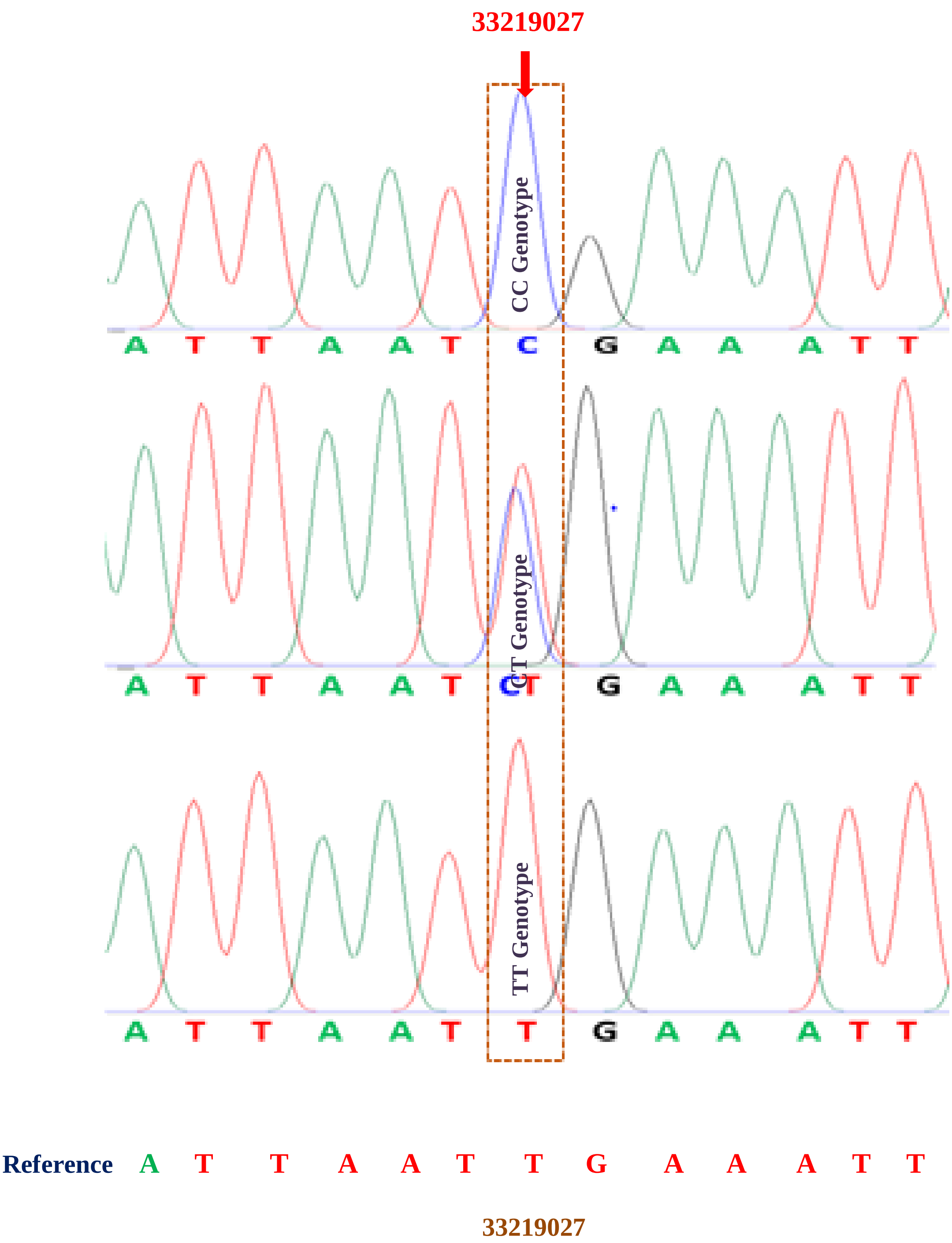


Figure 4.12 Chromatograph showing the SNP locus [C33464963A] in FAM19A1 gene in Sahiwal and Karan Fries cows

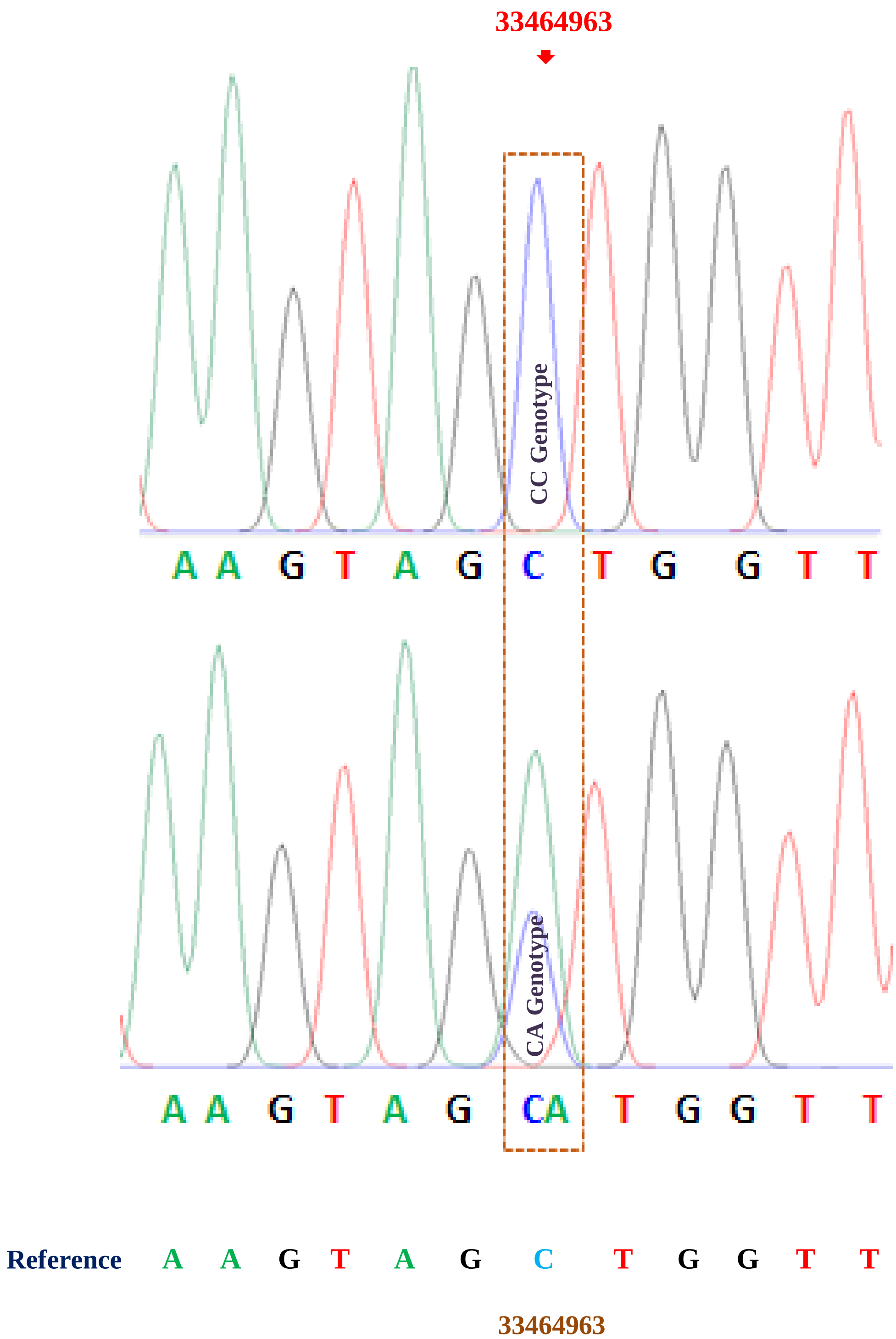
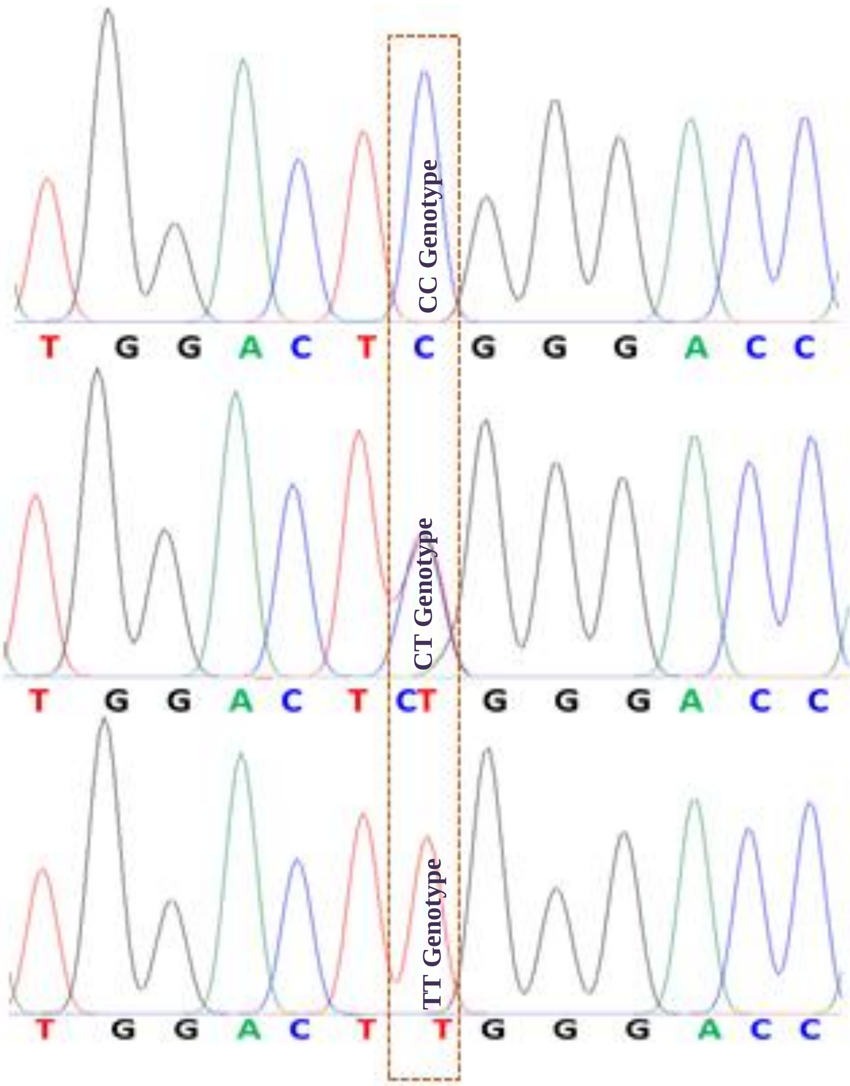


Figure 4.13 Chromatograph showing the SNP locus [C33471720T] in FAM19A1 gene in Sahiwal and Karan Fries cows

33471720



Reference T G G A C T C G G G A C C

33471720

Figure 4.14 Chromatograph showing the SNP locus [C33471980A] in FAM19A1 gene in Sahiwal and Karan Fries cows

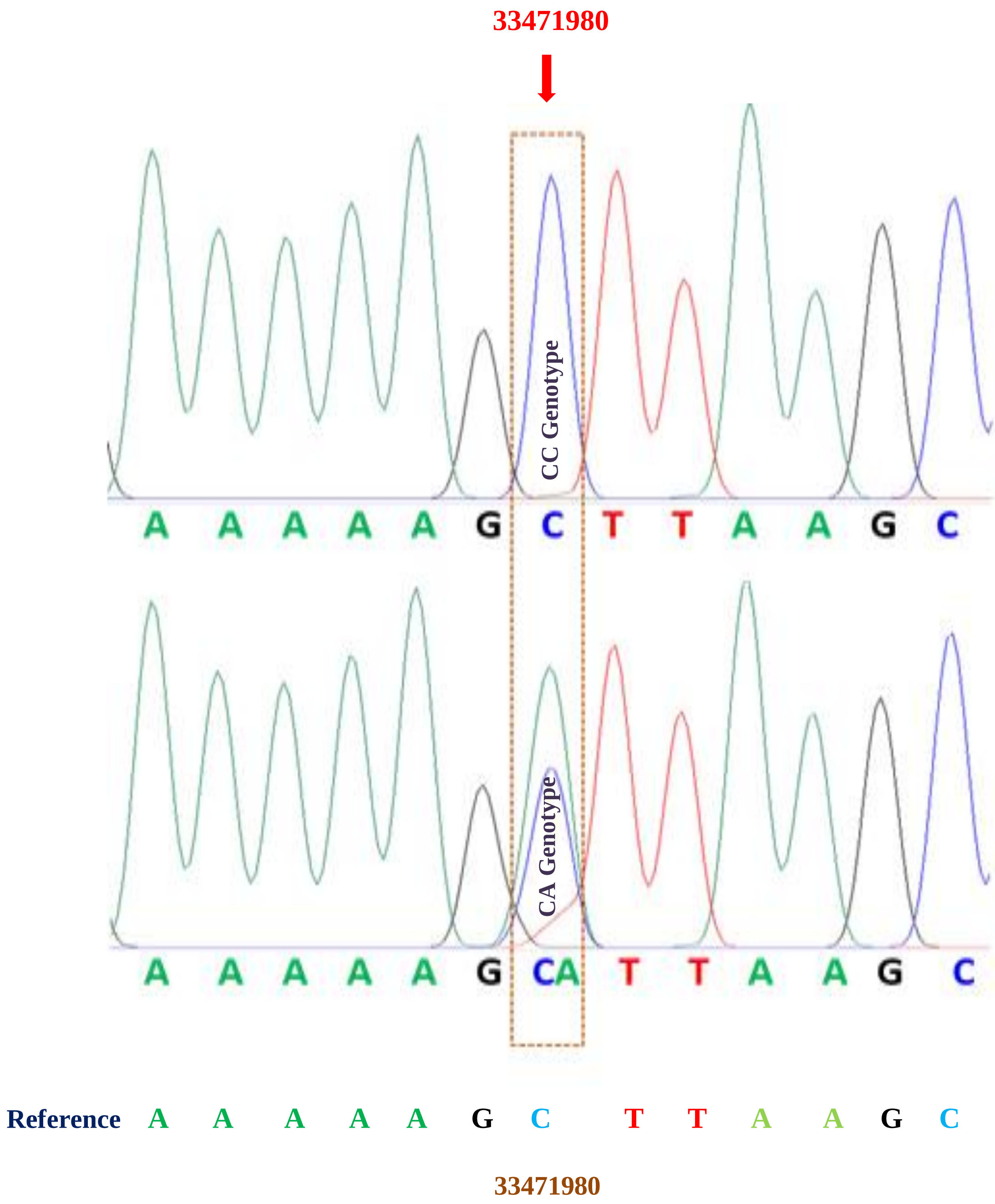
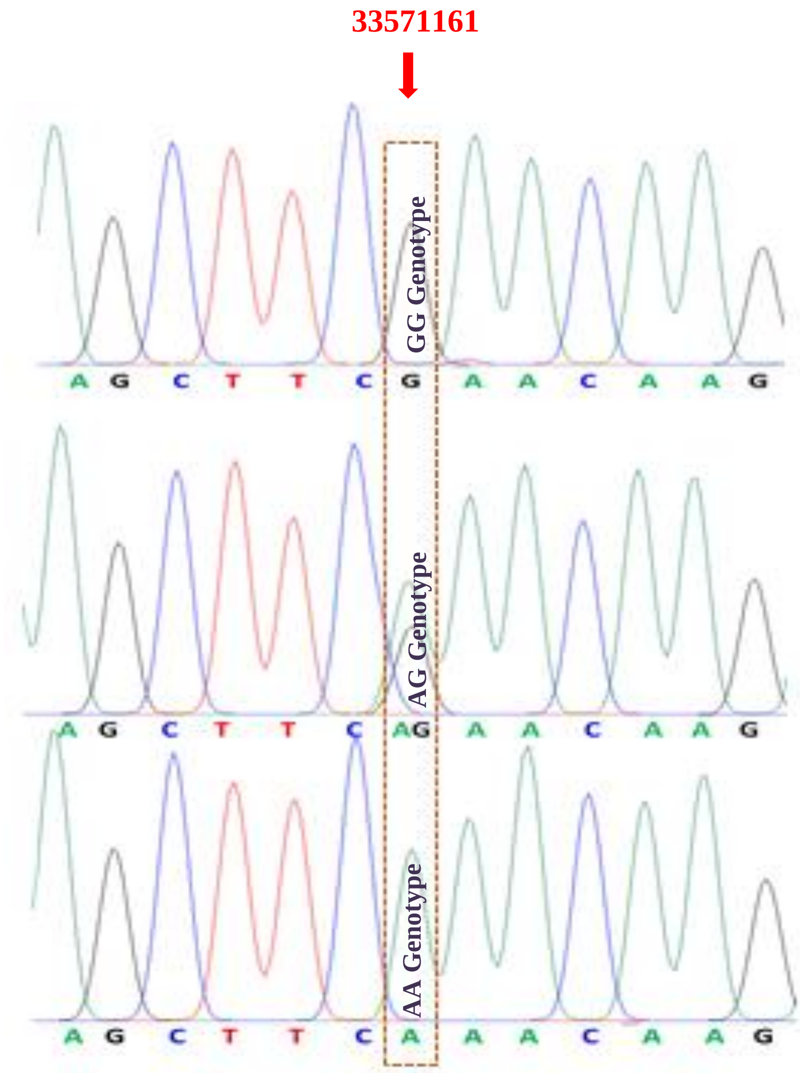


Figure 4.15 Chromatograph showing the SNP locus [G33571161A] in FAM19A1 gene in Sahiwal cows



Reference A G C T T C G A A C A A G

33571161

Figure 4.16 Chromatograph showing the SNP locus [G33571161A] in FAM19A1 gene in Sahiwal cows

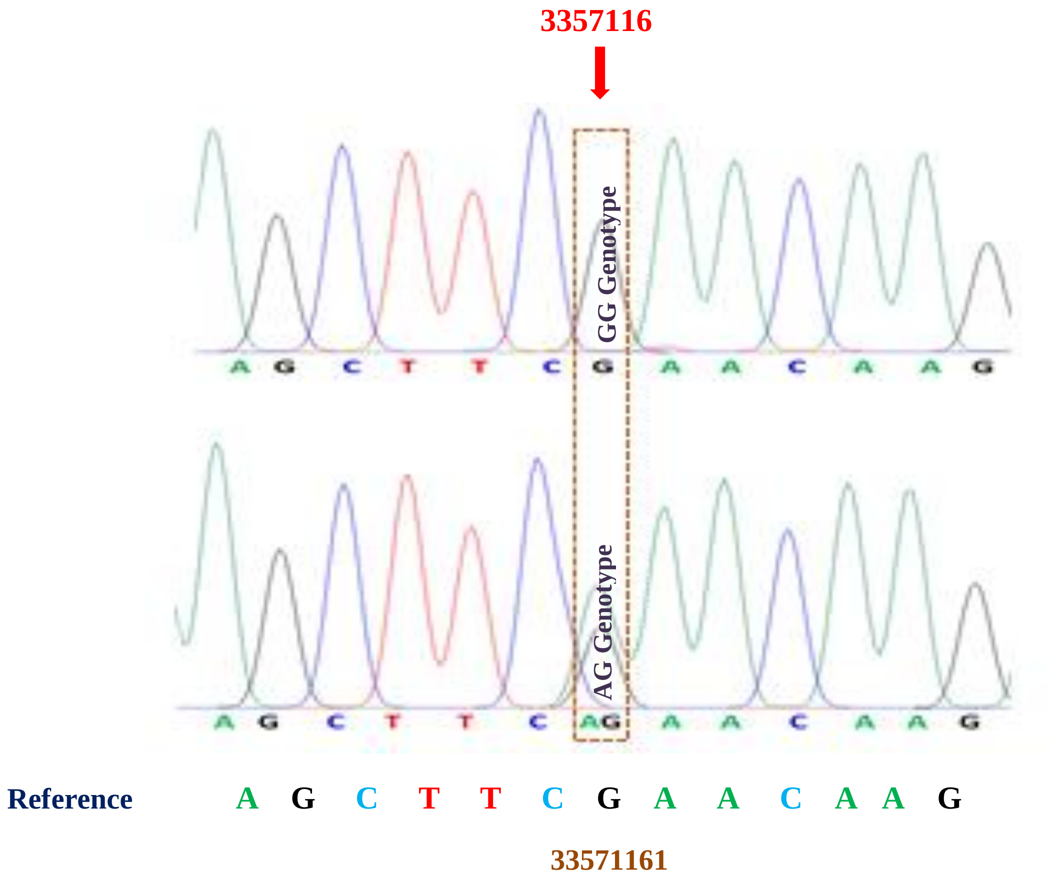


Figure 4.17 Chromatograph showing the SNP locus [G33589765A] in FAM19A1 gene in Karan Fries cows

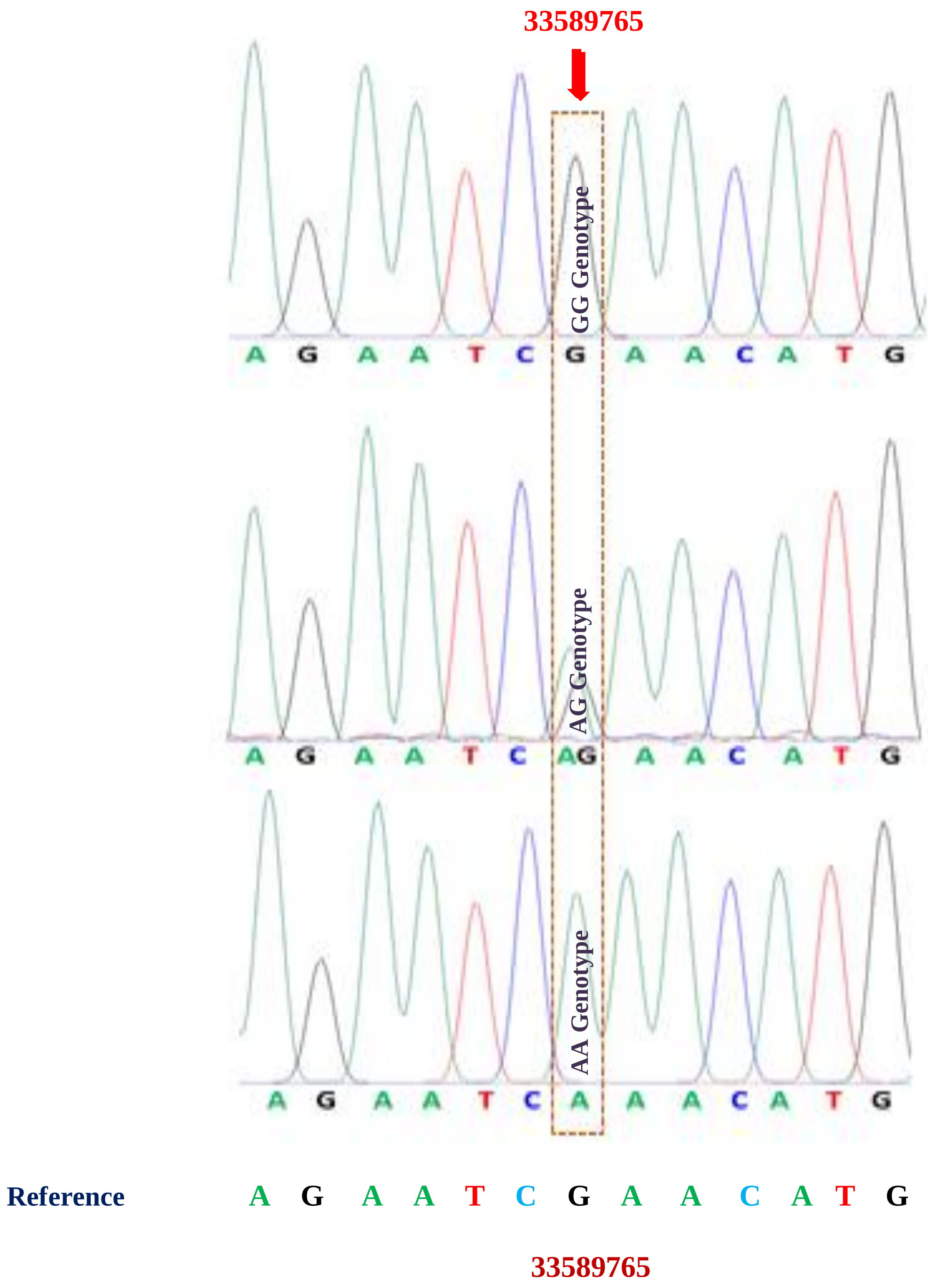
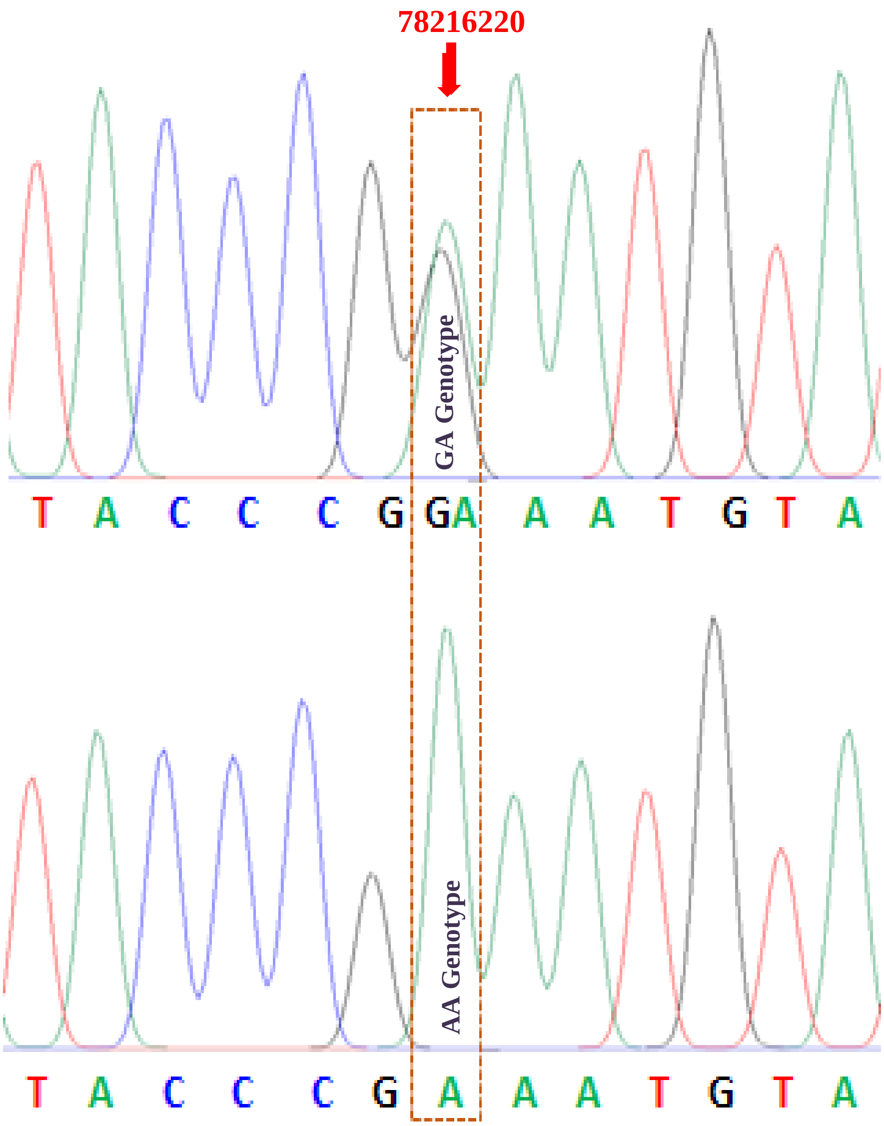


Figure 4.18 Chromatograph showing the SNP locus [G78216220A] in KCNB1gene in Sahiwal and Karan Fries cows



Reference T A C C C G A A A T G T A

78216220

Figure 4.19 Chromatograph showing the SNP locus [A78216335G] in KCNB1gene in Sahiwal and Karan Fries cows

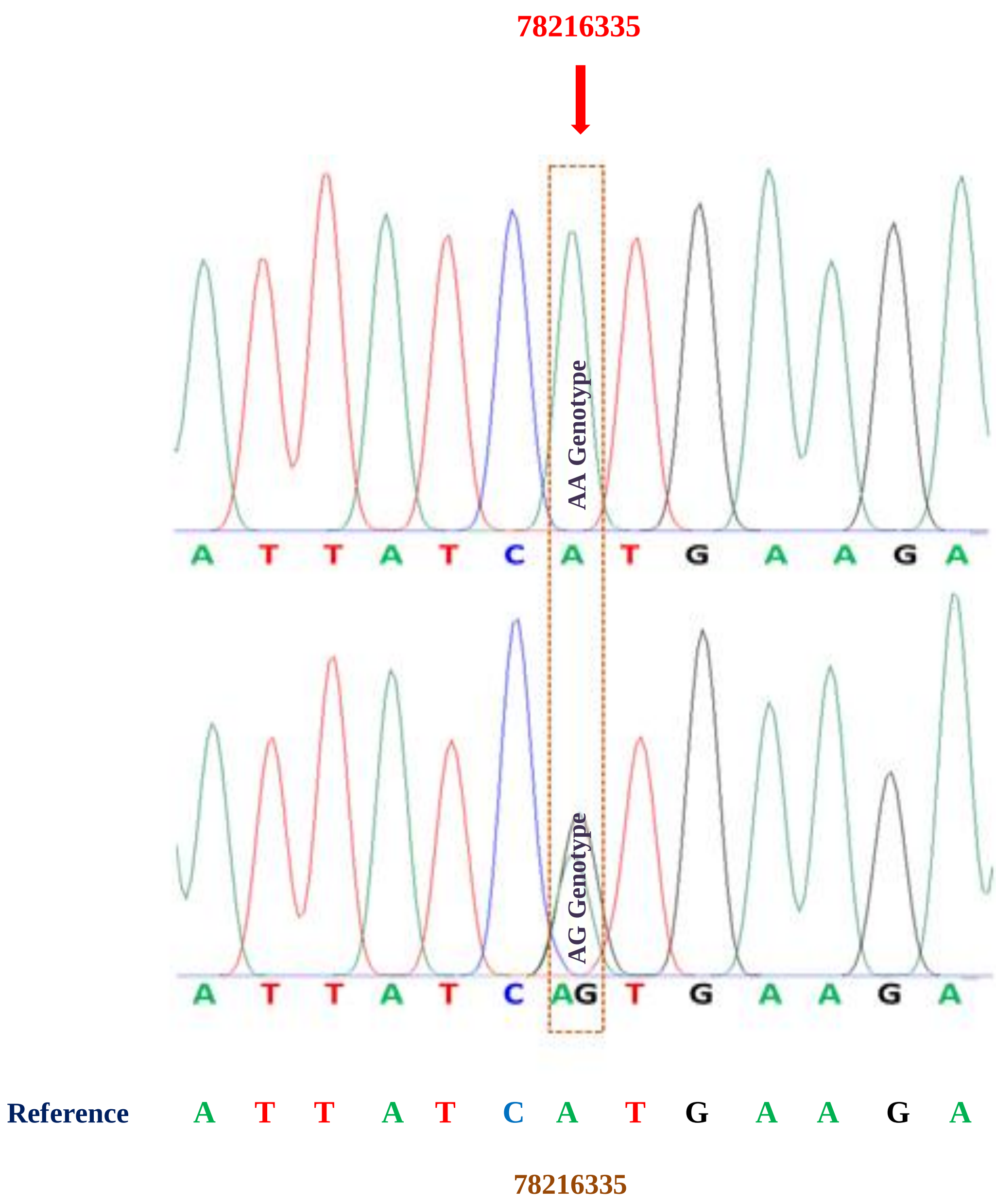
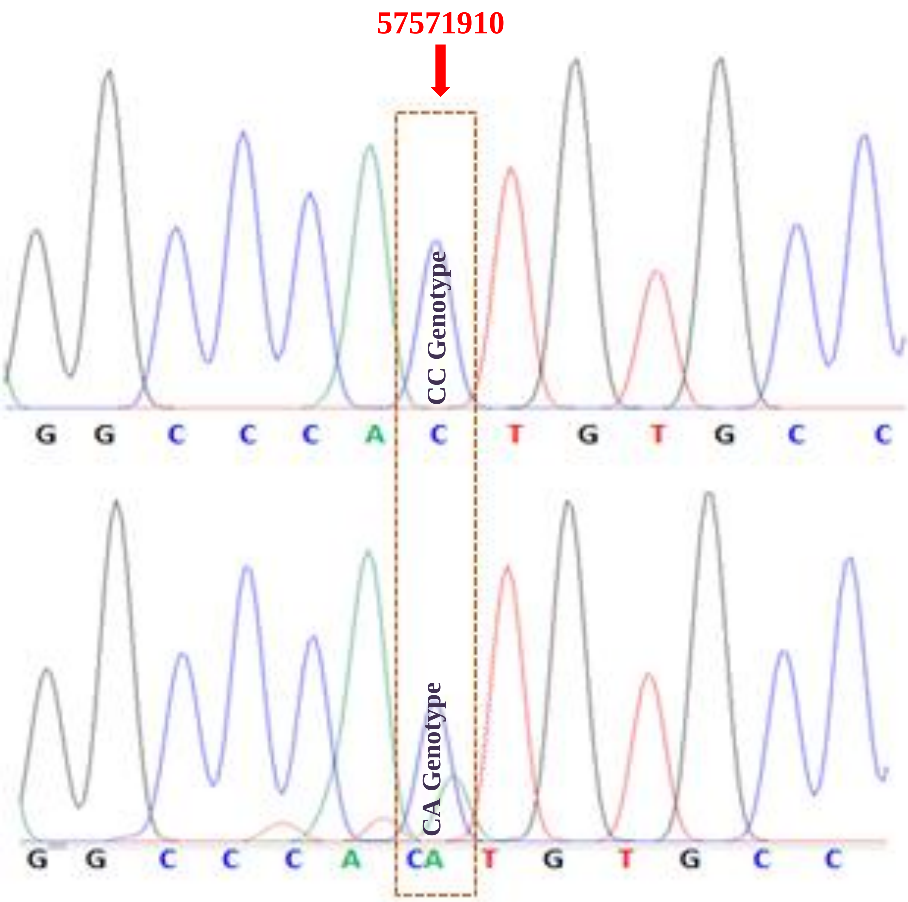


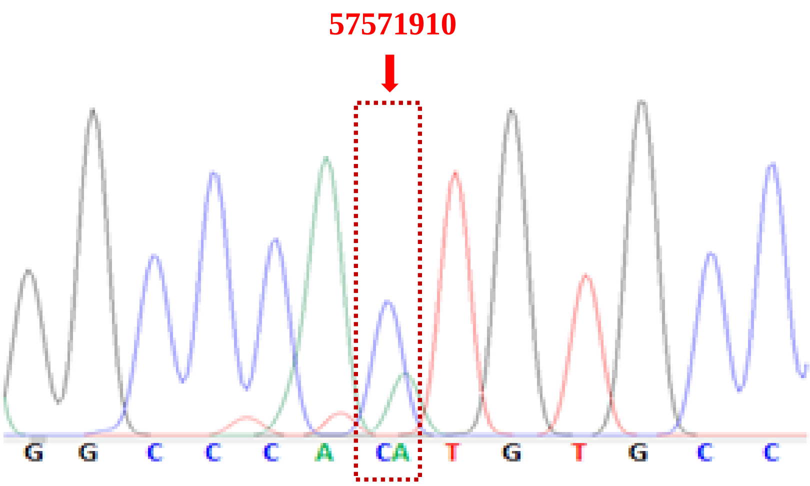
Figure 4.20 Chromatograph showing the SNP locus [C57571910A] in EDN3 gene in Sahiwal cows



Reference G G C C C A C T G T G C C

57571910

Figure 4.21 Chromatograph showing the SNP locus [C57571910A] in EDN3 gene in Karan Fries cows



Reference G G C C C A C T G T G C C

57571910

Sahiwal and Karan Fries cattle in Plate 4.29 and 4.30, respectively and their chromatograph showing nucleotide change is shown in Figure 4.14.

SNP at G33571161A locus in intron 3 (529 bp PCR product) of FAM19A1 gene was digested with *TaqI* enzyme. This showed three genotypes i.e. GG (329 and 200 bp), GA (529, 329 and 200 bp) and AA (529 bp) in Sahiwal cattle whereas, only two genotypes i.e. GG (329 and 200 bp) and GA (529, 329 and 200 bp) in Karan Fries cattle. These are shown in Plate 4.31 and 4.32 for Sahiwal and Karan Fries cows, respectively. The chromatograph showing nucleotide change is shown in Figure 4.15 and 4.16 for Sahiwal and Karan Fries cows, respectively.

PCR product of 368 bp harbouring SNP at locus G33589765A in 3'-UTR of FAM19A1 gene. This product was digested with *TaqI* enzyme and revealed three genotypes with three distinct band pattern in both Sahiwal and Karan Fries cattle. The different genotypes were GG (209 and 159 bp), GA (368, 209 and 159 bp) and AA (368 bp) which are depicted in Plate 4.33 and 4.34 for Sahiwal and Karan Fries cows, respectively and the chromatograph represented in Figure 4.17.

For PCR product of 505 bp present in intron 1 of KCNB1 gene, two SNPs were found. First SNP at locus G78216220A was digested with *MspI* enzyme and showed three possible genotype with three distinct band patterns after digestion in both Sahiwal and Karan Fries cattle. The three genotypes were GG (280 and 225 bp), GA (505, 280 and 225 bp) and AA (505 bp). These are shown in Plate 4.35 and 4.36 for Sahiwal and Karan Fries cows, respectively with their chromatograph in Figure 4.18.

