

**GENETIC POLYMORPHISM IN VDR, PTPRR AND HMGA2
GENE- THEIR ASSOCIATION WITH UDDER TYPE TRAITS
AND CLINICAL MASTITIS IN KARAN FRIES AND
SAHIWAL COWS**



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF**

**DOCTOR OF PHILOSOPHY
IN
ANIMAL GENETICS AND BREEDING
BY**

Dr. Rebeka Sinha
M.V.Sc. (Animal Genetics and Breeding)

**ANIMAL GENETICS & BREEDING DIVISION
ICAR - NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
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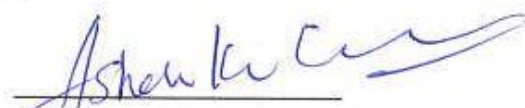
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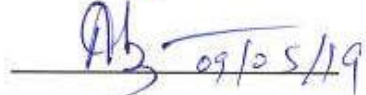

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(Director's nominee)









**ANIMAL GENETICS & BREEDING DIVISION
ICAR - NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)
KARNAL - 132001 (HARYANA), INDIA**

Dr. I. D. Gupta, Ph. D.
Principal Scientist

CERTIFICATE

This is to certify that the thesis entitled, "**Genetic Polymorphism in VDR, PTPRR and HMGA2 Gene- Their Association with Udder Type Traits and Clinical Mastitis in Karan Fries and Sahiwal Cows**" submitted by **Ms. Rebeka Sinha** 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 no part of the thesis has been submitted for any other degree or diploma.

Dated: ^{6th} -02-2019


(I. D. Gupta)
Major Advisor & Chairman

*Dedicated
To My
Respected Guide
&
Mentor
Dr. J. D. Gupta*



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***"No one who achieves success does so without acknowledging the help of others.
The wise and confident acknowledge this help with gratitude".
Alfred North Whitehead***

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ABBREVIATIONS

µg	:	Micro gram
µl	:	Micro litre
A	:	Adenine
bp	:	Base pair
BTA	:	<i>Bos taurus</i> 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	:	Deoxyribo nucleic acid
EDTA	:	Ethyelene Diamine Tetra acetic Acid
FTL	:	Front teat length
FTP	:	Front teat position
FTPl	:	Front teat placement
FUA	:	Fore udder attachment
G	:	Guanine
gm	:	Gram

HMGA2	: High mobility group AT-hook 2 Gene
HWE	: Hardy Weinberg Equilibrium
IL22	Interleukin 22
KF	: Karan Fries
LD	: Linkage disequilibrium
LTC TEC	: Long Term Changes in Teat-End Condition
M	: Molar
Mb	: Mega base pairs
MEGA	: Molecular Evolutionary Genetics Analysis
mg	: Milli gram
ml	: Millilitre
NCBI	: National Centre for Biotechnology Information
ng	: Nano gram
OD	: Optical Density
PCR	: Polymerase Chain Reaction
pmol	: Pico Mole
PTPRR	: Protein Tyrosine Phosphatase, Receptor Type R
RFLP	: Restriction Fragment Length Polymorphism
RE	: Restriction Enzyme
rpm	: Revolution Per Minute
RTP	: Rear teat Position

RTL	: Rear teat length
RTPl	: Rear teat Placement
RUH	: Rear udder height
RUW	: Rear udder width
SC	: Skin Condition
SCC	: Somatic cell count
SCS	: Somatic cell score
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
VDR	:	Vitamin D (1,25- Dihydroxyvitamin D3) Receptor
TCE	:	Total number of clinical mastitis episodes

Genetic Polymorphism in Bovine VDR, PTPRR and HMGA2 Gene - Their Association with Udder Type Traits and Clinical Mastitis in Karan Fries and Sahiwal Cows

A B S T R A C T

The current study was carried out in Sahiwal and Karan Fries cattle breeds with the objectives - To explore genetic polymorphism in bovine VDR, PTPRR and HMGA2 genes in Karan Fries and Sahiwal cows and; To find out the association of genetic variants with udder type traits, incidence of clinical mastitis and milk production. The SNPs reported of having significant effect on udder type traits situated in bovine VDR, PTPRR and HMGA2 genes were used. Total 253 lactating cows (Sahiwal = 87 and KF = 166) were genotyped by designing PCR-RFLP protocol on targeted SNPs, simultaneously, 14 udder type traits and 11 visual udder traits were recorded for each animal with accompanying milk production and incidence of clinical mastitis data. Before the final analysis the phenotypic data was adjusted for non-genetic factors. Population genetic parameters were calculated using Popgen v 1.32, association between genotypes and udder type traits/production traits was ascertained by SPSS and association between genotypes and incidence of mastitis was established using logistic regression (SAS university edition). Six SNPs rs454303072, rs135884509, rs382671389, rs435289107, rs136785910 and rs383429860 in KF cattle and four SNPs rs454303072, rs135884509, rs382671389, rs435289107 in Sahiwal cattle were found to be significantly deviating from HWE ($P \leq 0.05$). The loci were low to moderately polymorphic in Karan Fries ($PIC = 0.258$ to 0.375) and Sahiwal ($PIC = 0.138$ to 0.375) cattle. Four loci in Sahiwal breed were found to be in linkage disequilibrium ($P < 0.05$), however all the pairs were having low r^2 value. In Karan Fries cattle, genotype GG at SNP locus rs454303072, genotype AA at SNP locus rs383429860 and genotype AA at SNP locus rs135884509 were found to be less susceptible to mastitis and associated with udder type traits. Genotype AA at SNP locus rs382671389 was found to be associated with udder type traits. Genotype TT at SNP locus rs136785910 was found to be associated with type traits and milk production. Genotype CC at SNP locus rs435289107 was found to be associated with healthy udder and type traits. In Sahiwal cattle, the genotype AA at SNP locus rs454303072, rs135884509 and rs382671389 was found to be less susceptible to mastitis and significantly associated with major type traits. The genotype TT at SNP rs435289107 was found to have lower values of udder type traits (smaller udder and teat), milk yield (305d) and monthly milk yield. The genotype AA and AG at SNP rs383429860 were found to be less susceptible to mastitis and have positive effects on udder type traits. On the basis of the present study, it is recommended that the genotype AA at SNP locus rs135884509 and genotype AA at SNP locus rs383429860 in Karan Fries cattle and the genotype AA at SNP locus rs454303072 in Sahiwal cattle, may 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 और करण फ्राइज़ मवेशियों की नस्लों के साथ किया गया, जिसके निम्न उद्देश्य थे - करन फ्रीज़ व साहीवाल गौजातीय पशु में वि डी आर, पि टी पि आर आर, एवं एच एम जी ऐ 2 जीन में आनुवंशिक बहुरूपता का पता लगाने, एवं उक्त बहुरूपताओं का, अयन प्रकार, नैदानिक थनैला व दुग्ध उत्पादन के साथ सांख्यिकीय संगती का ज्ञात करना। इस शोध हेतु, गौजातीय वि डी आर, पि टी पि आर आर, एवं एच एम जी ऐ 2 जीन में वर्णित एकल न्यूक्लीओटाइड बहुरूपताओं का उपयोग किया गया। कुल 253 स्तनपान कराने वाली गायों (साहीवाल = 87 और केएफ = 166) को लक्षित एसएनपी पर पीसीआर-आरएफएलपी प्रोटोकॉल डिजाइन करके जीनोटाइप किया गया था, साथ ही, प्रत्येक पशु के दूध उत्पादन और नैदानिक थनैला की घटनाओं के साथ 14 अयन प्रकार लक्षण और 11 दृश्य अयन लक्षण दर्ज किए गए। अंतिम विश्लेषण से पहले फेनोटाइपिक डेटा को गैर-आनुवंशिक कारकों के लिए समायोजित किया गया था। पॉपजन v 1.32 का उपयोग करके जनसंख्या आनुवंशिक मापदंडों की गणना की गई, जीनोटाइप्स और अयन प्रकार के लक्षणों / उत्पादन के बीच संबंध एसपीएसएस द्वारा पता लगाया गया था और जीनोटाइप व मास्टिटिस के बीच संगती लॉजिस्टिक रीग्रेशन (एसएएस यूनिवर्सिटी संस्करण) का उपयोग करके स्थापित की गई थी। छह एसएनपी rs454303072, rs135884509, rs382671389, rs435289107, rs136785910 और rs383429860 करन फ्रीज़ मवेशियों में और चार SNPs rs454303072, rs135884509, rs382671389, rs4357989 साहीवाल मवेशियों में सांख्यिकीय रूप से हार्डी वीनबर्ग संतुलन से अभिव्यंजकतापूर्वक पृथक पाई गई। दोनों ही नस्लों, साहीवाल (PIC = 0.138 to 0.375) व करन फ्रीज़ (PIC = 0.258 to 0.375), में सभी पढ़े गए एस एन पी का बहुरूपी संसूचना सूचक काम से मध्यम रहा। साहीवाल नस्ल में चार लोकी लिंकेज डिसिपिलिब्रियम (पी ≤ 0.05) में पाए गए, हालांकि सभी जोड़े कम r^2 मूल्य वाले थे। करन फ्राइज़ मवेशियों में, एसएनपी लोकस rs454303072 में जीनोटाइप GG, एसएनपी लोकस rs383429860 पर जीनोटाइप AA और एसएनपी लोकस rs135884509 में जीनोटाइप AA थनैला रोग के लिए काम संवेदनशील और अयन प्रकार के साथ सांख्यिकीय रूप से युक्त पाया गया। एसएनपी लोकस rs136785910 पर जीनोटाइप TT को प्रकार के लक्षणों और दूध उत्पादन से सांख्यिकीय रूप से युक्त पाया गया। एसएनपी लोकस rs435289107 में जीनोटाइप CC स्वस्थ अयन और अयन प्रकार लक्षणों के साथ जुड़ा पाया गया था। साहीवाल मवेशियों में एसएनपी लोकस rs454303072, rs135884509 और rs382671389 पर जीनोटाइप AA को मास्टिटिस के लिए काम संवेदनशील और प्रमुख अयन प्रकार के लक्षणों के साथ सांख्यिकीय रूप से युक्त पाया गया। एसएनपी rs435289107 में जीनोटाइप TT में अयन प्रकार के लक्षण (छोटे अयन व स्तनग्रभाग), दूध की उपज (305d) और मासिक दूध की उपज के निम्न मूल्य पाए गए। एसएनपी rs383429860 पर जीनोटाइप AA और AG को मास्टिटिस के लिए कम संवेदनशील पाया गया और इनका अयन प्रकार लक्षणों पर सकारात्मक प्रभाव देखा गया। वर्तमान अध्ययन के आधार पर, यह अनुशंसा की जाती है कि एसएनपी लोकस rs135884509 पर जीनोटाइप एए और एसएनपी लोकस rs383429860 में जीनोटाइप एए, करन फ्राइज़ मवेशियों में एवं एसएनपी लोकस में जीनोटाइप AA साहीवाल मवेशियों में, उच्च दूध उत्पादन व सकारात्मक अयन प्रकार लक्षणों एवं थनैला के प्रति प्रतिरोधात्मकता के लिए चयन हेतु बड़ी आबादी में विधिमान्यकरण के पश्चात पशु सहायक के रूप में उपयोग किया जा सकता है।