Other SNP at locus A78216335G of 505 bp product was found in KCNB1 gene. This was digested with *BspHI* enzyme. After digestion two genotypes with two different patterns were generated for both breeds. The two genotypes were AA (396 and 109 bp) and AG (505, 396 and 109 bp). Plate 4.37 and 4.38 shows the banding pattern after digestion with enzyme for Sahiwal and Karan Fries cows, respectively with their chromatograph in Figure 4.19.

PCR product of 577 bp harbouring SNP at locus C57571910A in exon 1 of EDN3 gene in Sahiwal cattle. This PCR product was digested with enzyme *TspRI* and two genotypes were found in Sahiwal cattle i.e. CC (424 and 153 bp) and CA (577, 424 and 153 bp) (Plate 4.39 and Figure 4.20). Whereas, in Karan Fries cattle only monomorphic pattern were found. All the bands for genotype CA (577, 424 and 153 bp) was depicted in Plate 4.40 and Figure 4.21.

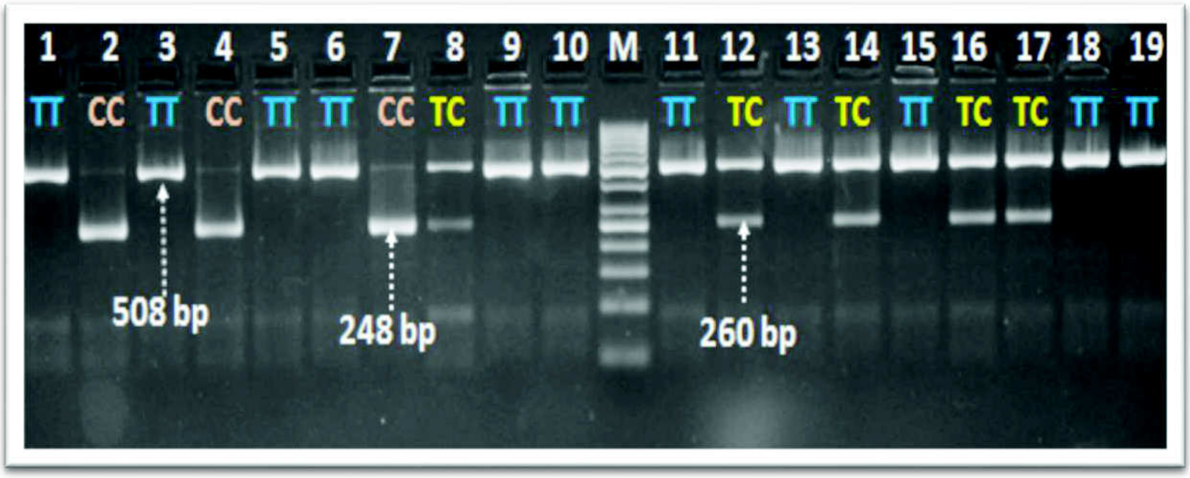
Table 4.3 Nucleotide changes in FAM19A1, KCNB1 and EDN3 gene in Sahiwal and Karan Fries cows as compared to *Bos taurus* Ref Seq

Gene	Position of Nucleotide	Location	Nucleotide changes		
			Sahiwal	Karan Fries	*Bos taurus* Ref Seq
FAM19A1	33069832	Intron 1	T/C	T/C	T
	33219027	Intron 1	C/T	C/T	C
	33464963	Intron 2	C/A	C/A	C
	33471720	Intron 2	C/T	C/T	C
	33471980	Intron 2	C/A	C/A	C
	33571161	Intron 3	G/A	G/A	G
	33589765	3' UTR	G/A	G/A	G
KCNB1	78216220	Intron 1	G/A	G/A	G
	78216335	Intron 1	A/G	A/G	A
EDN3	57571910	Exon 1	C/A	CA	C

4.5 Allelic and genotypic frequencies of FAM19A1, KCNB1 and EDN3 gene in Sahiwal and Karan Fries cows

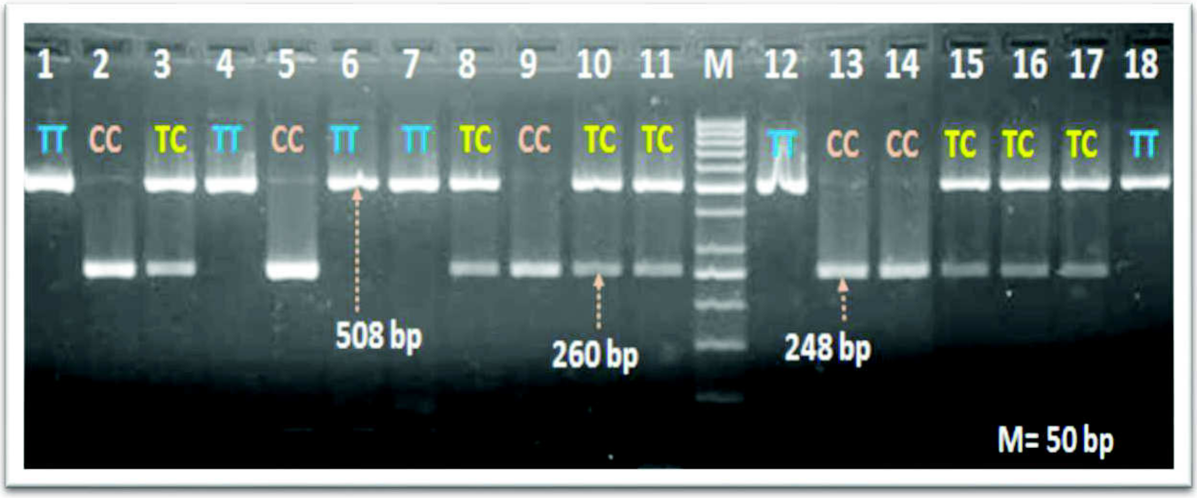
The allelic and genotypic frequencies of FAM19A1, KCNB1 and EDN3 gene were calculated using POPGENE software package (Yeh *et al*., 1999). The genotypic and allelic frequency of all the identified SNPs after digesting with respective restriction enzymes through PCR-RFLP protocol are presented in Table 4.4 and 4.5. Gene frequency values were used to calculate the PIC value. The PIC ranged from 0.1638 to 0.4550 for FAM19A1 gene, 0.3750 to 0.4800 for KCNB1 gene and 0.4200 for EDN3 gene in Sahiwal cows. In Karan Fries cattle PIC ranged from 0.1472 to 0.4608 in FAM19A1 gene, 0.4200 to 0.4662 in KCNB1 gene and 0.5000 in EDN3 gene. These value indicate that low to intermediate type

Plate 4.21 PCR-RFLP of SNP locus [T33069832C] in FAM19A1 gene in Sahiwal cows using *BsrI* restriction enzyme



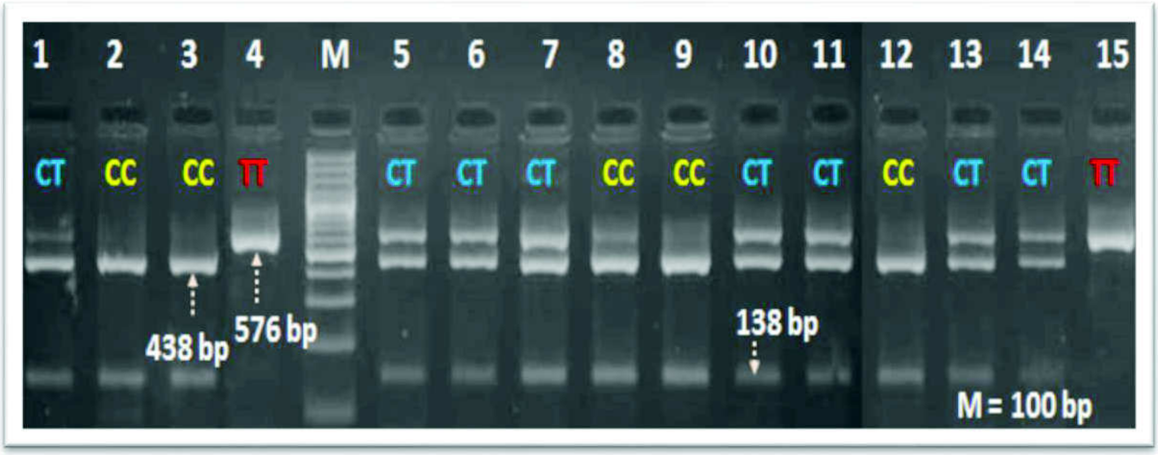
Lane 1, 3, 5, 6, 9, 10, 11, 13, 15, 18, 19 : TT Genotype 508 bp
Lane 8, 12, 14, 16, 17 : TC Genotype 508, 260, 248 bp
Lane 2, 4, 7 : CC Genotype 260, 248 bp
Lane M : 50 bp Marker

Plate 4.22 PCR-RFLP of SNP locus [T33069832C] in FAM19A1 gene in Karan Fries cows using *BsrI* restriction enzyme



Lane 1, 4, 6, 7, 12, 18 : TT Genotype 508 bp
Lane 3, 8, 10, 11, 15, 16, 17 : TC Genotype 508, 260, 248 bp
Lane 2, 5, 9, 13, 14 : CC Genotype 260, 248 bp
Lane M : 50 bp Marker

Plate 4.23 PCR-RFLP of SNP locus [C33219027T] in FAM19A1 gene in Sahiwal cows using *TaqI* restriction enzyme

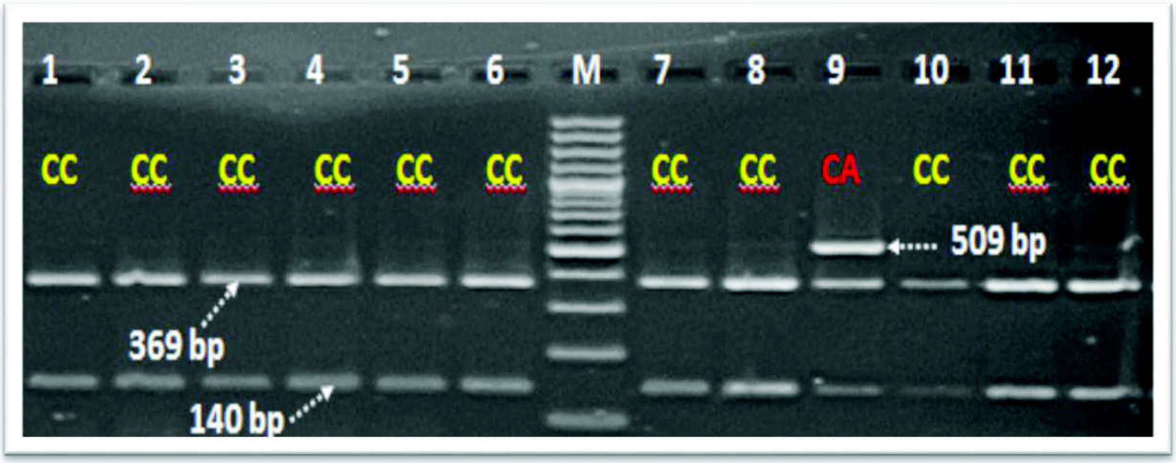


Lane 2, 3, 8, 9, 12	: CC Genotype 438, 138 bp
Lane 1, 5, 6, 7, 10, 11, 13, 14	: CT Genotype 576, 438, 138 bp
Lane 4, 15	: TT Genotype 576 bp
Lane M	: 100 bp Marker

Plate 4.24 PCR-RFLP of SNP locus [C33219027T] in FAM19A1 gene in Karan Fries cows using *TaqI* restriction enzyme

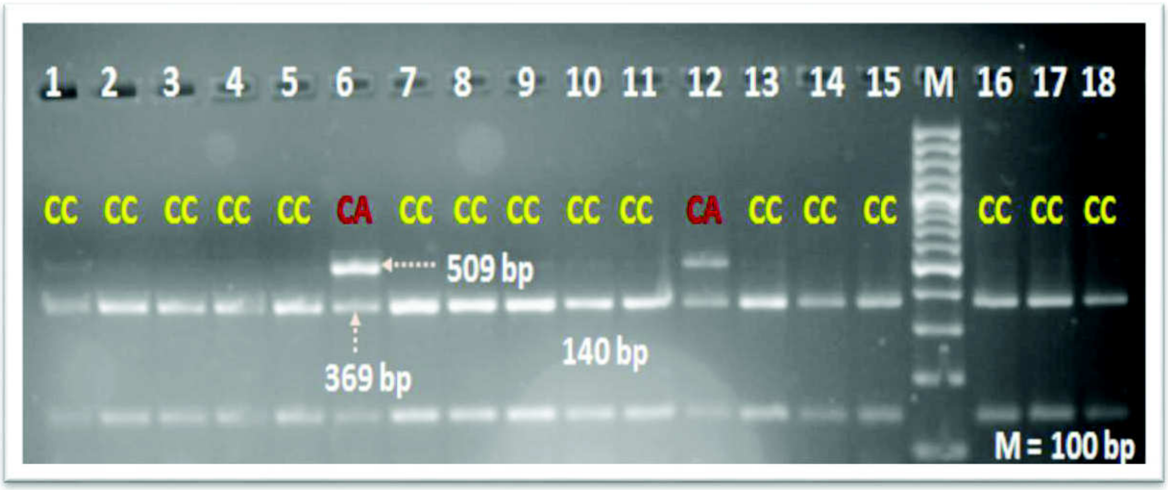
Lane 2, 3, 4, 12	: CC Genotype 438, 138 bp
Lane 1, 5, 6, 8, 9, 10, 11, 13, 14	: CT Genotype 576, 438, 138 bp
Lane 7	: TT Genotype 576 bp
Lane M	: 100 bp Marker

Plate 4.25 PCR-RFLP of SNP locus [C33464963A] in FAM19A1 gene in Sahiwal cows using *AluI* restriction enzyme



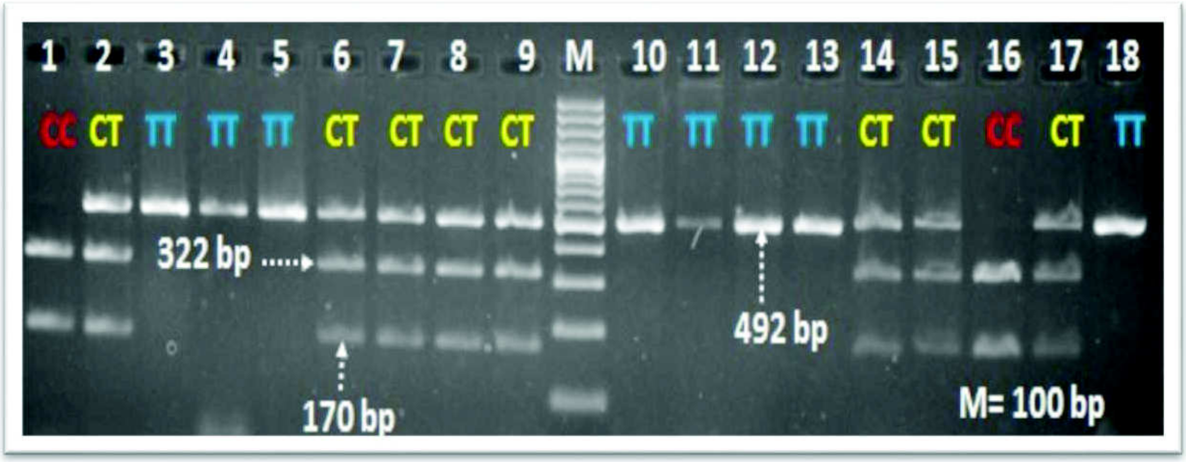
Lane 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12	**: CC Genotype 369, 140 bp**
Lane 9	**: CA Genotype 509, 369, 140 bp**
Lane M	**: 100 bp Marker**

Plate 4.26 PCR-RFLP of SNP locus [C33464963A] in FAM19A1 gene in Karan Fries cows using *AluI* restriction enzyme



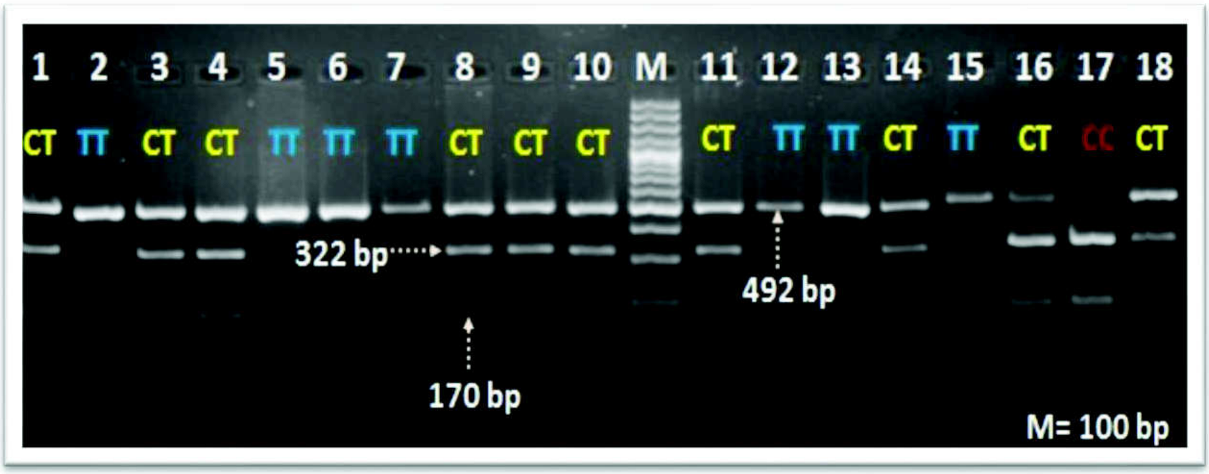
Lane 1, 2, 3, 4, 5, 7, 8, 10, 11, 13, 14, 15, 16, 17, 18	**: CC Genotype 369, 140 bp**
Lane 6, 12	**: CA Genotype 509, 369, 140 bp**
Lane M	**: 100 bp Marker**

Plate 4.27 PCR-RFLP of SNP locus [C33471720T] in FAM19A1 gene in Sahiwal cows using *HinfI* restriction enzyme



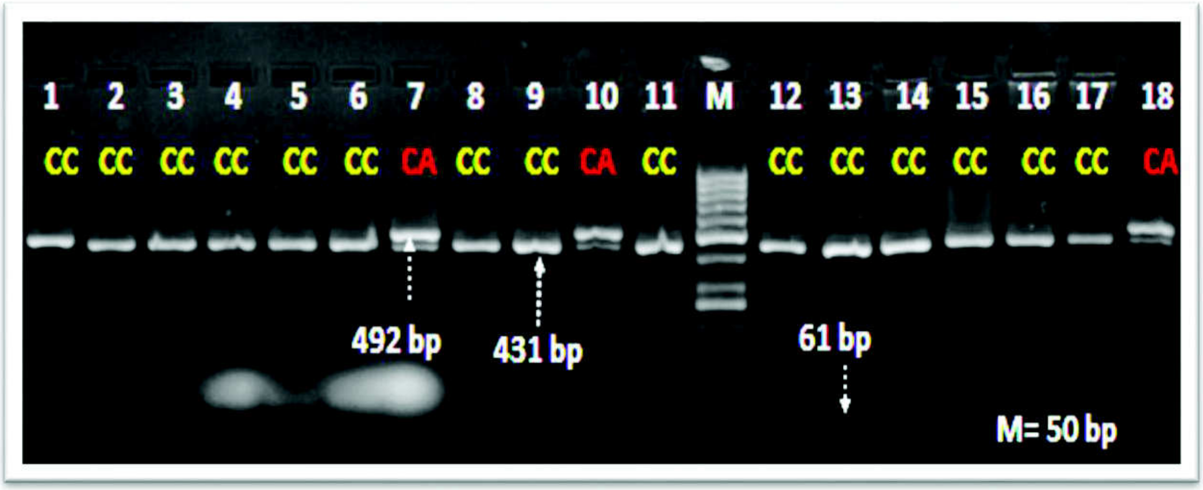
Lane 1, 16	: CC Genotype 322, 170 bp
Lane 2, 6, 7, 8, 9, 14, 15, 17	: CT Genotype 492, 322, 170 bp
Lane 3, 4, 5, 10, 11, 12, 13, 18	: TT Genotype 492 bp
Lane M	: 100 bp Marker

Plate 4.28 PCR-RFLP of SNP locus [C33471720T] in FAM19A1 gene in Karan Fries cows using *HinfI* restriction enzyme



Lane 17	: CC Genotype 322, 170 bp
Lane 1, 3, 4, 8, 9, 10, 11, 14, 16, 18	: CT Genotype 492, 322, 170 bp
Lane 2, 5, 6, 7, 12, 13, 15	: TT Genotype 492 bp
Lane M	: 100 bp Marker

Plate 4.29 PCR-RFLP of SNP locus [C33471980A] in FAM19A1 gene in Sahiwal cows using *HindIII* restriction enzyme



Lane 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 17 : CC Genotype 431, 61 bp
Lane 7, 10, 18 : CA Genotype 492, 431, 61 bp
Lane M : 50 bp Marker

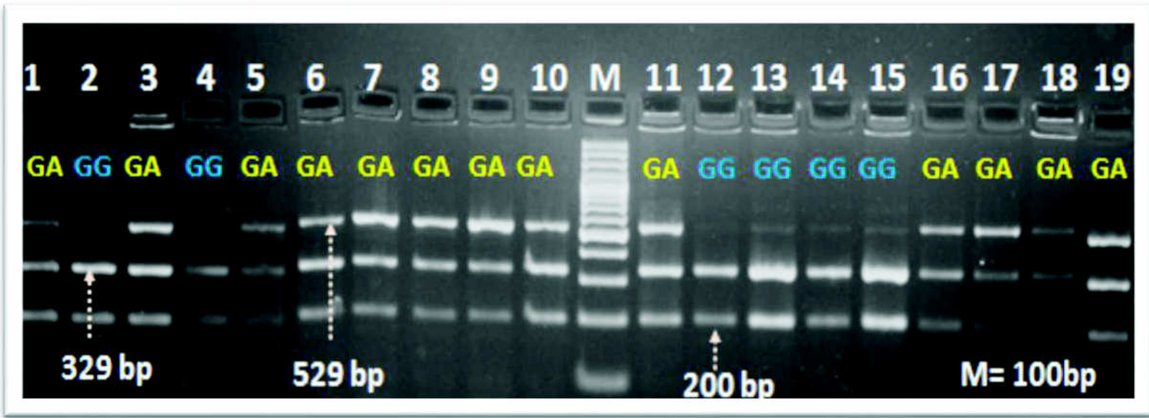
Plate 4.30 PCR-RFLP of SNP locus [C33471980A] in FAM19A1 gene in Karan Fries cows using *HindIII* restriction enzyme

Lane 1, 2, 3, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 : CC Genotype 431, 61 bp
Lane 4, 7, 16 : CA Genotype 492, 431, 61 bp
Lane M : 50 bp Marker

Plate 4.31 PCR-RFLP of SNP locus [G33571161A] in FAM19A1 gene in Sahiwal cows using *TaqI* restriction enzyme

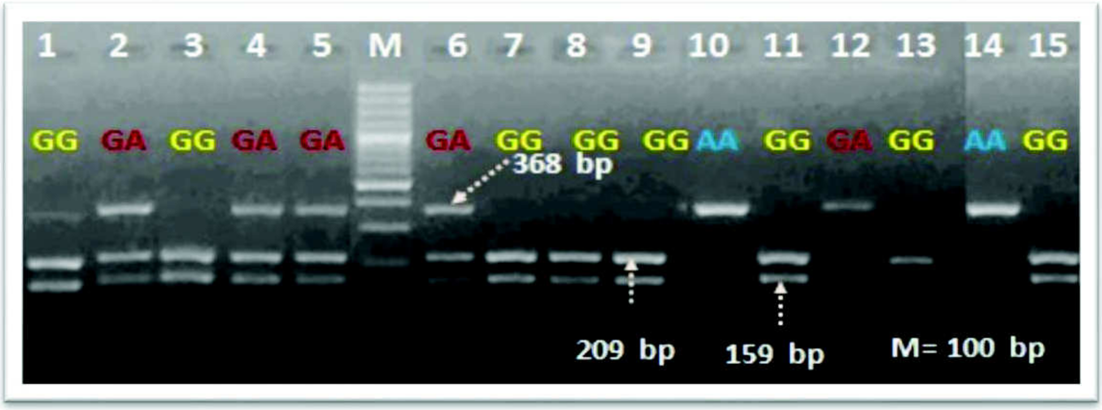
Lane 3, 11, 13	: GG Genotype 329, 200 bp
Lane 1, 2, 4, 5, 6, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19	: GA Genotype 529, 329, 200 bp
Lane 7	: AA Genotype 529 bp
Lane M	: 100 bp Marker

Plate 4.32 PCR-RFLP of SNP locus [G33571161A] in FAM19A1 gene in Karan Fries cows using *TaqI* restriction enzyme



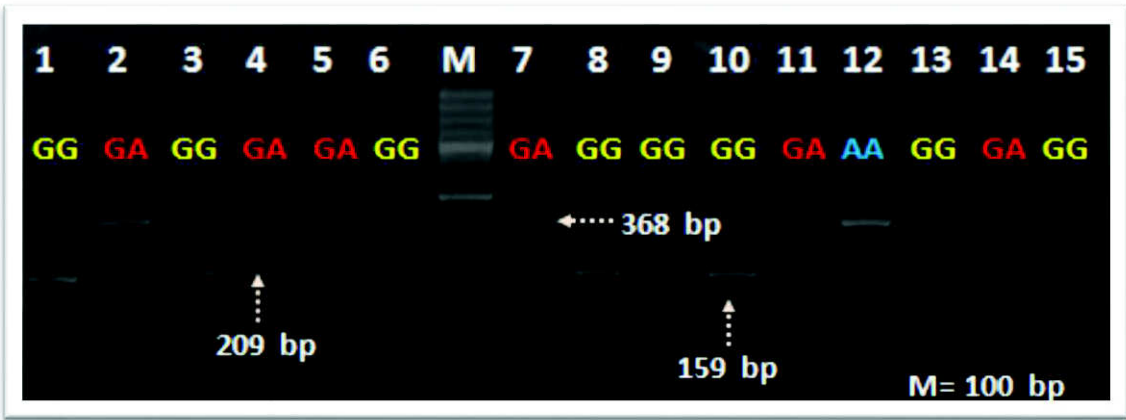
Lane 2, 4, 12, 13, 14, 15	: GG Genotype 329, 200 bp
Lane 1, 3, 5, 6, 7, 8, 9, 10, 11, 16, 17, 18, 19	: GA Genotype 529, 329, 200 bp
Lane M	: 100 bp Marker

Plate 4.33 PCR-RFLP of SNP locus [G33589765A] in FAM19A1 gene in Sahiwal cows using *TaqI* restriction enzyme



Lane 1, 3, 7, 8, 9, 11, 13, 15	: GG Genotype 209, 159 bp
Lane 2, 4, 5, 6, 12	: GA Genotype 368, 209, 159 bp
Lane 10, 14	: AA Genotype 368 bp
Lane M	: 100 bp Marker

Plate 4.34 PCR-RFLP of SNP locus [G33589765A] in FAM19A1 gene in Karan Fries cows using *TaqI* restriction enzyme

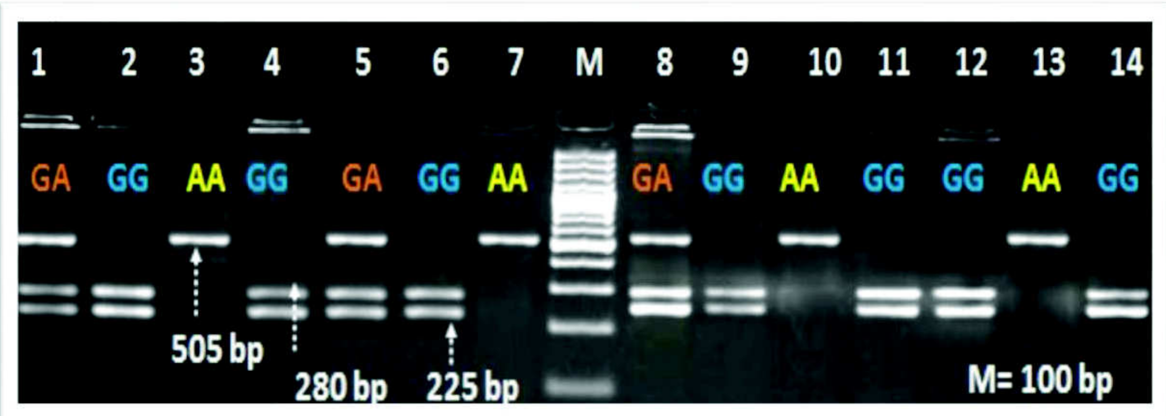


Lane 1, 3, 6, 8, 9, 10, 13, 15	: GG Genotype 209, 159 bp
Lane 2, 4, 5, 7, 11, 14	: GA Genotype 368, 209, 159 bp
Lane 12	: AA Genotype 368 bp
Lane M	: 100 bp Marker

Plate 4.35 **PCR-RFLP of SNP locus [G78216220A] in KCNB1gene in Sahiwal cows using *MspI* restriction enzyme**

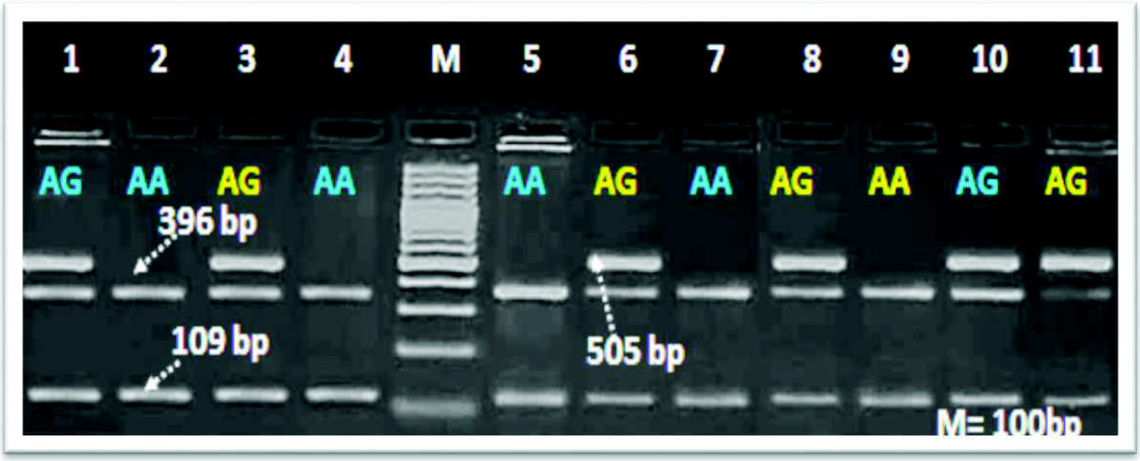
Lane 1, 3, 4, 6, 7, 10, 12, 13, 15	**: GG Genotype 280, 225 bp**
Lane 9	**: GA Genotype 505, 280, 225 bp**
Lane 2, 5, 8, 11, 14	**: AA Genotype 505 bp**
Lane M	**: 100 bp Marker**

Plate 4.36 **PCR-RFLP of SNP locus [G78216220A] in KCNB1gene in Karan Fries cows using *MspI* restriction enzyme**



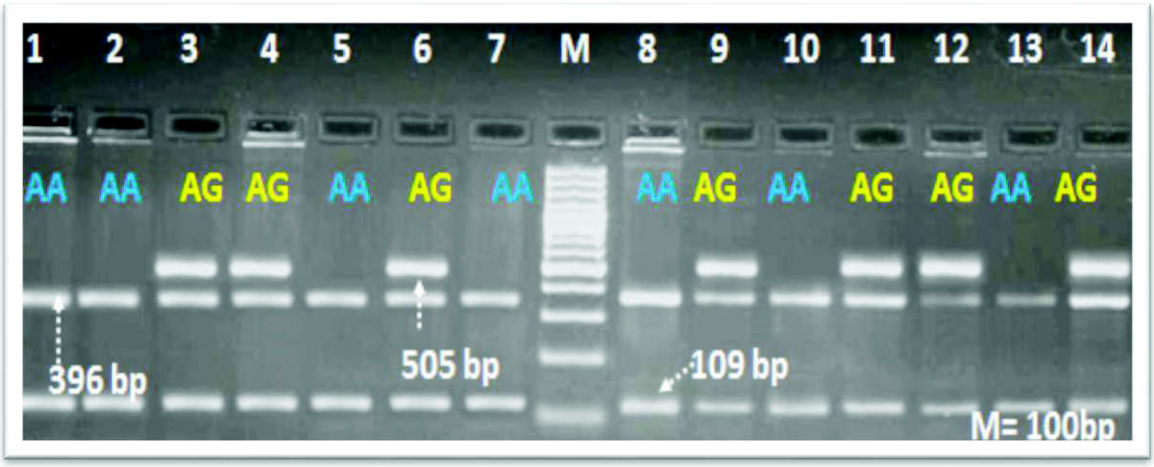
Lane 2, 4, 6, 9, 11, 12, 14	**: GG Genotype 280, 225 bp**
Lane 1, 5, 8	**: GA Genotype 505, 280, 225 bp**
Lane 3, 7, 10, 13	**: AA Genotype 505 bp**
Lane M	**: 100 bp Marker**

Plate 4.37 PCR-RFLP of SNP locus [A78216335G] in KCNB1gene in Sahiwal cows using *BspHI* restriction enzyme



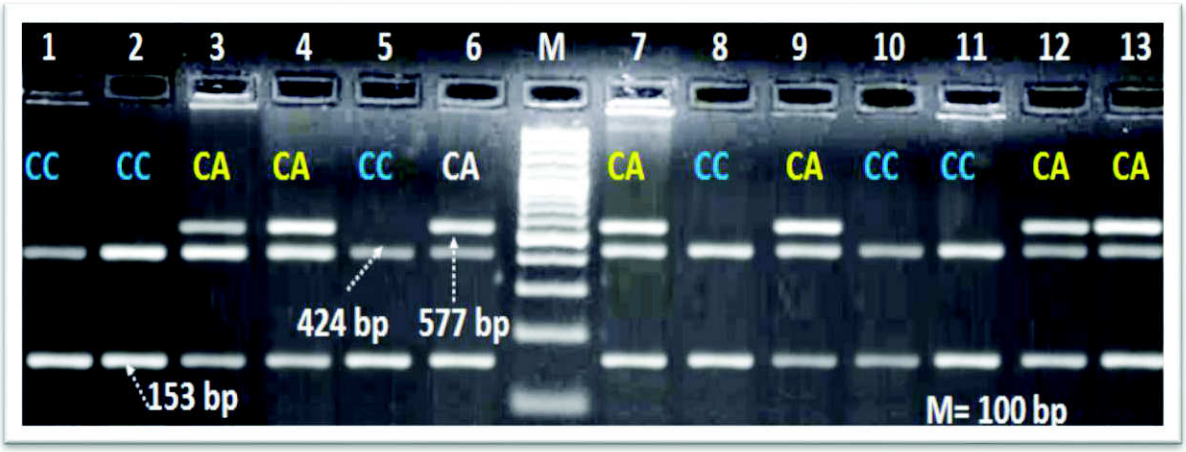
Lane 1, 2, 4, 5, 7, 10 : AA Genotype 396, 109 bp
Lane 3, 6, 8, 9, 11 : AG Genotype 505, 396, 109 bp
Lane M : 100 bp Marker

Plate 4.38 PCR-RFLP of SNP locus [A78216335G] in KCNB1gene in Karan Fries cows using *BspHI* restriction enzyme



Lane 1, 2, 5, 7, 8, 10, 13 : AA Genotype 396, 109 bp
Lane 3, 4, 6, 9, 11, 12, 14 : AG Genotype 505, 396, 109 bp
Lane M : 100 bp Marker

Plate 4.39 PCR-RFLP of SNP locus [C57571910A] in EDN3gene in Sahiwal cows using *TspRI* restriction enzyme



Lane 1, 2, 5, 8, 10, 11	: CC Genotype 424, 153 bp
Lane 3, 4, 6, 7, 9, 12, 13	: CA Genotype 577, 424, 153 bp
Lane M	: 100 bp Marker

Plate 4.40 PCR-RFLP of SNP locus [C57571910A] in EDN3gene in Karan Fries cows using *TspRI* restriction enzyme

Lane 1-18	: CA Genotype 577, 424, 153 bp
Lane M	: 100 bp Marker

of polymorphism were observed in FAM19A1 KCNB1 and EDN3 gene in both the breeds. Effective allele number (ne) and Shannon Index (I) were also calculated and the details are given in the Table 4.6.

Table 4.4 Genotypic frequency at each SNP locus of FAM19A1, KCNB1 and EDN3 Gene in Sahiwal and Karan Fries cows

Gene	SNPs	Genotype	Breed	
			Sahiwal	Karan Fries
FAM19A1	T33069832C	TT	0.15	0.60
		TC	0.28	0.21
		CC	0.57	0.19
	C33219027T	CC	0.47	0.53
		CT	0.35	0.25
		TT	0.18	0.22
	C33464963A	CC	0.62	0.84
		CA	0.38	0.16
	C33471720T	CC	0.15	0.17
		CT	0.34	0.37
		TT	0.51	0.46
	C33471980A	CC	0.82	0.67
		CA	0.18	0.33
	G33571161A	GG	0.11	0.74
		GA	0.65	0.26
		AA	0.24	-
	G33589765A	GG	0.57	0.63
		GA	0.29	0.15
		AA	0.14	0.22
KCNB1	G78216220A	GG	0.23	0.14
		GA	0.33	0.46
		AA	0.44	0.40
	A78216335G	AA	0.51	0.39
		AG	0.49	0.61
EDN3	C57571910A	CC	0.41	-
		CA	0.59	1.00

Table 4.5 Allelic frequency at each SNP locus of FAM19A1, KCNB1 and EDN3 gene in Sahiwal and Karan Fries cows

Gene	SNPs	Allele	Breed	
			Sahiwal	Karan Fries
FAM19A1	T33069832C	T	0.29	0.70
		C	0.71	0.30
	C33219027T	C	0.65	0.65
		T	0.35	0.35
	C33464963A	C	0.81	0.92
		A	0.19	0.08
	C33471720T	C	0.32	0.36
		T	0.68	0.64
	C33471980A	C	0.91	0.84
		A	0.09	0.16
	G33571161A	G	0.44	0.87
		A	0.56	0.13
	G33589765A	G	0.72	0.71
		A	0.28	0.29
KCNB1	G78216220A	G	0.40	0.37
		A	0.60	0.63
	A78216335G	A	0.75	0.70
		G	0.25	0.30
EDN3	C57571910A	C	0.70	0.50
		A	0.30	0.50

Table 4.6 Population genetics analysis of different polymorphic loci of FAM19A1, KCNB1 and EDN3 gene in Sahiwal and Karan Fries cows

Gene	Variation	Sahiwal			Karan Fries		
		ne	I	PIC	ne	I	PIC
FAM19A1	T33069832C	1.693	0.599	0.411	1.716	0.608	0.420
	C33219027T	1.926	0.673	0.455	1.825	0.644	0.455
	C33464963A	1.774	0.628	0.307	1.176	0.283	0.147
	C33471720T	1.774	0.628	0.435	1.837	0.648	0.460
	C33471980A	1.200	0.307	0.163	1.369	0.440	0.268
	G33571161A	1.968	0.685	0.492	1.293	0.387	0.226
	G33589765A	1.679	0.594	0.403	1.724	0.610	0.411
KCNB1	G78216220A	1.955	0.681	0.480	1.883	0.661	0.466
	A78216335G	1.600	0.526	0.375	1.731	0.613	0.420
EDN3	C57571910A	1.896	0.581	0.420	-	-	0.500

4.6 Estimates of udder and teat measurements

The mean and standard error of different udder and teat type traits *viz.*, fore udder attachment (FUA), rear udder width (RUW), rear udder height (RUH), udder balance (UB), udder depth (UD), udder length (UL), udder width (UW), udder circumference (UC), central ligament (CL), teat circumference (TC), fore teat length (FTL), rear teat length (RTL), distance between fore and rear teat (DFR), distance between left and right teat (DLR), shortest distance of floor from fore teat (SDF), shortest distance of floor from rear teat (SDR) and teat diameter (TD) in Sahiwal and Karan Fries cows were measured and presented in Table 4.7. The mean of FUA, RUW, RUH, UB, UD, UL, UW, UC, CL, TC, FTL, RTL, DFR, DLR, SDF, SDR and TD in Sahiwal cows were 107±2.10 cm, 8.99±0.32 cm, 22.69±0.99 cm, -1.83±0.48 cm, 19.43±0.88 cm, 51.30±0.94 cm, 63.45±1.20 cm, 142.64±1.80 cm, 3.40±0.19 cm, 8.64±0.88 cm, 7.14±1.00 cm, 6.15±1.12 cm, 4.59±0.81 cm, 5.81±0.88 cm, 42.46±1.00 cm, 41.28±1.04 cm and 2.71±0.08 cm, respectively. The mean of FUA, RUW, RUH, UB, UD, UL, UW, UC, CL, TC, FTL, RTL, DFR, DLR, SDF, SDR and TD in Karan Fries cows were 131±2.02 cm, 12.87±0.24 cm, 23.67±0.49 cm, 0.75±0.53 cm, 21.22±0.56 cm, 60.34±0.68 cm, 71.47±0.83 cm, 142.58±2.28 cm, 3.18±0.13 cm, 7.50±0.14 cm, 5.33±0.14 cm, 4.62±0.10 cm, 6.56±0.36 cm, 8.86±0.44 cm, 46.99±0.52 cm, 47.00±0.51cm and 2.22±0.03 cm, respectively.

4.7 Frequency of different udder and teat type traits under 9 score points in Sahiwal and Karan Fries cows

Frequency of different udder and teat type traits under 9 score points were assessed through ICAR recommendations and presented in Table 4.8 and 4.9 in Sahiwal and Karan Fries cows, respectively.

4.7.1 Fore udder attachment

Fore udder attachment was assessed as an angle between the abdominal wall and front part of udder. In the present study, FUA was categorized into three group *viz.*, loose, intermediate and strong groups according to 1 to 9 point scale scoring system of ICAR (2012). Generally strong attachment is preferred better than others which indicate better milk production. The maximum frequency for this trait in both Sahiwal and Karan Fries was observed in intermediate group followed by strong and loose groups. The frequencies in percentage for all the groups *viz.*, loose, intermediate and strong were 2.30, 55.17 and 42.53, respectively in Sahiwal cattle whereas, 14.46, 69.28 and 16.26, respectively for Karan Fries cattle. The average value for the trait in Sahiwal and Karan Fries cows were found to be 107±2.10 cm and 131±2.02 cm, respectively. Dubey (2010) found higher measurements for

FUA (127.38±1.56 cm) in Sahiwal cattle as compared to present finding in Sahiwal. They also reported the same trend in respect of frequencies with intermediate having the highest frequency.

Table 4.7 Estimates of different udder and teat type traits (Mean±SE) in Sahiwal and Karan Fries cows

Type of traits	Sahiwal		Karan Fries	
	Mean±SE	Range	Mean±SE	Range
Fore udder attachment	107±2.10	33-150	131±2.02	82-174
Rear udder width (cm)	8.99±0.32	4.82-15.25	12.87±0.24	5.59 - 20.32
Rear udder height (cm)	22.69±0.99	8.89-36.87	23.67±0.49	7.62 - 41.91
Udder Balance (cm)	-1.83±0.48	-11.43-5.12	0.75±0.53	-12.95-23.11
Udder Depth (cm)	19.43±0.88	9.69-23.61	21.22±0.56	14.24-29.00
Udder Length (cm)	51.30±0.94	39.37-68.61	60.34±0.68	40.64- 93.98
Udder Width (cm)	63.45±1.20	48.76-85.65	71.47±0.83	36.07- 99.06
Udder Circumference(cm)	142.64±1.8	110.24-168.91	142.58±2.28	74.16-225.29
Central ligament (cm)	3.40±0.19	0.0-6.35	3.18±0.13	0.0-8.12
Teat circumference(cm)	8.64±0.88	6.32-14.78	7.50±0.14	5.54-11.68
Fore teat length (cm)	7.14±1.00	3.17-11.68	5.33±0.14	2.03-11.68
Rear teat length (cm)	6.15±1.12	2.79-11.04	4.62±0.10	2.41-7.49
Distance between fore and rear teat (cm)	4.59±0.81	1.39-11.38	6.56±0.36	1.78-13.97
Distance between left and right teat (cm)	5.81±0.88	2.29-15.42	8.86±0.44	0.88-20.32
Shortest distance of floor from front teat (cm)	42.46±1.00	28.44-55.88	46.99±0.52	35.43-64.00
Shortest distance of floor from rear teat (cm)	41.28±1.04	25.40-53.34	47.00±0.51	27.56-62.48
Teat Diameter (cm)	2.71±0.08	1.80-4.49	2.22±0.03	1.50-3.71

Table 4.8 Frequencies of different udder and teat type traits under 9 point score of Sahiwal cows (*n*=87)

Type of Traits	Score group (1-9 score points)		
	1-3	4-6	7-9
Fore udder attachment	**Loose** 2.30 (2)	**Intermediate** 55.17 (48)	**Strong** 42.53 (37)
Rear udder width	**Narrow** 42.53 (37)	**Intermediate** 47.13 (41)	**Wide** 10.34 (9)
Rear udder height	**Low** 16.09 (14)	**Intermediate** 63.22 (55)	**High** 20.69 (18)
Udder Balance	**Deep rear udder** 11.49 (10)	**Same level** 67.82 (59)	**Deep front udder** 20.69 (18)
Udder Depth	**Deep** 65.51 (57)	**Intermediate** 28.74 (25)	**Shallow** 5.75 (5)
Udder Length	**Short** 37.93 (33)	**Intermediate** 47.13 (41)	**Long** 14.94 (13)
Udder Width	**Narrow** 41.38 (36)	**Intermediate** 43.68 (38)	**Wide** 14.94 (13)
Udder Circumference	**Small** 17.24 (15)	**Intermediate** 52.87 (46)	**Large** 29.89 (26)
Central ligament	**Weak** 9.20 (8)	**Intermediate** 56.32 (49)	**Strong** 34.48 (30)
Teat circumference	**Thin** 50.57 (44)	**Intermediate** 35.63(31)	**Thick** 13.80 (12)
Rear teat length	**Short** 52.87(46)	**Intermediate** 35.63 (31)	**Long** 11.50 (10)
Fore teat length	**Short** 55.17 (48)	**Intermediate** 37.93(33)	**Long** 6.90 (6)
Distance between fore and rear teat	**Short** 39.08 (34)	**Intermediate** 50.57 (44)	**Long** 10.35 (9)
Distance between left and right teat	**Short** 49.43 (43)	**Intermediate** 39.08 (34)	**Long** 11.49 (10)
Shortest distance of floor from front teat	**Short** 19.54 (17)	**Intermediate** 63.22 (55)	**Long** 17.24 (15)
Shortest distance of floor from rear teat	**Short** 10.34 (9)	**Intermediate** 44.83 (39)	**Long** 44.83 (39)
Teat Diameter	**Thin** 63.22 (55)	**Intermediate** 26.44 (23)	**Thick** 10.34 (9)

Table 4.9 Frequencies of different udder and teat type traits under 9 point score of Karan Fries cows (*n*=166)

Type of Traits	Score group (1-9 score points)		
	1-3	4-6	7-9
Fore udder attachment	**Loose** 14.46 (24)	**Intermediate** 69.28 (115)	**Strong** 16.26 (27)
Rear udder width	**Narrow** 22.89 (38)	**Intermediate** 62.04 (103)	**Wide** 13.25 (22)
Rear udder height	**Low** 28.92 (48)	**Intermediate** 65.06 (108)	**High** 6.02 (10)
Udder Balance	**Deep rear udder** 46.98 (78)	**Same level** 43.37 (72)	**Deep front udder** 9.63 (16)
Udder Depth	**Deep** 24.09 (40)	**Intermediate** 66.26 (110)	**Shallow** 9.63 (16)
Udder Length	**Short** 48.19 (80)	**Intermediate** 43.37 (72)	**Long** 8.43 (14)
Udder Width	**Narrow** 10.84 (18)	**Intermediate** 73.49 (122)	**Wide** 15.66 (26)
Udder Circumference	**Small** 22.89 (38)	**Intermediate** 69.27 (115)	**Large** 7.83 (13)
Central ligament	**Weak** 12.05 (20)	**Intermediate** 69.28 (115)	**Strong** 18.67 (31)
Teat circumference	**Thin** 57.23 (95)	**Intermediate** 34.34 (57)	**Thick** 8.43 (14)
Rear teat length	**Short** 38.56(64)	**Intermediate** 48.19 (80)	**Long** 13.25(22)
Fore teat length	**Short** 52.41(87)	**Intermediate** 39.76 (66)	**Long** 7.83 (13)
Distance between fore and rear teat	**Short** 39.76 (66)	**Intermediate** 52.41(87)	**Long** 7.83(13)
Distance between left and right teat	**Short** 29.52(49)	**Intermediate** 62.05 (103)	**Long** 8.43 (14)
Shortest distance of floor from front teat	**Short** 28.31 (47)	**Intermediate** 48.80 (81)	**Long** 22.89 (38)
Shortest distance of floor from rear teat	**Short** 13.85 (23)	**Intermediate** 46.39 (77)	**Long** 39.76 (66)
Teat Diameter	**Thin** 56.63 (94)	**Intermediate** 40.36 (67)	**Thick** 3.01 (5)

4.7.2 Rear udder width

This trait was assessed at the point where the "horizontal lines" between the rear udder starts and stops. RUW was categorized into three group *viz.*, narrow, intermediate and wide groups having the score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively for each group accordingly 1 to 9 point scale scoring system of ICAR (2012). The average value for the trait in Sahiwal and Karan Fries cows were 8.99±0.32 cm and 12.87±0.24 cm, respectively. In both the breeds the maximum frequency for this trait was observed in intermediate group followed by narrow and wide. In Sahiwal frequencies in percentage for narrow, intermediate and wide were 42.53, 47.13 and 10.34, respectively whereas, in Karan Fries cows these frequencies were 22.89, 62.05 and 15.06, respectively.

4.7.3 Rear udder height

Rear udder height was measured as the distance between the top of milk secreting tissue to the bottom of the vulva. It was classified as low, intermediate and high groups having score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively for each group according to 1-9 point scale scoring system. As per the recommendation of ICAR, the high RUH is considered better than other groups which might be due to the reason that there would be more space available for secretory tissues, which are known to synthesize milk (Dubey, 2010). The maximum frequency was observed in intermediate group for both Sahiwal and Karan Fries cattle. This was followed by high and low frequency in Sahiwal while low and high in Karan Fries cattle. In Sahiwal cattle the frequencies in percentage for low, intermediate and high groups were 16.09, 63.22 and 20.69, respectively while in Karan Fries cattle the values were 28.92, 65.06 and 6.02, respectively. These finding of frequencies were in accordance to the finding of Dubey (2010) who found the percentage for low, intermediate and high groups as 11.9, 71.4 and 16.8, respectively in Sahiwal cattle. The average value for this trait in Sahiwal and Karan Fries cattle were 22.69±0.99 cm and 23.67±0.49 cm, respectively. Dubey (2010) found lower measurements for RUH (12.65 ±0.23 cm) in Sahiwal cattle as compared to present finding.

4.7.4 Udder balance

Udder balance represents depth of the rear udder when assessed in relation to depth of the front udder. This trait was classified into three groups *viz.*, deep rear udder, both front and rear udder on same level and deep front udder with score point range of 1 to 3, 4 to 6 and 7 to 9 respectively in 1 to 9 point scale scoring system of ICAR (2012). The balance between the front and the rear udder and the overall udder's exterior form and location largely depends upon the attachment of central ligament with the udder (Atkins, 2007). The highest frequency of the trait in Sahiwal was for same level group whereas, in Karan Fries maximum was for deep rear udder. The frequencies of deep rear udder, both front and rear udder on same level and deep front udder in Sahiwal cattle were 11.49, 67.82 and 20.69, respectively while in Karan Fries cattle the frequency was 46.99, 43.37 and 9.64, respectively. Dubey (2010) found highest frequency for deep udder (46.5) followed by same level (42.6) and deep front udder groups (10.9) in Sahiwal cows. The average value of this trait in this study was -1.83±0.48 cm and 0.75±0.53 cm for Sahiwal and Karan Fries cows, respectively. Dubey (2010) in Sahiwal cows reported the average value of udder balance as 0.54±0.19 cm which is higher as compared to present study in Sahiwal cows.

4.7.5 Udder depth

The udder depth is distance from lowest part of the udder floor to the hock. Larroque and Ducrocq (2001) reported that the udder traits (especially the height of the udder above the hock) were found to positively influence the length of productive life of animal as udder depth with milking ease accounted for about 84 per cent of the total contribution of type traits to functional longevity. Udder depth was classified into three groups viz., deep, intermediate and shallow with score point range of 1 to 3, 4 to 6 and 7 to 9, respectively, for each group in 1 to 9 point scale scoring system of ICAR (2012). The frequency of udder depth in Sahiwal cows was higher in deep group being 65.51 per cent followed by intermediate (28.74 per cent) and shallow (5.75 per cent) group. Dubey (2010) reported the higher frequency of intermediate group being 68.4 per cent followed by shallow (25.7 per cent) and below hock (5.9 per cent) groups. In Karan Fries cows the highest frequency was for intermediate (66.27 per cent) followed by deep (24.10 per cent) and shallow (9.63 per cent) group. The average value of this trait in this study was 19.43±0.88 cm and 21.22±0.56 cm for Sahiwal and Karan Fries cows, respectively.

Dubey (2010) and Meingoas *et al*. (2017) reported a lower mean value of udder depth in Sahiwal cows and Zebu cows, respectively. In crossbred cows Patel and Trivedi (2018) reported a higher mean value (23.06±0.34 cm) while, Waghmore and Siddiqui (2000) and Kshatriya *et al.* (2009) reported a lower mean value of 14.65±0.20 cm and 20.30±0.86 cm, respectively. In Gir cattle mean value of udder depth was reported as 20.29 ±1.04 cm by Singhai *et al*. (2013) and 25.62±0.43 cm by Modh *et al*. (2017). Singh *et al*. (2009) reported a range of 19.4±0.4 cm to 28.5±0.7 cm in Vrindavani cows for udder depth.

4.7.6 Udder length

Udder length is represented from the point where the front udder base meets the body to rear udder end. This trait is not recommended by ICAR. In the present investigation, this was categorized into three groups as short, intermediate and long groups having score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively for each group in 1 to 9 scale scoring system. In Sahiwal cows the maximum frequency was for Intermediate (47.13 %) followed by short (37.93 %) and long (14.94 %) group.

However, in Karan Fries cows the values were maximum for short (48.19 percent), followed by intermediate (43.37 %) and long (8.44 %) group. The average value of this trait in this study was 51.30±0.94 cm and 60.34±0.68 cm for Sahiwal and Karan Fries cows, respectively. In the study the mean value in Karan Fries cows was higher than the crossbred cows were reported by different researchers (Waghmore and Siddiqui, 2000; Kshatriya *et al.*, 2009 and Patel and Trivedi, 2018). In Gir cows Singhai *et al*. (2013) reported the mean value as 45.99±2.07 cm while Modh *et al*., 2017 reported the value as 61.95±1.20 cm in Gir cows. In Vrindavani cows this value was ranged between 49.2±0.8 cm to 62.9±1.2 cm.

4.7.7 Udder width

This trait is assessed by measuring from one side of the udder to the other. Udder width was classified into three groups as narrow, intermediate and wide groups having the score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively for each group in 1 to 9 point scale scoring system. In both Sahiwal and Karan Fries cows highest frequency was observed for intermediate group. The frequency in percentage for all the three groups *viz.,* narrow, intermediate and wide groups were 41.38, 43.68 and 14.94 percent, respectively in Sahiwal cows while the value in Karan Fries were found 10.85, 73.49 and 15.66 percent, respectively. The mean value of this trait in the present study for Sahiwal and Karan Fries cows were 63.45±1.20 cm and 71.47±0.83 cm, respectively. In present investigation the mean value of udder width in Karan Fries cows was higher than the crossbred cows as reported by various workers (Waghmore and Siddiqui, 2000; Kshatriya *et al.*, 2009 and Patel and Trivedi, 2018). Modh *et al*. (2017) reported approximately same mean value (62.99±1.17 cm) of Udder width in Gir cows as compared to Sahiwal cows. Singh *et al*. (2009) in Vrindavani cattle reported that mean value of udder width ranged between 55.6±0.90 cm to 73.5±1.30 cm.

4.7.8 Udder circumference

Udder circumference is assessed by measuring a whole circle at base of the udder. In present investigation it was categorized as small, intermediate and large groups having score range of 1 to 3, 4 to 6 and 7 to 9 score point respectively for each group in 1 to 9 point scale scoring system. The highest frequency was observed for Intermediate group in both Sahiwal and Karan Fries cows. The frequency in percentage for all the three groups viz., small, intermediate and large groups were 17.24, 52.87 and 29.89, respectively in Sahiwal cows while the value in Karan Fries were 22.89, 69.28 and 7.83, respectively. The mean value of udder circumference in the present study was approximately same in both Sahiwal (142.64±1.80 cm) and Karan Fries (142.58±2.28 cm) cows.

4.7.9 Central ligament or udder cleft

It represents the depth of the cleft which is measured at the base of the udder, caused by central suspensory ligament. The udder cleft had the largest influence on true and functional longevity and is considered as one of the fundamental traits related to udder health (Vollema *et al*., 2000; Larroque and Ducrocq, 2001 and Schneider *et al*., 2003). ICAR (2012) recommended central ligament with deep definition better in cattle because these ligament can more easily bear the weight of the udder in a better way. The udder cleft was classified as weak (broken), intermediate and strong with score points of 1 to 3, 4 to 6 and 7 to 9, respectively in 1 to 9 point scale scoring system of ICAR (2012). The highest frequency for udder cleft was observed in intermediate group followed by strong and weak (broken) group in both Sahiwal and Karan Fries cows.

These finding of frequency were in accordance to the finding of Dubey (2010) who also reported maximum frequency for intermediate group followed by strong and weak (broken) group. The frequency in percentage for weak, intermediate and strong groups in Sahiwal cows were 9.20, 56.32 and 34.48, respectively while in Karan Fries cows these values were 12.05, 69.28 and 18.67, respectively. In present study the mean value of this trait was 3.40±0.19 cm and 3.18±0.13 cm for Sahiwal and Karan Fries cows, respectively which was higher than the mean value as reported in Sahiwal cows (1.76 ±0.07 cm) by Dubey (2010). Udder cleft involves the assessment of strength of ligaments that is responsible for attachment of udder to the pelvic floor. The productive life of the cow will be seriously compromised without adequate strength in this area (Atkins, 2007).

4.7.10 Teat circumference

It refers to the measurement at the middle of the teats encircling through tape. The teat circumference was classified as thin, intermediate and thick with score points of 1 to 3, 4 to 6 and 7 to 9, respectively in 1 to 9 point scale scoring system. In present investigation, the average value for this trait was found to be 8.64±0.88 cm in Sahiwal cows and 7.50±0.14 cm in Karan Fries cows. The highest frequency was observed in thin group followed by intermediate group in both Sahiwal and Karan Fries cows. Frequency in percentage observed for thin, intermediate and thick groups were 50.57, 35.63 and 13.80 in Sahiwal cows while 57.23, 34.34 and 8.43 in Karan Fries cows.

4.7.11 Teat length

It indicates the length of the teat from the base to the end of the teat. Teat length were categorized as short, intermediate and long with score points of 1 to 3, 4 to 6 and 7 to 9 score point, respectively in 1 to 9 point scale scoring system. As per the recommendation of ICAR (2012), intermediate teats is preferred in cattle because long teats are more easily damaged due to increased susceptibility to injury, which further increases the chances of infection and are genetically predisposed to a higher incidence of mastitis (Van Dorp *et al*., 1998), whereas short teats cause problem for milking machines.

In Sahiwal the highest frequency was observed in short group followed by intermediate and long group for both fore and rear teat length. These results were in accordance with finding of Dubey (2010) who reported similar trends in fore teat length of Sahiwal cows with frequency (in percentage) of short, intermediate and long group was 50.5, 45.6 and 3.9, respectively. In Karan Fries the highest frequency was found in short group for front teat length and in intermediate group for rear teat length. The frequencies in percentage of front teat length for short, intermediate and long groups were 55.17, 37.93 and 6.90, respectively in Sahiwal cows while, 52.41, 39.76 and 7.83, respectively in Karan Fries cows. The frequencies in percentage of rear teat length for short, intermediate and long groups were 52.87, 35.63 and 11.50, respectively in Sahiwal cows while 38.56, 48.19 and 13.25, respectively in Karan Fries cows.

The average value for fore and rear teat length in Sahiwal cows were 7.14±1.00 cm and 6.15±1.12 cm, respectively. Dubey (2010) and Singhai *et al*. (2013) reported approximately similar mean value of teat length in Sahiwal (6.92±0.17 cm) and Gir (7.07 ±0.42 cm) cows, respectively. In Karan Fries the average value for fore and rear teat length was 5.33±0.14 cm and 4.62±0.10 cm, respectively. Patel and Trivedi (2018) reported the mean value of this trait was 5.73±0.06 cm in Crossbred cows while, Kuczaj (2003) reported average value for fore and rear teat length in Red and White, Poland cows were 5.72 ±0.82 cm and 4.72 ±0.74 cm, respectively which are of same range as in the present study. Waghmore and Siddiqui (2000) reported a higher value of fore (7.32±0.09 cm) and rear (6.72±0.09 cm) teat length in Crossbred cows as compared to Karan Fries cows in present study.

4.7.12 Distance between teats

The distance between teats were taken between fore and rear teats and left and right teat. It was categorized as short, intermediate and long groups having score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively in 1 to 9 point scale scoring system. Squarely placed teat on the udder are recommended by ICAR for better milk productivity and udder health as these teats may be the reason for higher milk production. The maximum frequency for distance between fore and rear teat in Sahiwal and Karan Fries cows were observed in intermediate group whereas, for left and right teat maximum frequency was in short group for Sahiwal and intermediate group in Karan Fries cows.

The value in percentage of distance between fore and rear teat were 39.08, 50.57 and 10.35 in Sahiwal cows while, 39.76, 52.41 and 7.83 in Karan Fries cows. The value in percentage of distance between left and right teat in Sahiwal cows were 49.43, 39.08 and 11.49 whereas, in Karan Fries it was 29.52, 62.05 and 8.43. These data suggests rectangular placement of teats on the udder in Sahiwal cows whereas squarely placed in Karan Fries cows. The mean value of distance between fore and rear teat and left and right teat in Sahiwal cows were found of 4.59±0.81 cm and 5.81±0.88 cm, respectively while in Karan Fries it was 6.56±0.36 cm and 8.86±0.44 cm, respectively.

4.7.13 Shortest distance of teat from floor

It was assessed by measuring from tip of the fore and rear teats to the ground floor. It was categorized as short, intermediate and long groups having score range of 1 to 3, 4 to 6 and 7 to 9 score point, respectively in 1 to 9 point scale scoring system. The highest frequency for fore teat from floor in both Sahiwal and Karan Fries cows and rear teat from floor in Karan Fries cows was observed in intermediate group. For Sahiwal the frequency of shortest distance of floor from rear teat was equal in intermediate and long group.

The value in percentage of shortest distance of floor from fore teat for short, intermediate and long groups in Sahiwal cows were 19.54, 63.22 and 17.24, respectively while, in Karan Fries the values were 28.31, 48.80 and 22.89, respectively. The value in percentage of shortest distance of floor from rear teat for short, intermediate and long groups were 10.34, 44.83 and 44.83, respectively in Sahiwal cows whereas, 13.85, 46.39 and 39.76 respectively in Karan Fries cows. The average value of shortest distance of fore and rear teat from floor in Sahiwal cows were 42.46±1.00 cm and 41.28±1.04 cm while, 46.99±0.52 cm and 47.00±0.51 cm in Karan Fries cows. Kuczaj (2003) and Nakov *et al*. (2014) reported that higher mean value shortest distance of fore and rear teat from floor in Red and White, Poland cows were 53.90 ±5.01 cm from fore teat and 53.45 ±5.46 cm from rear teat however, in Dairy cows 51.26 ±5.98 cm from fore teat and 51.13±6.3 cm from rear teat as compared to recording of both Sahiwal and Karan Fries cows in the present study.

4.7.14 Teat diameter

It was classified as thin, intermediate and thick group with the corresponding score point range of 1 to 3, 4 to 6 and 7 to 9 points in 1 to 9 point scale scoring system of ICAR (2012). The maximum frequency for this trait was observed in thin group followed by intermediate and thick group in both Sahiwal and Karan Fries cows. Same trend was observed by Dubey (2010) in Sahiwal cows with the frequency in percentage for thin, intermediate and thick groups were 80.3, 18.8 and 0.9, respectively. In Present study the frequency in percentage for thin, intermediate and thick groups were 63.22, 26.44 and 10.34, respectively in Sahiwal cows while, 56.63, 40.36 and 3.01, respectively in Karan Fries cows. The mean value of the trait in Sahiwal and Karan Fries cows were observed as 2.71±0.08 cm and 2.22±0.03 cm, respectively. Dubey (2010) also reported the mean value of trait was 2.78±0.06 cm. But, Waghmore and Siddiqui (2000) reported a large difference in mean value of fore (7.32±0.09 cm) and rear (1.47±0.03 cm) teat diameter in Crossbred cows. However, Patel and Trivedi (2018) reported teat diameter (2.68±0.03 cm) comparable to the present study in Crossbred cows.

4.8 Effect of non-genetic factors on udder and teat type traits

4.8.1 Effect of season

Season has significant effect ($p<0.05$) on teat circumference while, other traits revealed no any significant effect of season in Sahiwal cows (Table 4.10 to 4.13). Teat circumference in winter (14.81±1.55 cm) was found significantly ($p<0.05$) higher than the other seasons. Teat circumference during summer, rainy and autumn were found of 10.02±1.05 cm, 4.75±1.92 cm and 4.99±2.61 cm, respectively. However, there is no any significant difference was found between rainy and autumn season measurements in Sahiwal cows. Similarly, Season did not have any significant effect on the udder and teat type traits in Karan Fries cows (Table 4.14 to 4.17).

4.8.2 Effect of parity

Parity had significant effect on different udder type traits viz., fore udder attachment, rear udder width, udder depth, udder width and teat diameter in Sahiwal cows (Table 4.10 to 4.13) whereas, in Karan Fries cows it has effect on rear udder height, udder depth, udder length, udder width, teat circumference, fore teat length, shortest distance of floor from teats and teat diameter (Table 4.14 to 4.17). Khan and Khan (2016) also found the stage of lactation, parity and herd as a significant source of variation for udder length, udder width, udder depth, udder circumference, 305 days milk yield and test day yield ($P<0.01$) in Sahiwal cows.

Fore udder attachment found to differ significantly ($p<0.01$) between different parities in Sahiwal cows. A higher FUA means tight attachment of the udder to the body wall whereas, loose attachment of udder to the body wall is indicated by a lower FUA. Fore udder attachment was higher in first parity and then decreases as the parity increases in the present study. Least square mean of FUA in first parity was 138.02±1.62 which decreases to 96.69±2.08 when cow is in fifth parity. The same type of pattern in FUA, a lower value in older cows was reported by Marinov *et al*. (2015).

Parity was found to significantly ($p<0.01$) effect RUW in Sahiwal cows. This trait showed an increasing trend with the increase of parity. Least square means for RUW with increasing parity from first to fifth or greater than fifth parity were 7.85±0.49 cm, 8.45±0.55 cm, 8.92±0.54 cm, 9.68±0.71 cm and 10.05±0.62 cm, respectively.

Udder width was found to be significantly ($p<0.01$) affected by parity in Sahiwal cows. UW showed an increasing trend up to fourth parity, after that it decreased for fifth or higher parity. This might suggests that multiparous cows have wider udder than primiparous cows. Least mean square for udder width from first to fifth or greater than fifth parity were 58.21±1.78 cm, 61.28±2.02 cm, 64.97±1.99 cm, 68.69±2.62 cm and 64.10±2.29 cm, respectively in Sahiwal cows. In Karan Fries cows UW found to differ significantly ($p<0.01$) among themselves except the udder width of 2^{nd} and 4^{th} parity. Least mean square for udder width from first to fifth or greater than fifth parity in Karan Fries cows were 62.54±1.23 cm, 70.01±1.31 cm, 73.44±1.96 cm, 71.18±1.76 cm and 80.19±2.49 cm, respectively. These findings are approximately closer with the finding of Singh *et al*. (2009) in Vrindavani cattle.

Udder width in Karan Fries cows were ranged from 62.54±1.23 cm to 80.19±2.49 cm in first to fifth or higher parity.

Udder depth (UD) found to differ among different parities significantly in both Sahiwal ($p<0.01$) and Karan Fries ($p<0.01$) cows. However, there was no significant difference in udder depth of 3rd and 4th parity in Karan Fries cows. Highest udder depth (23.99±1.70 cm) found in the first parity and the lowest (19.07±0.84 cm) in above fifth parity in Karan Fries cows while, in Sahiwal cows these value were 21.18±1.31 cm in first parity to 18.03±1.69 cm in above fifth parity. Zwertvaegher *et al*. (2012) and Mingoas *et al*. (2017) reported that udder height and udder depth significantly increased ($p<0.05$) with parity while both the traits decreased ($p<0.05$) at the 3rd stage of lactation. They also reported that there was no significant variation of teat size according to parity and stage of lactation.

Parity had significant effect on teat diameter in both Sahiwal ($P<0.05$) and Karan Fries ($P<0.01$) cows. Teat diameter increases upto third parity of cow then decreases in fourth parity and again increases for higher parity. Least square mean from first to fifth or greater than fifth parity were 1.99±0.04 cm, 2.20±0.05 cm, 2.30±0.07 cm, 2.24±0.06 cm and 2.36±0.09 cm, respectively in Sahiwal cows. There was no any significant difference between 2nd, 3rd, 4th and 5th or greater parity for teat diameter of Sahiwal cows. In Karan Fries cow least square means for first to fifth or greater than fifth parity were 2.51±0.12 cm, 2.70±0.13 cm, 2.64±0.13 cm, 2.63±0.17 cm and 3.07±0.10 cm, respectively. Similarly, in Karan Fries cow there was no any significant difference between 1st, 2nd, 3rd, and 4th for teat diameter. These findings are contrary to the findings of Mingoas *et al*. (2017) who reported no significant effect of parity on teat diameter but the findings are similar to findings of Singh *et al*. (2009) in Vrindavani cattle who also reported increase of teat diameter.

Parity was found to have significant ($p<0.01$) effect on RUH in Karan Fries cows while parity did not have any significant effect on RUH in Sahiwal cows. The value of RUH increases as the parity increases. Least square mean for rear udder height from first to fifth or greater than fifth parity were 20.99±0.74 cm, 21.75±0.78 cm, 24.05±1.17 cm, 24.49±1.05 cm and 27.08±1.49 cm, respectively in Karan Fries cows. There was no any significant difference between 1st, 2nd, 3rd, and 4th parity in Karan Fries cows.

Parity had significant ($p<0.01$) effect on udder length in Karan Fries cows. Least square mean for rear udder height from first to fifth or greater than fifth parity were 54.08±1.01 cm, 60.58±1.08 cm, 62.00±1.61 cm, 62.13±1.44 cm and 62.92±2.05 cm, respectively in Karan Fries cows. It indicates udder height increases with increase in parity. There was no any significant difference found between third, fourth and fifth parity. This study is in conformity to the study done in Vrindavani cattle by Singh *et al*. (2009).

Parity had significant ($p<0.01$) effect on front teat length in Karan Fries cows. Teat length increase from first to third parity than decreases again increases for higher parity. However, the decrease was not significant. Least square mean of teat length from first to fifth or greater than fifth parity were 4.47±0.21 cm, 5.25±0.22 cm, 5.59±0.33 cm, 5.48±0.29 cm and 5.86±0.42 cm, respectively in Karan Fries cows. There was significant difference among 1st, 2nd and 5th or greater parity. Similar, report found by Singh *et al*. (2009) and Olusayo *et al*. (2016), increase in teat length with parity.

Parity had significant ($p<0.01$) effect on shortest distance of floor from both fore and rear teats in Karan Fries cows. Distance of both fore and rear teats from floor decreases as parity of cow increases, which is due to increase in teat length in subsequent parity. Least square mean from first to fifth parity for SDF were 43.24±1.54 cm, 42.89±1.75 cm, 40.96±1.72 cm, 39.34±2.27 cm and 39.96±1.99 cm, respectively whereas, least square mean from first to fifth parity for SDR were 45.12±1.49 cm, 44.79±1.69 cm, 42.04±1.66 cm, 40.65±2.18 cm and 39.69±1.91 cm, respectively. There was no any significant difference between 1st, 2nd and 3rd parity and also between 4th and 5th parity for SDF in Karan Fries cows. For SDR there was no any significant difference between 1st and 2nd parity and 4th and 5th parity.