CHAPTER –1

Introduction

1. Introduction

India ranks first in milk production, accounting for 18.5% of world total milk production. The annual output has reached 163.7 million tons during 2016-17 as compared to 155.5 million tons during 2015-16, recording a growth of 5.3% per cent (Basic Animal Husbandry Fisheries Statistics, 2017). One of the most important reasons for this tremendous increase in milk production is continuous improvisation on selection for production traits. Higher selection pressure on production traits has been associated with occurrence of various production diseases in our cattle population. One of the most important diseases among them is mastitis.

Mastitis is the infection of mammary gland resulting in significant economic losses and an important threat affecting the world's dairy industry (Wallenberg *et al.*, 2002). It brings down milk yield, causes economic loss due to treatment and shortens the productive life of the cow. More than 100 recent studies spreading over 32 states of India indicate that the overall prevalence of mastitis ranges from 25 to 97% with an average prevalence of 45% (Anonymous, 2012a). Bovine mastitis directly or indirectly affects the economy of the farmers and ultimately affects the economy of the country. In India, the economic losses due to mastitis have increased about 115 folds in the last five decades from Rs. 529 million per annum in 1963 (Dhanda and Sethi, 1962) to Rs. 7165.51 crore per annum in 2012 (Anonymous, 2012a). Mastitis is a global problem and every country including developed ones suffers huge financial losses. In United States the total loss from mastitis was \$ 2 billion in the year of 2012 (Anonymous, 2012b). Almost 50% of milch animals, both crossbred and high producing indigenous cows are affected with clinical and sub-clinical mastitis. So, both genetic and proper phenotypic selection against mastitis should be carried together (Garg, 2001).

The morphological characteristics of udder and teat not only affect milk production, but also enhance the risk of intramammary infections (Chrystal *et al.*, 2001; Amin *et al.*, 2002). Dairy cows with long and thick teats have a higher risk of developing sub-clinical mastitis (Uzmay *et al.*, 2003). The udder and teat conformation traits were found to be associated with mastitis resistance (Klein *et al.*, 2005) and milk somatic cell count (Nash *et al.*, 2002; Sharma *et al.*, 2011). Therefore, it has been suggested that selection of cows with

desirable udder and teat morphology will help to reduce the incidence of mastitis and improve the milk quality (Juozaitiene *et al.*, 2006).

The udder of the cow is one of the most important criteria used to predict production performance. Udder type traits can be recorded relatively early in life of animals and are medium to high heritable (VanRadeq *et al.*, 1990), which makes selection relatively more efficient. Selection based on udder type traits that may give better production and also healthy udder seems to be the desirable condition for better production as well as reducing the udder related diseases specially mastitis. For better dairy breed selection the Body Condition Score (BCS) are relied upon that consists of udder and teat related traits and physical status. In dairy cattle judging, the mammary system (udder and teats) are assigned 30 points (Trimberger *et al.*, 1985). Under which udder size, teat measurement and shape are given weightage. It has been reported that udder shape has correlation with milk yield, milk protein and lactose, whereas, the udder circumference and length of udder were found to have correlation with milk yield (Rao, 2006). Udder is the most important site for judgment of milking ability of animals by local brokers or animal husbandry men in our country. So it is important to have knowledge of morphology of udder and teats and its relation with milk production.

Crossbred/Exotic and indigenous cattle contribute 25% and 20% to the total milk output, respectively (BAHS, 2017). The Karan Fries is a crossbred strain having good productivity, but these animals are also susceptible to mastitis. Sahiwal is one of the well established indigenous dairy cattle breed of tropical and subtropical countries known to have good genetic potential to produce substantially large amount of milk in their productive life span. The unique characteristics which make them different from high producing exotic/cross breeds such as Holstein-Friesian, Jersey etc. is their adaptability to extreme temperature fluctuations and parasitic resistance. It contributes 3.2% to the total indigenous cattle population of our country (Breed Survey, 2013). Indigenous cows are less prone to mastitis but due to intense selection of these cows for more milk production their susceptibility to mastitis has increased. Studies in linear type udder traits in purebred Sahiwal cows found that these traits need to be strengthened for improving the production sustainability (Dubey *et al.*, 2012).

To date, a large number of candidate genes influencing clinical mastitis have been reported in different indigenous breed of cattle. For udder conformation traits, the interest has been mainly focused on genes involved in udder growth, udder health and milk production.

According to the findings of different genome wide association studies, udder type trait related genes can be found on BTA 1, 2, 3, 4, 5, 6, 7, 11, 12, 13, 17, 20, 21, 22 and 25. Some of these genes are related to udder type traits and udder health together, some are related to udder type traits and milk production where as some of them are related to udder type trait, udder health and milk production. A majority of these genes, related to udder type traits and udder health, are located on BTA 3, 5 and 6. SNPs associated with udder support and average teat diameter were located on BTA 5 (Tolleson, 2016).

The genes viz., VDR [Vitamin D (1, 25-Dihydroxyvitamin D3) Receptor], PTPRR (Protein Tyrosine Phosphatase, Receptor Type R Gene), HMGA2 (High Mobility Group AT-hook 2 Gene) and Interleukin-22 (IL-22) are known for udder growth but have been found to be associated with clinical mastitis and milk production. Association of variants of these genes with udder type traits and incidence of mastitis can be used as an aid to selection of cows against mastitis. Till date, studies have not been attempted to investigate the influence of these candidate genes on clinical mastitis and udder type traits together in Karan Fries and Sahiwal Cattle. Information regarding association of udder type traits with incidence of mastitis and milk production is scanty in Karan Fries and Sahiwal cattle. Keeping the foregoing in consideration, the present study has been carried out with the following objectives:

1. To explore genetic polymorphism in VDR, PTPRR and HMGA2 genes in Karan Fries and Sahiwal cows.
2. To find out the association of genetic variants with udder type traits, incidence of clinical mastitis and milk production.

CHAPTER –2

Review of Literature

2. Review of Literature

2.1 Linear traits: History and development

The ultimate goal of a dairy farmer is getting profitability from the dairy cows. Dairy animals which complete more successful parities without any problems and having high milk production contribute significantly in achieving profitability of dairy farm. The dairy farmers will only keep those animals in the herd which meet their selection criteria each year. Selection for production is an important reason in the culling of dairy cows.

In the year 1906 a dairy cattle improvement program was initiated in the United States and since then it has been the basis for collecting the data necessary for national genetic improvement of production traits (Voelker, 1981). Comparison of the yield of a bull's daughters with yield of their dams was started in the 1930's. This daughter dam comparison procedure did account for the merit of the dam but variation in the environment between the time of the dam's and daughter's production was left unexplored. The daughter-dam comparison was replaced by herd mate comparison in 1961. This programme was designed to improve the milk production of national sire evaluation using herd-mate comparison which were introduced in April 1962 and also focused on improving milk production.

The first young sire sampling programme was started in the United States in 1950s'. Breeders wanted to improve the type of the animal in that programme and proofs for overall type score were available by 1970. There was no much interest among the dairy farmers on traits other than production and type in those days, probably because of the prevalence of low producing cow with poor udder and/or foot and leg problem. In this context, the U.S. Department of Agriculture (USDA) started national type evaluations using a sire model for Jerseys in 1978. This program was extended to Guernsey and Holstein breed in 1979. Previously, daughter averages or a herd-mate comparison system was used by breed associations (Wiggans, 1991).