4.8.3 Effect of stage of lactation

Stage of lactation had significant ($p<0.05$) effect on different udder and teat type traits *viz.,* udder circumference, teat circumference, distance between fore-rear teats, distance between left-right teats and shortest distance of floor from rear teat (Table 4.10 to 4.13) while, in Karan Fries cows stage of lactation had significant ($p<0.05$) effect on udder circumference only (Table 4.14 to 4.17). Marinov *et al*. (2015) reported reduction in value of scores for fore udder attachment, rear udder height and width and teat position with the increase in stage of lactation. Deng *et al.* (2012) found that stage of lactation did not affect

Table 4.10 ANOVA of fixed effects for udder type traits in Sahiwal cows

Source of Variance	d.f	FUA	RUW	RUH	UB	UD	UL	UW	UC	CL
Season	3	47.11	3.13	1.98	4.12	74.01	21.17	45.66	72.4	2.11
Parity	4	6311.91***	11.64**	84.94	12.88	156.77**	69.04	217.78**	140.33	1.2
Stage of lactation	2	54.43	1.27	7.21	0.57	66.24	40.39	28.4	412.81*	1.72
Error	77	44.26	4.02	36.42	8.63	29.1	33.44	53.62	120.85	1.45

*Significant at 5% level of significance; ** Significant at 1% level of significance; *** Significant at 0.1% level of significance

Table 4.11 ANOVA of fixed effects for teat type traits in Sahiwal cows

Source of Variance	d.f	TC	FTL	RTL	DFR	DLR	SDF	SDR	TD
Season	3	144.80*	3.66	0.31	44.04	52.05	13.38	24.63	0.09
Parity	4	27.4	36.18	29.44	10.91	16.72	39.57	80.5	0.60*
Stage of lactation	2	183.28*	25.9	13.14	80.76*	132.13**	82.63	108.67*	0.01
Error	77	29.08	37.72	46.97	24.74	29.15	40.18	37.22	0.24

* Significant at 5% level of significance; ** Significant at 1% level of significance

Table 4.12 Least squares means of subclasses of different fixed effects for udder type traits in Sahiwal cows

Effects		FUA (degree)	RUW (cm)	RUH (cm)	UB (cm)	UD (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
Overall mean (87)		**107±2.10**	**8.99±0.32**	**22.69±0.99**	**-1.83±0.48**	**19.43±0.88**	**51.30±0.94**	**63.45±1.20**	**142.64±1.80**	**3.40±0.19**
Season	**Summer (52)**	120.98±1.30	9.55±0.39	22.80±1.18	-1.88±0.57	18.84±1.05	51.71±1.13	65.21±1.43	143.94±2.15	3.81±0.23
	Rainy (13)	118.26±2.37	8.28±0.71	22.15±2.15	-2.73±1.05	21.09±1.05	49.47±2.06	60.76±2.61	138.65±3.93	3.22±0.43
	Autumn (6)	123.54±3.23	8.49±0.97	22.44±2.93	-1.10±1.42	18.94±1.92	53.14±2.80	64.60±3.55	144.71±5.33	2.53±0.58
	Winter (16)	119.02±6.17	9.64±0.57	23.36±1.73	-1.63±0.84	18.85±2.61	50.91±1.66	63.23±2.10	143.28±3.16	4.02±0.34
Parity	**1(29)**	138.02^{e}±1.62	7.85^{a}±0.49	22.24±1.47	-2.94±0.71	21.18^{c}±1.30	48.33±1.41	58.21^{a}±1.78	138.14±2.68	3.10±0.29
	2(22)	132.45^{d}±1.84	8.45^{ab}±0.55	21.39±1.67	-2.41±0.81	19.50^{b}±1.49	51.40±1.60	61.28^{b}±2.02	142.08±3.04	3.06±0.33
	3(16)	119.54^{c}±1.81	8.92^{b}±0.54	23.80±1.64	-1.87±0.80	19.79^{b}±1.47	51.18±1.57	64.97^{c}±1.99	145.01±2.99	3.63±0.32
	4(9)	105.36^{b}±2.38	9.68^{c}±0.71	27.04±2.16	-1.53±1.05	18.65^{a}±1.93	51.77±2.07	68.69^{c}±2.62	143.91±3.94	3.73±0.43
	>5(11)	96.69^{a}±2.08	10.05^{c}±0.62	18.97±1.89	-0.42±0.92	18.03^{a}±1.69	53.83±1.81	64.10^{d}±2.29	144.08±3.45	3.46±0.37
Stage of Lactation	**1(40)**	119.36±1.84	8.72±0.55	22.59±1.66	-2.04±0.81	19.64±1.48	52.43±1.59	64.76±2.01	147.03^{b}±3.59	3.22±0.33
	2(21)	118.84±2.17	8.92±0.65	21.86±1.97	-1.75±0.96	20.36±1.76	52.46±1.88	62.34±2.39	142.99^{a}±3.03	3.09±0.39
	3(26)	123.15±1.58	9.33±0.47	23.60±1.43	-1.71±0.69	18.29±1.28	49.02±1.37	63.26±1.74	137.91^{c}±2.61	3.88±0.28

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.13 Least squares means of subclasses of different fixed effects for teat type traits in Sahiwal cows

Effects		TC (cm)	FTL (cm)	RTL (cm)	DFR (cm)	DLR (cm)	SDF (cm)	SDR (cm)	TD (cm)
Overall mean (87)		**8.64±0.88**	**7.14±1.00**	**6.15±1.12**	**4.59±0.81**	**5.81±0.88**	**42.46±1.00**	**41.28±1.04**	**2.71±0.08**
Season	**Summer (52)**	10.02^{b}±1.05	6.79±1.20	4.66±0.17	6.01±0.60	8.11±0.74	46.43±0.88	47.31±0.86	2.21±0.05
	Rainy (13)	4.75^{a}±1.92	7.39±2.19	4.54±0.23	5.25±0.81	8.05±1.0	48.84±1.19	48.76±1.16	2.20±0.07
	Autumn (6)	4.99^{a}±2.61	8.18±2.98	4.43±0.22	7.18±0.74	9.13±0.92	46.76±1.10	46.37±1.07	2.22±0.06
	Winter (16)	14.81^{c}±1.55	6.19±1.76	4.85±019	7.80±0.65	10.16±0.81	45.93±0.96	45.56±0.94	2.23±0.05
Parity	**1(29)**	9.12±1.31	5.13±1.50	3.89±0.15	7.41±0.53	9.35±0.66	53.53±0.78	53.39±0.77	1.99^{a}±0.04
	2(22)	6.97±1.49	8.24±1.70	4.44±0.16	6.63±0.57	9.33±0.71	48.48±0.84	48.33±0.82	2.20^{b}±0.05
	3(16)	9.94±1.46	7.82±1.67	4.99±0.25	6.01±0.85	8.14±1.06	46.69±1.25	46.88±1.22	2.30^{b}±0.07
	4(9)	7.45±1.93	6.65±2.20	4.83±0.22	5.96±0.76	8.67±0.94	44.65±1.12	45.52±1.09	2.24^{b}±0.06
	>5(11)	9.73±1.69	7.85±1.92	4.95±0.32	6.81±1.08	8.83±1.35	41.60±1.59	40.89±1.55	2.36^{b}±0.09
Stage of Lactation	**1(40)**	8.23^{b}±1.48	8.50±1.69	4.70±0.20	7.32^{c}±0.70	10.19^{b}±0.87	45.81±1.0	45.63^{a}±1.01	2.28±0.06
	2(21)	4.46^{a}±1.76	6.76±2.00	4.36±0.18	5.68^{a}±0.62	8.10^{a}±0.77	47.82±0.91	48.07^{c}±0.90	2.18±0.05
	3(26)	13.23^{c}±1.28	6.15±1.46	4.80±015	6.69^{b}±0.53	8.30^{a}±0.66	47.34±0.78	47.31^{b}±0.76	2.20±0.04

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.14 ANOVA of fixed effects for udder type traits in Karan Fries cows

Source of Variance	d.f	FUA	RUW	RUH	UB	UD	UL	UW	UC	CL
Season	3	152.37	0.377	31.57	43.26	59	81.47	36.47	412.49	1.8
Parity	4	1173.68	14.93	140.6***	23.29	605.9***	529.28***	1121.0***	580.83	0.27
Stage of lactation	2	255.8	3.13	17.54	0.97	6.99	149.68	182.82	1810.45*	0.04
Error	156	231.37	6.78	27.94	32.17	36.1	52.51	77.73	588.1	2.12

*Significant at 5% level of significance; ******* Significant at 0.1% level of significance

Table 4.15 ANOVA of fixed effects for teat Type Traits in Karan Fries cows

Source of Variance	d.f	TC	FTL	RTL	DFR	DLR	SDF	SDR	TD
Season	3	1.16	1.68	1.09	36.3	31.86	41.27	50.23	0.005
Parity	4	13.31***	9.63**	7.43	12.56	7.27	607.71**	581.25**	0.68**
Stage of lactation	2	1.81	1.18	2.14	23.06	43.98	35.85	50.62	0.08
Error	156	2.47	2.24	1.27	14.67	22.59	31.62	30.29	0.11

Significant at 5% level of significance; ***** Significant at 0.1% level of significance

Table 4.16 Least squares means of subclasses of different fixed effects for udder type traits in Karan Fries cows

Effects		FUA (degree)	RUW (cm)	RUH (cm)	UB (cm)	UD (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
Overall Mean (166)		**131±2.02**	**12.87±0.24**	**23.67±0.49**	**0.75±0.53**	**21.22±0.56**	**60.34±0.68**	**71.47±0.83**	**142.58±2.28**	**3.18±0.13**
Seasons	**Summer (52)**	120.98±1.30	12.98±0.41	22.66±0.83	2.31±0.89	21.01±0.94	62.07±1.14	72.01±1.38	140.62±3.8	3.01±0.22
	Rainy (38)	118.26±2.37	12.96±0.55	25.20±1.12	-0.70±1.20	21.90±1.27	59.92±1.53	71.00±1.86	146.49±5.1	3.13±0.30
	Autumn (32)	123.54±3.23	12.73±0.50	23.70±1.03	0.59±1.10	21.40±1.17	60.78±1.41	72.54±1.72	137.92±4.7	3.07±28
	Winter (44)	119.02±6.17	12.82±0.44	23.13±0.90	0.82±0.96	20.56±1.02	58.59±1.23	70.34±1.50	143.71±4.1	3.49±0.24
Parity	**1(57)**	148.02±1.62	11.82±0.36	20.99^{a}±0.74	0.01±0.79	23.79^{d}±1.70	54.08^{a}±1.01	62.54^{a}±1.23	139.93±3.40	3.04±0.41
	2(48)	132.45±1.84	12.89±0.38	21.75^{a}±0.78	1.11±0.84	21.98^{c}±1.20	60.58^{b}±1.08	70.01^{b}±1.31	148.25±3.62	3.20±0.29
	3(22)	119.54±1.81	12.94±0.58	24.05^{b}±1.17	0.21±1.26	20.71^{b}±1.34	62.00^{bc}±1.61	73.44^{c}±1.96	141.17±5.40	3.23±0.32
	4(26)	105.36±2.38	13.02±0.52	24.49^{b}±1.05	2.16±1.13	20.34^{b}±1.20	62.13^{bc}±1.44	71.18^{b}±1.6	144.81±4.8	3.25±0.21
	>5(13)	96.89±2.08	13.69±0.73	27.08^{c}±1.49	0.25±1.60	19.27^{a}±0.84	62.92^{c}±2.05	80.19^{d}±2.49	136.76±6.8	3.17±0.20
Stage of lactation	**1(53)**	119.36±1.83	13.15±0.47	22.87±0.97	0.94±1.04	21.42±1.10	62.50±1.33	73.82±1.62	150.71^{b}±4.46	3.21±0.26
	2(53)	118.84±2.17	12.88±0.42	23.93±0.86	0.61±0.92	21.05±0.98	59.99±1.18	71.17±1.44	137.51^{a}±3.96	3.18±0.23
	3(60)	123.15±1.58	12.59±0.36	24.22±0.73	0.69±0.79	21.18±0.83	58.53±1.01	69.43±1.23	138.34^{a}±3.38	3.14±0.20

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.17 Least squares means of subclasses of different fixed effects for teat type traits in Karan Fries cows

Effects		TC (cm)	FTL (cm)	RTL (cm)	DFR (cm)	DLR (cm)	SDF (cm)	SDR (cm)	TD (cm)
Overall mean (166)		**7.50±0.14**	**5.33±0.14**	**4.62±0.10**	**6.56±0.36**	**8.86±0.44**	**46.99±0.52**	**47.00±0.51**	**2.22±0.03**
Season	**Summer (52)**	7.75±0.24	5.06±0.23	5.94±1.34	7.02±0.97	8.69±1.06	40.37±1.24	41.25±1.19	2.61±0.09
	Rainy (38)	7.29±0.33	5.35±0.31	6.35±2.45	3.00±1.77	4.40±1.93	42.80±2.26	44.38±2.18	2.75±0.17
	Autumn (32)	7.52±0.30	5.33±0.29	6.31±3.32	1.31±2.41	2.19±2.62	40.63±3.07	41.42±2.96	2.89±0.23
	Winter (44)	7.42±0.26	5.58±0.25	6.00±1.97	7.04±1.43	7.94±1.55	41.31±1.82	42.78±1.75	2.51±0.14
Parity	**1(57)**	6.52^{a}±0.22	4.47^{a}±0.21	4.87±1.67	5.44±1.21	6.22±1.31	43.24^{b}±1.54	45.12^{c}±1.49	2.51^{b}±0.12
	2(48)	7.20^{b}±0.23	5.25^{b}±0.22	7.69±1.89	3.64±1.37	4.14±1.49	42.89^{b}±1.75	44.79^{c}±1.69	2.70^{b}±0.13
	3(22)	7.85^{c}±0.35	5.59^{c}±0.33	5.68±1.86	4.70±1.35	6.37±1.47	40.96^{b}±1.72	42.04^{b}±1.66	2.64^{b}±0.13
	4(26)	7.78^{c}±0.31	5.48^{bc}±0.29	5.21±2.45	4.14±1.78	5.87±1.93	39.34^{a}±2.27	40.65^{a}±2.18	2.63^{b}±0.17
	>5(13)	8.13^{c}±0.44	5.86^{d}±0.42	7.31±2.15	5.06±1.56	6.43±1.69	39.96^{a}±1.99	39.69^{a}±1.91	3.07^{a}±0.1
Stage of lactation	**1 (53)**	7.29±0.28	5.54±0.27	7.11±1.89	3.04±1.37	3.99±1.48	43.65±1.74	45.07±1.68	2.74±0.13
	2 (53)	7.75±0.25	5.16±0.24	5.99±2.23	2.90±1.62	3.43±1.76	41.13±2.07	42.77±1.99	2.70±0.16
	3 (60)	7.45±0.21	5.29±0.20	5.35±1.63	7.83±1.18	9.99±1.28	39.05±1.50	39.54±1.45	2.68±0.10

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

the udder measurements. Mingoas *et al*. (2017) found that udder depth significantly decreased ($p<0.05$) at third stage of lactation while there was no significant variation ($p<0.05$) of teat size according to the stage of lactation.

Udder circumference was found to differ significantly ($p<0.05$) between different stage of lactation in both Sahiwal and Karan Fries cows. In Sahiwal cows udder circumference shows a decreasing trend with the advancement of lactation while in Karan Fries cows it increased in the second stage and then after decreased in the third stage of lactation. Least square means of udder circumference for first to third stage of lactation were 147.03±3.59 cm, 142.99±3.03 cm and 137.91±2.61 cm, respectively in Sahiwal cows whereas, 150.71±4.46 cm, 137.51±3.96 cm and 138.34±3.38 cm, respectively in Karan Fries cows. This might suggest that with the reduction in milk yield after middle of lactation, the capacity of udder also decreases.

Teat circumference was found to differ significantly ($p<0.05$) between different stage of lactation in Sahiwal cows. Teat circumference first decreases in 2^{nd} stage of lactation then again it increases in the third. Least square mean of teat circumference for first to third stages were 8.23±1.48 cm, 4.46±1.76 cm and 13.23±1.28 cm, respectively. Mingoas *et al*. (2017) also reported that the thickness of teat increased significantly from first to third lactation, for front and rear teats for 11.11% and 16.08%, respectively.

For distance between teats in Sahiwal cow same trend were followed between fore and rear teats or left and right teat. Distance between teat decreases in 2^{nd} stage and then again increases in the third stage of lactation. There was no significant difference between all stage of lactation in distance between fore and rear teat. Least square mean for distance between fore and rear teat for first to third stages were 7.32±0.70 cm, 5.68±0.62 cm and 6.69 ±0.53 cm, respectively while least square mean for distance between left and right teat for first to third stages were 10.19±0.87cm, 8.10±0.77 cm and 8.30±0.66 cm, respectively for Sahiwal cows.

Shortest distance of rear teat from floor found to differ significantly ($p<0.05$) between different stage of lactation in Sahiwal cows. Least square mean of SDR for first to third stages of lactation was 45.63±1.01 cm, 48.07±0.90 and 47.31±0.76 cm, respectively.

4.9 Estimates of production traits

The mean and standard error of different milk production traits *viz*., Monthly milk yield (MMY), test-day milk yield (TDMY), 305 days milk yield (305DMY) and total milk yield (TMY) in Sahiwal and Karan Fries cows are presented in Table 4.18. The mean of MMY, TDMY, 305DMY and TMY in Sahiwal cows were 213.92±20.33 kg, 6.93±0.67 kg, 2016.13±144.97 kg and 2124.650±166.93 kg, respectively. In Karan Fries cows the mean of MMY, TDMY, 305DMY and TMY were 375.69±13.7 kg, 13.20±0.45 kg, 3525.97±103.2 kg and 4180.01±166.8 kg, respectively.

Table 4.18 Estimates of different production traits (Mean±SE) in Sahiwal and Karan Fries cows

Production Traits	Sahiwal	Karan Fries
	Mean±SE	Mean±SE
Monthly milk yield (Kg)	213.92±20.33	375.69±13.70
Test-day milk yield (Kg)	6.93±0.67	13.20±0.45
305 days milk yield (Kg)	2016.13±144.97	3525.97±103.20
Total milk yield (Kg)	2124.650±166.93	4180.01±166.80

4.10 Effect of non-genetic factors on production traits in Sahiwal and Karan Fries cows

Effect of different non-genetic factors such as season, parity and stage of lactation on various milk production traits *viz.*, monthly milk yield, test day milk yield, 305 days milk yield and total milk yield were revealed through analysis of variance and presented in Table 4.19 and 4.20 for Sahiwal cows while in Table 4.22 and 4.23 for Karan Fries cows. The least square means and standard error for Sahiwal and Karan Fries cows were shown in Table 4.21 and 4.24, respectively.

4.10.1 Effect of season

In the present study, season of calving did not have significant effect on any production traits in both Sahiwal and Karan Fries cows. Kumar *et al.* (2012) and Singh *et al*. (2014) also reported non-significant effect of season of calving on 305DMY in Sahiwal and

Frieswal cattle, respectively. Katok and Yanar (2012) reported significant effect (P<0.05) of season of calving on 305DMY. Ratwan *et al*. (2016) observed significant effect of period and season of calving in Jersey crossbred cattle. Dubey *et al*. (2008) found all the monthly test day (MTDY) milk yields to be highly significant in Karan Fries cattle for the season of calving. Significant effect of year and season of calving was reported by Atil *et al*. (2001) in Holstein Friesian cattle. Least square mean of total milk yield and 305DMY was found to be higher in rainy season than the other seasons in the study while, for monthly milk yield and test day milk yield summer season had the highest value as compared to other seasons. The non- significant effect of season of calving may be due to insufficient number of animals in each season in the study. Maximum number of calving of animals occurred in summer season.

4.10.2 Effect of parity

Parity had significant effect on monthly milk yield (p<0.01), test-day milk yield (p<0.05) and 305 days milk yield (p<0.01) in Sahiwal cows whereas, in Karan Fries cows it had significant (p<0.05) effect only on 305 days milk yield. Jinger *et al*. (2014) and Japheth *et al*. (2015) also reported significant (P<0.01) influence of parity on milk yield in Karan Fries cows.

Monthly milk yield and test day milk yield showed an increasing trend up to 4th parity and decreases after that as parity increases in Sahiwal cattle. Least square mean of monthly milk yield from first to fifth or greater than fifth parity in Sahiwal cows were 155.07±30.27 kg, 189.95±34.33 kg, 168.05±33.75 kg, 334.63±44.44 kg and 221.89±38.89 kg, respectively. There was significant difference in monthly milk yield between 1st, 4th and 5th parity in Sahiwal cattle. Least square mean of test day milk yield from first to fifth or greater than fifth parity in Sahiwal cows were 5.00±1.00 kg, 5.91±1.13 kg, 5.59±1.11 kg, 9.4±1.47 kg and 8.76±1.28 kg, respectively and highest test day milk yield was found in 4th parity.

Parity had significant effect on 305DMY in both Sahiwal and Karan Fries cattle. In Sahiwal cattle 4th parity had the highest value of 305 days milk yield. The least square mean of 305 days milk yield from first to fifth or greater than fifth parity in Sahiwal cows were 1491.16±216.39 kg, 1974.18±233.67 kg, 1651.56±262.73 kg, 2787.62±347.86 kg and 2176.13±304.70 kg, respectively which, indicates significant difference between 305 days milk yield in 1st, 4th and 5th parity in Sahiwal cattle. Significant effect of parity on 305DMY was also reported by Rehman and Khan (2012), Khan and Khan (2016) and Kakati *et al*. (2017). Kakati *et al*. (2017) reported highly significant (P<0.01) effect of parity on 305DMY in Freiswal cattle. He reported that fourth parity had the highest 305 days milk yield of 3136.51±134.15 kg while first parity had the lowest 305 days milk yield of 2649.66±124.77 kg which is similar to the present finding in Sahiwal cattle. Fahim *et al.* (2017) also found the same in crossbred cows. Wondifraw *et al.* (2013) and Narwaria *et al.* (2015) also observed the highly significant effect of parity on 305DMY.

The least square mean of 305 days milk yield from first to fifth or greater than fifth parity in Karan Fries cows were 3510.17±155.65 kg, 3521.06±166.34 kg, 3418.46±241.22 kg, 2962.18±223.12 kg and 4217.99±318.59 kg, respectively. In this milk yield was highest in animals of above 5th parity which might be due to culling of low yielders and only high yielders reach the above fifth parity and also there might be sampling error due to less number of observation.

Table 4.19 ANOVA of fixed effects for monthly milk yield and test-day milk yield in Sahiwal cows

Source of Variance	d.f	MMY	TDMY
Season	3	11152.52	14.71
Parity	4	56787.77**	50.25*
Stage of lactation	2	12925.52	21.74
Error	77	15344.040	16.85

***** Significant at 5% level of significance; ****** Significant at 1% level of significance

Table 4.20 ANOVA of fixed effects for 305 days milk yield and total milk yield in Sahiwal cows

Source of Variance	d.f	305DMY	TMY
Season	3	1468587.36	2220604.60
Parity	4	3312608.25**	2867858.98
Error	79	969767.94	1285734.67

****** Significant at 1% level of significance

Table 4.21 Least squares means of subclasses of different fixed effects for production traits in Sahiwal cows

Effect		MMY (Kg)	TDMY(Kg)	305DMY(Kg)	TMY(Kg)
Overall mean (87)		213.92±20.33	6.93±0.67	2016.136±144.97	2124.650±166.93
Season	**Summer (52)**	242.89±24.33	8.55±0.80	1771.45±153.21	1813.25±176.42
	Rainy (13)	199.99±44.30	6.19±1.46	2414.91±278.64	2616.86±320.84
	Autumn (6)	229.33±60.15	6.06±1.99	1850.07±411.09	2041.20±473.35
	Winter (16)	183.45±35.65	6.93±1.18	2028.10±250.60	2027.27±287.94
Parity	**1(29)**	155.07^{a}±30.27	5.00^{a}±1.00	1491.16^{a}±216.39	1663.97±249.16
	2(22)	189.95^{ab}±34.33	5.91^{a}±1.13	1974.18^{a}±233.67	2084.63±269.06
	3(16)	168.05^{a}±33.75	5.59^{a}±1.11	1651.56^{a}±262.73	1734.81±302.52
	4(9)	334.63^{c}±44.44	9.4^{b}±1.47	2787.62^{c}±347.86	2838.02±400.54
	>5(11)	221.89^{b}±38.89	8.76^{b}±1.28	2176.13^{b}±304.70	2301.79±350.84
Stage of Lactation	**1(40)**	225.08±34.15	7.85±1.13		
	2(21)	244.14±40.48	7.64±1.34		
	3(26)	172.53±29.48	5.31±0.97		

Figures in parenthesis are number of animals;

Figures with dissimilar superscript differ significantly

Table 4.22 ANOVA of fixed effects for monthly milk yield and test-day milk yield in Karan Fries cows

Effect	d.f	MMY	TDMY
Season	3	16986.74	38.77
Parity	4	42789.93	28.92
Stage of lactation	2	286146.94***	358.53***
Error	156	21977.32	23.55

*** Significant at 0.1% level of significance

Table 4.23 ANOVA of fixed effects for monthly milk yield and test-day milk yield in Karan Fries cows

Effect	d.f	MY	TMY
Season	3	830956.78	4303410.92
Parity	4	3409786.95*	6392304.06
Error	158	1266339.4	3303208.09

* Significant at 5% level of significance

4.10.3 Stage of lactation

Stage of lactation did not have any significant effect on the production traits *viz.*, monthly milk yield and test day milk yield in Sahiwal cattle whereas, in Karan Fries stage of lactation had highly significant ($p<0.01$) effect on monthly milk yield and test day milk yield. Highly significant ($p<0.01$) effect of stage of lactation on TDMY in Sahiwal, Karan Fries, Tharparkar cows and Murrah buffaloes were reported by Sarkar *et al*. (2006) and in Holstein dairy cows by Cobanglu *et al*. (2017). Khan and Khan (2016) also reported the effect of stage of lactation on test day milk yield in Sahiwal cattle. In present study, both the traits i.e. monthly milk yield and test day milk yield showed a decreasing trend with lactation stage in Karan Fries cattle. Both the traits differ significantly ($p<0.01$) between different stage of lactation in Karan Fries cows. Least square mean of monthly milk yield for first to third stages were 442.54± 27.29 kg, 401.54±24.24 kg and 283.00±20.71 kg, respectively whereas, for test day milk yield it was 15.82±0.89 kg, 13.81±0.79 kg and 9.98±0.67 kg, respectively in Karan Fries cattle.

However, the least square mean in Sahiwal cows varied from 225.08±34.15 kg in first stage of lactation to 172.53±29.48 kg in third stage of lactation. The highest monthly milk yield (244.14±40.48 kg) obtained in 2nd stage of lactation. Test day milk yield showed a decreasing trend with the advance stage of lactation. Least square mean ranged from 7.85±1.13 kg in first stage of lactation to 5.31±0.97 kg in third stage of lactation.

In Karan Fries cows, least square mean of MMY and TDMY differed significantly among themselves with least in 3rd lactation i.e. 283.00±20.71kg and 9.98±0.67kg, respectively.

Table 4.24 Least squares means of subclasses of different fixed effects for production traits in Karan Fries cows

Effect		MMY(Kg)	TDMY(Kg)	305DMY(Kg)	TMY(Kg)
Overall mean (166)		**375.69±13.7**	**13.20±0.45**	**3525.97±103.2**	**4180.01±166.8**
Season	**Summer (52)**	365.49± 23.33	13.09±0.76	3510.61± 151.73	3889.17±245.05
	Rainy (38)	391.24± 31.41	14.14±1.02	3599.45± 216.28	4278.51±349.31
	Autumn (32)	398.03± 28.99	13.67±0.94	3669.54± 208.82	4616.49±337.26
	Winter (44)	348.01± 25.33	11.91±0.82	3324.29± 184.66	3935.87±298.24
Parity	**1(57)**	379.80± 20.79	12.99±0.68	3510.1^{b}±155.65	3946.21±251.38
	2(48)	401.46± 22.15	14.25±0.72	3521.0^{b}±166.34	4172.15±268.66
	3(22)	335.77± 33.06	11.73±1.08	3418.46^{b}±241.22	3928.51±389.59
	4(26)	325.40± 29.62	12.20±0.96	2962.18^{a}±223.12	3424.62±360.35
	>5(13)	436.03± 41.98	14.84±1.37	4217.9^{c}±318.59	5028.55±514.55
Stage of Lactation	**1(53)**	442.54^{c}±27.29	15.8^{c}±0.89		
	2(60)	401.5^{b}±24.24	13.81^{b}±0.79		
	3(53)	283.0^{a}±20.71	9.9^{a}±0.67		

Figures in parenthesis are number of animals;

Figures with dissimilar superscript differ significantly

4.11 Effect of non-genetic factors on incidence of clinical mastitis in Sahiwal and Karan Fries cows

The odd ratios for calving season, parity and level of production associated with clinical mastitis were assessed with logistic models and the estimates are presented in Table 4.25 and 4.26 in Sahiwal and Karan Fries cows, respectively. In Sahiwal cows parity and level of production had significant effects on clinical mastitis. There was no significant effect of season on incidence of mastitis in both the breeds in present study. There was increase in the relative risk of mastitis as parity of animals increases. Animals in 4^{th} parity had significantly ($p<0.05$) highest odd ratio of 5.17 in Sahiwal cattle. Prevalence of udder quarters affected with clinical mastitis tended to increase with the increase in parity, from first to third parity, and after that it slightly begins to decline (Nakov and Trajcev, 2012). The present study is generally consistent about the reports that with the risk of increasing clinical mastitis with increasing parity (Sargeant *et al.,* 1998; Heikkila *et al.,* 2012; Breen *et al.,* 2009; Boujenane *et al*., 2015).

In terms of level of production, high yielders was found to have higher odd ratio of 8.22 in Sahiwal cows which means they have higher risk of incidence of clinical mastitis than the low yielders animals. Jingar *et al*. (2014) reported significant ($p<0.01$) effect of level of production on incidence of clinical mastitis in Karan Fries, Karan Swiss, Sahiwal cows and Murrah buffaloes while, non-significant effect in Tharparkar cows.

Parity had highly significant effect on incidence of mastitis however, season of calving and level of production did not show any significant effect on mastitis in Karan Fries cattle. Highly significant effect of parity ($p<0.01$) on mastitis was revealed by Wald chi square with a value of 11.83 in Karan Fries cows. There was increase in the relative risk of incidence of mastitis during 2^{nd} and 3^{rd} parity of animals with odd ratio of 5.66 and 7.79, respectively. Abebe *et al*. (2016) reported that cows with four or more parities had pendulous udder and prone for mastitis. Awale *et al*. (2012) found that that cows with pendulous quarters appear to be most susceptible to mammary infections as pendulous udder exposes the teat and udder to injury and pathogens can easily adhere to the teat and gain access to the gland tissue.

In a logistic regression study the relative risk of clinical mastitis was lower for primiparous cows which increased with further parity (Nakov *et al.,* 2014). Increasing parity number was also one of the predictors noted to associate with the presence of mastitis (Katsande *et al*., 2013; Abrahmsen *et al.,* 2014; Mureithi *et al*., 2016). They reported that likelihood of mastitis as 24.8 times higher in multiparous cows having four or more calvings compared with primiparous cows.

Table 4.25 Effect of non-genetic factors on incidence of clinical mastitis in Sahiwal cows

Effect		Mastitis			
		Estimate±SE	**Wald Chi sq.**	**Odds ratio**	**95% CI**
Intercept		-	**6.07**	-	-
Season	**Summer**	0.33-0.86	0.14	1.38	0.26-7.47
	Rainy	0.69±1.07	0.42	1.99	0.25-16.12
	Autumn	0.40±1.52	0.07	1.49	0.08-29.18
	Winter	-	0.43	-	-
Parity	**Parity (1)**	-20.26±0.85	0.00	0.00	0.00
	Parity (2)	-0.12±0.95	0.02	0.89	0.14-5.67
	Parity (3)	-0.66±1.01	0.43	0.52	0.07-3.74
	Parity (4)	1.64±1.07	2.35*	5.17	0.63-42.23
	Parity (>5)	-	6.07	-	-
Level of production	**High (1)**	2.11±0.84	6.35**	8.22	1.59- 42.29
	Medium (2)	2.04±0.96	4.53*	7.68	1.17- 50.24
	Low(3)	-	6.95	-	-

***** Significant at 5% level of significance; ****** Significant at 1% level of significance

There was no significant effect of season on incidence of mastitis in both Sahiwal and Karan Fries cows in present study. However, rainy season was found to have relatively higher risk of incidence for the mastitis in both the breeds. Nakov *et al.* (2014) reported that spring season was with the highest percentage of diagnosed cases of clinical mastitis with the exception of farm, where fall of the season was with the highest percentage of cases.

Table 4.26 Effect of non-genetic factors on incidence of clinical mastitis in Karan Fries cows

Effect		Mastitis			
		Estimate±SE	Wald Chi sq	Odds ratio	95% CI
Intercept		-	11.83***	-	-
Season	Summer	0.36-0.61	0.35	1.43	0.44- 4.72
	Rainy	0.62±0.57	1.20	1.87	0.61-5.68
	Autumn	-0.05±0.58	0.008	0.95	0.30-2.97
	Winter	-	1.77	-	-
Parity	Parity (1)	0.69±0.63	1.22	2.001	0.58-6.86
	Parity (2)	1.73±0.66	6.86**	5.66	1.55-20.72
	Parity (3)	2.05±0.64	10.15***	7.79	2.20-27.59
	Parity (4)	0.58±0.94	0.38	1.79	0.28-11.23
	Parity (>5)	-	13.52**	-	-
Level of production	High (1)	-0.54±0.46	1.36	0.59	0.24-1.44
	Medium (2)	0	3.02	0.285	0.07-1.17
	Low (3)	-	3.45	-	-

****** Significant at 1% level of significance; *******Significant at 0.1% level of significance

4.12 Incidence of clinical mastitis under different categories of non genetic factors in Sahiwal and Karan Fries cows

In the present study rainy season was found to have highest incidence of clinical mastitis in both the breeds. However, mean of incidence of clinical mastitis in Sahiwal cows (20.6%) were higher as compare to Karan Fries cattle (20.4%). The incidence of mastitis was observed maximum in rainy season i.e. 30.4% followed by, summer (24.9%), autumn

(21.0%) and lowest was found in winter (17.1%) season in Sahiwal cattle (Table 4.27). Similar report was found in Karan Fries cattle rainy season having maximum incidence of clinical mastitis i.e. 30.50% followed by autumn (23.4%), summer (19.3%), and winter (19.3%) season (Table 4.27).

With respect to parity, incidence varied from 3 % in first parity to 52.80 % in the fourth parity which further decreases to 27.20 % in fifth and above parities in Sahiwal cows. Whereas, in Karan Fries cattle incidence varied from 8% in first parity to 41.10 % in the fourth parity.

Our results indicated that high producing animal having highest percentage of incidence of clinical mastitis i.e. 31 % in both Sahiwal and Karan Fries cows.

Table 4.27 Incidence of clinical mastitis under different categories of non-genetic factors in Sahiwal and Karan Fries cows

Non-Genetic Factors		Sahiwal		Karan Fries	
		No. of Obs. (87)	Incidence (%)	No. of Obs. (166)	Incidence (%)
Season	Summer	52	24.90	62	19.30
	Rainy	13	30.40	28	30.50
	Autumn	6	21.00	32	23.40
	Winter	16	17.10	44	19.30
Parity	Parity(1)	24	3.00	57	8.00
	Parity(2)	20	19.62	48	15.70
	Parity(3)	18	17.30	22	34.30
	Parity(4)	14	52.80	26	41.10
	Parity(>5)	11	27.20	13	15.40
Level of Production	High(1)	27	31.30	58	31.00
	Medium (2)	34	22.20	82	23.00
	Low (3)	26	16.54	26	14.00

Nakov and Trajcev (2012) reported a significant association between the breed and the presence of mastitis. The likelihood of mastitis was found to be 16.4 times higher in Holstein-Friesian x zebu crosses than the pure local zebu cattle. This shows that pure local breeds are more resistant to contracting mastitis than the European breeds. Similar result reported by Sanotharan *et al*. (2016) susceptibility to mastitis depends up on breed difference.

4.13 Morphological characteristics of udder and teats

Visual appraisal was made to evaluate the udder shape, teat shape and skin condition of udder and teat as well as their percentage of occurrence in Sahiwal and Karan Fries cows is presented in Table 4.28.

4.13.1 Udder shape

The udder shape was classified into pendulous, trough and round types and their frequencies in Sahiwal and Karan Fries cattle are shown in Table 4.28. The frequencies of pendulous, trough and round types in Sahiwal cattle *viz.*, 20.69 %, 25.29 % and 54.02 %, respectively whereas, in Karan Fries cows the frequency *viz.*, 28.92 %, 43.37% and 27.71 %, respectively. In Sahiwal cow round shaped udders were most common while in Karan fries cattle trough shaped had the highest frequency of occurrence. Contrary to the present findings Singh (2006) and Ahlawat (2007) reported that trough shaped udder to be more common in Sahiwal cattle. Singh (2005), Kamboj *et al.* (2007) and Patel and Trivedi (2018) found trough shaped udders to be common in Karan Fries cattle. Rao (2006) reported round shaped udders (50 %) to be most frequent in Karan Fries cows followed by trough (43.75 %) and pendulous (6.25 %) udders

4.13.2 Udder suspension

Udder suspension was categorized as very tight and tight in one class, then intermediate and pendulous type. The frequencies of very tight or tight, intermediate and pendulous type were 34.48 %, 44.83 % and 20.69 %, respectively in Sahiwal cattle whereas, 30.12%, 56.63 % and 13.25 %, respectively in Karan Fries cows. Intermediate type udder suspension was most common followed by tight and pendulous type in both the breeds. Weak suspension means weak median suspensory ligament in the udder which indicates a lack of support in ligament that ties the udder to the cattle body wall. Over the time, weakness in the ligament will allow the udder to hang down too far from the body which further subject the udder to serious problems and increased potential for injury to udder.