Linear scoring of type appraisal traits on a 50-point scale was started for Ayrshire, Jersey and Guernsey breed by their respective associations in the year 1980. Afterwards, in January 1981 by milking Shorthorn, Red and White breed association and in the year 1982 by Brown Swiss breed association also initiated its own sire model evaluation system. They also implemented the 50 point scale in January 1983 (Norman *et al.*, 1983).

In the year 1985, linear assessment of type, with traits scored on their actual appearance (from one biological extreme to another) rather than desirability was introduced in the United Kingdom. Holstein-Friesian breed of cattle were assessed on a 1-9 scale for a total of 15 separate linear traits to describe the degree of type shown by cows and heifers in the United Kingdom. This system was more accurate and objective as per evaluation of dairy cattle than traditional desirability based schemes. Linear classification is conducted by breed society through appointed classifiers for herds wishing to assess individual animal strengths and weaknesses, and AI companies' progeny testing sires (www.ddc-wales.co.uk). The European Holstein Friesian Federation (EHFF) established a working group to examine harmonization of type classification systems in 1986 with the objectives of recommendations for the harmonization of type classification, including definition of traits, classification systems, publication of type proofs and evaluation of AI cows. In 1988 World Holstein Friesian Federation adopted the European Holstein Friesian Confederation type harmonization programme which had established 12 linear standard traits such as stature, chest and body, dairy character, rump width, rear leg set, feet, fore udder, rear udder height, udder support, udder depth, teat placement and teat length. The quality and robustness of the programme was demonstrated in the Multiple Across Country Evaluation (MACE) for type evaluations, producing inter country correlations, which at one time were considered impossible to achieve. Type traits were conformation traits of dairy animal which helped in determining dairy characteristics features of any dairy animal (WHFF, 2005). The International Committee of Animal Recording (ICAR) has adopted the principle of the WHFF type harmonization programme and it is logical, considering the quality of the results, to fully integrate the recommendations of both organizations into an international standard for type evaluation, administration and presentation. The data collected within these recommended standards qualifies for MACE evaluation (Hewitt, 2001). In New Zealand, data were extracted from national database on all primiparous cows of Holstein Friesian, Jersey or Holstein Friesian x Jersey crossbreds. Out of total 17 type traits assessed on a scale of 1-9; 4 traits were scored by the dairy farmer (adaptability to the milk routine, speed of milking, temperament and overall farming opinion) and the remaining 13 by industry trained classifiers primarily from the breed societies (stature, capacity, rump angle, rump width, legs, live weight, udder support, fore udder attachment, rear udder height, front teat placement, rear teat placement and two composite traits udder overall and dairy conformation) (Hewitt, 2001).

2.2 Type harmonization or linear conformation scoring in India

The genetic improvement of Indian breed of cattle through selective breeding is an important aspect for conservation and propagation of valuable germplasm. Numbers of studies on confirmation traits of the animals of developed countries have attracted the Indian scientist to incorporate the type appraisal programme in propagation of valuable germplasm of this country. Presently, two breeds of cattle such as Karan Fries, Sahiwal and Murrah breed of buffaloes have been studied in this direction.

2.3 Udder and teat type traits

2.3.1 Type traits definition

Linear type traits are the basis of all modern type classification systems, and are the foundation of all systems for describing the dairy cow. Linear classification is based on measurements of individual type traits instead of opinions. It describes the degree of trait not the desirability (ICAR &WHFF, 2012). Advantages of linear scoring are:

- Traits are scored individually
- Scores cover a biological range
- Variation within traits is identifiable
- Degree rather than desirability is recorded

2.3.2 International standard traits

According to ICAR (2012) & WHFF (2005), the International standard traits satisfy the following definitions:

- Linear in a biological sense
- Single trait
- Heritable
- Economic value: Direct or indirect with reference to the breeding goal
- Possible to measure instead of score
- Variation within the population
- Each linear trait should describe a unique part of the cow which is not covered by a combination of the other linear traits

2.3.3 Standard trait definition

The precise description of each trait should be well defined and it is essential to know the full range of linear scores for identifying the intermediate and extremes of each trait. The

assessment parameters for the calculations should be based on the expected biological extremes of a cow in the first lactation. The linear scale used, must cover the expected biological extremes of the population in the country of assessment (ICAR &WHFF, 2012).

2.3.4 Internationally accepted standards for udder and teat type traits

The following standards are accepted internationally for scoring of udder and teat type traits:

- ICAR (2012) & WHFF (2005) (International Committee for Animal Recording and World Holstein Friesian Federation, 2012) has a 1-9 point scale scoring system.
- WCGALP, 2014 (Proceedings of 10th World Congress of Genetics Applied to Livestock Production, 2014) (Flury *et al.*, 2014)
- **H.A.U, 2016** (Holstein Association USA, 2016) has a 1 - 50 point scale scoring system

Different internationally accepted udder and teat type traits recommended by these 3 standards are described as follows (Table 2.1):

Table 2.1 Different international standards of udder and teat type traits

Type traits	Class and Description	Measurement
ICAR (2012) & WHFF (2005)		
Fore udder attachment (degree)	Weak and loose	Angle between abdominal wall and udder
	Intermediate acceptable	
	Strong and tight	
Rear udder height(cm)	Very low	Distance between bottom of vulva and milk secreting tissue
	Intermediate	
	High	
Udder depth (cm)	Below hocks	Distance from lowest part of udder floor to hock
	Level with hock	
	Intermediate	
	Shallow	
Udder Balance (cm)	Deep rear udder	Depth of rear udder was assessed in relation to depth of front udder
	Front and rear udder on same level	
	Deep front udder	

Table 2.1 contd...

Type traits	Class and Description	Measurement
ICAR (2012) & WHFF (2005)		
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	
Central Ligament (udder cleft) (cm)	Convex to flat floor, broken ligament	The depth of cleft at the base of the rear udder
	Slight definition (intermediate)	
	Deep cleft/strong ligament	
Fore teat placement (Visual)	Wide	The position of the front teat from centre of quarter
	Intermediate	
	Close groups	
Rear teat Placement (Visual)	Wide	The position of the rear teat from
	Intermediate	
	Close groups	
Teat thickness (cm)	Thin	Thickness of the teat in the middle of the teat.
	Intermediate	
	Thick	
Front Teat Position (Visual)	Outside of quarter	Position of the centre of front teat placement at the point of the udder as viewed from rear
	Middle of quarter	
	Inside of quarter	
Rear teat Position (Visual)	Outside	Position of the centre of rear teat placement at the point of the udder as viewed from rear
	Mid point	
	Inside of quarter	
Teat length (cm)	Short	Distance between bases of the teat to the tip of the teat
	Intermediate	
	Long	

Table 2.1 contd...

Saini and Gill (1989); Ahmed and Barbary (2000); Rao (2006)		
Udder shape	Pendulous	Visual Observation
	Trough	
	Round	
Udder suspension	Very tight	Subjective assessments of udder support
	Tight	
	Intermediate/medium	
	Pendulous	
	Very Pendulous/Broken floor	
Udder length (cm)	Long	Distance between rear & fore attachment of udder along the median line
	Intermediate	
	Short	
Udder circumference (cm)	Large/long	Measuring around the base of udder
	Intermediate	
	Short	
Britt and Farnsworth (2011)		
Teat Size (Visual Observation)	Very small	Subjective assessments of teat length and circumference
	Small	
	Intermediate/medium	
	Large	
	Very large/balloon shaped	
Skin Condition	Normal – Smooth and healthy	Visual Appraisal
	Dry – No cracking	
	Rough-Dryness + cracking	
Teat shape	Funnel/Conical	Visual Appraisal
	Cylinder	
	Wide/Flat	
	Bottle	
Teat end shape /Teat tip shape	Flat	Visual Observation
	Round	
	funnel shape (inverted)	
	Disk	
	Pointed (hyperkeratosis)	

Table 2.1 contd...

Britt and Farnsworth (2011); Mein <i>et al.</i> (2001);		
Long-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 & cracking	
	Open skin, haemorrhage, trauma / abnormal condition	
WCGALP (2014); Flury <i>et al.</i> (2014)		
Teat circumference (cm)	Long	Measured at the midpoint of teat length
	Medium	
	Short	
Shortest distance from front and rear teat ends to floor	>55 cm	Distance between teat tip to the ground
	50-55 cm	
	<50 cm	
Distance between teats (cm)	Long	Distance between teats at the midpoint of teat length
	Intermediate	
	Short	

2.4 Effect of Age, Parity, Sire, Breed, Stage of lactation, Season, Farm and herd on milk production and different Udder type traits

2.4.1 Effect of Age

Frisch (1982) found that incidence of bottle teats in Sahiwal crossbred cows was higher in age of 5 yr or more than in young cows. Riley *et al.* (2004) stated that the structure and quality of the dam's udder was potentially one of the most important age dependent factors in Brahman calves. In Sahiwal cows, the effect of age was significant on udder cleft (Dubey, 2010). Udder support scores decreased as cows aged. Age of the cow affected udder length, width, depth and circumference. The age of the cow was also a significant source of variation for 305-day milk yield and test day yield in Sahiwal cows (Khan and Khan, 2016). The age of mastitic cows was significantly associated with mean daily milk reduction (kg)

and it was revealed that mastitis was higher in cows with age above 5 years (2.09 ± 0.25 kg) than their counterparts (1.19 ± 0.31 kg) in dairy cows of India (Bangar *et al.*, 2018).