Table 4.28 Frequency of different visual traits in Sahiwal and Karan Fries cows

Traits	Code	Frequency	
		Sahiwal	Karan Fries
Udder Shape	Pendulous (1)	18 (20.69 %)	48 (28.92 %)
	Trough (2)	22 (25.29 %)	72 (43.37%)
	Round (3)	47 (54.02%)	46 (27.71 %)
Udder Suspension	Tight+ very tight (1)	30 (34.48 %)	50 (30.12%)
	Intermediate (2)	39 (44.83 %)	94 (56.63 %)
	Pendulous (3)	18 (20.69 %)	22 (13.25 %)
Fore Teat Placement	Inside (1)	28 (32.18 %)	67 (40.36 %)
	Intermediate (5)	48 (55.17 %)	87 (52.41 %)
	Outside (9)	11(12.65 %)	12 (07.23%)
Rear Teat Placement	Inside (1)	13 (14.94 %)	49 (29.52 %)
	Intermediate (5)	40 (45.98 %)	80 (48.19 %)
	Outside (9)	34 (39.08 %)	37 (22.29%)
Teat Size	Small+ very small (1)	19 (21.84 %)	42 (25.30 %)
	Intermediate (2)	39 (44.83 %)	77 (46.39 %)
	Long + very Long (3)	29 (33.33 %)	47 (28.31 %)
Teat End Shape	Flat (1)	8 (09.20 %)	35 (21.08 %)
	Round (2)	45 (51.72 %)	86 (51.81 %)
	Pointed (3)	34 (39.08 %)	45 (27.11 %)
Teat Shape	Funnel (1)	48 (55.17 %)	56 (33.73 %)
	Cylindrical (2)	33 (37.93 %)	83 (50.00 %)
	Bottle (3)	6 (06.90 %)	27 (16.27%)
Skin Condition	Normal (1)	65 (74.71 %)	65 (39.16 %)
	Dry (2)	22 (25.29 %)	46 (27.71 %)
	Rough + dry + Crack (3)	-	55 (33.13 %)
Longer Term Changes In Teat-end Shape	No Ring (1)	24 (27.58%)	46 (27.71 %)
	Rough Ring (2)	17 (19.54 %)	76 (45.78 %)
	Smooth Ring (3)	41 (47.13 %)	32 (19.28 %)
	Very Rough Ring (4)	5 (05.75 %)	12 (07.23 %)

4.13.3 Fore teat placement

This was grouped into inside (close), intermediate and outside (wide) type. More than 50 % were of intermediate type placement followed by inside type and lastly outside type in both Sahiwal and Karan Fries cattle. WHFF defined intermediate teat placement as squarely placed teat under each quarter which are properly spaced from side and rear views. The frequencies of inside (close), intermediate and outside (wide) type were 32.18 %, 55.17 % and 12.65 %, respectively in Sahiwal cows whereas, 40.36 %, 52.41 % and 07.23%, respectively in Karan Fries cattle. Contrary to the present findings, Susanta *et al*. (2013) found higher percentage of closer (inside) teat placement in desi cows in comparison to crossbred cattle.

4.13.4 Rear teat placement

Rear teat placement was categorized into inside (close), intermediate (squarely placed) and outside (wide) type. In both the breeds intermediate placement were of highest percentage. The frequencies of inside (close), intermediate and outside (wide) type were 14.94 %, 45.98 % and 39.08%, respectively in Sahiwal cow while in Karan Fries it was 29.52 %, 48.19 % and 22.29%, respectively.

4.13.5 Teat size

Teat size was grouped into small, intermediate and long type with the frequency of 21.84 %, 44.83 % and 33.33%, respectively in Sahiwal where as in Karan Fries the percentages were 25.30 %, 46.39 % and 28.31 %, respectively. In both the breeds intermediate teat size were in maximum frequency followed by long and small size of teat.

4.13.6 Teat end shape

Teat end shape was classified as flat, round and pointed in both the breeds. More than 50 % teat end shape were of round type followed by pointed and flat type in both Sahiwal and Karan Fries cow. Kamboj *et al.* (2007) and Susanta *et al*. (2013) reported pointed teat to be comparatively higher in desi cows. The frequency in percentage of teat end shape were 09.20 %, 51.72 % and 39.08 % for flat, round and pointed type, respectively in Sahiwal cows while in Karan Fries frequency were 21.08 %,51.81 % and 27.11 %, respectively.

4.13.7 Teat shape

Teat shape was classified as funnel, cylindrical and bottle shape in present study. The frequencies in percentage of funnel, cylindrical and bottle shaped teats were 55.17, 37.93 and 6.89%, respectively in Sahiwal while in Karan Fries frequency were 50.00%, 33.73%, and 16.27%, respectively. In both the breeds, funnel shaped teats were most common followed by cylindrical and bottle shaped teats. Singh (2006) and Ahlawat (2007) also found funnel shaped teats highest number in Sahiwal cattle whereas, Singh (2005), Kamboj *et al.* (2008) and Patel and Trivedi (2018) reported that same in Karan Fries cattle. Rao (2006) reported cylindrical teat shape (75 %) to be in higher frequency than conical/ funnel shaped (25 %) teat in Karan Fries cow. Tikli *et al*. (2005) found that cows of similar lactation stage and age having funnel shaped teat produced more milk (10.9 to 15.4%) than the cows having cylindrical shaped teats. In cylindrical shaped teats there is a higher incidence of mastitis (Prajapati *et al.*, 1995).

4.13.8 Skin condition

Skin condition was grouped into normal and dry in Sahiwal cattle whereas, in Karan Fries cattle it was grouped as normal, dry and roughness along with dry. Normal skin condition was most frequent in both Sahiwal and Karan Fries cattle. The frequency of normal and dry skin was 74.71 % and 25.29 %, respectively in Sahiwal cattle while in Karan Fries cattle the frequency of normal, dry and roughness along with dry skin was 39.16 %, 27.71 % and 33.13 %, respectively. In some severe cases of mastitis, the skin of the udder and teat peels off which might be due to high milk production in Karan Fries cattle prone to mastitis. Machine milking further exacerbates the problems of chapping or cracking of the skin (Mein *et al*., 2001).

4.13.9 Longer term changes in teat end condition

It was classified into no ring, rough ring, smooth ring and very rough ring according to International Teat Club. The frequencies of no ring, rough ring, smooth ring and very rough ring obtained were 27.58 %, 19.54 % 47.13 %, and 5.75 %, respectively in Sahiwal cows whereas 27.71%, 45.78 %, 19.28 % and 7.23 %, respectively in Karan Fries cattle. In the present study higher percentage of rough rings were found in Karan Fries cattle which might be due to higher milk production. Juozaitiene *et al*. (2019) found that lower percentage of rough ring *i.e.*11.8% and very rough ring *i.e.* 2.9 % of the teats whereas, 44.3 % of the teats were grouped into no ring and 41.1% were present in smooth ring group in cattle.

4.14 Estimation of phenotypic correlations (r_p) between production traits and udder/ teat type traits

The estimate of Pearson correlation between production traits and udder/teat type traits, between udder and teat type traits, among udder traits and among teat traits are presented in Table 4.29 to 4.32 in Sahiwal cows and from Table 4.33 to 4.36 in Karan Fries cows.

In Sahiwal cattle correlation between rear udder height (0.18), rear udder width (0.18), central ligament (0.18), rear (0.23) and fore teat length (0.22) with 305DMY were positively significant ($p<0.05$). Negative correlation of udder length (-0.02), udder width (-0.03) and shortest distance of teat from floor with 305 days milk yield were found in present study. A positive and significant ($p<0.05$) correlation of total milk yield with fore udder attachment (0.18), rear udder height (0.20), udder circumference (0.18), central ligament (0.19), fore (0.19) and rear (0.19) teat length were also found in Sahiwal cattle.

Positive correlation of milk yield with most of the udder type traits suggest that the less depth udder will have more circumference, so that it can possesses more number of mammary tissue, which synthesize milk inside the udder, and this ultimately increases the milk production level (Dubey, 2010). Monthly milk yield had positive and highly significant ($p<0.01$) correlation with rear udder height (0.34), rear udder width (0.30) and udder circumference (0.30) while significant ($p<0.05$) with udder length (0.27) in Sahiwal cows. A positive and highly significant ($p<0.01$) correlation of udder length (0.33), width (0.30) and circumference (0.42) with test day milk yield was present in Sahiwal cows. Distance between fore and rear teat also show a significant ($p<0.05$) correlation (0.19) with test day milk yield in Sahiwal cattle. Khan and Khan (2016) also reported that the udder length, width, circumference and depth increases as test day milk yield increases in Sahiwal cattle.

In Karan Fries cows a positive and highly significant ($p<0.01$) correlation of udder length, circumference and central ligament with all the production traits taken in the present study were found. Highly significant ($p<0.01$) and negative correlation of udder depth with production traits was present. Rear udder height had positive and highly significant ($p<0.01$) correlation with 305 days milk yield and total milk yield while rear udder width showed negative and highly significant ($p<0.01$) correlation with 305 days milk yield and total milk yield. Monthly milk yield showed positive and significant ($p<0.05$) correlation with fore udder attachment and udder width.

Distance between fore and rear teat was found to be significantly ($p<0.05$) associated with 305 days milk yield and total milk yield. In Crossbred cows udder length, width, depth and fore teat diameter were positively associated with milk yield (Patel *et al.*, 2016). All these three udder measurements should be the important criteria for selection of dairy cows as the udder length, width and depth decides the capacity of udder which further reflects the milk yield. Rao (2006) found significant and positive correlation of teat length with milk yield and a negative significant correlation between rear teat diameter and milk yield in Karan Fries cows. Correlation coefficient of teat length and diameter with milk production were not encouraging in Karan Fries Cattle (Gupta *et al.*, 1991) whereas a positive and significant ($p<0.05$) association of fore teat diameter with milk yield in karan Fries cow was reported by Patel *et al.* (2016). All phenotypic correlations between the type traits and the yield traits were small to moderate as reported by Brotherstone (1994) in Holstein-Friesian dairy cattle, this is similar to present study.

The estimate of correlation between udder and teat type traits is presented in Table 4.30 and 4.34 for Sahiwal and Karan Fries cattle, respectively. Distance between fore and rear teat was found to be significantly ($p<0.05$) correlated with udder length (0.23) and width (0.26) in Sahiwal cows. In Karan Fries cattle shortest distance of floor from teat was found to be highly significantly ($p<0.01$) correlated with udder length, width and circumference and significantly ($p<0.05$) correlated with udder width. Distance between teat had significant ($P<0.05$, $P<0.01$) correlation with udder length and width. Teat diameter had positive and significant correlation with rear udder width and udder width while teat circumference had positive and highly significant ($p<0.01$) correlation with udder balance. A non significant phenotypic correlation between udder (udder length, udder depth) and teat traits (teat length, teat diameter) was reported by Mingoas *et al*. (2017).

The estimate of correlation among udder type traits are presented in Table 4.31 and 4.35 for Sahiwal and Karan Fries cows, respectively. In the present study rear udder height was highly significantly ($p<0.01$) correlated with rear udder width and udder width in Sahiwal cows. Udder length was positively and significantly correlated with udder width and circumference. Udder depth had significant ($p<0.05$) correlation with fore udder attachment, central ligament and udder balance. Udder width had positive and significant ($p<0.05$) correlation with udder circumference. In Karan Fries cows there was negative and significant correlation of udder depth with udder length (-0.18), width (-0.22), circumference (-0.21) and central ligament (-0.20). Fore udder length has positive and significant ($p<0.05$) correlation with udder circumference (0.15). Udder length had significant correlation with udder width (0.44) and udder circumference (0.30). Central ligament has positive and significant ($p<0.01$) correlation rear udder height (0.22) and udder circumference (0.23). A positive and significant ($p<0.01$) was also present between udder circumference and udder width in Karan Fries cows.

Table 4.29 Pearson correlation between production traits and udder and teat type traits in Sahiwal cows

Traits	305DMY	MMY	TDMY	TMY
FUA	0.06	-0.06	-0.11	0.18*
RUW	0.18*	0.30**	0.09	0.16
RUH	0.18*	0.34**	0.10	0.19*
UB	0.03	-0.02	0.01	0.09
UD	0.15	0.05	0.01	0.13
UL	-0.02	0.27*	0.33**	0.01
UW	-0.03	0.10	0.30**	-0.13
UC	0.16	0.30**	0.42**	0.18*
CL	0.18*	0.10	0.02	0.19*
TC	0.15	0.06	0.07	0.16
RTL	0.23*	0.04	0.13	0.19*
FTL	0.22*	0.06	0.14	0.19*
DFR	0.05	0.13	0.19*	-0.01
DLR	0.06	0.04	-0.09	0.02
SDF	-0.14	-0.07	0.06	-0.16
SDR	-0.11	-0.11	0.03	-0.13
TD	0.04	-0.01	0.05	0.02

***** Significant at 5 % level of significance; ****** Significant at 1 % level of significance

Table 4.30 Pearson correlation between udder type traits and teat type traits in Sahiwal cows

Traits	FUA	RUW	RUH	UB	UD	UL	UW	UC	CL
TC	0.03	-0.06	-0.06	0.12	-0.08	-0.04	-0.04	-0.15	0.08
FTL	-0.07	-0.02	0.00	0.17	0.08	-0.01	-0.07	0.02	-0.05
RTL	-0.15	-0.07	-0.06	0.15	0.11	-0.09	-0.11	-0.10	-0.03
DFR	-0.13	0.04	0.03	-0.06	0.02	0.23*	0.26*	0.01	0.06
DLR	-0.05	-0.01	-0.02	0.04	0.01	0.08	0.12	-0.05	0.19
SDF	0.04	-0.03	-0.07	0.03	0.04	-0.10	0.04	0.10	-0.01
SDR	-0.02	0.04	0.00	0.00	0.10	-0.19	-0.06	0.02	0.01
TD	-0.04	-0.20	-0.17	0.13	0.02	0.08	0.00	0.02	0.16

***** Significant at 5 % level of significance

Table 4.31 Pearson correlation among udder type traits in Sahiwal cows

Traits	FUA	RUH	RUW	UD	UB	UL	UW	UC	CL
FUA	1								
RUH	0.15	1							
RUW	0.13	0.99**	1						
UD	0.21*	0.14	0.14	1					
UB	0.06	-0.10	-0.14	0.25*	1				
UL	0.01	0.04	-0.18	-0.12	0.06	1			
UW	-0.07	-0.23*	-0.20	-0.17	-0.06	0.85**	1		
UC	0.08	-0.08	-0.11	-0.14	0.08	0.43*	0.30*	1	
CL	0.09	0.02	0.01	0.24*	0.19	0.01	-0.09	0.15	1

***** Significant at 5 % level of significance; ****** Significant at 1 % level of significance

Table 4.32 Pearson correlation among teat type traits in Sahiwal cows

Traits	TC	RTL	FTL	DFR	DLR	SDF	SDR	TD
TC	1							
RTL	0.02	1						
FTL	0.08	0.96**	1					
DFR	-0.12	-0.01	-0.02	1				
DLR	-0.14	-0.06	-0.07	0.73**	1			
SDF	0.06	0.03	0.02	-0.56**	-0.65**	1		
SDR	0.02	-0.01	-0.03	-0.51**	-0.68**	0.91**	1	
TD	0.03	0.04	0.04	-0.13	0.04	-0.24*	-0.34**	1

***** Significant at 5 % level of significance; ****** Significant at 1 % level of significance

Table 4.33 Pearson correlation between production traits and udder and teat type traits in Karan Fries cows

Traits	305DMY	MMY	TDMY	TMY
FUA	0.11	0.18*	0.19*	0.10
RUH	0.32**	-0.01	-0.02	0.34**
RUW	-0.23**	-0.13	-0.07	-0.21**
UD	-0.30**	-0.24**	0.197*	-0.31**
UB	0.07	0.04	-0.01	0.05
UL	0.27**	0.25**	0.21**	0.28**
UW	0.07	0.17*	0.14	0.07
UC	0.21**	0.34**	0.34**	0.21**
CL	0.39*	0.27**	0.26**	0.38
TC	0.05	0.15	0.09	0.05
RTL	0.06	-0.02	-0.05	0.06
FTL	-0.07	-0.04	-0.06	-0.08
DFR	0.16*	0.01	-0.02	0.16*
DLR	0.12	0.00	-0.03	0.12
SDF	-0.11	-0.12	-0.07	-0.11
SDR	-0.01	-0.01	-0.05	-0.01
TD	0.03	0.01	-0.03	0.03

***** Significant at 5 % level of significance; ****** Significant at 1 % level of significance

Table 4.34 Pearson correlation between udder type traits and teat type traits in Karan Fries cows

Traits	FUA	RUW	RUH	UB	UD	UL	UW	UC	CL
TC	0.04	0.00	0.00	0.21**	0.07	0.01	0.09	0.05	0.00
RTL	0.07	0.15	0.02	0.06	-0.06	0.10	0.11	0.00	-0.05
FTL	0.08	0.08	-0.11	0.03	-0.02	-0.03	0.15	-0.02	0.02
DFR	0.02	0.08	0.02	0.07	-0.03	0.18*	0.20**	0.13	0.01
DLR	0.01	0.04	0.08	0.02	-0.07	0.23**	0.41**	0.12	0.02
SDF	0.07	0.03	-0.03	0.04	0.39*	-0.33**	-0.36**	-0.21**	-0.19*
SDR	0.04	0.13	-0.02	0.00	0.31*	-0.33**	-0.54**	0.25**	-0.14
TD	-0.02	0.19*	0.03	0.15	-0.08	0.14	0.21**	0.05	0.04

*Significant at 5 % level of significance; **Significant at 1 % level of significance

Table 4.35 Pearson correlation among udder type traits in Karan Fries cows

Traits	FUA	RUH	RUW	UD	UB	UL	UW	UC	CL
FUA	1								
RUH	-0.04	1							
RUW	0.00	0.06	1						
UD	0.09	-0.04	0.00	1					
UB	0.09	-0.06	0.07	0.09	1				
UL	0.06	-0.06	0.07	-0.18*	0.06	1			
UW	-0.04	-0.06	0.00	-0.22**	0.04	0.44**	1		
UC	0.15*	-0.11	0.10	-0.21**	0.01	0.30**	0.22**	1	
CL	0.12	0.22**	-0.02	-0.20**	0.05	0.04	0.05	0.23**	1

***** Significant at 5 % level of significance; ******Significant at 1 % level of significance

Table 4.36 Pearson correlation among teat type traits in Karan Fries cows

Traits	TC	RTL	FTL	DFR	DLR	SDF	SDR	TD
TC	1							
RTL	0.12	1						
FTL	0.06	0.53**	1					
DFR	-0.01	-0.01	0.01	1				
DLR	0.12	0.02	0.04	0.70**	1			
SDF	-0.13	-0.16*	-0.05	-0.18*	-0.20*	1		
SDR	-0.05	-0.09	-0.09	-0.24**	-0.35**	0.75**	1	
TD	0.44**	0.30**	0.27**	0.10	0.12	-0.09	-0.06	1

***** Significant at 5 % level of significance; ******Significant at 1 % level of significance

Pearson correlation among teat type traits are presented in Table 4.32 and 4.36 in Sahiwal and Karan Fries cows, respectively. In Sahiwal cows negative significant ($p<0.01$) correlation was observed distance between floor from teat with distance between teat and teat diameter. Positive and highly significant ($p<0.01$) correlation was present between fore and rear teat length (0.96), distance between left-right and fore-rear teat (0.73) and shortest distance from floor of rear teat and fore teat (0.91) in Sahiwal cows.

Similarly, in Karan Fries cows also negative significant ($p<0.01$) correlation was found distance between floor from teat with distance between teat whereas, positive and significant ($p<0.01$) correlation of teat diameter with teat circumference and teat length. In Karan Fries cows also positive and highly significant ($p<0.01$) correlation was present between fore and rear teat length (0.53), distance between left-right and fore-rear teat (0.70) and shortest distance from floor of rear teat and fore teat (0.75). Mingoas *et al*. (2017) reported in zebu cow's positive correlation between udder diameter and height with length and diameter of teats.

4.15 Point biserial correlation between incidence of clinical mastitis, milk production and udder type taits in Sahiwal and Karan Fries cows

Correlation between categorical trait i.e. incidence of clinical mastitis with other continuous traits that is production traits and udder type traits had been assessed through point biserial correlation using SPSS 22.0 Version. The value of their estimation is shown in Table 4.37 and 4.38 for Sahiwal and Karan Fries cows, respectively.

4.15.1 Correlation between incidence of clinical mastitis and milk production

In present study a positive and significant correlation of clinical mastitis was found with total milk yield and 305 days milk yield in both the breeds. This suggests that high yielders are more prone to mastitis. A low correlation of clinical mastitis was found with monthly milk yield and test day milk yield. In Sahiwal a negative correlation was present between monthly milk yield and clinical mastitis in the study which might suggest lowering of the quantity of milk due to infection in that particular period. Nakov *et al*. (2014) also reported that cows with clinical mastitis, before contracting the disease yielded more milk than the healthy cows. The difference between milk yield of the healthy cows and the mastitic cows after clinical mastitis was significantly differ.

4.15.2 Correlation between incidence of clinical mastitis and udder/teat type traits

In present study Sahiwal had negative and highly significant ($p<0.01$) correlation of clinical mastitis with fore udder attachment ($r_{p,}$-0.26) and central ligament ($r_{p,}$ -0.23). This suggest that incidence of clinical mastitis was reduced by strong fore udder attachment. Distance between teat and shortest distance of floor from front teat was also positively significantly correlated with clinical mastitis. In Karan Fries udder depth, teat circumference and teat length was positively significantly ($p<0.05$) correlated with clinical mastitis. Haghkhah *et al.* (2011) found that subclinical mastitis occurrence was highest for the cows with long and thick teats. Few study have also showed higher value of somatic cell score for shorter teats (Nemcova *et al.,* 2007). Bharti *et al.* (2015) reported that increase in teat length and diameter was found significantly associated with the degree of intra-mammary infection with somatic cell count.

Table 4.37 Point biserial correlation between mastitis, production and udder type traits in Sahiwal and Karan Fries cows

Traits		Sahiwal		Karan Fries	
		No. of obs.	Mastitis, r_{pb}	No. of obs.	Mastitis, r_{pb}
Production Traits	Monthly Milk Yield	87	-0.09	166	0.05
	Test day Milk Yield	87	0.02	166	0.02
	Total Milk Yield	87	0.32**	166	0.19*
	305 Days Milk Yield	87	0.33**	166	0.17*
Udder Type Traits	Fore Udder Attachment	87	-0.26**	166	-0.07
	Rear Udder Height	87	0.11	166	-0.01
	Rear Udder Width	87	-0.04	166	-0.06
	Udder Depth	87	0.09	166	0.16*
	Udder Balance	87	-0.06	166	0.08
	Udder Length	87	0.04	166	0.09
	Udder Width	87	0.16	166	0.03
	Udder Circumference	87	0.04	166	0.05
	Central Ligament	87	-0.23**	166	0.03
Teat Type Traits	Teat Circumference	87	0.09	166	0.18*
	Rear Teat Length	87	0.08	166	0.17*
	Front Teat Length	87	0.09	166	0.19*
	Distance between fore and rear teat	87	0.29**	166	-0.08
	Distance between left and right teat	87	0.23*	166	0.02
	Shortest distance of floor from front teat	87	0.21*	166	-0.08
	Shortest distance of floor from rear teat	87	-0.15	166	0.08
	Teat Diameter	87	0.1	166	0.09

*Significant at 5 % level of significance; **Significant at 1 % level of significance

Table 4.38 Association of visual traits with mastitis through chi-square in Sahiwal and Karan Fries cows

Traits	Sahiwal		Karan Fries	
	d.f	χ2 value	d.f	χ2 value
Udder shape	2	2.61	2	0.89
Udder suspension	2	5.72	2	0.82
Fore teat placement	2	6.88*	2	2.82
Rear teat placement	2	2.59	2	0.90
Teat size	2	6.04*	2	6.53*
Teat end shape	2	9.50**	2	6.43*
Teat shape	2	6.89*	2	1.70
Skin condition	1	0.11	2	6.98*
Long-term Changes In Teat-end Condition	3	0.50	3	11.04*

*Significant at 5 % level of significance; **Significant at 1 % level of significance

4.16 Association of visual traits with incidence of clinical mastitis

The effect of both categorical traits i.e. effect of visual observation on incidence of clinical mastitis has been done using chi-square (χ2) analysis and results are presented in Table 4.38. In the present study significant association of clinical mastitis was found with fore teat placement, teat size, teat end shape and teat shape in Sahiwal cattle. In Karan Fries cows clinical mastitis has been significantly ($p<0.05$) associated with teat size, teat end shape, skin condition and long term changes in teat end condition (hyperkeratosis).

Uzmay *et al.* (2003) reported the risk of sub-clinical mastitis was highest for the cows with long, thick and cylindrical teats. Patel (2014) and Kamboj *et al.* (2008) also reported the highest incidence of subclinical mastitis in cows having cylindrical teats. Bottle shaped teats and less distance of anterior and posterior teat from ground are associated with Somatic cell score (SCC) in Sahiwal cattle (Ahlawat, 2007). Singh *et al*. (2013) also found decreasing teat-end to floor distance is a risk factor for clinical mastitis. They also found strong association between teat end shape and clinical mastitis. The cows having flat teat or round teat ends were found 7.6 or 3.2 times more likely to have mastitis than the cows having pointed teat ends. Bharti *et al*. (2015) found significant association of somatic cell count with teat length, teat end shape and teat diameter in Jersey cross breed.

Juozaitiene *et al*. (2019) reported that the number of clinical mastitis cases were higher for the udder quarters having very high hyperkeratosis scores. Similar to the present study they also found increased risks of mastitis in dairy herds associated with different levels of teat hyperkeratosis. Contrary to the present study no statistical significance between teat shape/teat tip shape and SCC was found by Okano *et al*. (2015). A significant relationship between teat dimensions, teat anatomical characteristics, hyperkeratosis and subclinical mastitis was reported by Guarin *et al*. (2017).

4.17 Association of identified genetic variants with production, incidence of clinical mastitis and udder type traits in Sahiwal and Karan Fries cows

In the present study genetic polymorphism (SNPs) in candidate genes were identified. Through, PCR-RFLP technique genotypes of animals were obtained. Relationship of each SNP locus with the production traits and udder type traits in Sahiwal and Karan Fries cows was analyzed using GLM procedure. For relationship of SNP locus with the incidence of mastitis was done through Chi-square (χ^2) analysis.

4.17.1 Association of genetic variants of FAM19A1 gene with production traits, udder and teat type traits in Sahiwal and Karan Fries cows

In the present study total seven SNPs viz., T33069832C, C33219027T, C33464963A, C33471720T, C33471980A, G33571161A, and G33589765A were found in FAM19A1 gene of Sahiwal and Karan Fries cows. Out of these seven SNPs two SNPs i.e. T33069832C, C33219027T were present in intron 1, three SNPs i.e.C33464963A, C33471720T, C33471980A in intron 2, SNP G33571161A in intron 3 and G33589765A SNP in 3’ UTR region. SNP C33219027T and C33471980A in Sahiwal cow (Table 4.39) while SNPs C33219027T, C33471980A G33571161A, and G33589765A were found to have significant effect on the production traits in Karan Fries cattle (Table 4.40). However, in Sahiwal cows all SNPs except G33571161A and G33589765A were significantly associated with any of the udder/teat traits (Table 4.43) whereas in Karan Fries all the SNPs have association with any of the studied udder/teat traits (Table 4.44)

Table 4.39 Effect of identified genetic variants on production traits in Sahiwal cows

Gene	SNPs	305DMY	MMY	TDMY	TMY
FAM19A1	**T33069832C** ***BsrI***	517414.26	7234.63	18.13	629418.30
	C33219027T ***TaqI***	591261.56	72103.44**	43.08*	209252.47
	C33464963A ***AluI***	156699.87	5682.97	13.78	402452.56
	C33471720T ***HinfI***	663704.41	6465.43	1.22	945588.69
	C33471980A ***HindIII***	1110110.49	490.28	81.82*	823952.75
	G33571161A ***TaqI***	205408.37	10175.44	18.39	45711.97
	G33589765A ***TaqI***	1447793.72	20055.89	24.82	1844867.18
KCNB1	**G78216220A** ***MspI***	1858727.022	26427.849	45.16*	2667393.22
	A78216335G ***BspHI***	3546.76	7835.00	0.09	1.93
EDN3	**C57571910A** ***TspRI***	657782.51	13149.17	16.61	586712.75

*Significant at 5% level of significance; ** Significant at 1% level of significance

BsrI was used as restriction enzyme for PCR-RFLP analysis of SNP T33069832C, and three genotypes were obtained i.e. TT (508 bp), TC (508, 260 and 248 bp) and CC (260 and 248 bp) in both the breeds. This SNP did not have significant effect on any of the studied milk production traits in both Sahiwal and Karan Fries cows. SNP T33069832C was found to be significantly ($p<0.05$) associated with teat circumference in both Sahiwal and Karan Fries cattle. In Sahiwal cows, TT genotypes ($11.76^{b}\pm1.41$ cm) having more teat circumference than TC ($7.24^{a}\pm1.04$ cm) and CC genotypes ($8.49^{ab}\pm0.72$ cm) (Table 4.49). Whereas, in Karan

Fries cows heterozygous TC animals were having thicker teat (7.75^{b}±0.25 cm) compared to TC (7.67^{b}±0.15 cm) and CC (6.89^{a}±0.26 cm) genotypes (Table 4.50).

Table 4.40 Effect of identified genetic variants on production traits in Karan Fries cows

Gene	SNPs	305DMY	MMY	TDMY	TMY
FAM19A1	**T33069832C *BsrI***	2218063.28	19079.60	34.91	2215094.12
	C33219027T *TaqI*	978012.30	22209.72	73.06*	751807.27
	C33464963A *AluI*	4862304.78	3937.53	21.57	6063215.38
	C33471720T *HinfI*	3490061.11	18614.60	23.84	3889283.32
	C33471980A *HindIII*	13502454.25*	9483.26	20.49	10355259.42*
	G33571161A *TaqI*	23704752.22**	12294.21	14.65	24186533.77**
	G33589765A *TaqI*	817444.81	65550.19*	46.77	255842.99
KCNB1	**G78216220A *MspI***	367253.99	24504.91	16.25	726150.88
	A78216335G *BspHI*	154418.17	40754.56	125.17*	61659.15

*Significant at 5% level of significance; **Significant at 1% level of significance

For SNP C33219027T, *TaqI* enzyme was used for digestion of product and three genotypes viz., CC (438 and 138 bp), CT (576, 438 and 138 bp) and TT (576 bp) were obtained in both Sahiwal and Karan Fries cattle. In Sahiwal cattle, it has highly significant ($p<0.01$) effect for monthly milk yield while significant ($p<0.05$) effect for test day milk yield in both the breeds. Monthly milk yield (277.56^{b}±31.99 Kg) and test day milk yield (9.66^{b}±1.02 Kg) were higher for animals with homozygous TT genotype (Table 4.41) in Sahiwal cow while heterozygous animals with CT genotype produced more test day milk yield (14.30±0.74 Kg) than either of the homozygotes in Karan Fries cows (Table 4.42). SNP C33219027T had significant ($p<0.01$) effect on udder circumference in Sahiwal cows while in Karan Fries cows it had significant effect on rear udder height, udder width and shortest distance of teat from floor.

Table 4.41 Association of genetic variants of FAM19A1 gene with production traits in Sahiwal cows

SNPs	Genotypes	305DMY (Kg)	TMY(Kg)	MMY (Kg)	TDMY (Kg)
T33069832C *BsrI* (FAM19A1)	TT(13)	2176.94±270.87	2079.07±331.86	224.57±37.13	9.39±1.15
	TC(24)	1848.06±199.36	1696.04±244.24	185.41±27.32	7.31±0.84
	CC(50)	1887.62±138.12	1862.54±169.21	207.68±18.93	8.02±0.58
C33219027T *TaqI* (FAM19A1)	CC(41)	1961.58±152.38	1859.22±187.52	207.36^{ab}±19.98	7.07^{a}±0.63
	TC(30)	1772.59±178.14	1770.85±219.22	160.35^{a}±23.36	8.47^{ab}±0.74
	TT(16)	2089.51±243.94	1969.15±300.18	277.56^{b}±31.99	9.66^{b}±1.02
C33464963A *AluI* (FAM19A1)	CC(54)	1965.31±132.73	1887.18±162.57	200.91±18.18	7.99±0.56
	CA(33)	1845.70±169.79	1786.42±207.96	209.21±23.26	8.09±0.72
C33471720T *HinfI* (FAM19A1)	CC(13)	2010.93±270.38	1983.50±330.99	185.26±37.14	7.95±1.16
	CT(30)	2058.23±177.98	2004.21±217.88	219.58±24.45	8.26±0.76
	TT(44)	1798.77±146.96	1703.36±179.91	199.03±20.19	7.90±0.63
C33471980A *HindIII* (FAM19A1)	CC(71)	1866.32±115.17	1802.76±141.42	205.19±15.86	7.57^{a}±0.48
	CA(16)	2157.89±242.61	2053.96±297.91	199.06±33.42	10.07^{b}±1.01
G33571161A *TaqI* (FAM19A1)	GG(10)	1878.94±310.04	1764.00±380.21	176.45±42.25	7.82±1.31
	GA(56)	1968.95±131.01	1866.92±160.67	200.08±17.85	7.64±0.55
	AA(21)	1808.78±213.95	1841.52±262.37	227.81±29.15	9.18±0.90
G33589765A *TaqI* (FAM19A1)	GG(50)	1836.89±136.50	1777.23±167.50	186.32±18.77	7.38±0.58
	GA(25)	1867.73±193.05	1745.86±236.88	221.70±26.54	8.92±0.82
	AA(12)	2374.78±278.64	2362.66±341.90	241.25±38.31	8.88±1.19
G78216220A *MspI* (KCNB1)	GG(20)	2227.21±214.70	2210.87±262.98	247.75±29.55	9.81^{b}±0.91
	GA(29)	1985.54±178.30	1935.37±218.39	183.00±24.54	7.55^{a}±0.75
	AA(38)	1708.16±155.76	1592.53±190.79	197.14±21.43	7.46^{a}±0.66
A78216335G *BspHI* (KCNB1)	AA(44)	1913.63±147.30	1848.81±180.26	194.68±20.10	8.00±0.63
	AG(43)	1926.40±149.01	1849.11±182.34	213.66±20.34	8.06±0.63
C57571910A *TspRI* (EDN3)	CC(36)	1816.45±162.19	1751.22±198.80	189.43±22.19	7.51±0.69
	CA(51)	1992.99±136.27	1917.96±167.02	214.39±18.64	8.40±0.58

Figures in parenthesis are number of animals;

Figures with dissimilar superscript differ significantly

Table 4.42 Association of genetic variants of FAM19A1 gene with production traits in Karan Fries cows

SNPs	Genotypes	305DMY (Kg)	TMY (Kg)	MMY (Kg)	TDMY (Kg)
T33069832C *BsrI* (FAM19A1)	TT(99)	4264.74±181.55	4206.20±185.16	372.02±14.97	12.78±0.49
	TC(35)	3975.85±305.35	3952.31±311.41	393.93±25.18	14.32±0.82
	CC(32)	3892.31±319.34	3812.81±325.68	346.18±26.34	12.62±0.86
C33219027T *TaqI* (FAM19A1)	CC(88)	4111.19±193.01	4099.84±196.91	369.35±15.87	13.10^{ab}±0.51
	TC(42)	4299.09±279.39	4175.11±285.02	396.10±22.97	14.30^{b}±0.74
	TT(36)	3988.12±301.78	3905.94±307.86	348.77±24.81	11.56^{a}±0.80
C33464963A *AluI* (FAM19A1)	CC(139)	4207.47±1806.28	4161.06±1840.56	373.80±149.94	13.23±4.82
	AC(27)	3743.72±1767.79	3643.20±1798.66	360.61±145.62	12.25±5.18
C33471720T *HinfI* (FAM19A1)	CC(28)	3710.86 ± 340.57	3642.26±347.11	364.90±28.16	12.35±0.92
	CT(61)	4313.39±230.74	4278.893±235.17	391.10±19.08	13.74±0.62
	TT (77)	4141.52±205.37	4074.79±209.31	358.72±16.98	12.80±0.55
C33471980A *HindIII* (FAM19A1)	CC(112)	4330.07^{b}±168.71	4250.26^{b}±172.62	376.91±14.09	13.32±0.46
	CA(54)	3721.30^{a}±242.98	3717.13^{a}±248.60	360.77±20.29	12.57±0.66
G33571161A *TaqI* (FAM19A1)	GG(123)	4355.47^{b}±159.41	4302.52^{b}±162.62	376.75±13.44	13.25±0.44
	GA(43)	3492.92^{a}±269.62	3431.25^{a}±275.04	357.10±22.73	12.57±0.74
G33589765A *TaqI* (FAM19A1)	GG(104)	4171.20±177.60	4115.66±181.29	392.91^{b}±14.42	13.63±0.47
	GA(25)	4236.15±362.24	4058.52±369.76	347.33^{ab}±29.41	12.49±0.97
	AA(37)	3951.62±297.76	3980.06±303.94	328.37^{a}±24.18	11.88±0.79
G78216220A *MspI* (KCNB1)	GG(24)	4018.156±370.02	3937.97±377.07	356.56±30.37	12.83±.99
	GA(76)	4103.68±207.93	4025.65±211.89	358.06±17.06	12.68±0.56
	AA(66)	4206.11±223.13	4186.27±227.38	392.80±18.31	13.61±0.60
A78216335G *BspHI* (KCNB1)	AA(65)	4170.06±224.27	4100.86±228.71	352.13±18.42	11.99^{a}±0.59
	AG(101)	4107.57±179.92	4061.37±183.47	384.23±14.77	13.77^{b}±0.48

Figures in parenthesis are number of animals;
Figures with dissimilar superscript differ significantly

In Sahiwal cows both the homozygotes were significantly different from each other Table 4.45) with homozygous TT having wider udder circumference (148.07±2.63 cm) than the other genotypes. CC genotype was found to have higher rear udder height (24.28±0.55 cm) than the other genotypes while heterozygous CT genotype was having wider udder (Table 4.46) in Karan Fries cows. In case of both rear and fore teat in Karan Fries cattle CC genotype was having shorter distance of teat from floor (Table 4.50).

HindIII enzyme was used for PCR-RFLP analysis of SNP C33471980A and showed two genotypes i.e. CC (431and 61 bp) and CA (431, 61 and 492 bp) in both Sahiwal and Karan Fries cattle. It had significant ($p<0.05$) effect on test day milk yield in Sahiwal cattle (Table 4.39) and on 305 days milk yield and total milk yield in Karan Fries cattle (Table 4.40). Test day milk yield was higher for animals with CA genotype having 10.07^{b}±1.01 kg while CC genotypes animals were having 7.57^{a}±0.48 kg test day milk in Sahiwal cow (Table 4.41). However, CC genotypes animal produced more 305 days milk yield (4330.07^{b}±168.71 kg) and total milk yield (4250.26^{b}±172.62 kg) than CA genotypes in Karan Fries cows (Table 4.42). For udder type traits this SNP had significant ($p<0.05$) effect on udder width, length, teat length and highly significant ($p<0.01$) effect on udder circumference in Sahiwal cows (Table 4.43).

In Karan Fries cows it had significant ($p<0.05$) effect on udder depth and distance between fore and rear teat (Table 4.44). In Sahiwal cow animal with CA genotype were having udder, wider (55.26^{b}±1.53 cm), longer (53.62^{b}±1.51 cm) and with more circumference (147.97^{b}±2.66 cm) than the CC genotype animals (Table 4.45). In case of teat measurement, fore (9.94^{b}±1.46 cm) and rear (9.46^{b}±1.61 cm) teats were also found larger in CA genotype than the CC genotype animals in Sahiwal cows (Table 4.49). Similarly, in Karan Fries cows CA genotypes having deeper udder (55.76^{b}±0.80 cm) (Table 4.46) and more distance between fore and rear teat (7.48^{b}±0.52 cm) than the CC genotype (Table 4.50).

For SNP C33464963A, *AluI* restriction enzyme was used for PCR-RFLP analysis and after digestion two genotypes i.e. CC (369 and 140 bp) and CA (509, 369 and 140 bp) were obtained in both the breeds. SNP C33464963A did not have significant effect on any of the studied milk production traits in both Sahiwal and Karan Fries cows. However, It had significant ($p<0.05$) effect on udder balance, teat circumference and distance between teat in Sahiwal cattle while, in Karan Fries cattle only significant effect ($p<0.05$) were found in front teat length.

In heterozygous animal there was more difference between fore and rear udder (-3.03^{b}±0.50 cm) than CC genotype in Sahiwal cattle (Table 4.45) and Animals with CC genotypes were having thicker teat (9.43^{b}±0.70 cm) than CA genotype (7.33^{a}±0.89 cm). In Sahiwal cattle, CA genotype animals were having more distance between fore- rear teat (7.40^{b}±0.88 cm) and left-right teat (8.59^{b}±0.93 cm) (Table 4.49) and these animals were also having longer fore teat (5.82^{b}±0.28 cm) as compared with CC genotype animals in Karan Fries cows (Table 4.50).

HinfI restriction enzyme was used for PCR-RFLP analysis of SNP C33471720T for FAM19A1 gene revealed three types of genotypes i.e. CC (322 and 170 bp), CT (492, 322 and 170 bp) and TT (492 bp) for Sahiwal and Karan Fries cattle. This SNP did not show any significant effect on production traits in Sahiwal and Karan Fries cattle. However, It had highly significant ($p<0.01$) effect on rear udder height, rear udder width, distance between left-right teat, shortest distance of rear teat from floor while significant ($p<0.05$) effect on teat circumference in Sahiwal cattle. CT genotype was having higher rear udder height (24.03^{b}±1.05 cm) and width (24.21^{b}±1.07 cm) as compared to both homozygotes in Sahiwal cattle (Table 4.45).

For teat circumference and for distance between left-right teat traits CC genotype was having highest dimension than other genotype while in TT genotype animals rear teat were having more distance from ground (44.24^{b}±0.90 cm) in Sahiwal cow (Table 4.49). SNP C33471720T in Karan Fries cattle had significant ($p<0.05$) effect on udder balance, udder width and central ligament (udder cleft). CT genotype of SNP C33471720T in Karan Fries cattle had wider udder (73.66^{b}±1.11 cm), larger cleft (3.56^{b}±0.18 cm) and less difference was found between fore and rear udder (-0.38^{a}±0.71 cm) than other homozygous (CC and TT) genotypes (Table 4.46).

Strillacci *et al.* (2014) also reported this SNP (rs110064285) to be associated with udder depth in Valdostana Red Pied cattle which is similar to present finding in Karan Fries cattle.

TaqI was used as restriction enzyme for PCR-RFLP analysis of SNP G33571161A, and three genotypes i.e. GG (329 and 200 bp), GA (529, 329 and 200 bp) and AA (529 bp) were obtained in Sahiwal cattle, whereas, only two genotypes i.e. GG (329 and 200 bp) and GA (529, 329 and 200 bp) in Karan Fries cattle. This SNP has highly significant ($p<0.01$) effect on 305milk yield and total milk yield in Karan Fries cattle (Table 4.40) while no any significant effect was found for production trait (Table 4.39) and udder type trait in Sahiwal cattle (Table 4.43). In Karan Fries cattle, GG genotype produced significantly higher 305milk yield (4355.47^{b}±159.41 kg) and total milk yield (4302.52^{b}±162.62 kg) than GA genotype (Table 4.42). For udder type traits this SNP has significant effect ($p<0.05$) on udder circumference and distance between fore and rear teat in Karan Fries cattle (Table 4.44). GG genotype has significantly higher circumference of udder (146.66^{b}±2.26 cm) than GA genotype (135.24^{a}±3.83 cm) (Table 4.46). GG genotype animals were also having significantly more distance between fore and rear teat (7.12^{b}±0.34 cm) than GA genotype (5.68^{a}±0.58 cm) in Karan Fries cattle (Table 4.50).

For SNP G33589765A, TaqI restriction enzyme was used for PCR-RFLP analysis and after digestion three different genotypes i.e. GG (209 and 159 bp), GA (368, 209 and 159 bp) and AA (368 bp) genotypes were found in both breeds. This SNP G33589765A also did not have any significant effect on production and udder type trait in Sahiwal cattle. However, in Karan Fries cattle it has significant ($p<0.05$) effect on monthly milk yield and udder width. GG genotype was having significantly higher monthly milk yield (392.91^{b}±14.42 kg) than AA genotype (328.37^{a}±24.18 Kg) (Table 4.42). Similarly, Udder width of GG genotype animals were significantly higher (72.78^{b}±0.85 cm) than GA genotype (69.05^{a}±1.73 cm) and AA genotype (69.16^{a}±1.42 cm) in Karan Fries cattle (Table 4.46).

Strillacci *et al.* (2014) also found SNPs rs135018045 and rs133223316 to be associated with udder type trait. In present study both the breeds were monomorphic for these SNPs. There are no earlier reports available to compare or contrast present finding about other SNPs in FAM 19A1 gene.

4.17.2 Association of genetic variants of KCNB1 gene with production traits, udder and teat type traits in Sahiwal and Karan Fries cows

Two SNPs G78216220A and A78216335G were found in intron 1 of KCNB1 gene in a product of 505 bp. *MspI* enzyme was used for PCR-RFLP analysis of SNP G78216220A and gave three genotypes i.e. GG (280 and 225 bp), GA (505, 280 and 225 bp) and AA (505 bp) in both Sahiwal and Karan Fries cows. This SNP has significant ($p<0.05$) effect on test day milk yield in Sahiwal cattle (Table 4.39) whereas it did not have any significant effect on production traits in Karan Fries cattle (Table 4.40). GG genotype (9.81^{b}±0.91) has significantly higher test day milk yield than GA genotype (7.55^{a}±0.75) and AA genotype (7.46^{a}±0.66) in Sahiwal cattle (Table 4.41). SNP G78216220A has significant ($p<0.05$) effect on teat diameter and distance between fore and rear teat and highly significant ($p<0.01$) effect on udder circumference (Table 4.43).

GG genotype was having significantly higher udder circumference ($148.07^{b}\pm2.35$ cm) than AA genotype ($139.57^{a}\pm1.70$ cm) animals (Table 4.47). Teat diameter was also higher for GG genotype ($28.25^{b}\pm1.10$ cm) than GA ($24.91^{a}\pm0.91$ cm) and AA ($25.83^{ab}\pm0.79$ cm) genotypes while distance between teat was significantly larger for GA genotype ($7.82^{b}\pm0.93$ cm) than GG ($4.76^{a}\pm1.12$ cm) and AA ($5.46^{a}\pm0.81$) genotypes in Sahiwal cow (Table 4.51). In Karan Fries cattle, it has highly significant ($p<0.01$) effect on udder length and width and significant ($p<0.05$) effect on shortest distance of rear teat from floor (Table 4.44). GG genotype animals were having significantly larger ($61.52^{b}\pm1.41$ cm) and wider ($71.07^{ab}\pm1.74$ cm) udder than GA genotype but comparable to AA genotype (Table 4.48). GA genotype was having value larger distance of floor from rear udder (Table 4.52) in Karan Fries cattle.

This association in Karan Fries cattle is similar to the findings of Strillacci *et al.* (2014) who also reported this SNP (rs41710487/ G78216220A) having association with milk yield, udder attachment, udder depth, udder height, udder width in Valdostana Red Pied cattle.

BspHI restriction enzyme was used for PCR-RFLP analysis of SNP A78216335G and two genotypes viz., AA (396 and 109 bp) and AG (505, 396 and 109 bp) were obtained in both the breed. SNP A78216335G has significant ($p<0.05$) effect on test day milk yield in Karan Fries cattle (Table 4.40) while it did not have any significant effect on production traits in Sahiwal cattle (Table 4.39). AG genotype ($13.77^{b}\pm0.48$ cm) was superior to AA genotypes ($11.99^{a}\pm0.59$ cm) in terms of higher test day milk production in Karan Fries cattle (Table 4.42). In Sahiwal cattle it was having highly significant ($p<0.05$) effect on rear udder height and rear udder width (Table 4.43). Heterozygous AG genotype was having significantly higher rear udder height ($25.03^{b}\pm0.85$ cm) and width ($25.41^{b}\pm0.85$ cm) as compared to AA genotype (Table 4.47). In Karan Fries cattle it has significant ($p<0.05$) effect on rear udder height, rear udder width, udder circumference and shortest distance of fore teat from floor (Table 4.44).

Whereas, in Karan Fries cattle AA genotype was having significantly higher rear udder height ($27.12^{b}\pm0.54$ cm) and width ($13.18^{b}\pm0.32$ cm) as compared to AG genotype but for circumference GA genotype ($146.27^{b}\pm2.48$ cm) was having significantly larger udder circumference than AA genotype ($137.25^{a}\pm3.09$ cm) (Table 4.48). Similarly, AA genotypes have fore teat closer to ground ($45.70^{a}\pm0.67$ cm) than AG genotype ($47.71^{b}\pm0.54$ cm) in

Table 4.43 Effect of identified genetic variants on udder and teat type traits in Sahiwal cows

Traits	T33069832C *BsrI*	C33219027T *TaqI*	C33464963A *AluI*	C33471720T *HinfI*	C33471980A *HindIII*	G33571161A *TaqI*	G33589765A *TaqI*	G78216220A *MspI*	A78216335G *BspHI*	C57571910A *TspRI*
FUA	381.12	233.23	232.20	14.44	95.67	57.55	361.32	56.11	141.90	88.74
RUH	15.02	23.36	8.76	176.66**	1.93	37.37	48.05	22.95	536.18**	79.41
RUW	13.17	19.88	12.00	170.01**	2.23	56.32	56.61	21.48	558.71**	60.20
UD	23.49	40.65	2.89	64.99	25.59	42.85	1.26	14.57	10.34	14.18
UB	16.65	1.32	103.5*	5.20	0.51	3.12	1.12	0.36	1.18	9.64
UL	6.06	24.32	0.21	15.96	154.05*	39.25	95.83	17.72	41.90	136.96*
UW	5.27	52.63	5.10	7.57	182.25*	0.86	88.29	23.61	40.63	312.92**
UC	7.50	473.77**	86.81	157.21	656.56**	70.43	271.79	482.13**	2.20	21.80
CL	0.30	2.06	0.15	1.18	0.52	1.74	0.82	0.32	0.11	0.42
TC	87.35*	48.81	75.82*	65.36*	0.44	7.86	12.51	17.37	2.83	26.73
RTL	11.53	17.77	24.73	29.65	218.89*	6.35	15.54	31.96	72.14	27.91
FTL	5.26	33.67	14.04	18.25	183.75*	2.37	6.57	30.36	11.57	11.27
DFR	9.47	46.32	73.56*	40.50	2.71	2.98	20.92	71.68*	12.70	29.04
DLR	14.45	52.62	70.07*	126.43**	53.01	13.88	12.98	38.09	4.93	3.50
SDF	11.62	10.61	22.70	60.99	0.40	21.92	1.84	33.97	2.12	44.80
SDR	7.39	3.74	15.29	175.70**	1.57	8.04	0.78	34.10	19.26	27.91
TD	13.82	20.27	12.00	34.83	5.56	13.66	48.23	72.26*	17.64	3.05

*Significant at 5% level of significance; **Significant at 1% level of significance

Table 4.44 Effect of identified genetic variants on udder and teat type traits in Karan Fries cows

Traits	T33069832C *BsrI*	C33219027T *TaqI*	C33464963A *AluI*	C33471720T *HinfI*	C33471980A *HindIII*	G33571161A *TaqI*	G33589765A *TaqI*	G78216220A *MspI*	A78216335G *BspHI*
FUA	374.30	292.52	3.17	18.82	608.96	85.04	52.00	202.87	295.75
RUH	1.24	124.11**	51.88	4.54	0.92	.54	12.13	0.37	1448.61**
RUW	0.79	7.79	4.94	3.37	2.93	13.89	2.46	11.60	33.88*
UD	11.64	53.31	63.12	32.49	100.38*	42.21	57.11	78.30	24.87
UB	23.19	5.88	9.29	103.71*	10.86	44.48	34.04	5.39	16.88
UL	43.74	39.52	40.63	85.38	28.20	4.03	36.79	699.74**	22.95
UW	13.46	298.43*	34.10	245.07*	62.41	36.03	261.55*	480.19**	7.16
UC	63.02	1038.38	125.24	571.38	44.13	1751.08*	879.90	271.00	3215.23*
CL	2.04	.29	0.17	5.10*	0.43	0.00	0.44	0.12	1.52
TC	8.42*	.29	2.20	2.67	0.03	1.13	0.81	0.16	2.94
FTL	0.96	3.52	8.84*	2.55	4.41	0.42	0.45	1.65	2.48
RTL	0.10	0.59	2.86	1.43	0.00	0.51	1.51	0.96	1.08
DFR	11.92	32.89	1.18	0.28	42.71*	66.81*	10.31	4.75	15.21
DLR	4.73	24.59	1.12	17.16	0.26	2.78	8.22	0.76	4.67
SDF	58.21	113.12*	23.85	0.04	21.38	23.96	4.69	65.96	160.01*
SDR	57.10	163.10**	8.86	7.82	14.43	52.49	5.55	112.32*	53.61
TD	0.16	0.03	0.28	0.18	0.00	0.28	0.01	0.21	0.01

*Significant at 5% level of significance; **Significant at 1% level of significance

Table 4.45 Association of identified genetic variants of FAM19A1 gene with udder type traits in Sahiwal cows

SNPs	Genotypes	FUA (degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
T33069832C	TT(13)	113.84 ± 5.35	23.91±1.69	24.15±1.71	48.09±1.51	-.87±.80	51.03±1.72	71.54±0.89	143.17±3.06	3.71±0.33
	TC(24	104.54 ± 3.93	22.17±1.25	22.64±1.26	49.88±1.11	-2.84±0.59	50.21±1.26	52.51±1.29	142.09±2.25	3.63±0.24
	CC(50)	106.58 ± 2.72	22.32±.86	22.60±0.87	48.29±0.77	-2.33±0.41	51.05±0.87	52.27±0.89	141.97±1.56	3.50±0.17
C33219027T	CC(41)	109.46±19.32	23.02±.95	23.26±0.96	49.57±0.84	-2.07±.46	50.22±0.96	51.16±0.97	139.20^{a}±1.64	3.72±0.18
	TC(30)	105.63±19.07	21.51±1.11	21.92±1.12	47.41±0.99	-2.38±.54	50.86±1.12	52.67±1.14	143.12^{ab}±1.92	3.26±0.21
	TT(16)	103.81±20.13	23.13±1.52	23.52±1.54	48.89±1.35	-2.47±.74	52.27±1.54	54.05±1.56	148.07^{b}±2.63	3.71±0.29
C33464963A	CC(54)	107.63±2.64	22.18±.83	22.43±0.84	48.67±0.74	-1.78^{a}±0.39	51.14±0.84	52.15±.85	142.87±1.49	3.58±0.16
	CA(33)	106.24±3.37	23.07±1.06	23.53±1.07	48.75±.95	-3.03^{b}±0.50	50.29±1.07	52.31±1.09	141.06±1.90	3.54±0.20
C33471720T	CC(13)	108.08±5.41	17.88^{a}±1.60	18.24^{a}±1.62	45.85±1.49	-2.69±0.82	49.56±1.71	51.93±1.75	139.17±3.02	3.77±0.33
	CT(30)	107.47±3.57	24.03^{b}±1.05	24.21^{b}±1.07	49.55±0.98	-1.79±0.54	51.43±1.13	51.74±1.15	144.58±1.99	3.71±0.21
	TT (44)	106.57±2.94	22.86^{b}±0.87	23.27^{b}±0.88	48.96±0.81	-2.44±0.44	50.77±0.93	52.62±.95	141.44±1.64	3.40±0.18
C33471980A	CC(71)	106.60±2.30	22.59±0.72	22.92±0.73	48.44±0.64	-2.29±0.35	50.19^{a}±0.71	51.52^{a}±.72	140.88^{a}±1.26	3.53±0.14
	CA(16)	109.31±4.84	22.20±1.52	22.51±1.54	49.84±1.36	-2.09±0.74	53.62^{b}±1.51	55.26^{b}±1.53	147.97^{b}±2.66	3.73±0.30
G33571161A	GG(10)	105.10±6.16	21.48±1.92	21.73±1.92	47.57±1.71	-1.70±0.93	50.47±1.94	51.82±2.00	143.89±3.47	3.75±0.37
	GA(56)	107.94±2.60	23.20±0.81	23.68±0.81	49.43±0.72	-2.44±0.39	50.25±0.82	52.25±0.84	141.24±1.46	3.66±0.15
	AA(21)	105.81±4.25	21.18±1.32	21.14±1.33	47.27±1.18	-2.01±0.64	52.50±1.34	52.28±1.38	143.90±2.39	3.21±0.26
G33589765A	GG(50)	108.60±2.73	22.29±0.85	22.63±0.86	48.84±0.77	-2.36±0.42	49.58±0.85	51.00±0.87	140.10±1.52	3.51±0.17
	GA(25)	102.64±3.86	21.75±1.21	21.96±1.21	48.46±1.10	-2.22±0.59	52.86±1.20	54.06±1.23	144.33±2.15	3.76±0.24
	AA(12)	110.167±5.57	25.08±1.75	25.60±1.76	48.60±1.59	-1.88±0.86	51.75±1.74	53.40±1.78	146.38±3.11	3.36±0.34

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.46 Association of identified genetic variants of FAM19A1 gene with udder type traits in Karan Fries cows

SNPs	Genotype	FUA(degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
T33069832C	TT(99)	131.93±1.58	23.41±0.53	12.56±0.26	53.62±0.60	0.69±0.57	60.81±0.75	71.54±0.89	142.03±2.55	3.14±0.14
	TC(35)	127.68±2.67	23.29±0.90	12.80±0.44	53.12±1.01	2.03±0.96	59.06±1.26	70.67±1.49	143.60±4.29	2.93±0.24
	CC(32)	134.12±2.79	23.67±0.94	12.58±0.46	54.29±1.05	0.99±1.00	60.90±1.32	71.84±1.56	143.99±4.49	3.42±0.25
C33219027T	CC(88)	131.93±1.68	24.28^{b}±0.55	12.86±0.27	54.29±0.63	0.91±0.60	59.82±0.79	70.55^{a}±0.92	140.27±2.68	3.18±0.15
	TC(42)	133.38±2.44	21.36^{a}±0.80	12.55±0.40	52.35±0.91	1.48±0.88	61.05±1.15	74.63^{b}±1.33	148.73±3.88	3.04±0.22
	TT(36)	128.08±2.64	23.80^{ab}±0.86	12.09±0.43	53.56±0.99	0.79±0.95	61.35±1.24	69.77^{a}±1.44	141.78±4.19	3.18±0.24
C33464963A	CC(139)	131.43±15.86	23.68±0.44	12.69±0.22	53.37±0.50	0.93±0.48	60.68±0.63	71.21±0.75	143.12±2.14	3.13±0.12
	AC(27)	131.77±16.11	22.17±1.01	12.22±0.50	55.04±1.14	1.57±1.09	59.34±1.43	72.44±1.70	140.77±4.87	3.22±0.27
C33471720T	CC(28)	130.64±17.13	23.37±1.00	12.32±0.49	54.18±1.12	2.44^{b}±1.06	62.35±1.40	70.26^{a}±1.64	146.84±4.78	3.10^{ab}±0.26
	CT(61)	132.00±15.39	23.16±0.68	12.86±0.33	54.24±0.76	-0.38^{a}±0.71	60.76±0.95	73.66^{b}±1.11	144.20±3.23	3.56^{b}±0.18
	TT (77)	131.34±15.93	23.68±0.60	12.53±0.29	52.97±0.67	1.64^{ab}±0.63	59.54±0.84	70.05^{a}±0.99	140.08±2.88	2.81^{a}±0.16
C33471980A	CC(112)	130.13±1.49	23.49±0.50	12.71±0.24	52.10^{a}±0.56	0.85±0.53	60.17±0.70	71.84±0.83	143.09±2.39	3.18±0.13
	CA(54)	134.22±2.14	23.33±0.72	12.42±0.35	55.76^{b}±0.80	1.40±0.77	61.05±1.01	70.53±1.20	141.99±3.45	3.07±0.19
G33571161A	GG(123)	131.04±1.43	23.40±0.47	12.79±0.23	53.34±0.53	1.34±0.51	60.37±0.67	71.69±0.79	146.66^{b}±2.26	3.14±0.13
	GA(43)	132.67±2.42	23.53±0.81	12.13±0.39	54.49±0.90	0.16±0.86	60.72±1.14	70.62±1.34	135.24^{a}±3.83	3.15±0.21
G33589765A	GG(104)	132.06±1.56	23.16±0.52	12.72±0.25	53.19±0.58	0.98±0.55	60.89±0.73	72.78^{b}±0.85	144.0±2.47	3.09±0.14
	GA(25)	130.84±3.19	24.16±1.06	12.23±0.52	55.57±1.18	2.39±1.13	60.45±1.49	69.05^{a}±1.73	135.01±5.04	3.28±0.28
	AA(37)	130.22±2.62	23.73±0.87	12.58±0.43	53.59±0.97	0.26±0.93	59.25±1.23	69.16^{a}±1.42	144.41±4.14	3.21±0.23

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.47 Association of identified genetic variants of KCNB1 and EDN3 gene with udder type traits in Sahiwal cows

SNP	Genotype	FUA(degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
G78216220A (KCNB1)	GG(20)	108.00±4.35	21.78±1.36	22.15±1.37	47.65±1.22	-2.23±0.66	51.94±1.38	53.49±1.40	148.07^{b}±2.35	3.41±0.27
	GA(29)	108.17±3.62	21.95±1.13	22.28±1.14	48.89±1.01	-2.14±0.55	50.73±1.15	52.14±1.17	141.55^{ab}±1.95	3.64±0.22
	AA(38)	105.81±3.16	23.34±0.99	23.64±1.00	49.10±0.88	-2.35±0.48	50.30±1.00	51.59±1.02	139.57^{a}±1.70	3.58±0.19
A78216335G (KCNB1)	AA(44)	105.84±2.92	20.06^{a}±0.84	20.34^{a}±0.84	48.36±0.82	-2.14±0.44	51.50±0.92	52.89±0.94	142.34±1.65	3.60±0.18
	AG(43)	108.40±2.95	25.03^{b}±0.85	25.41^{b}±0.85	49.05±0.83	-2.37± 0.45	50.12±0.93	51.52±0.95	142.02±1.67	3.53±0.18
C57571910A (EDN3)	CC(36)	108.30±3.23	21.38±1.00	21.85±1.01	48.22±0.91	-2.65±0.49	52.26^{b}±1.01	54.47^{b}±1.00	142.78±1.83	3.48±0.20
	CA(51)	106.25±2.71	23.32±0.84	23.54±0.85	49.04±0.76	-1.97±0.41	49.80^{a}±0.85	50.62^{a}±0.84	141.76±1.53	3.62±0.16

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.48 Association of identified genetic variants of KCNB1 gene with udder type traits in Karan Fries cows

SNP	Genotype	FUA (degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)	UW (cm)	UC (cm)	CL (cm)
G78216220A	GG(24)	133.25±3.24	23.27±1.08	12.220±0.53	54.89±1.20	1.517±1.16	61.52^{b}±1.41	71.07^{ab}±1.74	143.03±5.17	3.23±0.29
	GA(76)	132.55±1.82	23.46±0.61	12.350±0.29	54.26±0.67	1.115±0.65	57.39^{a}±0.79	69.05 ^{a}± 0.97	140.87±2.91	3.14±0.16
	AA(66)	129.56±1.95	23.47±0.65	13.07±0.32	52.47±0.72	0.768±0.70	63.61^{b}±0.85	74.26^{b}±1.05	144.78±3.12	3.12±0.17
A78216335G	AA(65)	129.80±15.26	27.12^{b}±0.54	13.18^{b}±0.32	53.16±0.74	0.63±0.70	60.92±0.92	71.15±1.09	137.25^{a}±3.09	3.27±0.17
	AG(101)	132.54±16.20	21.07^{a}±0.43	12.25^{a}±0.25	53.95±0.59	1.29±0.56	60.16±0.74	71.58±0.88	146.27^{b}±2.48	3.07±0.14

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Karan Fries cattle (Table 4.52). There are no earlier reports available to compare or contrast the present findings of SNP A78216335G.

4.17.3 Association of genetic variants of EDN3 gene with production traits, udder and teat type traits in Sahiwal cows

In EDN3 gene, SNP C57571910A was found in Exon 1 of Sahiwal cattle. *TspRI* enzyme was used for PCR-RFLP analysis of SNP C57571910A which gave two genotypes in Sahiwal cattle i.e. CC (424 and 153 bp) and CA (577, 424 and 153 bp) whereas, in Karan Fries cattle monomorphic pattern of genotype i.e. CA (577, 424 and 153 bp) was found. This SNP did not have significant effect on production traits (Table 4.39) however it has significant effect on udder length and width in Sahiwal cattle (Table 4.43). The CC genotype having longer ($52.26^b \pm 1.01$ cm) and wider udder ($54.47^b \pm 1.00$ cm) as compared to CA genotype in Sahiwal cattle (Table 4.47). There are no earlier reports available to compare or contrast the present finding with regards to association of C57571910A SNP of EDN3 gene with production traits, udder and teat type traits in cattle.

4.18 Association of identified genetic variants with incidence of clinical mastitis in Sahiwal and Karan Fries cows

The animals whose udder measurements were taken grouped as mastitis affected and non-affected animals. Within each group animals were assigned respective genotypes of the obtained by PCR-RFLP analysis for different genes. Association study was done using Chi-square (χ^2) analysis. The association results are presented in Table 4.53 and 4.54 for Sahiwal and Karan Fries cows, respectively.

In the present study SNP C33464963A present in intron 2 of FAM19A1 gene was significantly ($p<0.05$) associated with the incidence of mastitis in both Sahiwal (Table 4.53) and Karan Fries cattle (Table 4.54) and SNP C57571910A present in Exon 1 of EDN3 gene significantly ($p<0.05$) associated with incidence of mastitis in Sahiwal cattle (Table 4.53). In both Sahiwal and Karan Fries cows CC genotype of SNP C33464963A was less susceptible to mastitis than CA genotype.

CC genotype of SNP C57571910A present in exon 1 of EDN3 gene was more susceptible to incidence of mastitis in Sahiwal cattle as compared to CA genotype.

Wu *et al.* (2015) performed genome-wide association studies for the detection of quantitative trait loci in Danish Holsteins breed and reported EDN3 to be a candidate gene for mastitis susceptibility. Sahana *et al.* (2014) also performed genome-wide association studies for clinical mastitis traits in Holstein and Nordic Red cattle and found that best region on BTA 13 significant for clinical mastitis was located at 57.60 to 57.85 Mb. This region includes EDN3 gene. This SNP was rs136962405 found in 5′-UTR region. But in the present study both the breeds were monomorphic for this SNP. There is no earlier reports are available to compare or contrast the present findings with regard to association of genetic variants of FAM19A1 and EDN3 gene with clinical mastitis in cattle.

Table 4.49 Association of identified genetic variants of FAM19A1 gene with teat type traits in Sahiwal cows

SNPs	Genotype	TC (cm)	FTL (cm)	RTL (cm)	DFR (cm)	DLR (cm)	SDF (cm)	SDR (cm)	TD (cm)
T33069832C	TT(13)	11.76^{b}±1.41	6.73±1.68	5.54±. 1.85	5.83±1.43	6.11±1.52	41.29±1.74	42.57±1.76	27.33±1.40
	TC(24)	7.24^{a}±1.04	6.37±1.24	5.51±1.36	5.42±1.05	6.82±1.12	42.92±1.28	43.74±1.29	26.18±1.03
	CC(50)	8.49^{ab}±0.72	7.16±0.86	6.57±0.94	6.47±0.73	7.65±0.77	42.14±.089	42.93±0.89	25.70±0.71
C33219027T	CC(41)	9.65±0.81	6.29±0.94	5.59±1.04	5.73±0.79	6.84±0.84	42.00±0.98	42.79±0.99	26.18±0.78
	TC(30)	8.18±0.94	8.09±1.10	6.99±1.21	5.42±0.93	6.44±0.98	42.88±1.14	43.38±1.16	26.67±0.92
	TT(16)	6.88±1.29	6.12±1.50	5.87±1.66	8.24±1.27	9.47±. 1.35	41.60±1.57	43.37±1.59	24.71±1.26
C33464963A	CC(54)	9.43^{b}±0.70	5.75±0.90	6.60±0.82	5.28^{a}±0.68	6.33^{a}±0.73	42.36±0.85	43.12±0.86	26.65±0.68
	CA(33)	7.33^{a}±0.89	6.74±1.15	7.34±1.05	7.40^{b}±0.88	8.59^{b}±0.93	42.01±1.09	43.08±1.10	25.15±0.87
C33471720T	CC(13)	11.35^{b}±1.42	6.45±1.68	6.29±1.84	8.27±1.41	11.25^{b}±1.45	39.41±1.71	38.38^{a}±1.67	27.68±1.38
	CT(30)	7.60^{a}±0.94	7.77±1.10	7.18±1.21	5.30±0.93	6.55^{a}±0.95	42.60±1.13	43.47^{b}±1.10	26.57±0.91
	TT(44)	8.53^{ab}±0.77	6.40±0.91	5.36±1.00	5.98±0.77	6.42^{a}±0.79	42.81±0.93	44.24^{b}±0.90	25.27±0.75
C33471980A	CC(71)	8.60±0.62	6.19^{a}±0.69	5.37^{a}±0.76	6.00±0.61	7.56±0.64	42.20±0.74	43.16±0.75	25.96±0.60
	CA(16)	8.78± 1.31	9.94^{b}±1.46	9.46^{b}±1.61	6.46±1.29	5.54± 1.35	42.37±1.56	42.82±1.58	26.61±1.26
G33571161A	GG(10)	8.81±1.67	6.85± 1.92	6.00±2.11	5.37±1.64	7.14±1.73	43.62±1.98	43.77±2.00	26.28±1.59
	GA(56)	8.88±0.70	7.03±0.81	6.39±0.89	6.20±0.69	7.56±0.73	42.38±0.83	43.25±0.84	25.69±0.67
	AA(21)	7.88±1.15	6.48±1.32	5.48±1.45	6.12±1.13	6.21± 1.19	41.17±1.36	42.40±1.38	27.02±1.10
G33589765A	GG(50)	9.09±0.74	7.21±0.86	6.62±0.94	6.48±0.72	7.57±0.77	42.09±0.89	43.03±0.90	25.33±0.70
	GA(25)	8.06±1.05	6.35±1.21	5.60±1.33	6.11±1.03	7.01±1.09	42.30±1.26	43.09±1.27	26.45±0.99
	AA(12)	7.92±1.52	6.62±1.75	5.13±1.92	4.40±1.48	5.96±1.58	42.69±1.82	43.43±1.83	28.41±1.43

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.50 Association of identified genetic variants of FAM19A1 gene with teat type traits in Karan Fries cows

SNPs	Genotype	TC (cm)	FTL (cm)	RTL (cm)	DFR (cm)	DLR (cm)	SDF (cm)	SDR (cm)	TD (cm)
T33069832C	TT(99)	7.67^{b}±0.15	5.25±0.14	4.67±0.11	7.06±0.38	8.98±0.48	46.29±0.55	46.26±0.56	2.19±0.03
	TC(35)	7.75^{b}±0.25	5.22±0.24	4.59±0.19	6.34±0.65	8.65±0.81	47.35±0.92	46.74±0.94	2.30±0.05
	CC(32)	6.89^{a}±0.26	5.52±0.26	4.67±0.19	6.23±0.68	9.40±0.84	48.41±0.96	48.44±0.99	2.20±0.05
C33219027T	CC(88)	7.50±0.16	5.35±0.15	4.70±0.12	6.17±0.40	8.48±0.50	48.02b±0.57	48.09^{b}±0.58	2.23±0.03
	TC(42)	7.64±0.23	4.97±0.22	4.51±0.17	7.26±0.59	9.60±0.73	45.87^{a} ±0.83	45.07^{a} ±0.84	2.18±0.05
	TT(36)	7.50±0.25	5.54±0.24	4.72±0.18	7.58±0.63	9.53±0.79	45.49^{a} ±0.90	45.59^{a}±0.91	2.23±0.05
C33464963A	CC(139)	7.49±0.13	5.20^{a}±0.12	4.60±0.09	6.79±0.32	8.95±0.40	46.76±0.46	46.68±0.48	2.20±0.02
	CA(27)	7.80±0.29	5.82^{b}±0.28	4.95±0.21	6.56±0.74	9.17±0.92	47.78±1.06	47.31±1.08	2.31±0.06
C33471720T	CC(28)	7.27±0.29	5.68±0.27	4.94±0.21	6.63±0.73	7.99±0.90	46.88±1.04	46.71±1.07	2.28±0.06
	CT(61)	7.43±0.19	5.27±0.18	4.64±0.14	6.74±0.49	9.26±0.61	46.92±0.71	46.42±0.72	2.16±0.04
	TT (77)	7.72±0.17	5.18±0.16	4.56±0.12	6.80±0.44	9.14±0.54	46.95±0.63	47.10±0.64	2.24±0.03
C33471980A	CC(112)	7.55±0.14	5.18±0.13	5.18±0.13	6.40^{a}±0.36	9.02±0.45	46.68±0.52	46.58±0.53	2.22±0.03
	CA(54)	7.52±0.21	5.53±0.19	5.53±0.19	7.48^{b}±0.52	8.93±0.65	47.44±0.75	47.21±0.76	2.22±0.04
G33571161A	GG(123)	7.59±0.13	5.27±0.13	4.62±0.10	7.12^{b}±0.34	8.91±0.43	46.70±0.49	46.45±0.50	2.24±0.03
	GA(43)	7.40±0.23	5.38±0.22	4.75±0.17	5.68^{a}±0.58	9.21±0.73	47.57±0.84	47.73±0.85	2.15±0.05
G33589765A	GG(104)	7.61±0.15	5.34±0.14	4.76±0.11	6.57±0.37	9.12±0.47	46.95±0.54	46.62±0.55	2.22±0.03
	GA(25)	7.48±0.31	5.13±0.29	4.44±.22	6.52±.77	8.24±.95	46.41±1.10	46.78±1.13	2.20±0.06
	AA(37)	7.37±0.25	5.29±0.24	4.51±0.18	7.41±0.63	9.13±0.78	47.20±0.91	47.25±0.93	2.21±0.05

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.51 Association of identified genetic variants of KCNB1 and EDN3 gene with teat type traits in Sahiwal cows

SNPs	Genotype	TC	FTL	RTL	DFR	DLR	SDF	SDR	TD
G78216220A (KCNB1)	GG (20)	8.32±1.17	6.50±1.34	5.48±1.48	4.76^{a}±1.12	6.05±1.21	42.94±1.39	42.90±1.41	28.25^{b}±1.10
	GA (29)	9.52±.97	8.05±1.12	7.34±1.23	7.82^{b}±0.93	8.44±1.01	40.98±1.16	42.01±1.17	24.91^{a}±0.91
	AA (38)	8.12±0.85	6.19±0.97	5.54±1.07	5.46^{a}±0.81	6.83±0.88	42.81±1.01	44.04±1.02	25.83^{ab}±0.79
A78216335G (KCNB1)	AA(44)	8.81±0.79	7.24±0.91	7.02±0.99	5.71±0.77	6.95±0.82	42.38±0.94	43.57±0.95	26.52±0.75
	AG(43)	8.45±0.80	6.51±0.92	5.20±1.00	6.47±0.78	7.43±0.83	42.07±0.95	42.62±0.96	25.62±0.76
C57571910A (EDN3)	CC (36)	7.97±0.87	6.45±1.00	5.45±1.10	5.40±0.85	7.43±0.91	43.08±1.04	43.77±1.05	25.85±0.84
	CA (51)	9.10±0.73	7.18±0.84	6.60±0.92	6.57±0.72	7.02±0.76	41.63±0.87	42.62±0.88	26.23±0.70

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.52 Association of identified genetic variants of KCNB1 gene with teat type traits in Karan Fries cows

SNPs	Genotype	TC	FTL	RTL	DFR	DLR	SDF	SDR	TD
G78216220A	GG(24)	7.48±0.31	5.62±0.30	4.613±0.22	6.60±0.79	8.92±0.98	47.16±1.12	47.19^{ab}±1.13	2.23±0.67
	GA(76)	7.51±0.17	5.20±0.16	4.56±0.12	6.54±0.44	8.91±0.55	47.78±0.62	47.88^{b}±0.63	2.16±0.04
	AA(66)	7.59±0.19	5.29±0.18	4.79±0.13	7.04±0.47	9.11±0.59	45.86±0.67	45.38^{a}±0.68	2.27±0.04
A78216335G	AA(65)	7.79±0.19	5.45±0.18	4.76±0.13	6.10±0.47	8.78±0.59	45.70^{a}±0.67	46.07±0.69	2.23±0.04
	AG(101)	7.37±0.15	5.20±0.14	4.59±0.11	7.17±0.38	9.12±0.47	47.71^{b}±0.54	47.24±0.56	2.21±0.03

Figures in parenthesis are number of animals; Figures with dissimilar superscript differ significantly

Table 4.53 Association of polymorphic patterns of identified genetic variants with incidence of mastitis in Sahiwal cows

Gene	SNPs	Genotype	Non-affected (69)	Affected (18)	χ^2 value
			Number of cows		
FAM19A1	T33069832C *BsrI*	TT(13)	9	4	3.22
		TC(24	17	7	
		CC(50)	43	7	
	C33219027T *TaqI*	CC(41)	34	7	0.35
		TC(30)	22	8	
		TT(16)	13	3	
	C33464963A *AluI*	CC (54)	47	7	5.17*
		CA (33)	22	11	
	C33471720T *HinfI*	CC(13)	9	4	0.98
		CT(30)	24	6	
		TT (44)	36	8	
	C33471980A *HindIII*	CC(71)	57	14	0.22
		CA(16)	12	4	
	G33571161A *TaqI*	GG(10)	8	2	0.06
		GA(56)	44	12	
		AA (21)	17	4	
	G33589765A *TaqI*	GG(50)	41	9	1.39
		GA(25)	20	5	
		AA(12)	8	4	
KCNB1	G78216220A *MspI*	GG (20)	16	4	1.28
		GA (29)	21	8	
		AA (38)	32	6	
	A78216335G *BspHI*	AA(44)	36	8	0.34
		AG(43)	33	10	
EDN3	C57571910A *TspRI*	CC (36)	24	12	5.98*
		CA (51)	45	6	

Figures in parenthesis are number of animals; *Significant at 5% level of significance

Table 4.54 Association of polymorphic patterns of identified genetic variants with incidence of mastitis in Karan Fries cows

Gene	SNPs	Genotype	Non-Affected (132)	Affected (34)	χ^2 value
			Number of cows		
FAM19A1	T33069832C *BsrI*	TT(99)	80	19	0.26
		TC(35)	27	8	
		CC(32)	25	7	
	C33219027T *TaqI*	CC(88)	74	14	3.98
		TC(42)	29	13	
		TT(36)	29	7	
	C33464963A *AluI*	CC(139)	115	24	5.43*
		CA(27)	17	10	
	C33471720T *HinfI*	CC(28)	23	5	0.39
		CT(61)	47	14	
		TT (77)	62	15	
	C33471980A *HindIII*	CC(112)	85	27	2.78
		CA(54)	47	7	
	G33571161A *TaqI*	GG(123)	96	27	0.63
		GA(43)	36	7	
	G33589765A *TaqI*	GG(104)	87	17	4.82
		GA(25)	16	9	
		AA(37)	29	8	
KCNB1	G78216220A *MspI*	GG(24)	21	3	1.16
		GA(76)	60	16	
		AA(66)	51	15	
	A78216335G *BspHI*	AA(65)	50	15	0.44
		AG(101)	82	19	

Figures in parenthesis are number of animals; *Significant at 5% level of significance

4.19 Association of identified genetic variants with observational traits in Sahiwal and Karan Fries cows

In the present study few significant association of observational traits with identified SNPs were present in Sahiwal and Karan Fries cattle which are presented in Table 4.55 and 4.56. The code 1, 2 and 3 stands for very tight or tight, intermediate and pendulous type udder suspension, respectively. For teat end shape code 1, 2 and 3 stands for flat, round and pointed teat, respectively and for teat shape it stands for funnel, cylindrical and bottle shape teat, respectively.