2.4.2 Effect of Parity

Anderson (1985) reported that production of cow reached the peak around fifth parity. In Karan Fries cattle, parity was found to have significant effect on dairy character, fore udder attachment, rear udder width and udder depth (Mitra *et al.*, 1998). Kuczaj (2003) showed that the parity significantly affected almost all udder traits and central ligament measurement had lowest increase in relation to parity. Kuczaj (2003) also reported that the fore udder depth and fore udder width increased from 26.92 to 32.67 cm and 36.65 to 42.00 cm, respectively, while fore teat length, rear teat length, fore teat thickness and rear teat thickness increased from 5.72 to 6.27 cm, 4.72 to 5.16 cm, 2.88 to 3.20 cm and 2.86 to 3.32 cm, respectively, with the advancement of parity in Holstein-Friesian cattle. The increased incidence of mastitis with increased parity suggests multiparous cows consistently suffer more severe yield losses than primiparous cows due to higher milk yield (Wilson *et al.*, 2004). Angelova (2006) also found significant effect of parity on teat position in Brown cattle of Bulgaria. Petkov and Stoyanova (2006) established significant effect of parity on fore udder attachment, udder depth, teat length, and udder balance. They also reported that the fore udder score was reduced, the udder depth was increased, central ligament became more pronounced, and the slope of the udder bottom was increased because of reducing the area of attachment of the fore udder with advancing of the lactation values in Black and white cows of Bulgaria. Singh *et al.* (2010) reported that in Vrindavani crossbred cattle the mean udder length, width and depth increased from 49.2 ± 0.8 to 62.9 ± 1.2 cm; 55.6 ± 0.9 to 73.5 ± 1.3 cm and 19.4 ± 0.4 to 28.5 ± 0.7 cm respectively, from first to fifth parity, while teat length and teat diameter from 5.0 ± 0.1 to 6.4 ± 0.2 cm and 2.1 ± 0.05 cm to 2.7 ± 0.1 cm, respectively, from first parity to fifth parity. Almost all udder and teat dimensions differed significantly among different parities (Singh *et al.*, 2010). Prasad *et al.* (2010b) reported that the mean udder length, width and depth of Murrah buffaloes increased from 52.21 ± 0.61 to 54.12 ± 0.73 cm, 48.61 ± 0.60 to 50.71 ± 1.02 cm and 15.46 ± 0.17 to 15.51 ± 0.26 cm, respectively from first parity to fifth parity. The average teat length and diameter increased from 6.90 ± 0.20 cm to 8.37 ± 0.36 cm and 2.61 ± 0.05 to 2.82 ± 0.06 cm, respectively from first parity to fourth parity (Prasad *et al.*, 2010b). Dubey (2010) reported a significant effect of parity on teat length in Sahiwal cattle. This may be due to the effectiveness of streak canal

as barrier to infection decreases as parity increases (Khate and Yadav, 2010). Abdullah *et al.* (2013) studied Nili Ravi buffaloes (N = 200) and reported that, the udder length showed the pattern of increasing size as lactation number increases. The distance between teats also increased with consecutive parity. Japheth *et al.* (2015) reported significant influence ($P < 0.01$) of parity on the 305 day milk yield and total milk yield in Karan Fries cows. Jingar *et al.* (2014) reported parity number significantly influenced mastitis incidence in indigenous ($P < 0.05$) and crossbred cows ($P < 0.01$). Wondifraw *et al.* (2013) and Narwaria *et al.* (2015) also observed highly significant effect of parity on 305 day milk yield in Holstein x Deoni crossbred cows and Sahiwal cows, respectively. Emre and Alaçam (2015) reported that the development of hyperkeratosis was more prevalent in the fifth lactation and above. In Holstein cattle of Bulgaria, Marinov *et al.* (2015) found that after third lactation the length of the teat was increased compared to those on first lactation, with an increment of 9.61% and 9.32% for front and rear teats, respectively. Also the teat thickness was increased significantly from first towards third lactation, showing an increment of 11.11% and 16.08%, for front and rear teat, respectively (Marinov *et al.*, 2015). Marinov *et al.* (2015) showed that parity had highly significant effect on fore udder attachment, rear udder height, udder depth and balance, teat length and thickness in Holstein cattle of Bulgaria. However, the effect of the parity was having lower significance in rear udder width (Marinov *et al.*, 2015). The parity was a significant source of variation for udder length, width, depth, circumference, 305 day milk yield and test day yield in Sahiwal cows (Khan and Khan, 2016). Kakati *et al.* (2017) studied the same in Frieswal cows and revealed a highly significant ($P < 0.01$) effect of parity on 305 day milk yield. Mingoas *et al.* (2017) observed that udder morphologic characteristics varied significantly ($P < 0.005$) according to parity in zebu cows; the udder depth significantly increased at the 3rd parity while the udder height increased at the 2nd parity and there was no significant variation of teat size according to parity (Mingoas *et al.*, 2017). In Indian dairy cows, the prevalence of mastitis was significantly higher in multiparous cows (30.34 to 40.16%) than in primiparous cows (16.36 to 22.00%) (Bangar *et al.*, 2018). According to Juozaitiene, V. (2019), there were significant differences between front and rear teats for the prevalence of hyperkeratosis in first, second and third (≥ 3 lactations) lactation cows ($P < 0.001$). The development of hyperkeratosis varies based on a number of lactation. It was found that multiparous cows had increased degrees of hyperkeratosis compared to younger ones ($P < 0.01$).

2.4.3 Effect of Farm and herd management

The effect of farm was significant on fore udder attachment, udder height and teat thickness in Sahiwal cows (Dubey, 2010). The possible reason for significant influence of farm is due to prevalence of different management and nutritional practices on different farms, thus affecting the expression of conformation traits. The herd was a significant source of variation for udder length, width, depth, circumference, 305 day milk yield and test day yield (Khan and Khan, 2016) in the same breed. Effect of farm was studied on prevalence of mastitis and milk yield in indigenous and crossbred cows at three organized dairy farms situated at ICAR-IVRI, Izatnagar, ICAR-NDRI, Karnal and MPKV, Rahuri (Bangar *et al.*, 2018). The farm had significant effect on mean daily reduction of milk in mastitic cows (Bangar *et al.*, 2018). The highest reduction in milk yield (kg/day) was observed at NDRI farm (2.46 ± 0.25 kg) followed by MPKV (1.25 ± 0.36 kg) and IVRI farm (1.20 ± 0.30 kg) (Jingar *et al.*, 2014; Bangar *et al.*, 2018).

2.4.4 Effect of Sire and Breed

Sori *et al.* (2005), Khate and Yadav (2010) and Sharma and Maiti (2010) reported significant effect of breed on mastitis incidence in cows and buffaloes. Effect of sire was significant on teat length in Sahiwal cattle (Dubey, 2010). The incidence of mastitis was more (6.97 to 21.47%) in Karan Fries and Karan Swiss cows, (4.76 to 26.57%) and was lower (2.61 to 28.48%) in Sahiwal and Tharparkar (4.94 to 26.01%) cattle (Jingar *et al.*, 2014). The incidence of mastitis varied from 6.31 to 15.71% in Murrah buffaloes during different stages of lactation (Jingar *et al.*, 2014). A significant effect ($P < 0.01$) of breed on incidence of mastitis was found in cattle vs. buffaloes ($P < 0.01$), crossbred vs. indigenous cows ($P < 0.01$), crossbred vs. buffaloes ($P < 0.01$) and indigenous cows vs. buffaloes ($P < 0.05$) (Jingar *et al.*, 2014). Mingoas *et al.* (2017) observed that udder morphologic characteristics varied significantly ($P < 0.005$) according to breed.

2.4.5 Effect of Stage of lactation

Krastanov (1995) established a significant effect of the stage of lactation on rear udder width and height in dairy cattle of Bulgaria. The stage of lactation significantly influenced dairy character and rear udder width in Karan Fries cattle (Mitra *et al.*, 1998). Angelova (2006) also found statistically significant effect of the lactation stage on udder depth, teat position and central ligament in brown cattle of Bulgaria. Petkov and Stoyanova (2006) studied the impact of the stage of lactation on udder traits and established a significant

effect only on fore udder attachment and central ligament. Zwervaeher *et al.* (2012) reported that in Holstein cows, udder depth and height significantly decreased at third lactation stage, while there were no significant variations of teats morphology with respect to lactation stage. This was associated to involution phenomenon that occurs in udder according to lactation stage (Abdou *et al.*, 2012). Mazza *et al.* (2013) found that with advancement of the lactation stages the value of the scores for fore udder attachment, teat position, rear udder height and width were reducing in Valdostana cattle. The incidence of clinical mastitis was highest in early lactation in indigenous cows and buffaloes and was lowest in late stage of lactation, but in crossbred cows incidence was more in mid stage of lactation (Jingar *et al.*, 2014). It was also observed that prevalence of clinical mastitis decreased as stage of lactation increased in Murrah buffaloes, Sahiwal and Tharparkar cows, whereas, the crossbred cows suffered more during mid-lactation (Jingar *et al.*, 2014). The stage of lactation had significant effect on rear udder width and front and rear teat position in Holstein cattle of Bulgaria (Marinov *et al.*, 2015). The stage of lactation was a significant source of variation for udder length, width, depth, circumference, 305 day milk yield and test day yield in Sahiwal cows (Khan and Khan, 2016). Mingoas *et al.* (2017) observed that udder morphologic characteristics varied significantly ($P<0.005$) according to lactation stage in zebu cows. The udder depth significantly decreased at the 3rd stage of lactation while the udder height decreased at the 3rd stage of lactation. There was no significant variation of teat size according to stage of lactation in zebu cows (Mingoas *et al.*, 2017).

2.4.6 Effect of season

Ray *et al.* (1992) showed that the highest milk yield was obtained in animals calving in winter season followed by rainy and summer season. Also, milk production was decreased for cows calving in summer and fall (Ray *et al.*, 1992). Mayer *et al.* (1999); Payne and Wilson (1999) and West (2003) have shown that hot climate contributes significantly to reduced milk production indirectly through its effect on feed intake. In crossbred Ayrshire cows, the differences in average milk production during the three seasonal categories (rainy, summer and winter) were significant (Epaphras *et al.*, 2004). However, pair-wise comparison of seasonal categories showed that only milk production between the long and short rain seasons differed (Epaphras *et al.*, 2004). December to February were the critical months when milk production was lowest, compared to other calendar months (Epaphras *et al.*, 2004). According to Pawar *et al.* (2012), the total lactation milk yield (TLMY) and 305 day milk yield were found to be significantly affected by season in Murrah buffaloes.

2.4.7 Effect of level of production on incidence of clinical mastitis

Capuco *et al.* (2001) reported that the clinical mastitis in early lactation restricted differentiation of secretory cells and increase in milk yield until peak lactation, thus resulting in more severe reduction of milk yield. Singh *et al.* (2001) observed that the higher incidence of mastitis during early lactation in crossbred cows attribute due to poor pre partum management, changed udder environment and increased udder size, which renders mammary gland more vulnerable to mastitis. Chishty *et al.* (2007) and Kavitha *et al.* (2009) reported higher incidence of mastitis (43.42 to 51.81%) in late lactation in crossbred cows and buffaloes. According to Jingar *et al.* (2014), the level of milk production significantly affected incidence of clinical mastitis in Karan Fries, Karan Swiss, Sahiwal cows and Murrah buffaloes, but effect was non-significant in Tharparkar cows. The animals having higher or lower (due to mastitic history) milk yield suffered more with incidence of mastitis in comparison to medium producers (Jingar *et al.*, 2014). However, medium producing Tharparkar cows had more incidences of mastitis than the crossbred Karan Fries and Karan Swiss cows and Murrah buffaloes (Jingar *et al.*, 2014). The frequency of clinical mastitis in relation to level of milk production varied from 53.04 to 30.53, 57.75 to 26.56, 58.97 to 27.37, 68.33 to 26.37 and 51.34 to 18.75 % in Karan Fries, Karan Swiss, Sahiwal, Tharparkar cows and Murrah buffaloes, respectively (Jingar *et al.*, 2014) and similar results were also obtained in the earlier reports in cattle and buffaloes (Biffa *et al.*, 2005; Kocak, 2006; Zwald *et al.*, 2006; Fadlemula *et al.*, 2009; Corbett, 2009; Steeneveld *et al.*, 2008; Sharma, *et al.*, 2011; Grohn *et al.*, 2004; Wilson *et al.*, 2004). The incidence of mastitis was highest in early lactation in Sahiwal cattle (52.94%) and Murrah buffaloes (52.30%) probably due to non-adaptation of animals to the milking methods, more udder pressure and weaning associated milking behavior (Jingar *et al.*, 2014). A decreasing mastitis incidence in order of early, mid and late lactation was observed in indigenous (Sahiwal and Tharparkar) cows and Murrah buffaloes, but crossbred cows exhibited more mastitis in early lactation due to higher milk yield followed by late and mid lactation (Jingar *et al.*, 2014).

2.5 Udder type traits in Indigenous and crossbred cattle in India

Several studies in the past two decades have been conducted in various breeds of cattle and buffalo on different udder type traits including visual observations as well as measurable traits. The same have been summarized in Table 2.2 and 2.3.

Table 2.2 Studies on different udder types (visual observations) in cattle breeds

Breed	Udder types		References
	Shape	Percentage	
FH (Holstein Friesian + Hariana) cattle	Trough	25.0	Singh <i>et al.</i> , 1997
	Round	65.6	
	Pendulous	09.4	
FBH(½ Holstein Friesian + ¼ Brown Swiss + ¼ Hariana) cattle	Trough	24.1	
	Round	75.9	
	Pendulous	-	
FJH (½ Holstein Friesian + ¼ Jersey + ¼ Hariana) cattle	Trough	38.50	
	Round	61.50	
	Pendulous	-	
BFH (½ Brown Swiss + ¼ Holstein Friesian + ¼ Hariana) cattle	Trough	28.60	
	Round	71.40	
	Pendulous		
Crossbred cattle	Bowl	Not available	Chaki <i>et al.</i> , 1999
	Round		
	Goat		
Crossbred cattle	Bowl	35.48	Sonwane <i>et al.</i> , 2002
	Round	16.12	
	Trough	6.45	
	Pendulous	41.43	
Holstein cattle	Trough	55.9	Uzmay <i>et al.</i> , 2003
	Ball Shaped	25.9	
	Rear-Heavy	11.5	
	Pendulous	6.7	
Local cattle (Bangladesh)	Round Udder	-	Bhuiyan <i>et al.</i> , 2004
Karan Fries cattle	Trough	45.94	Singh, 2005
	Round	11.94	
	Goat	15.09	
	Pendulous	19.47	
	Unbalanced	7.51	
Karan Fries cattle	Round	-	Rao, 2006
Sahiwal cattle	Trough	50.0	Singh, 2006
	Round	42.8	
	Pendulous	7.14	
Sahiwal cattle	Trough	50.0	Ahlawat, 2007
	Trough	44.4	
	Round	33.3	

Table 2.2 contd...

Breed	Udder types		References
	Shape	Percentage	
Crossbred cattle	Globular	53.64	Gajbhiye <i>et al.</i> , 2007
	Trough	37.69	
	Pendulous	02.58	
Karan Fries cattle	Trough	45.94	Kamboj <i>et al.</i> , 2007
	Pendulous	19.47	
	Goaty	15.09	
	Round	11.94	
	Unbalanced	7.51	
Gaolao strain of Nagpuri buffaloes	Bowl	52.6	Tayade <i>et al.</i> , 2010
	Round	36.2	
	Goat	11.2	
Gaolao cattle	Round	10-30	Chachare and Walkunde, 2011
	Bowl	60-90	
	Cup / Goat	10-20	
Gaolao buffaloes	Bowl	53.40	Gubbawar <i>et al.</i> , 2012
	Round	38.60	
	Goat	18.00	
Marathwadi buffalo	Round	35.95	Sonwane, 2015
	Trough	35.48	
	Bowl	25.24	
	Pendulous	03.33	
Crossbred cattle	Trough	56.50	Patel <i>et al.</i> , 2016
	Round	19.50	
	Pendulous	16.50	
	Goat	07.50	

Table 2.3 Studies on different udder type traits (measurements) in cattle and buffalo breeds

Breed	Salient Findings		References
	Udder type traits	Mean	
Kankrej cattle	Udder length	59.58 ± 0.36	Prajapati <i>et al.</i> , 1995
	Udder width	63.18 ± 0.45	
	Udder depth	24.95 ± 0.22	
Karan Fries cattle	Dairy character	79.89±0.75	Mitra <i>et al.</i> , 1998
	Fore udder attachment	80.22±0.96	
	Rear udder height	82.72±0.55	
	Rear udder width	80.07±0.74	
	Udder depth	82.15±0.89	
	Suspensory ligament	86.34±0.8	
	Teat placement	78.29±0.95	
Holdeo crossbred cattle	Udder length	49.87 ± 0.56	Waghmore and Siddiqui, 2000
	Udder depth	14.65 ± 0.20	
	Udder width	51.08 ± 0.58	
Murrah Buffaloes	Udder length	54.2 ± 0.34	Prasad <i>et al.</i> , 2010
	Udder width	50.6 ± 0.36	
	Udder depth	15.6 ± 0.10	
Surti Buffaloes	Udder length	45.0 ± 0 0.7	Lavania <i>et al.</i> , 2011
Gir cattle	Udder length	45.99 ± 2.07	Singhai <i>et al.</i> , 2013
	Udder width	20.51 ± 0.99	
	Udder depth	20.29 ± 1.04	
Crossbred cattle	Udder length	58.24 ± 0.68	Patel <i>et al.</i> , 2016
	Udder width	65.45 ± 0.70	
	Udder depth	23.06 ± 0.34	
Gir cattle	Udder length	61.95 ± 1.20	Modh <i>et al.</i> , 2017
	Udder width	62.99 ± 1.17	
	Udder depth	25.62 ± 0.43	
Buffaloes of Gujarat	Udder length	58.24 ± 0.68	Khatri <i>et al.</i> , 2017

2.6 Teat type traits in Indigenous and crossbred cattle in India

A number of investigations have been conducted in breeds of cattle and buffalo on different teat type traits including visual observations and measurable traits, which have been summarized in Table 2.4 and 2.5.