Skin condition was coded as 1 and 2 as normal and dry skin in Sahiwal cattle whereas, in Karan Fries cattle it was coded as 1, 2 and 3 into normal, dry and roughness along with dry skin. For teat placement code 1, 5 and 9 were used which stands for inside (close), intermediate and outside (wide) placement. Code 1, 2, 3 and 4 stands for no ring, rough ring, smooth ring and very rough ring for longer term changes in teat end condition trait.

4.19.1 Association of identified genetic variants of FAM19A1 with observational traits in Sahiwal and Karan Fries cows

SNP C33219027T of FAM19A1gene was found to have significant association with udder suspension and teat end shape in Karan Fries cattle while no significant association of this SNP was found with of the visual observations in Sahiwal cattle. CC genotype has highest number of intermediate suspension and round end shaped teat in comparison to CT and CC genotypes. CT genotype has maximum percentage of flat teat end shape than CC and TT genotype animals. Flat teat had significant correlation with SCC counts (Bharti *et al.*, 2015)

SNP C33464963A present in intron 2 of FAM19A1 gene was found to be significantly ($p<0.05$) associated with skin condition in Karan Fries cattle. CA genotype has higher percentage of rough skin (51.85 %). The present observation suggests animal with CA genotype are more susceptible to mastitis than the CC genotype in which the percentage of rough skin was 29.49 %. This SNP did not affect significantly any observational traits in Sahiwal cows.

SNP C33464963A of FAM19A1 gene has significant ($p<0.05$) effect on teat end shape and longer term changes in teat end condition while highly significant ($p<0.01$) effect on teat shape in Sahiwal cattle. Whereas, in Karan Fries cattle it has significant ($p<0.05$) effect on teat end shape and rear teat placement. CC genotype has mostly round type teat in Sahiwal cattle while, in Karan Fries cattle round type was mostly common in CA genotype.

Flat type teat in low percentage for both genotypes in both the breeds. Bharti *et al.* (2015) reported a significant correlation of flat teat end with SCC counts. CC genotype has higher percentage of cylindrical teat (40.85 %) than CA genotype (25.00 %). Prajapati (1995) reported that cylindrical shaped teats there is a higher incidence of mastitis in Sahiwal cattle.

In present study, CC genotype animals are more susceptible to mastitis. CC genotype has 52.11 % rough ring and 4.23 % very rough ring in Sahiwal cows. So CC genotype having mostly cylindrical teat and more than 50 % rough ring are more susceptible to mastitis than CA genotype in Sahiwal cows. CC genotypes had maximum percentage (56 %) of intermediate placed teat which is favorable for milk production and also it is less susceptible to mastitis in Karan Fries cattle. There is significant difference between milk production between CA and CC genotype in Karan Fries cows with CC having maximum production.

SNP C33464963A present in Intron 3 of FAM19A1 gene has significant ($p<0.05$) effect on udder suspension and skin condition in both Sahiwal and Karan Fries cows. In both the breeds GG genotype has maximum number of tight and intermediate suspension of udder with normal skin condition. Therefore, GG genotype is desirable in both cases. In Karan Fries cows there is also significant difference between two genotypes in milk production with GG genotype having higher milk production than GA genotype.

4.19.2 Association of identified genetic variants of KCNB1 with observational traits in Sahiwal and Karan Fries cows

SNP G78216220A present in Intron 1 of KCNB1 gene was significantly ($p<0.05$) associated with teat shape in Sahiwal cattle and rear teat placement in Karan Fries cattle. All the three genotypes have maximum percentage of funnel shaped teat in Sahiwal cattle. In Karan Fries cattle AA genotype has maximum percentage of intermediate placed udder which is favorable for milk production.

SNP A78216335G present in Intron 1 of KCNB1 gene was significantly ($p<0.05$) associated with teat shape in Sahiwal cattle and teat end shape in Karan Fries cattle. AA genotype has maximum percentage (61.36 %) of funnel shaped udder in Sahiwal cows and this genotype has mostly round shaped teat in Karan Fries cows.

Table 4.55 Association of identified genetic variants with visual traits in Sahiwal cows

Gene	SNP	Genotype					χ^2 value
FAM19A1	C33471980A *HindIII*	**Teat Shape**					
		Genotype	1	2	3		
		CC (71)	39 (54.93 %)	29 (40.85 %)	3 (4.22 %)		15.60**
		CA (16)	6 (37.50 %)	4 (25.00 %)	6 (37.50 %)		
		Teat End Shape					
			1	2	3		7 .02*
		CC (71)	4 (5.63 %)	40 (56.34 %)	27 (38.03 %)		
		CA (16)	4 (25.00 %)	5 (31.25 %)	7 (43.75 %)		
		Longer Term Changes in Teat End condition					
			1	2	3	4	7.98*
		CC (71)	19 (26.76%)	37 (52.11 %)	12 (16.9 %)	3 (4.2%)	
		CA (16)	6 (37.50 %)	3 (18.75 %)	5 (31.25 %)	2 (12.5%)	
	G33571161A *TaqI*	**Udder Suspension**					
			1	2	3		10.15*
		GG (10)	3 (30 %)	3 (30 %)	4 (40 %)		
		GA (56)	22 (39.29 %)	21 (37.50 %)	13 (23.21 %)		
		AA (21)	5 (23.81 %)	15 (71.43 %)	1 (4.76 %)		
		Skin Condition					
			1	2			6.94*
		GG (10)	10 (100.00 %)	0 (0.00 %)			
		GA (56)	43 (76.79 %)	13 (23.21 %)			
		AA (21)	12 (57.14 %)	9 (42.86 %)			
KCNB1	G78216220A *MspI*	**Teat Shape**					
			1	2	3		10.53*
		GG (20)	10 (50.00 %)	9 (45.00 %)	1 (5.00%)		
		GA (29)	12 (41.38 %)	12 (41.38 %)	5 (17.24 %)		
		AA (38)	27 (71.05 %)	11 (28.95 %)	0		
	A78216335G *BspHI*	**Teat Shape**					
			1	2	3		6.77*
		AA (44)	27 (61.36 %)	17 (38.64 %)	0		
		AG (43)	21 (48.84 %)	16 (37.21 %)	6 (13.95 %)		
EDN3	C57571910A *TspRI*	**Teat Shape**					
			1	2	3		9.19*
		CC (36)	13 (36.11 %)	19 (52.78 %)	4 (11.11 %)		
		CA (51)	35 (68.63 %)	14 (27.45 %)	2 (3.92 %)		
		Udder Suspension					
			1	2	3		8.34*
		CC (36)	18 (50.00 %)	10 (27.78 %)	8 (22.22 %)		
		CA (51)	12 (23.53 %)	29 (56.86 %)	10 (19.61 %)		
		Fore Teat Placement					
			1	5	9		7.83*
		CC (36)	11 (30.56 %)	17 (47.22 %)	8 (22.22 %)		
		CA (51)	14 (27.45 %)	35 (68.63 %)	2 (3.92 %)		

*Significant at 5% level of significance; ** Significant at 1% level of significance

Table 4.56 Association of identified genetic variants with visual traits in Karan Fries cows

Gene	SNPs	Genotype	1	2	3	χ^2 value
		Udder Suspension				
FAM19A1	C33219027T *TaqI*	CC(88)	31(35.23 %)	53 (60.22 %)	4 (4.55 %)	18.36**
		CT(42)	15 (35.71%)	18 (42.86 %)	9 (21.43 %)	
		TT(36)	4 (11.11 %)	23 (63.89 %)	9 (25.00 %)	
		Teat End Shape				
			1	**2**	**3**	
		CC(88)	18 (20.45 %)	53 (60.23 %)	17 (19.32 %)	10.35*
		CT(42)	12 (28.57 %)	14 (33.33 %)	16 (38.10 %)	
		TT(36)	5 (13.89 %)	19 (52.78 %)	12 (33.33 %)	
	C33464963A *AluI*	**Skin Condition**				
			1	**2**	**3**	
		CC(139)	55 (39.57 %)	43 (30.94%)	41 (29.49 %)	6.65*
		CA(27)	10 (37.04 %)	3 (11.11 %)	14 (51.85 %)	
	C33471980A *HindIII*	**Rear Teat Placement**				
			1	**5**	**9**	
		CC(112)	26 (23.21 %)	56 (50.00%)	30 (26.79 %)	7.99*
		CA(54)	23 (42.59 %)	24 (44.45 %)	7 (12.96 %)	
		Teat End Shape				
			1	**2**	**3**	
		CC(112)	24 (21.43 %)	52 (46.43 %)	36(32.14 %)	6.16*
		CA(54)	11 (20.37 %)	34 (62.96 %)	9 (16.67 %)	
	G33571161A *TaqI*	**Udder Suspension**				
			1	**2**	**3**	
		GG(123)	44 (35.77 %)	65 (52.85 %)	14 (11.38 %)	7.48*
		GA(43)	6 (13.95 %)	29 (67.44 %)	8 (18.61 %)	
		Skin Condition				
			1	**2**	**3**	
		GG(123)	57 (46.34 %)	33 (26.83 %)	33 (26.83 %)	12.08**
		GA(43)	8 (18.60 %)	13 (30.23 %)	22 (51.17 %)	
KCNB1	G78216220A *MspI*	**Rear Teat Placement**				
			1	**5**	**9**	
		GG(24)	9 (37.50 %)	13 (54.17 %)	2 (8.33 %)	9.68*
		GA(76)	19 (25.00 %)	32 (42.11 %)	25 (32.89 %)	
		AA(66)	21 (31.82 %)	35 (53.03%)	10 (15.15 %)	
	A78216335G *BspHI*	**Teat End Shape**				
			1	**2**	**3**	
		AA(65)	18 (27.69 %)	36 (55.38 %)	11 (16.93 %)	6.56*
		AG(101)	17 (16.83 %)	50 (49.50 %)	34 (33.67 %)	

*Significant at 5% level of significance; ** Significant at 1% level of significance

4.19.3 Association of identified genetic variants of EDN3 with observational traits in Sahiwal cows

SNP C57571910A in exon 1 of EDN3 gene was significantly ($p<0.05$) associated with teat shape, udder suspension and fore teat placement in Sahiwal cows. CA genotype was having mostly intermediate udder suspension with intermediate placed teat. This condition is desirable for maximum milk production and is also less susceptible to incidence of mastitis. CA genotype was having more milk production than CC genotype in present study.

CHAPTER –5

Summary and Conclusions

5. SUMMARY AND CONCLUSIONS

The present study was conducted to explore genetic polymorphism in FAM19A1, KCNB1 and EDN3 gene and to detect the association of identified allelic variants with udder type traits, incidence of clinical mastitis and milk production in Karan Fries and Sahiwal cows. A total of 87 Sahiwal and 166 Karan Fries cows maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal were used in the study.

Genomic DNA was isolated from whole blood samples of Sahiwal and Karan Fries cows by phenol-chloroform method. The quality and quantity of DNA was checked after extraction of DNA. To amplify the targeted regions, seven sets of forward and reverse region-specific oligonucleotide primers for FAM19A1 gene and one set for each KCNB1 and EDN3 gene were designed using Primer3 software. The PCR amplification of each of the targeted regions were carried out after optimizing PCR conditions for each region. The targeted regions of each primer sets were custom sequenced in both the breeds and genotyped using PCR-RFLP technique and edited for identification of SNPs. For determining SNPs each edited sequence of Sahiwal and Karan Fries cows was aligned with corresponding reference sequence of *Bos taurus* using Clustal W multiple sequence alignment program for DNA.

All the primer sets in the study were found polymorphic except primer set 2 (intron 1) of FAM19A1 gene in both the breeds and EDN3 gene in Karan Fries cattle was found monomorphic. Total ten SNPs in Sahiwal cows (T33069832C, C33219027T, C33464963A, C33471720T, C33471980A, G33571161A, G335589765A, G78216220A, A78216335G, C57571910A) of FAM19A1, KCNB1 and EDN3 genes were observed whereas, in Karan Fries cows total nine SNPs (T33069832C, C33219027T, C33464963A, C33471720T, C33471980A, G33571161A, G33589765A, G78216220A, A78216335G) of FAM19A1 and KCNB1 genes were observed.

PCR-RFLP analysis with *BsrI* restriction enzyme revealed SNP at locus T33069832C in intron 1 (508 bp) of FAM19A1 gene, resolved into TT, TC and CC in Sahiwal and Karan Fries cattle. *TaqI* enzyme revealed SNP at C33219027T locus in intron 1 (576 bp) of FAM19A1 gene, resolved into CC, CT and TT in both the breeds. PCR-RFLP analysis with *AluI* enzyme unveiled SNP at locus C33464963A in intron 2 (509) of FAM19A1 gene, gave genotypes CC and CA in both Sahiwal and Karan Fries cattle. Two SNPs were found for another 492 bp PCR product in intron 2 of FAM19A1 gene in both the breeds. First one at locus C33471720T was digested with *HinfI* enzyme resolved into CC, CT and TT. Other SNP was at locus C33471980A, was digested with *HindIII* enzyme and revealed genotypes CC and CA. SNP at locus G33571161A in intron 3 (529 bp PCR product) of FAM19A1 gene was digested with *TaqI* enzyme showed GG, GA and AA in Sahiwal cattle while GG and GA genotypes in Karan Fries cattle. PCR product harbouring SNP at locus G33589765A in 3'-UTR (368 bp) of FAM19A1 gene was digested with *TaqI* enzyme, revealed GG, GA and AA genotypes. For PCR product of 505 bp present in intron 1 of KCNB1 gene, two SNPs were found. First SNP at locus G78216220A, was digested with *MspI* enzyme and showed GG, GA and AA genotype while other SNP at locus A78216335G was digested with *BspHI* enzyme resolved into AA and AG. PCR-RFLP analysis with *TspRI* restriction enzyme revealed SNP at locus C57571910A in exon 1 of EDN3 gene, showed CC and CA genotype in Sahiwal cattle.

Association of each SNP locus with production traits (305 milk yield, total milk yield, monthly milk yield and test day milk yield) and udder type traits *viz.*, fore udder attachment (FUA), rear udder width (RUW), rear udder height (RUH), udder balance (UB), udder depth (UD), udder length (UL), udder width (UW), udder circumference (UC), central ligament (CL), teat circumference (TC), fore teat length (FTL), rear teat length (RTL), distance between fore and rear teat (DFR), distance between left and right teat (DLR), shortest distance of floor from fore teat (SDF), shortest distance of floor from rear teat (SDR) and teat diameter (TD) was analyzed using GLM procedure of SPSS Version 22. Association of each SNP locus with incidence of mastitis and observational (visual traits) *viz.*, udder shape (US), udder suspension (USUS), teat shape (TS), teat end shape (TES), teat size (TSI), rear teat placement (RTP), fore teat placement (FTP), skin condition (SC) and longer term changes in teat end condition (LTCTEC) was analyzed using chi-square (χ^2) test. The effect of non-genetic factors (season of calving, parity and stage of lactation) on udder conformation traits, milk production and clinical mastitis were analyzed using least-squares analysis and the significant difference between means among subclasses *viz.*, season of calving, parity and stage of lactation were assessed through DMRT. The data were adjusted for the significant non-genetic factors to remove the environmental effects.

Season had significant ($p<0.05$) effect on TC while, stage of lactation had significant ($p<0.05$) effect on UC, TC, SDR and DFR in Sahiwal cattle. Parity was found to have highly significant ($p<0.01$) effect on FUA, RUW, UD, UW and significant ($p<0.05$) effect on TD in Sahiwal cows. In Karan Fries cows parity had highly significant ($p<0.01$) effect on RUH, UD, UL, UW, TC, FTL, SDF, SDR and TD whereas, stage of lactation had significant ($P<0.05$) effect on UC. Season did not have significant effect on udder type traits in Karan Fries cows.

As revealed by logistic regression, parity and level of production ($p<0.05$) had significant effects on clinical mastitis in Sahiwal cows. There was no significant effect of season on incidence of mastitis in both the breeds. Parity had highly significant ($p<0.01$) effect on incidence of mastitis.

In Sahiwal cattle correlation between RUH, RUW, CL, RTL and FTL with 305MY were positively significant ($p<0.05$). A positive and significant ($p<0.05$) correlation of TMY with FUA, RUH, UC, CL, RTL and FTL were found. MMY had positive and highly significant ($p<0.01$) correlation with RUH, RUW, UC while significant ($p<0.05$) with UL. A positive and highly significant ($p<0.01$) correlation of UL, UW, UC and significant ($p<0.05$) correlation of DFR with TDMY was found in Sahiwal cattle.

In Karan Fries cows a positive and highly significant ($p<0.01$) correlation of UL, UC and CL with all production traits were present. Highly significant ($p<0.01$) and negative correlation of UD with production traits was present. RUH had positive and highly significant ($p<0.01$) correlation with 305MY and TMY while RUW showed negative and highly significant ($p<0.01$) correlation with 305MY and TMY. MMY showed positive and significant ($p<0.05$) correlation with FUA and UW.

Positive and significant ($p<0.05$) point biserial correlation of clinical mastitis was found with TMY and 305MY in both the breeds. Sahiwal had negative and highly significant ($p<0.01$) effect of clinical mastitis with FUA, CL and positive association with DFR, DLR and SDF. In Karan Fries UD, TC and TL had positive and significant ($p<0.05$) effect on clinical mastitis.

The association analysis of SNPs with production traits revealed that SNPs at loci C33219027T and C33471980A of FAM19A1 gene and G78216220A of KCNB1 gene had significant ($p<0.05$) effect on TDMY in Sahiwal cattle. SNPs at locus C33219027T had significant ($p<0.05$) effect on MMY in Sahiwal cattle. In Karan Fries cattle, significant effect on 305MY and TMY was found for SNP at loci C33471980A ($p<0.05$) and G33571161A ($p<0.01$) of FAM19A1 gene. SNP at C33219027T locus of FAM19A1 gene and SNP locus A78216335G of KCNB1 gene had significant ($p<0.05$) effect on TDMY while SNP at

G33589765A locus of FAM 19A1 gene had significant (p<0.05) effect on MMY in Karan Fries cows.

The association analysis of SNPs of FAM19A1 gene with udder type traits revealed that SNP at locus T33069832C had significant (p<0.05) effect on TC in Karan Fries and Sahiwal cattle. Effect of SNP at locus C33219027T was highly significant (p<0.01) for UC and RUH in Sahiwal cows and SDR in Karan Fries cows whereas, significant (p<0.05) effect for UW, SDF in Karan Fries cattle. Effect of SNP at locus C33464963A was significant (p<0.05) for UB, TC, DFR, DLR in Sahiwal cows while for FTL in Karan Fries cows. SNP at locus C33471720T had highly significant (p<0.01) effect on RUH, RUW, TC, DLR and SDR in Sahiwal cattle whereas, in Karan Fries cattle significant (p<0.05) effect was on UB, UW, CL. Similarly, effect of SNP at locus C33471980A had significant (p<0.05) effect on UL, UW, UC, FTL and RTL in Sahiwal cattle whereas, on UD and DFR in Karan Fries cattle. SNPs G33571161A and G33589765A did not have any significant effect in Sahiwal cattle. However, in Karan Fries cattle SNP at locus G33571161A had significant (p<0.05) effect on UC and DFR while SNP at locus G33589765A had significant (p<0.05) effect on UW only.

The association analysis of SNPs of KCNB1 gene with udder type traits revealed that SNP at locus G78216220A had significant (p<0.05) effect on UC, DFR and TD in Sahiwal cattle and on UL, UW and SDR in Karan Fries cattle. SNP at locus A78216335G had highly significant (p<0.01) effect on RUH and RUW in both the breeds while significant (p<0.05) effect on UC in Karan Fries cattle.

SNP at locus C57571910A of EDN3 gene had highly significant (p<0.01) effect on UW and significant (p<0.05) effect on UL in Sahiwal cattle.

The chi-square analysis of identified genetic variants with mastitis revealed that SNP at locus C33464963A present in intron 2 of FAM19A1 gene had significant (p<0.05) effect on incidence of mastitis in both Sahiwal and Karan Fries cattle and SNP at locus C57571910A present in exon 1 of EDN3 gene had significant (p<0.05) effect on incidence of mastitis in Sahiwal cattle.

The association analysis of SNPs of FAM19A1 gene with observational (visual) trait revealed that SNP at locus C33219027T had highly significant (p<0.01) effect on USUS and significant (p<0.05) effect on TES in Karan Fries cattle. SNP at locus C33464963A was found to be significantly (p<0.05) associated with SC in Karan Fries cattle. SNP at locus C33471980A was found to be significantly (p<0.05) associated with TS, TES and LTCTEC in Sahiwal cattle and with RTP and TES in Karan Fries cattle. SNP at locus G33571161A had significant (p<0.05) effect on USUS and SC in both the breeds.

The association analysis of SNPs of KCNB1 gene with observational (visual) trait revealed that SNPs at loci G78216220A and A78216335G had significant (p<0.05) effect on TS in Sahiwal cattle. However, SNPs at loci G78216220A and A78216335G had significant (p<0.05) effect on RTP and TES, respectively in Karan Fries cattle.

SNP at locus C57571910A present in EDN3 gene had significant (p<0.05) effect on TS, USUS and FTP in Sahiwal cattle.

Conclusions:

On the basis of results observed in the present study, the following conclusions can be drawn:

- Genomic DNA samples of 253 (166 Karan Fries and 87 Sahiwal) cows were analyzed.
- A total of 9 primer sets (7 for FAM19A1, 1 for KCNB1 and 1 for EDN3 gene) were designed and amplified.
- Total 10 SNPs were found in Sahiwal cattle, 7 on FAM19A1, 2 on KCNB1 and 1 on EDN3 gene.
- In Karan Fries cattle 9 SNPs were revealed, 7 on FAM19A1 gene and 2 on KCNB1 gene.
- PCR-RFLP analysis was carried out using *BsrI, TaqI, AluI, HinfI, HindIII, MspI, BspHI* and *TspRI* restriction enzymes for all 253 DNA samples.
- Parity had highly significant (p<0.01) effect on FUA, RUW, UD, UW and significant (p<0.05) effect on TD whereas, stage of lactation had significant (p<0.05) effect on UC, TC, SDR and DFR and season was found significantly (p<0.05) associated with TC in Sahiwal cattle.
- In Karan Fries cattle, parity had highly significant (p<0.01) effect on RUH, UD, UL, UW, TC, FTL, SDF, SDR and TD while stage of lactation had significant (p<0.05) effect on UC. Season did not have significant effect on udder type traits in Karan Fries.
- Logistic regression revealed significant effect (p<0.05) of parity and level of production on clinical mastitis in Sahiwal cattle whereas, in Karan Fries cattle only parity had highly significant (p<0.01) effect on clinical mastitis.
- Point biserial correlation of clinical mastitis was found to be positive and significant (p<0.05) with TMY and 305MY in both the breeds. Negative and highly significant (p<0.01) correlation of clinical mastitis with FUA, CL and positive correlation with DFR,

DLR and SDF was found in Sahiwal cattle. In Karan Fries cattle, UD, TC and TL was significantly ($p<0.05$) correlated with clinical mastitis.

- Sahiwal cows with ‘TT’ genotype at SNP C33219027T locus recorded maximum UC, MMY and TDMY while in Karan Fries cows ‘CC’ genotype at SNP C33219027T locus recorded maximum RUH, SDF, SDR and lowest UW with maximum intermediate type USUS, round TES and average value of production traits.
- Karan Fries cows with ‘CC’ genotype at SNP C33464963A locus had lowest FTL and maximum SC of no ring type with lesser incidence of mastitis.
- Similarly, ‘CC’ genotype at SNP C33464963A locus in Sahiwal cows had minimum UB, DFR, DLR and maximum TC with minimum incidence of mastitis.
- ‘CT’ genotype at SNP locus C33471720T had maximum value of UW and CL with minimum UB in Karan Fries cows while this genotype had maximum RUH, RUW with lowest TC and average value of DLR and SDR in Sahiwal cows with highest production traits in both the breeds.
- Sahiwal cows with ‘CA’ genotype at locus C33471980A had higher UL, UW, UC, TL with maximum funnel shaped teat, round teat end and higher TDMY but with more number of rough ring than ‘CC’ genotype. However, in Karan Fries cows, ‘CC’ genotype had higher 305MY, TMY but minimum UD, DFR and with intermediate placement of rear teat.
- ‘GG’ genotype of SNP at locus G33571161A recorded higher UC, DFR with maximum number of intermediate type USUS, normal SC and higher 305MY, TMY than ‘GA’ genotype in Karan Fries cows while in Sahiwal cows with ‘GG’ genotype at locus G33571161A exhibit normal SC.
- Karan Fries cows with ‘GG’ genotype at locus G33589765A had significantly ($p<0.05$) highest MMY and UW than genotypes ‘GA’ and ‘AA’.
- ‘GG’ genotype at locus G78216220A of KCNB1 gene had maximum UC, TD and minimum DFR with maximum percentage of funnel shaped teat and highest TDMY in Sahiwal cows while in Karan Fries cows ‘AA’ genotype at locus G78216220A is associated with highest UW, UL and lowest SDR with maximum intermediate placed fore teat.
- Sahiwal cows with ‘AA’ genotype at locus A78216335G of KCNB1 gene had significantly ($p<0.01$) lower RUH and RUW with maximum percentage funnel shaped teat while in

Karan Fries cows 'AA' genotype of this SNP had recorded maximum value of RUH, RUW and minimum UC, SDF and TDMY with mostly round teat end shape.

- 'CA' genotype at locus C57571910A of EDN3 gene had lower UL, UW but with intermediate USUS type, FTP type and funnel shaped teat and was also less susceptible to mastitis compared to 'CC' genotype.

Recommendations

It is inferred from the present investigation that 'CA' genotype at locus C33471980A and 'GG' genotype at locus G33571161A of FAM19A1 gene and 'AG' genotype at locus A78216335G of KCNB1 gene in Karan Fries cattle and 'CA' genotype at C57571910A locus in EDN3 gene and 'CC' genotype at C33464963A locus of FAM19A1 gene in Sahiwal cattle were identified as favourable genotypes. Hence, these genotypes can be used as an aid to selection for higher milk production with desired udder conformation and lesser susceptibility to mastitis after substantiation of the findings in a larger population.

Bibliography

BIBLIOGRAPHY

Abebe, R., Hatiya, H., Abera, M., Megersa, B. and Asmare, K. 2016. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet. Res.,* **12**: 270

Abrahmsen, M., Persson, Y., Kanyima, B.M. and Bage, R. 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Trop. Anim. Health Prod.*, **46**: 99–105.

Ahlawat, K. 2007. Effect of udder characteristics on milk somatic cell counts and incidence of mastitis in Sahiwal cows. M.V.Sc. Thesis. ICAR-National Dairy Research Institute, Karnal, India.

Ahlawat, K., Dang, A.K. and Singh, C. 2008. Relationship of teat and udder shape with milk SCC in primiparous and multiparous Sahiwal cows. *Indian J. Dairy Sci.,* **61**(2): 152-156.

Ahmed, J.U., Sutradhar, S. and Rahman, M.M. 2005. Morphological characteristics of udders and teats in relation to mastitis and milk yield in crossbred dairy cows. The Bangladesh Veterinarian, **22**: 23-28.

Amin, A., Gere, T. and Kishk, W. 2002. Genetic and environmental relationship among udder conformation traits and mastitis incidence in Holstein Friesian into two different environments. *Arch. Tierz*., **45**(1): 129-138

Asaf, V.N.M., Kumar, A., Rahim, A., Sebastian, R., Mohan, V., Dewangan, P. and Panigrahi, M. 2014. An overview on single nucleotide polymorphism studies in mastitis research. *Vety. World,* **7**(6): 416-421.

Atil, H., Khatt, S.A. and Yakupoglu, C. 2001. Genetic analysis for milk traits in different herds of Holstein Friesian cattle in Turkey. *J. Biol. Sci*., **1**: 737-741.

Atkins, G. 2007. Long life milkers. *The Aust. Holst. J.,* 52- 55

Atkins, G. and Shannon, J. 2002. Minimizing Lameness through Genetic Selection. *Adv. Dairy Techno.,* **14**: 93

Awale, M.M., Dudhatra, G.B., Avinash, K., Chauhan, B.N. and Kamani, D.R. 2012. Bovine Mastitis: A Threat to Economy. *Sci. Rep*., **1**: 295.

Bajwa, I.R., Khan, M.S., Khan, M.A. and Gondal, K.Z. 2004. Environmental factors affecting milk yield and lactation length in Sahiwal cattle. *Pakistan Vet. J.*, **24**(1): 23-27.

Bardakcioglu, H.E., Sekkin, S. and Oraltoplu, H.D. 2011. Relationship between some teat and body measurements of Holstein cows and sub-clinical mastitis and milk yield. *J. Anim. Vet. Adv*., **10**(13): 1735-1737.

Baynash, A.G., Hosoda, K., Giaid, A., Richardson, J.A., Emoto, N., Hammer, R.E. and Yanagisawa, M. 1994. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell*, **79**: 1277–85.

Bermingham, M., Berry, D.P. and Cromie, A.R. 2007. Irish Holstein-Friesian cows are taller than a decade ago. In: Agricultural Research Forum, Tullamore, pp: 77.

Bharti, P., Bhakat, C., Pankaj, P.K., Bhat, S.A., Prakash, M.A., Thul, M.R. and Japheth, K.P. 2015. Relationship of udder and teat conformation with intra-mammary infection in crossbred cows under hot-humid climate. *Vet. World,* **8**(7): 898-901.

Bhuiyan, M.M., Islam, M. R., Ali, M. L., Hossain, M.K., Kadir, M.A., Lucky, N.S. and Das, B.R. 2004. Importance of mammary system conformation traits in selecting dairy cows on milk yield in Bangladesh. *J. Biol. Sci.,* **4**(2): 100-102.

Bosma, M.M. and Hille, B. 1992. Electrophysiological properties of a cell line of the gonadotrope lineage. *Endocrinology,* **130**(6): 3411–3420.

Boujenane, I., El Aimani, J. and By, K. 2015. Incidence and occurrence time of clinical mastitis in Holstein cows. *Tur. J. Vet & Anim Sci*., **39**: 42–49.

Breen, J.E., Bradley, A.J. and Green, M. J. 2009. Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. *J. Dairy Sci.,* **92**: 3106-3115.

Britt, J.S. and Farnsworth, R. 2011. Hoard's Dairyman. http://milkquality.wisc.edu.

Brotherstone, S. 1994. Genetic and phenotypic correlations between linear type traits and production traits in Holstein-Friesian dairy cattle. *Anim. Prod*., **59**: 183-187.

Brotherstone, S., White, I.M., Coffey, M., Downs, S.H., Mitchell, A.P., Clifton-Hadley, R.S., More, S.J., Good, M. and Woolliams, J.A. 2010. Evidence of genetic resistance of cattle to infection with Mycobacterium bovis. *J. Dairy Sci.*, **93**(3): 1234-42.

Campos, R.V., Cobuci, J.A., Kern, E. L., Costa, C.N. and McManus, C.M. 2015. Genetic parameter for linear type traits and milk, fat and protein product. *Asian Australas. J. Anim. Sci*., **48**(4): 476-484.

Chachare, R.A. and Walkunde, T.R. 2011. Udder and teat measurements influenced by milking, udder shape and texture in Gaolao cows. *Res. J. Anim. Husbandry Dairy sci.*, **2**(12): 40-42.

Cobanoglu, O., Gurcan, E.K., Cankaya, S., Kul, E., Abaci, S.H. and Ulker, M. 2017. Effects of lactation month and season on test-day milk yield and milk components in Holstein cows. *Indian J. Anim. Res.*, **51** (5) : 952-955

Cole, J.B., Wiggans, G.R., Ma, L., Sonstegard, T.S., Lawlor, T.J. and Crooker, B.A. 2011. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. *BMC Genomics*. **12**: 408.

DAHD (Department of Animal Husbandry, Dairying and Fisheries) Annul Report. 2017-18. Ministry of Agriculture, Government of India. (http://dahd.nic.in/dahd/statistics/animal-husbandry-statistics.aspx).

Dahiya, S.P., Rathi, S.S. and Narula, H.K. 2006. Linear scoring of type conformation for milk production in Sahiwal cows. *Indian J. Dairy Sci.*, **59** (1): 46-48.

Deng, M.P., Badri, T.M., Atta, M. and Hamad, M.E. 2012. Relationship between udder dimensions and milk yield of Kenana × Friesian crossbred cows. Research Opinions in Animal & Veterinary Science, **2**(1):49-54

Duan, X.L., Zhang, X.S. and Li, G.W. 2003. Clinical relationship between EDN-3 gene, EDNRB gene and Hirschsprung's disease. *World J. Gastroenterolgy,* **9** (12): 2839-2842.

Dube, B., Dzama, K., Banga, C.B. and Norris, D. 2008. An analysis of the genetic relationship between udder health and udder conformation traits in South African Jersey cows. *Animal*, **3** (4): 494-500.

Dubey, A., Mishra, S., Khune, V., Gupta, P.K., Sahu, B.K. and Nandanwar, A.K. 2010. Improving linear type traits to improve production sustainability and longevity in purebred Sahiwal cattle. *J. Agr. Sci. Tech.,* **5**: 636–639.

Durr, J.W., Cue, R.I., Monardes, H.G., Moro-Mendez, J. and Wade, K.M. 2008. Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. *Livest. Sci.*, **117**: 225-232.

Fahim, A., Kamboj, M.L., Sirohi, A.S., Bhakat, M. and Mohanty, T.K. 2017. Factors affecting milk quality of crossbred dairy cows in automated Herringbone milking system. *Indian J. Dairy Sci.,* **87** (11): 1396–1401.