Table 2.4 Studies on different teat types (visual observations) in cattle breeds

Breed	Salient Findings		References
	Shape of teat	Percentage	
FH (Holstein Friesian + Hariana) cattle	Cylindrical	75.0	Singh <i>et al.</i> , 1997
	Conical	25.0	
FBH (½ Holstein Friesian + ¼ Brown Swiss + ¼ Hariana) cattle	Cylindrical	75.80	
	Conical	24.20	
FJH (½ Holstein Friesian + ¼ Jersey + ¼ Hariana) cattle	Cylindrical	92.30	
	Conical	7.70	
BFH (½ Brown Swiss + ¼ Holstein Friesian + ¼ Hariana) cattle	Cylindrical	71.30	Sonwane <i>et al.</i> , 2002
	Conical	28.60	
Crossbred cattle	Cylindrical	51.61	
	Funnel	12.90	Uzmay <i>et al.</i> , 2003
	Pear	35.48	
Holstein cattle	Cylindrical	73.3	
	Funnel	18.8	
	Pear	3.2	
	Long and thick	4.7	
Karan Fries cattle	Conical/Funnel	78.1	Singh, 2005
	Cylindrical	11.9	
	Bottle	10.0	
Karan Fries cattle	Conical	25.0	Rao, 2006
	Cylindrical	75.0	
	Supernumerary	18.7	
Sahiwal cattle	Conical	78.5	Singh, 2006
	Cylindrical	21.5	
Sahiwal cattle	Conical	44.4	Ahlawat, 2007
	Cylindrical	38.8	
	Bottle	16.7	

Table 2.4 contd...

Breed	Salient Findings		References
	Shape of teat	Percentage	
Karan Fries cattle	Conical /Funnel	48.27	Kamboj <i>et al.</i> , 2007
	Cylindrical	37.42	
	Bottle	14.31	
Indigenous cattle	Closer teat placement, cylindrical teats and pointed teat end were comparatively higher		Susanta <i>et al.</i> ,2013
Crossbred cattle	Funnel	51.11	Patel <i>et al.</i> , 2016
	Cylindrical	25.55	
	Pear	18.05	
	Bottle	2.27	
Zebu cattle	Fore teats more developed than hind teats		Mingoas <i>et al.</i> , 2017

Table 2.5 Studies on different teat type traits (measurements) in cattle and buffalo breeds

Breed	Salient Findings		References
	Teat type traits	Mean value (cm)	
Holdeo crossbred cattle	front teat length	7.32 ± 0.09	Waghmore and Siddiqui, 2000
	front teat diameter	1.59 ± 0.03	
	rear teat length	6.72 ± 0.09	
	rear teat diameter	1.47 ± 0.03	
	mean teat length for left fore	7.37 ± 0.14	
	mean teat length for right fore	7.53 ± 0.1	
	mean teat length for left hind	8.23 ± 0.18	
	mean teat length for right hind	8.12 ± 0.16	
Mediterranean buffalo	average teat length	6.11	Campanile <i>et al.</i> , 2003

Table 2.5 contd...

Breed	Salient Findings		References
	Teat type traits	Mean value (cm)	
Murrah Buffalo	teat diameter for left fore teat	2.62 ± 0.02	Prasad <i>et al.</i> , 2010
	teat diameter for right fore teat	2.71 ± 0.02	
	teat diameter for left hind teat	2.80 ± 0.03	
	teat diameter for right hind teat	2.89 ± 0.03	
Surti Buffalo	front teat length	6.9 ± 0.3	Lavania <i>et al.</i> , 2011
	rear teat length	7.7 ± 0.4	
Indigenous Cattle of Punjab	length of fore teats	4.77 ± 0.51	Hussain <i>et al.</i> , 2012
	diameter for fore teats	1.68 ± 0.13	
	length of rear teats	4.66 ± 0.54	
	diameter for rear teats	1.70 ± 0.08	
Gir cattle	teat length	7.07 ± 0.42	Singhai <i>et al.</i> , 2013
	teat diameter	3.49 ± 0.21	
Crossbred cattle	The average length of fore teats was found to be non significantly longer than rear teats (6.07 cm vs. 5.39 cm)		Patel <i>et al.</i> , 2016
Murrah Buffalo	Teat length	8.23 ± 0.18	Sahu <i>et al.</i> , 2016
Buffaloes of Gujarat	The average length of fore teat was found to be non significantly longer than rear teats (6.07 cm vs. 5.39 cm)		Khatri <i>et al.</i> , 2017.
Gir cattle	The average length of fore teat was found to be non significantly longer than rear teats (9.26 cm vs 8.64 cm)		Modh <i>et al.</i> , 2017

2.7 Correlation among udder type traits

Short *et al.* (1991) reported high genetic correlations between teat length and other udder size traits in Holstein cows. Dubey (2010) studied linear type traits in Sahiwal and showed that udder depth was positively correlated with rear udder height, fore udder attachment, front and rear teat placement. The rear udder height was found to be positively

correlated with udder depth, udder balance, teat length, front teat placement and teat thickness and negative correlation exists between udder cleft, fore udder attachment and rear teat placement. The positive and significant correlation exists with fore teat length (0.21) and significantly negative correlation exists between rear teat placement (0.28) and udder cleft (0.25). The udder balance was found to be positively correlated with udder height, teat length and thickness and negative correlation was recorded with udder depth, udder cleft, fore udder attachment, front and rear teat placement. The positive significant correlation ($P < 0.01$) was observed with teat length (0.38) however, negative significant correlation exists with rear and front teat placement at $P < 0.01$ and $P < 0.05$, respectively (Dubey, 2010). The udder cleft was found to be positively correlated with fore udder attachment, teat length, front and rear teat placement and negative correlation was recorded with udder height, udder balance and teat thickness. The fore udder attachment was found to be positively associated with udder depth, udder cleft, and rear teat placement and negative correlation was recorded with udder height, udder balance, teat length, front teat placement and teat thickness (Dubey, 2010). All the correlations were low and statistically non-significant. The significant positive correlation was recorded with teat length and udder height (0.21) at $P < 0.05$ and with udder balance (0.38) and teat thickness (0.27) at $P < 0.01$ while rest of the correlations are low and statistically non-significant. The association between front teat placement and udder balance (0.19) was found to be negatively significant ($P < 0.05$) while rest of the correlations are very low and statistically non significant. The significant negative association ($P < 0.01$) was recorded with rear udder height (0.28) and udder balance (0.30) while rest of the correlations are low and statistically non significant. The teat thickness showed the positive significant correlation ($P < 0.01$) with teat length (0.27) while rest of the correlations were low and statistically non significant (Dubey, 2010). Ahlawat *et al.* (2008) in Sahiwal cows, Prasad *et al.* (2010a) in Murrah buffaloes and Singhai *et al.* (2013) in Gir cows, observed positive and significant ($P < 0.05$) to highly significant ($P < 0.01$) association of udder width and udder depth with average rear teat length, fore teat diameter, rear teat diameter, overall teat length and overall teat diameter. Prasad *et al.* (2010a) reported highly significant ($P < 0.01$) correlation between the teat length and teat diameter. Kasap *et al.* (2014) reported that udder depth was found to be in strong negative genetic correlation with fore udder attachment ($r = -0.436$). An almost negligible negative genetic correlation between udder depth and rear udder width was determined ($r = -0.086$). Genetic correlations between all other traits were positive and low to moderate ($r = 0.15 - 0.54$). In zebu cows, highly positive genetic correlations were found among all udder measurements and ranged from 0.71 ± 0.05 between udder length and

udder depth to 0.84 ± 0.02 between udder length and udder width (Mingoas *et al.*, 2017). A strong positive correlation ($r_p=0.60$) between the diameter (240.21 ± 28.58 mm) and height (131.12 ± 23.64 mm) of udders ($p < 0.001$) and between length (39.51 ± 6.44 mm) and diameter (19.85 ± 3.08 mm) of teats ($r_p=0.78$) were found in the zebu cows (Mingoas *et al.*, 2017). A positive and highly significant correlation ($r_p=0.60$) was found between udder depth and height. Teat length and diameter were positively and highly correlated ($r_p=0.78$) to each other (Mingoas *et al.*, 2017). Khatri *et al.* (2017) reported the correlations observed between various teat measurements, viz., fore teat length, rear teat length, fore teat diameter and rear teat diameter were also highly significant ($P < 0.01$) in Murrah buffalo.

2.8 Correlation between udder type traits and milk production traits

Norman *et al.* (1988) reported that most phenotypic correlations between udder type and first lactation milk yield ranged from 0.18 to 0.38. Among the linear type traits, correlation with first lactation yield had greatest absolute value for dairy character (0.19 to 0.53), udder depth (-0.26 to -0.30) and rear udder width (0.20 to 0.31). Multiple correlations of all type appraisal traits with first lactation milk yield ranged from 0.41 to 0.59 (Norman *et al.*, 1988). The genetic correlation between milk and final score was positive for Guernsey (0.25) and Jerseys (0.21) (Norman *et al.*, 1988). The genetic correlation between yield and most linear type traits were low to moderate except for dairy character (0.53 to 0.77) (Norman *et al.*, 1988). Saiyad and Patel (1989) studied the milk yield was significantly correlated with the udder length, width, depth and circumference (0.46, 0.59, 0.35 and 0.62 respectively). Gupta *et al.* (1991) while working on Karan Fries cow, reported that all four teat diameter were almost same and hence no significant correlation between teat diameter and milk yield could be established. Sekerden and Erdem (1993) observed that the 100 days lactation milk yield of 57 Jersey cows was correlated with rear udder width and rear height on day 20 of lactation which was 0.19 ± 0.13 and 0.18 ± 0.13 , respectively. Singh *et al.* (1999) reported that the correlation of milk flow rate with milk yield (0.49) and teat length (0.39) was highly significant and positive. The highest milk flow rate was observed in cows having trough udder and cylindrical teat shape (Singh *et al.*, 1999). Waghmore and Siddiqui (2000) reported strong correlation of milk yield with udder length (0.49), width (0.44) and depth (0.52) in Holdeo crossbred cows. Udder with poor udder cleft caused pendulous udders, which were more prone to injury and infection (Atkins and Shannon, 2002), thus decreasing the level of milk production level of animal. MacNeil and Mott (2006) stated mean estimate

of the phenotypic variance of udder score was 1.89 in Hereford cattle (1 - 9 scale, with 1 being very pendulous with balloon teats and 9 being an ideal mammary system) and the estimates of phenotypic correlations between pre-weaning gain and udder score, and milk production and udder score were $- 0.07 \pm 0.04$ and $- 0.09 \pm 0.05$, respectively. Also, the genetic correlation of milk production with udder score was estimated as $- 0.36 \pm 0.16$ (MacNeil and Mott, 2006). Atkins (2007) reported that the udder with more depth could be attributed due to a weakness of the suspensory ligament which was known to attach udder with ventral abdominal wall and the pelvic floor and was fundamental to udder health and longevity (Atkins, 2007). Bermingham *et al.* (2007) studied relationships between some type traits and milk yield, calving interval, true and functional survival and lifespan. Taller, wider, deeper and more angular cows with deep udders produced more milk but had genetically inferior fertility and survival. Singh *et al.* (2010) reported strong correlation of milk yield with udder length, width and depth in Vrindavani cattle. Dubey (2010) studied correlation between udder type traits and milk production traits in Sahiwal and reported that the traits such as udder cleft, fore udder attachment, teat length, fore teat placement, rear teat placement and teat width were positively correlated with first lactation milk yield. This might be due to the fact that udder with less depth will have more circumference, so that it can possess the more number of mammary tissue, which is known to synthesize milk inside the udder, thus ultimately increasing the level of milk production (Dubey, 2010). Also, udder with more depth produces lesser amount of milk, which was indicated by the negative correlation between the rear udder depth and first lactation milk yield. Dubey (2010) also reported moderate level of correlation between teat length and first lactating milk yield, also the teat length and rear teat placement were significantly ($P < 0.01$) and positively correlated with udder balance which was subsequently found to be positively associated with first lactation yield. The positive correlation observed may be due to the fact that rear teats which were closer facilitates much easier milking and consequently resulted in more first lactation yield. Also, the first lactation yield and rear udder height showed negative correlation (Dubey, 2010). Prasad *et al.* (2010a) reported significant ($P < 0.05$) and positive correlation of udder width, length, depth with average test day milk yield in Murrah buffaloes. Prasad *et al.* (2010a) also reported positive and highly significant correlation of average teat diameter with milk yield in Murrah buffaloes. Deng *et al.* (2012) also reported significant ($P < 0.05$) correlation of udder length (0.64) with milk yield in Kenana x Friesian crossbred cows. According to Khan and Khan (2016), the genetic correlation of 305 day milk yield with udder length, udder width, udder depth and udder circumference were 0.38 ± 0.02 , 0.41 ± 0.02 , 0.35

± 0.02 and 0.36 ± 0.02 , respectively, and the phenotypic correlation between 305 day milk yield with udder length was 0.45 ± 0.05 and with udder width was 0.48 ± 0.05 . The udder length had the highest phenotypic correlation with test day yield 0.45 ± 0.02 at first stage of lactation followed by udder width (0.39 ± 0.03), udder circumference (0.31 ± 0.03) and udder depth (0.29 ± 0.03). The udder length, udder width, udder depth and udder circumference had genetic correlation of 0.40 ± 0.02 , 0.43 ± 0.04 , 0.36 ± 0.03 and 0.37 ± 0.03 with test day yield at first stage of lactation (Khan and Khan, 2016). Modh *et al.* (2017) studied the correlations between milk yield and udder width (0.194) and was found positive and significant ($P < 0.05$). Khatri *et al.* (2017) reported correlations between milk yield and various udder measurements traits viz., udder length (0.499), udder width (0.413) and udder depth (0.178), which were found to be positive and significant ($P < 0.05$; $P < 0.01$) in buffaloes. There was significant ($P < 0.001$) correlations between udder diameter ($r_p = 0.541$) and height ($r_p = 0.549$) with milk yield found in the Zebu cows (Mingoas *et al.*, 2017). In zebu cows, there was significant correlations between udder diameter ($r_p = 0.541$) and height ($r_p = 0.549$) with milk yield (Mingoas *et al.*, 2017). The udder size (depth and height) was also strong and positively correlated ($r_p = 0.541$ and $r_p = 0.549$, respectively) to milk yield in native African cattle breed (Mingoas *et al.*, 2017). Teat length and diameter were not significantly correlated to milk yield (Mingoas *et al.*, 2017).

2.9 Heritability of different Udder type traits

Thompson *et al.* (1981) reported the heritability estimate of 0.27 in Holstein cattle for udder depth. Fuerst-Waltl *et al.* (1998) reported heritability for udder depth, fore udder attachment, and rear udder height as 0.31, 0.26 and 0.22, respectively. The heritability for rear udder height, fore udder attachment was previously estimated as 0.08, 0.01, respectively in Brown Swiss cattle (Norman *et al.*, 1988). Norman *et al.* (1988) also studied heritability estimate for rear teat placement in Brown Swiss, Guernsey, HF and Jersey cattle, and they were found to be 0.25, 0.21, 0.23 and 0.27, respectively. Chrystal *et al.* (1999) reported estimate of heritability for teat diameter, which was 0.23, 0.27 and 0.35 for 1st, 2nd, and all lactations combined, respectively. Fuerst-Waltl *et al.* (1998) and Schrooten *et al.* (2000) reported heritabilities for teat length as 0.25 and 0.45, respectively in dairy cattle. Schrooten *et al.* (2000) also reported heritability for udder depth, fore udder attachment and rear udder height with values of 0.45, 0.35, and 0.35, respectively. MacNeil and Mott (2006) reported a similar heritability estimates for udder score in Hereford cattle (0.23). In Sahiwal cattle, the

heritability estimates for most of the linear type traits were moderate to high in magnitude indicating scope for improvement in this breed (Dahiya *et al.*, 2006). Whereas, the heritability of different udder type traits in Sahiwal cows estimated by Dubey (2010) were low to high (0.014 to 0.713). In case of udder depth, rear udder height and fore udder attachment the estimated value of heritability was 0.11 ± 0.38 , 0.03 ± 0.37 and 0.014 ± 0.35 , respectively in Sahiwal (Dubey, 2010). The heritability estimates of teat length and fore teat placement were estimated by Dubey (2010) in Sahiwal cattle, and reported the values to be low to medium i.e., 0.26 ± 0.41 and 0.29 ± 0.44 for teat length and fore teat placement, respectively. Similarly, the heritability estimates of rear teat placement was 0.20 ± 0.40 , teat thickness was 0.016 ± 0.35 and linear type traits was 0.01 to 0.37 in Sahiwal cattle (Dubey, 2010). Estimated heritability ranged from 0.15 to 0.27 for fore udder attachment, rear udder height, rear udder width and udder depth in Holstein cattle (Kasap *et al.*, 2014). Bradford *et al.* (2015) reported an estimated heritability of 0.32 for udder suspension. Bradford *et al.* (2015) estimated genetic parameters for udder traits in Hereford cattle and reported a heritability estimate of 0.28 for teat size. Recently, Khan and Khan (2016) have reported the univariate animal model heritability estimates for udder length, width, depth and circumference, which were found to be 0.68 ± 0.03 , 0.66 ± 0.03 , 0.54 ± 0.04 and 0.60 ± 0.04 , respectively in Sahiwal.

2.10 Effect of udder type traits on milk production in Karan Fries and Sahiwal cattle

Pander and Chopra (1989) observed in crossbred cows that udder depth, semilunar circumference, udder width affected the milk flow rate significantly and the udder shape and all other measurement affect the daily milk yield and 100 day milk yield. The udder which were shallow and having low rear udder height were reported to be low in milk production as compared to those having high rear udder height and deeper udder (Foster *et al.*, 1989; Misztal *et al.*, 1992; Short and Lawlor, 1992). Ghosh and Prasad (1998) studied udder shapes in 24 Jersey x Red Sindhi crosses and found that higher milk yield in pendulous udder compared to round and goat type of udder, whereas, the bowl type of udder had significantly higher milk yield than that of goat type of udder. Singh *et al.* (1999) studied the milk producing ability of different shapes of udder of 132 crossbred cows and reported that the percentage of trough, round and pendulous udder was 25, 72 and 3%, respectively. The milk yield for three udder shapes were 9.68, 7.04 and 5.05 kg per day respectively and milk flow rate was 0.920, 0.823 and 0.691 kg per minute, respectively.