Flury, C., Boschung, C., Denzler, M., Bapst, B., Schnyder, U. and Gredler, B. 2014. Genome-wide association study for 13 udder traits from linear type classification in cattle. In: *Proceedings of the 10th World Congress on Genetics Applied to Livestock Production:* 17-22.

Foster, W.W., Freeman, A.E. and Berger, P.J. 1989. Association of Type Traits Scored Linearly with Production and Herdlife of Holsteins. *J. Dairy Sci.*, **72**: 2651.

Foster, W.W., Freeman, A.E., Berger, P.J. and Kuck, A. 1988. Linear type trait analysis with genetic parameter estimation. *J. Dairy Sci.,* **71**: 223-231.

Fujimori, Y., Uchide, T., Temma, K., Sasaki, T., Kizaki, K., Hara, Y. and Saida, K. 2004. Complete cDNA sequence and mRNA expression of dog preproendothelin-3. *J. Vet . Med. Sci.,* **66** (10): 1251-1254.

Gajbhiye, A.R., Wanjari, B.V., Chavan, M.S., Jadhao, S.G. and Sahare, T.Y. 2007. Udder measurements and its correlation with milk productivity in crossbred cattle. *Indian J. Field Vet*., **3**(1): 39-40.

Gallo-Hendrikx, E., Murray, S.A., Vonderhaar, B.K. and Xiao, Z.J. 2001. Vanadate Disrupts Mammary Gland Development in Whole Organ Culture. *Dev. Dynam*., **222**: 354-367.

Gao, Y.L., Dong, M., Wei, L., Aimin, L., Xianyong, C., Zhang, C. and Chen, H. 2013. Two novel SNPs in the coding region of bovine VDR gene and their associations with growth traits. *J. Genet.,* **92**: 53-59.

Ghosh, B. and Prasad, J. 1998. Milk yield and composition as influenced by udder measurements in Jersey x Red Sindhi crosses. *Indian J. Anim. Prod. Man*., **14**: 23-25.

Gibson, K. 2015. Genetic evaluation of Brown Swiss cattle in the US. M.Sc. *Thesis* . The Pennsylvania a state university.

Gonzalez, M.E., Gonzalez, V.M., Montano , M.F., Medina, G.E., Mahadevan, P., Villa, C. and Villa, R. 2017. Genome-wide association analysis of body conformation traits in Mexican Holstein cattle using a mix of sampled and imputed SNP genotypes. *Genet. Mol. Res.*, **16**(2): 1-9

Guarín, J.F., Paixao, M.G. and Ruegg, P.L. 2017. Association of anatomical characteristics of teats with quarter-level somatic cell count. *J. Dairy Sci.*, **100**: 643–652.

Gupta, R., Singh, R.P. and Tomar, S. 1991. Udder and Teat measurement and their association with milk production in Karan-Fries cows. *Indian J. Anim. Res.*, **25**(1): 23-28.

Haghkhah, M., Ahmadi, M.R., Gheisari, H.R. and Kadivar, A. 2011. Preliminary bacterial study on subclinical mastitis and teat condition in dairy herds around Shiraz. *Turk. J. Vet. Anim. Sci*., **35**: 387-394.

Hagnestam, C., Emanuelson, U. and Berglund, B. 2007. Yield losses associated with clinical mastitis occurring in different weeks of lactation. *J. Dairy Sci.*, **90**: 2260-2270.

Harvey, W.R. 1990. User’s Guide for LSMLMW and MIXMDL, Mixed Model Least - Squares and Maximum Likehood Computer Program. PC-2 version. The Ohio State University, Columbus, USA.

Heikkila, A.M., Nousiainen, J.I. and Pyorala, S. 2012. Costs of clinical mastitis with special reference to premature culling. *J. Dairy Sci.*, **95**: 139-150

Hiendleder, S., Thomsen, H., Reinsch, N., Bennewitz, J., Leyhe-Horn, B., Looft, C., Xu, N., Medjugorac, I., Russ, I., Kuhn, C., Brockmann, G.A., Blumel, J., Brenig, B., Reinhardt, F., Reents, R., Averdunk, G., Schwerin, M., Forster, M., Kalm, E. and Erhardt, G. 2003. Mapping of QTL for body conformation and behavior in cattle. *J. Hered.*, **94**(6): 496–506

Hussain, R., Khan, A., Javed, M.T. and Rizvi, F. 2012. Possible risk factors associated with mastitis in indigenous cattle in Punjab, Pakistan. *Pakistan Vet. J*., **32**: 605-608.

ICAR. 2012. Conformation Recording of Dairy Cattle. Available at: www.icar.org/ Documents/…./ Guidelines/……/ Section/205.0-5.10.pdf.

Japheth, K.P., Mehla, R.K. and Bhat, S.A. 2015. Effect of non-genetic factors on various economic traits in Karan Fries crossbred cattle. *Indian J. Dairy Sci.,* **68**(2): 163-169

Jingar, S. C., Mehla, R. K., Singh, M. and Singh, P.K. 2014. Effect of stages and level of milk production on mastitis incidence in cows and Murrah buffaloes. *J. Bio. Innov.,* **3**(3): 117-123.

Juozaitiene, V., Juozaitis, A., Zymantiene, J., Oberauskas, V., Aniuliene, A., Kajokiene, L., Yilmaz, A. and Simokaitiene, A. 2019. The effect of different levels of teat-end hyperkeratosis on mammary infrared thermograph and mastitis in dairy cows. *Ankara Univ. Vet. Fak. Derg.,* **66**: 21- 26.

Kakati, P., Panchal, D., Patel, A., Bahuguna, P., Joshi, R. and Rank, D. 2017. Genetic parameters of production and reproduction traits and factors affecting it in Frieswal cattle. *Inter. J. Livest. Res*., **7**(7): 190- 199.

Kamboj, M.L., Singh, A. and Prasad, S. 2007. Effect of udder and teat shapes and their measurements on somatic cell counts in milk of Karan Fries cows. *Res. J. Anim. Husb. Dairy Sci*., **60**(6): 435- 440.

Kamboj, M.L., Singh, A. and Prasad, S. 2008. Effect of udder and teat characteristics on incidences of subclinical mastitis in crossbred cows. *Indian Vet. J.,* **85**(8): 846-848.

Katok, N. and Yanar, M. 2012. Milk traits and estimation of genetic, phenotypic and environmental trends for milk and milk fat yields in Holstein Friesian Cows. *Int. J. Agric. Biol*., **14**: 311-314.

Katsande, S., Matope, G., Ndengu, M. and Pfukenyi, D.M. 2013. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *J. Vet. Res*., **80**: 523.

Khan, M. A. and Muhammad, K. 2015. Non-genetic factors affecting linear type traits in Sahiwal cows. *J. Anim. Plant Sci.*, **25**(1):29- 36.

Khan, M.A. and Khan, M.S. 2016. Genetic parameters of udder traits and their relationship with milk yield in Sahiwal cows of Pakistan. *J. Anim. Plant Sci.*, **26**(4): 880- 886.

Khatri, S.B., Trivedi, M.M., Patel, Y.G. and Rajpura, R.M. 2017. Udder and teat measurements and their relation with milk production in buffaloes. International Journal of Advanced Biological Research **7**(3): 582-584.

Kim, S.J., Widenmaier, S.B., Choi, W.S., Nian, C., Ao, Z. and Warnock, G. 2012. Cell Death. Differ. Pancreatic *β*-cell prosurvival effects of the incretin hormones involve post-translational modification of Kv2.1 delayed rectifier channels. **19**(2): 333-344.

Klein, D., Flock, M., Khol, J.L., Franz, S., Stuger, H.P. and Baumgartner, W. 2005. Ultrasonographic measurement of the bovine teat: breed differences, and the significance of the measurements for udder health. *J. Dairy Res.*, **72**: 296-302.

Kshatriya, P.S., Trivedi, M.M. and Dhami, A.J. 2009. Association of udder biometry and skin thickness with milk yield in Kankrej and crossbred cows. *Indian J. Field Veterinarian,* **5**: 11–13

Kuczaj, M. 2003. Analysis of changes in udder size of high yielding cows in subsequent lactations with regard to mastitis. *Electronic J. Pol. Agric. Univ. Ser. Anim. Husb*., **6**(1): 02

Kumar, A.V., Rao, L.V., Kumar, M.K., Srinu, B. and Rao, T.M. 2012. Efficacy of udder disinfectants on reduction of bacterial load and certain pathogens of public health significance. *J. Microbiol. Biotech. Res*., **2**: 147-151.

Kumar, M., Vohra, V., Ratwan, P., Chopra, A. and Chakravarty, A. K. 2017. Influence of FASN gene polymorphism on milk production and its composition traits in Murrah buffaloes. *Indian J. Anim. Res*., **51** (4): 640-643

Kumar, M., Vohra, V., Ratwan, P., Valsalan, J., Patil, C. S. and Chakravarty, A. K. 2016. Estimates of genetic parameters for fat yield in Murrah buffaloes. *Vety. World*, **9**(3): 295-298.

Larroque, H. and Ducrocq, V. 2001. Relationship between type and longevity in the Holstein breed. *Genet. Sel. Evol*., **33**: 39- 59.

Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A. and Silanikove, N. 2007. Changes in milk composition as affected by subclinical mastitis in sheep. *J. Dairy Sci.,* **87**: 46-52.

Lescourret, F., Genest, M., Barnouin, J., Chassagne, M. and Faye, B. 1993. Data modeling for database design in production and health monitoring systems for dairy herds. *J. Dairy Sci*., **76**: 1053-I062.

Liu, S.B., Huize, T., Lu, Y. and Anmingy, J. 2014. Genetic parameter estimates for selected type traits and milk production traits of Holstein cattle in Southern China. *Turk. J. Vet. Anim. Sci.,* **38**: 552–556.

Marinov, I., Penev, T. and Gergovska, Z.H. 2015. Factors affecting linear type traits in Black-and-White cows. *Int. J. Curr. Microbiol. App. Sci.,* **4**(10): 374- 383.

Mein, G.A, Reinemann, D.J, Schuring, N. and Ohnstad, I. 2001. Milking machines and mastitis risk: A storm in a teat-cup. USA: Proceedings of the 43rd Annual Meeting of the National Mastitis Council.

Meingoas, P.J.K., Ndukum, A.J., Dakyang, H. and Zoli, A.P. 2017. Effects of body conformation and udder morphology on milk yield of zebu cows in North region of Cameroon. *Vety. World*, **10**(8): 901- 905.

Merete, A., Lund, M. S., Boichard, D. and Ramayo-Caldas, Y. 2018. A system-based analysis of the genetic determinism of udder conformation and health phenotypes across three French dairy cattle breeds. *PLoS One*, **13**(7): 100-110

Meyer, K. and Burnside, E.B. 1987. Scope for a Subjective Assessment of Milking Speed. *J. Dairy Sci*., **70**: 1061–1068.

Miglior, F., Muir, B L. and Doormaal, B J. 2005. Selection indices in Holstein cattle of various countries. *J. Dairy Sci.,* **88**: 1255- 1263.

Misztal, I., Lawlor, T.J., Short, T.H. and VanRaden, P.M. 1992. Multiple-trait estimation of variance components of yield and type traits using an animal model. *J. Dairy Sci.,* **75**: 544-551

Mitra, A., Joshi, B.K. and Kale, M.M. 1998. Genetic parameters of linear type traits in Karan-Fries cattle. *Indian J. Ani. Prod. Mgt.,* **14** (3): 170-174.

Modh, R.H., Islam, M.M., Patel, Y.G., Modi, K .J. and Wadhwani, K.N. 2017. Studied the effect of parity on udder and teat biometry and its association with milk yield in Gir cows. *Inter. J. Sci., Environ. and Tech.,* **6**(3): 2068-2073

Moore, R. K., Higgins, S., Kennedy, B.W. and Burnside, E.B. 1981. Relationships of teat conformation and udder height to milk flow rate and milk production in Holsteins. Can. *J. Anim. Sci.,* **61**: 493.

Mureithi, D.K. and Njuguna, M.N. 2016. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Livest. Res. Rural. Dev*., **28**: 13

Nakov, D. and Trajcev, M. 2012. Udder quarter risk factors associated with prevalence of bovine clinical mastitis. *Mac. Vet. Rev.*, **35**(2): 55-64.

Nakov, D., Hristov, S., Andonov, S. and Trajchev, M. 2014. Udder-related risk factors for clinical mastitis in dairy cows. *Vet. Arhiv*., 84: 111-127.

Narwaria, U.S., Mehla, R.K., Verma, K.K., Lathwal, S.S., Yadav, R. and Verma, A.K. 2015. Study of short lactation in Sahiwal cattle at organized farm. *Vet. World,* **8**(5): 690-694.

Nash, D.L., Rogers, G.W., Cooper, J.B., Hargrove, G.L. and Keown, J.F. 2002. Relationships among severity and duration of clinical mastitis and sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. *J. Dairy Sci.,* **85:** 1273-1284.

Nemcova, E., Stipkova, M., Zavadilova, L., Bouska, J. and Vacek, M. 2007. The relationship between somatic cell count, milk production and six linearly scored type traits in Holstein cows. *Czech J. Anim. Sci*., **52**(12): 437-446.

Norman, H.D., Powell, R.A., Wright, J.R. and Cassel, B.G. 1988. Phenotypic and Genetic Relationship between Linear Functional Type Traits and Milk Yield of Five Breeds. *J. Dairy Sci.,* **71**(7): 1880-1896.

Nyman, A.K. 2007. Epidemiological studies of risk factors for bovine mastitis. Doctoral thesis. Swedish University of Agricultural Sciences. Uppsala, Sweden. Electronic version available at http://epsilon.slu.se/eng.

Ogorevc, J., Kunej, T., Razpet, A. and Dovc, P. 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. *Anim. Genet.,* **40**: 832- 851.

Okano, W., Junior, C.K., Bogado, A.L.G. and Filho, L.C.N. 2015. Relationship between Shape of Teat and Teat Tip and Somatic Cell Count (SCC) in Dairy Cows. *Acta Scientiae Veterinariae,* **43**: 1276

Olusayo, A., Machebe, N.S., Ezekwe, A.G. and Agaviezor, O.B. 2016. Effect of parity on changes in udder traits, milk yield and composition of West African dwarf sheep during lactation. *Anim. Prod. Sci.,* http://dx.doi.org/10.1071/AN15241

Pal, S.K., Takimoto, K., Aizenman, E. and Levitan E.S. 2006. Apoptotic surface delivery of K^+ channels. *Cell Death Differ,* **13**(4): 661- 667.

Patel, Y. and Trivedi, M. 2018. Morphometric Characteristics of Udder and Teats in Crossbred Cows. *Int. J. Livest. Res.*, **8**(6): 251-257

Patel, Y.G. 2014. Studies of association between morphometric characteristics of udder and teats and incidences of sub-clinical mastitis in crossbred cows maintained at dairy farms in Anand district, Thesis, Anand Agricultural University, Anand.

Patel, Y.G., Trivedi, M.M., Rajpura, R.M., Savaliya, F.P. and Parmar, M. 2016. Studied udder and teat measurement and their relation with milk production in crossbred cows. *Int. J. Sci. Env. Tech.,* **5**(5): 3048- 3054.

Pausch, H., Emmerling, R., Schwarzenbacher, H. and Fries, R. 2016. A multi-trait meta-analysis with imputed sequence variants reveals twelve QTL for mammary gland morphology in Fleckvieh cattle. *Genet. Sel. Evol*., **48**: 14

Pawlina, E., Kruszynski W. and Kuczaj, M. 2000. An investigation of changes in udder size of Red and White cows in first and third lactation. *Med. Weter*., **56**: 672-674

Prajapati, K.B., Ashwar, J.P., Patel, J.P., Patel J.B. and Singh, D.V. 1995. Size and shape of udder and teats in Kankrej cows. *Indian J. Anim. Prod. Mgmt*., **11**(1): 43-48.

Ranjeet, S.R., Dutt, T., Singh, M. and Kumar, A. 2010. Association of udder and teat dimensions with production traits in Vrindavani cattle. *Indian J. Dairy Sci*., **63**(6): 455-458.

Rao, T.K.S. 2006. Udder and teat dimension and their relationship with milk yield and composition in Karan Fries cows. M.V.Sc. *Thesis,* ICAR-National Dairy Research Institute, Karnal, India.

Rasmussen, M.D. and Larsen, H.D. 1998. The effect of post milking teat dip and suckling on teat skin condition, bacterial colonization, and udder health. *Acta Vet. Scand*., **39**: 443-452

Ratwan, P., Mandal, A. and Kumar, M. 2016. Genetic analysis of lactation traits in Jersey crossbred cattle. *Ind. J. Dairy Sci.*, **69**(2): 1- 4.

Rehman, Z and Khan, M.S. 2012. Environmental factors affecting performance traits of Sahiwal cattle in Pakistan. *Pak. Vet. J.*, **32**: 229- 233

Rehman, Z.U., Khan, M.S., Bhatti, S.A., Iqbal, J. and Iqbal A. 2008. Factors affecting first lactation performance of Sahiwal cattle in Pakistan. *Arch Tierz Dummerstorf*, **51**: 305–317.

Riera-Nieves, M., Rodriguez-Marquez, J.M., Perozo-Prieto, E., Rizzi, R. and Cefis, A. 2005. Morphometric characterization of the teats in Carora cows. *Revista Cientifica- Facultad De Ciencias Veterinarias,* **15**(5): 421-428.

Rupp, R. and Boichard, D. 1999. Genetic parameters for clinical mastitis, somatic cell score, production, udder type traits, and milking ease in first lactation Holsteins. *J. Dairy Sci.*, **82**(10): 2198- 2204

Sabin George, H. C., Suman C. L., Rathore, R. S. and Bisht, G. S. 2007. Incidences of subclinical mastitis in crossbred cattle herd. *Indian J. Anim. Prod Mgmt.*, **23**: 1-4

Sahana, G., Guldbrandtsen, B., Thomsen, B. and Lund, M.S. 2013. Confirmation and fine-mapping of clinical mastitis and somatic cell score QTL in Nordic Holstein cattle. *Anim. Genet*., **44**(6): 620–626.

Sahana, G., Guldbrandtsen, B., Thomsen, B., Holm, L.E., Panitz, F., Brondum, R.F., Bendixen, C. and Lund, M.S. 2014. Genome wide association study using high-density single nucleotide polymorphism arrays and whole-genome sequences for clinical mastitis traits in dairy cattle. *J. Dairy Sci*., **97**: 7258–7275.

Saini, A.L. and Gill, R.S. 1989. Proceedings of II World Buffalo Congress on Milk Production in Relation to Variation in Size and Shape of Udder and Teats in Murrah Buffaloes. **12** (16): 70-75.

Sambrook, J. and Russell, D. 2001. Molecular cloning: A laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York.

Sanotharan, N., Pagthinathan, M. and Nafees, M.S.M. 2016. Prevalence of bovine subclinical mastitis and its association with bacteria and risk factors in milking cows of Batticaloa district in Sri Lanka. *Int. J. Sci. Res. Innov. Technol.*, **3**: 137-150

Sargeant, J.M., Scott, M., Leslie, M., Ireland, K.E. and Bashiri, M.J.A. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. *Can. Vet. J.*, **39**: 33-38.

Sarkar, U., Gupta, A.K., Sarkar, V., Mohanty, T.K., Raina, V.S. and Prasad, S. 2006. Factors affecting test day milk yield and milk composition in dairy animals. *J. Dairying, Foods & H.S.* **25**(2): 129-132

Schneider, M.P., Durr, J.W., Cue, R.I. and Monardes, H.G. 2003. Impact of type traits on functional herd life of quebec Holsteins assessed by survival analysis. *J. Dairy Sci*., **86**: 4083-4089.

Shahid, M., Sabir, N., Ahmed, I., Khan, R. W., Irshad, M., Rizwan, M. and Ahmed, S. 2011. Diagnosis of subclinical mastitis in bovine using conventional methods and electronic detector. *ARPN J. Agri. Biol. Sci.*, **6**(11)**:** 18-22

Shamay, A., Shapiro, F., Barash, H., Bruckental, I. and Silanikove, N. 2000. Effect of dexamethasone on milk yield and composition in dairy cows. Annals de Zootechnie, **49**: 343-352.

Sharma, N., Rho, G.Y., Hong, Y.H., Lee, T.Y., Hur, T.Y. and Jeong, D.K. 2012. Bovine mastitis: an Asian perspective. *Asian J. Anim. Vet. Adv.*, **7**: 454-476.

Sharma, N., Singh, N.K., Singh, O.P., Pandey, V. and Verma, P.K. 2011. Oxidative stress and antioxidant during transition period in dairy cow. *Asian-Australasian J. Anim. Sci.*, **24**(40): 479-484.

Shook, G.E. 1989. Selection for disease resistance. *J. Dairy Sci.,* **72**: 1349-1362.

Silanikove, N., Merin, U. and Leitner, G. 2006. Physiological role of indigenous milk enzymes: An overview of an evolving picture. *Int. Dairy J*., **16**: 533–545.

Silanikove, N., Rauch-Cohen, A., Shapiro, F., Arieli, A., Merin U. and Leitner, G. 2012. Lipopolysaccharide challenge of the mammary gland in cows induces nitrosative stress that impaired milk oxidative stability. *Animal,* **6**: 1451-1459.

Silanikove, N., Shapiro, F., Shamay, A. and Leitner, G. 2005. Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: Manipulation with casein hydrolyzates. *Free Radic. Biol. Med*., **38**: 1139–1151.

Singh, A. 2005. Factor affecting incidence of mastitis in crossbred cows. M.V.Sc. Thesis, ICAR-National Dairy Research Institute, Karnal, India.

Singh, D., Kumar, S., Singh, B. and Bardhan, D. 2014. Economic losses due to important diseases of bovines in central India. *Vet. World*, **7**(8): 579-585.

Singh, M. 2006. Udder biometry and its association with milk yield and quality in Sahiwal cows. M.V.Sc. Thesis, ICAR-National Dairy Research Institute, Karnal, India.

Singh, R.R., Dutt, T., Singh, M. and Kumar, A. 2009. Association of udder and teat dimensions with production traits in Vrindavani cattle. *Ind. J. Dairy Sci.,* **63**(6): 455-458.

Singh, R.S., Bansal, B.K., Gupta, D.K., Randhawa, S.S. and Dhaliwal, P.S. 2013. The relationship of teat end hyperkeratosis with teat morphology and subclinical mastitis in holstein friesian × sahiwal crossbred dairy cows. *Abstract* .15th International Conference on Production Diseases in farm animals (ICPD), At Uppsala, Sweden.

Singh, R.S., Bansal, B.K. and Gupta, D.K. 2010. Udder health in relation to udder and teat morphometry in Holstein Friesian × Sahiwal crossbred dairy cows. *Trop. Anim. Health Prod.*, **46** (1): 93- 98.

Singhai, S.K., Ravikala, K., Murthy, K.S., Gajbhiye, P.U., Vataliya, P. H. and Savsani, H.H. 2013. Udder teat morphology and body measurements and their relationship with milk yield and milking traits in Gir cows. *Indian J. Anim. Prod. Mgmt.*, **29**(1-2): 5-11.

Smith, S.P., Allaire, F.R., Taylor, W.R., Kaeser, H.E. and Conley, J. 1985. Genetic parameters and environmental factors associated with type traits scored on an ordered scale during first lactation. *J. Dairy sci*., **68**: 2058-71.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical methods (7th ed). Iowa State Univ

Sonwane, J.S., Karanjkar, P.L. and Karanjkar, L.M. 2002. Udder characterization of milch animals in Ambejogai tehsil. *Indian J. Anim. Res.*, **36**(1):55-57.

Sorensen, M.K., Jensen, J. and Christensen, L.G. 2010. Udder conformation and mastitis resistance in Danish first-lactation cows: heritability, genetic and environmental correlations. *Acta Agr. Scand*., **50**: 72-82.

Steeneveld, W., Hogeveen, H., Barkema, H.W., Vanden, B. and Huirne, R.B.M. 2008. The infuence of cow factors on the incidence of clinical mastitis in dairy cows. *J. Dairy Sci*., **91**: 1391-1402

Strapak, P., Antalík, P. and Szencziova, I. 2011. Milkability evaluation of Holstein dairy cows by Lactocorder. *J. Agrobiology*, **28**(2): 139-146.

Strillacci, M.G., Frigo, E., Schiavini, F., Samore, A.B., Canavesi, F., Vevey, M., Cozzi, M.C., Soller, M., Lipkin, E. and Bagnato, A. 2014. Genome-wide association study for somatic cell score in Valdostana Red Pied cattle breed using pooled DNA. *BMC Genet.*, **15**: 106

Susanta, P., Das, P. and Ghosh, R.K. 2013. Comparative Study on Udder and Teat of Desi and Crossbred Cows in Relation to Udder Immunity. *Indian J. Vet. Anat*., **25**(2): 101-115.

Tag, E.D., Genena, S.K., Salem, A.Y., El-Awady, H.G. and El-Hamady, W.A. 2011. Milk ability in relation to udder conformation, udder health and productive traits in Friesian cows under Egyption farm conditions. In Proceedings of the 4th Scientific Conference of Animal Wealth Research in the Middle East and North Africa, Foreign Agricultural Relations 95-106.

Tilki, M., Inal, S., Colak, M. and M. Garip. 2005. Relationships between milk yield and udder measurements in Brown Swiss cows. *Turk J. Vet. Anim Sci.,* **29**: 75-81.

Tolleson, M.W. 2016. Genome-wide association for udder composition traits in *Bos Indicus -Bos Taurus* cows. M. Sc. Thesis, Texas A&M University.

Uzmay, C., Kaya, Y., Akbas, Y. and Kaya, A. 2003. Effects of udder and teat morphology, parity and lactation stage on subclinical mastitis in Holstein cows. *Turk. J. Vet. Anim. Sci.,* **27**: 935-941.

Van Dorp, T.E., Dekkers, J.C.M., Martin, S.W. and Noordhuizen, J.P.T.M. 1998. Genetic Parameters of Health Disorders, Relations with 305- day Milk Yield and Conformation Traits of Registered Holstein Cows. *J. Dairy Sci.,* **81**: 2264-2270

Velazquez, M. 2000. Factors affecting teat length and its importance on milk yield and udder health. In: Udder health and milk composition, with special reference to beef cows. Swedish University of Agricultural Sciences Skara. ISBN: 91-576-6004-2, pp: 35.

Vollema, A.R., Van Der Beek, S., Harbers, A.G.F. and De Jong, G. 2000. Genetic evaluation for longevity of Dutch dairy bulls. *J. Dairy Sci*., **83**: 2629–2639.

Waghmore, P. and Siddiqui, M. F. 2000. Studies on correlation of different udder and teat measurements with lactation milk yield in case of Holde crossbred cows. *Karnataka J. Agric. Sci*., **13**(3): 802-804.

Wang, Y., Wu, C.M., Lu, L.M., Ren, G.W.N., Cao, X.Y. and Shen, J.Z. 2008. Macrolide-lincosamide-resistant phenotypes and genotypes of Staphylococcus aureus isolated from bovine clinical mastitis. *Vet. Microbiol*., **130**: 118- 125

Wattiaux, M.A., Nordheim, E.V. and Crump, P. 2005. Statistical evaluation of factors and interactions affecting dairy herd improvement milk urea nitrogen in commercial Midwest dairy herds. *J. Dairy Sci*., **88**: 3020- 3035.

WHFF, 2005. International Type Evaluation of Dairy Cattle. A Report Published on June.

Wondifraw, Z., Thombre, B.M. and Bainwad, D.V. 2013. Effect of non-genetic factors on milk production of Holstein Friesian x Deoni crossbred cows. *Afric. J. Dairy Farm. Milk Prod.,* **1**(4): 079-084.

Wu, X., Lund, M. S., Sahan, G., Guldbrandtsen, B., Sun, D., Zhang, Q. and Su, G. 2015 .Association analysis for udder health based on SNP-panel and sequence data in Danish Holsteins. *Genet. Sel. Evol*., **47**(1):50

Yakubu, A. 2011. Path analysis of conformation traits and milk yield of Bunaji cows in smallholder's herds in Nigeria. *Agricultura Tropica et Subtropica*, **44**(3):152-157

Yamashita, K., Fujinaga, T., Okumura, M., Takiguchi, M., Tsunoda, N. and Mizuno, S. 1991. Serum C-reactive protein (CRP) in horses: the effect of aging, sex, delivery and inflammations on its concentration. *J. Vet. Med. Sci.*, **53**(6): 1019-1024.

Yeh, F.C., Yang, R.C. and Boyle, T. 1999. POPGENE Version 1.31. Microsoft Windows-based freeware for population genetic analysis. http://www.ualberta.ca/~fyeh/fyeh/

Zwertvaegher, I., Van Weyenberg, S., Piepers, S., Baert, J. and De Vliegher, S. 2012. Variance components of teat dimensions in dairy cows and associated factors. *J. Dairy Sci*., **95**: 4978-4988

List of Websites visited and referred:

http:// www.holsteinusa.com/ genetic_ evaluations /ss_linear.html.

http://dahd.nic.in/dahd/statistics/animal-husbandry-statistics.aspx

http://*www.ebi.ac.uk/tools/msa/clustalw2*

http://www.ensembl.org/index.html

http://www.icar.org/ Documents/…./ Guidelines/……/ Section/205.0-5.10.pdf.

Appendices

Appendix-I

1. Chemicals		Source
Agarose (low EEO)	:	Himedia
Ammonium chloride	:	SRL
Amyl alcohol	:	SRL
Boric acid	:	Sigma
Bromophenol blue	:	Himedia
Chloroform	:	SRL
EDTA di sodium salt	:	SRL
Ethanol	:	Himedia
Ethidium bromide	:	SRL
Formaldeyde	:	SRL
Glacial acetic acid	:	SRL
Hydrochloric acid	:	SRL
Isoamyl alcohol (extra pure AR)	:	SRL
Isopropanol	:	SRL
Magnesium chloride	:	SRL
Nuclease free water	:	Thermo Scientific
Phenol (extra pure AR)	:	SRL
Potassium bicarbonate	:	SRL
Sodium acetate	:	SRL
Sodium chloride	:	SRL
Sodium dodecyl sulfate	:	SRL
Sodium hydroxide	:	SRL
Tris base	:	Sigma
Tris HCL	:	Sigma

2. Equipments		Source
Centrifuge - Refrigerated	:	Sigma
Deep Freezer (-20°C)	:	Bluestar
Distillation plant	:	Millipore
Gel documentation system	:	Biorad
Horizontal gel electrophoresis apparatus	:	Tarson
Hot air oven	:	Scientronic Instruments
Ice box	:	Tarsons
Incubator- BOD	:	ABC India
Magnetic stirrer	:	Scientronic instruments
Micro-centrifuge	:	Tarsons
Micropipette (all ranges)	:	Thermo Scientific
Spectrophotometer	:	Schimadzu Cooperation
Thermal cycler	:	Biorad

UV transilluminator	:	Dyna Light
Vortexer	:	GeNei
Water bath	:	Scientronic
Weighing balance (Digital)	:	Citizen

3. Lab wares **Source**

3.1 Glass wares

Beakers, conical flasks	:	Borosil
Measuring cylinders, Pasteur pipettes	:	Borosil
10 ml pipettes, reagent bottles	:	Borosil

3.2 Plastic wares

Polypropylene centrifuge tube	:	Tarsons
Eppendorf tube (1.5 ml and 0.5 ml)	:	Axygen USA
PCR tube (0.2 ml)	:	Axygen USA
Microtips (All ranges)	:	Axygen USA

4. Enzymes and Biologicals

BsrI restriction enzyme (10 U/µl)	:	Thermo Scientific
TaqI restriction enzyme (10 U/µl)	:	Thermo Scientific
AluI restriction enzyme (10 U/µl)	:	Thermo Scientific
HinfI restriction enzyme (10 U/µl)	:	Thermo Scientific
HindIII restriction enzyme (10 U/µl)	:	Thermo Scientific
MspI restriction enzyme (10 U/µl)	:	Thermo Scientific
BspHI restriction enzyme (10 U/µl)	:	Thermo Scientific
TspRI restriction enzyme (10 U/µl)	:	Thermo Scientific
Primers (100 pmole/µl)	:	Eurofins
100 bp DNA Ladder	:	Thermo Scientific
50bp DNA ladder	:	Thermo Scientific
PCR Master Mix (2X)	:	Thermo Scientific

5. Miscellaneous items **Source**

Disposable gloves	:	Microflex
Para film	:	Parafilm
pH paper	:	SDFCL

The chemicals used in this study were of molecular biology grade. The list of chemicals and the preparation of reagents are as follows:

Preparation of Reagents

Tris (1 M) pH 8.0

Tris base (121.10 gm) was dissolved in 800 ml of double distilled waterand the pH was adjusted to 8.0 by adding concentrated HCl (40 ml). The solution was allowed to cool to room temperature before making final volume to 1 liter and stored at 4 ^{0}C.

Sodium chloride (5 M)

Sodium chloride (29.40 gm) was dissolved in 80 ml of double distilled water and the final volume made up to 100 ml. Sterilized by autoclaving and stored at 4 ^{0}C.

Sodium acetate (3 M)

24.60 gm of Sodium acetate (anhydrous) was dissolved in 80 ml of double distilled water. pH was adjusted to 5.2 with glacial acetic acid and the final volume was adjusted to 100 ml. Sterilized by autoclaving and stored at room temperature.

EDTA (0.5 M)

Disodium salt of EDTA (18.60 gm) was dissolved in 80 ml of double distilled water by adding NaOH pellets. pH was adjusted to 8.0 and final volume made up to 100 ml Sterilized by autoclaving and stored at 4 ^{0}C.

Ammonium chloride (1 M)

Ammonium chloride (5.35 gm) was dissolved in 80 ml of double distilled water and final volume was made up to 100 ml. Sterilized by autoclaving and stored at 4 ^{0}C.

Sodium bicarbonate (1 M)

10.00 gm of sodium bicarbonate was dissolved in 80 ml of double distilled water and made up to a final volume of 100 ml. Sterilized by autoclaving.

Sodium dodecyl sulphate (SDS) 10 %

Dissolved 10.00 gm of Sodium dodecyl sulphate in 80 ml of autoclaved double distilled water and made up to a final volume of 100 ml.

RBC Lysis buffer (1X prepared freshly every time)

NH_4Cl (155 mM)	:	155 ml
$KHCO_3$ (10 mM)	:	10 ml
EDTA (0.1mM)	:	2 ml

Final volume was made up to 100ml by adding double distilled water. Autoclaved and stored at 4^0C

RBC Lysis buffer (1X prepared freshly every time)

NaCl (75 mM)	:	75 ml
Tris (10 mM)	:	10 ml
EDTA (2 mM)	:	4 ml

Final volume was made up to 100ml by adding double distilled water. Autoclaved and stored at room temperature

Phenol equilibration (Tris saturation)

Phenol crystals (500 gm) stored at –20 ^{0}C was liquefied by keeping in a water bath maintained at 65 ^{0}C for 1 hour. 8-hydroxyquinoline was added to a liquefied phenol at a final concentration of 0.1 %. Then equal volume (500 ml) of 0.5 M Tris (pH 8.0) was added and stirred for 4 hours on magnetic stirrer and pH was checked repeatedly till it reached 8.0. Finally, 0.1 M Tris was added to an equilibrated phenol and stirred well and stored in amber colored bottles at (4 ^{0}C).

Proteinase-K

Proteinase-k (20 mg) was dissolved in 1ml of double distilled water. Aliquots of 400µl per vial made and stored at -20 ^{0}C.

Ethanol 70 %

Ethanol 99.9 %	:	70 ml
Distilled water	:	30 ml

Phosphate Buffer Saline (1X) Ca^{++} and Mg^{++} free

NaCl	:	4.0 gm
KCl	:	0.1 gm
Na_2HPO_4	:	0.7 gm
KH_2PO_4	:	0.1gm

The pH was adjusted to 7.4 and final volume made up to 500 ml with double distilled water. Autoclaved and stored the buffer at room temperature.

TE buffer (10 mM)

Tris (1M, pH 8.0)	:	1.00 ml
EDTA (0.5M, pH 8.0)	:	200 µl
Final volume made up to	:	100 ml

Sterilized by autoclaving and stored the buffer at room temperature.

Agarose gel:

Agarose 0.8 %

Agarose	:	0.60 gm
TBE (10 X)	:	10 ml
Double distilled water	:	90 ml

Agarose 1.5 %

Agarose	:	1.50 gm
TBE (10X)	:	10 ml
Double distilled water	:	90 ml

Agarose 2.5 %

Agarose	:	2.5 gm
TBE (10X)	:	10 ml
Distilled water	:	90 ml

Ethidium bromide

Ethidium bromide (0.25 gm) was dissolved in 25 ml of double distilled water. Stirred on a magnetic stirrer for several hours to ensure that the dye is properly dissolved. Wrapped the container with aluminum foil and stored at 4 ^{0}C.

Preparation of 6X gel loading dye

Bromo phenol blue	:	0.25 % (w/v)
Xylene cyanol FF	:	0.25 % (w/v)
Glycerol in water	:	30 % (v/v)

TBE buffer (10X)

Tris base	:	108.0 gm
Boric acid	:	55.0 gm
EDTA (0.5 M, pH 8.0)	:	40 ml

Double distilled water was added to make the final volume 1000ml, filtered and autoclaved.

TAE buffer (50X)

Tris base	:	242.0 gm
Glacial acetic acid	:	57.1 ml
EDTA (0.5 M, pH 8.0)	:	100 ml

Double distilled water was added to make the final volume 1000ml, filtered and autoclaved.