DeGroot *et al.* (2002) concluded that udder capacity traits - high rear udder height and weak central ligament, i.e. connection with large udders, had a positive relationship with milk production. Das *et al.* (2002) reported that in Jersey CB cows pre-partum udder measurement had significant effect on milk yield. Bhuiyan *et al.* (2004) studied the same and reported that in local cows of Bangladesh maximum test day milk yield was found in bowl shaped udder, whereas, udder length, width and depth were significantly and positively correlated with milk yield on test day. Singh (2005) also reported that in Karan Fries cows trough shaped udders were related to more milk yield. In Sahiwal cattle, dairy character and most of the udder traits had high association with milk production. All the linear type traits accounted for 43.05 and 37.23 percent of the total variability in milk production up to first and third lactation, respectively (Singh, 2005). Dahiya *et al.* (2005) reported the index selection in Sahiwal cattle based on first lactation milk yield, dairy character and rear udder width resulted in expected gain of 242.28 kg and 2.21 points in total milk production up to third lactation and overall type score, respectively. It was also 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). Rao (2006) reported that trough shaped udder was related to more milk yield, milk protein and less lactose in Karan Fries cattle. Nemcova *et al.* (2007) reported that fore udder attachment had significant influence on milk production, but only in the first two test days of lactation. Also, the teat length showed a lower statistically significant influence on milk in the last part of lactation. Bermingham *et al.* (2007) reported taller, wider, deeper udders tended to produce more milk. Circumference and length of udder was also significantly and positively correlated with milk yield. Kshatriya *et al.* (2009) studied the association of udder and skin thickness with milk yield in Kankrej and crossbred and found mean udder length, width and depth in crossbred cows as 20.73 ± 0.89 , 23.31 ± 0.85 and 8.00 ± 0.34 inches respectively. Udder depth, central ligament, rear udder height and front teat placement had significant effect on milk production during the entire lactation in Holstein cows (Zwertvaegher *et al.*, 2012). Yosbanis *et al.* (2013) studied 492 buffaloes of two cattle farm in Cuba, udder measurements included: depth, length, width, volume, separation. It was found that the average value of udder length was 38.46 cm and it showed moderate to high correlation (0.24 to 0.54) with all the production variables. Patel *et al.* (2016) showed that the correlations between milk yield and various udder measurement traits viz., udder length (0.499), udder width (0.413) and udder depth (0.178) were positive and significant in crossbred cattle ($P < 0.05$; $P < 0.01$). Khan and Khan (2016) reported that in Sahiwal cows of Pakistan udder length had highest phenotypic correlation with test day yield

at first stage of lactation followed by udder width, udder circumference and udder depth. The magnitude of the phenotypic correlations for udder length and udder depth decreased in second and third stage of lactation (Khan and Khan, 2016). Mingoas *et al.* (2017) showed that udder size was strong and positively correlated to milk yield in local zebu cows and longer teats were found to be associated with weaker udder support and deeper udders. Also udder depth and udder height had highly significant effects on milk yield.

2.11 Effect of teat type traits on milk production

Donald (1960), Chyr (1973), and Freeman (1976) reported that cows with shorter teat length had higher milk production than cows with long teat lengths. Studies regarding milk production in dairy cattle, often report the relationship between larger teat diameter and increased milk production (Moore *et al.* 1981; Seykora and McDaniel, 1986). In addition, Rogers and Hargrove (1993) also reported Holsteins with larger dimensions of the udder and teats correspond with higher milk production. Gupta *et al.* (1991) and Rao (2006) reported that in Karan Fries cows, the teat length was significantly and positively correlated with milk yield.

2.12 Effect of udder type traits on mastitis in Karan Fries and Sahiwal cattle

Dairy cattle with shallow udders produced less milk, but udders that were too deep tend to have more mastitis (Young *et al.* 1960; Van Vleck and Norman, 1972; Chyr *et al.*, 1974). An intermediate udder depth appears to be preferable in the dairy industry. Seykora and McDaniel (1986) reported lower SCS for cows with higher udders and deeper central ligament. Studying genetic correlations, Boettcher *et al.* (1998) and Rupp and Boichard (1999) found deep udders and weak fore udder attachment associated with increased SCS. Hence, it has been suggested that some teat-end shapes act as risk factors for infection, and as they have high daughter-dam heritability, they can be eliminated by selective breeding (Chrystal *et al.*, 1999). Sorensen *et al.* (2000) reported that cows having weaker fore udder attachment and lower udder depth had high risk of clinical mastitis in Holstein cows. Rogers *et al.* (1991), DeGroot *et al.* (2002) and Kadarmideen (2004) concluded that selection for higher udders with tighter attachments would be favourable for reducing SCS. Higher SCS were determined in cows with larger as well as smaller distance between teats. In Holstein cows, Nash *et al.* (2002) reported that stronger fore udder attachment, shallower udders, deeper udder cleft was related to less number of TCE (total number of clinical mastitis episodes). Uzmay *et al.* (2003) reported that in Holstein cows trough shaped udders had

lowest risk of sub-clinical mastitis, pendulous udders had the highest risk. The deeper udders were found to be at higher risk of developing mastitis due to their increased tendency to become soiled (Lopez-Benavides *et al.*, 2005). Ahlawat (2007) studied the same and reported that in Sahiwal cows pendulous udder had highest SCC and also reported that udder characteristics had significant affect on the incidence of mastitis. Trajcev and Nakov (2010) reported 60.61% and 39.39% subclinical mastitis for the rear and front udder quarters, respectively. It was also reported that the rear udder quarters had a higher risk of clinical mastitis incidence when compared to the front udder quarters (Nakov *et al.*, 2014). Deng *et al.* (2012) reported that the mean udder length, udder depth was 44.8 cm and 23.7 cm, respectively, in Friesian x Kenana crossbred cows. Singh *et al.* (2014) reported that in HF x SW cows longer rear udder depth, deeper udder and less udder to floor distance were related to high risk of clinical mastitis. In Holstein cows, deep udders, weak central ligaments and fore attachments and low rear udder height showed the highest SCS (Somatic cell score) during the entire lactation. The deep udders were found to be more susceptible to mastitic lesions (Singh *et al.*, 2014). Bharti *et al.* (2015) reported pendulous shaped udder was more susceptible to intra-mammary infections in Jersey crossbred cattle.

2.13 Effect of teat type traits on mastitis in Karan Fries and Sahiwal cattle

Large teat diameter has been linked to increased mastitis in dairy cows (Hickman, 1964; Seykora and McDaniel, 1986) and also had larger teat canals, allowing for an increased rate of milk flow, but at the same time provided a larger passage for irritants or bacteria to enter the mammary system. Rogers *et al.* (1991) found a positive genetic correlation between teat length and SCC. DeGroot *et al.* (2002) preferred closer teats as favourable for selection for lower SCS. Less pointed and more inverted teat ends have been associated with increased susceptibility to mastitis as they retain milk, which could act as a substrate for bacterial growth (Chrystal *et al.*, 2001). The teats with larger diameter might act as predisposing factor for intra-mammary infections (Mein *et al.*, 2004). The cows with shorter teats showed a higher value of SCS. The higher value of the somatic cell score for shorter teats and low SCS appeared to be associated with an intermediate distance between the front teats and longer teats in dairy cattle (Nemcova *et al.*, 2007). Orban *et al.*, (2009) reported an association of inverted teat end with higher log SCC value and a negative correlation of somatic cell number with teat diameter.

Rogers *et al.* (1991) and Kadarmideen (2004) reported a negative genetic relationship between SCS and front teat placement. Ahlawat (2007) studied relation of teat types and mastitis in Sahiwal cows and reported that bottle shaped teats are related to high SCC. The probability of incidence of mastitis varies considerably between different teat shape, sizes, and morphology of the teat end (Bardakcioglu *et al.*, 2011). The occurrence of subclinical mastitis was highest for cows with long and thick teats (Singh *et al.*, 2014) and these were a potential risk factor for intra mammary infection. Bharti *et al.* (2015) reported flat and inverted teat end and long, thick teat were more susceptible to intra-mammary infections in Jersey crossbred cattle. Guarin and Ruegg (2016) reported that in Holstein cows bigger pre milking teat apex diameter was related to high occurrence of clinical mastitis. According to Juozaitiene, V. (2019), there was significant relationships between teat condition and udder quarter cases ($P<0.001$) and rear udder quarters had 1.28-1.40 times higher ($P<0.01$) frequency of subclinical and clinical mastitis cases.

2.14 Effect of Mastitis on production traits in Karan Fries & Sahiwal cattle

Hortet and Seegers (1998) reported that average daily milk loss in dairy cows due to mastitis varied from 0 to 3 kg in France. Rajala-Schultz *et al.* (1999) reported daily loss during the first 2 wk after the occurrence of mastitis, which varied from 1.0 to 2.5 kg, and the total loss over the entire lactation period varied from 110 to 552 kg in Holstein cows. In Holstein cows, Kocak (2006) reported the incidences of mastitis in first, second and third parity groups, as 19.94%, 33.74% and 40.74%, respectively. The daily milk losses due to mastitis in Holstein cattle varied between 0.76 kg/d and 4.56 kg/d. Also, the total milk loss was 600.87 kg for cows that became mastitic in the first six weeks of lactation and 503.86 kg for cows that became mastitic after the sixth week of lactation. Hagnestam *et al.* (2007) reported that the magnitude of yield loss varied from 0 to 902 kg (0 - 11 % of the 305 day milk yield) and a reduction of 1 to 8 kg daily milk yield from the day of onset of clinical mastitis. The crossbred cows were more susceptible to intra-mammary infection as compared to indigenous cows (Slettbakk *et al.*, 1995; Radostits *et al.*, 2000; Sharma and Maiti, 2010). Kumar *et al.* (2010) have also reported higher milk losses in crossbred cows as compared to indigenous cows. The high losses in crossbred cows may relate to high milk production. Boujenane *et al.* (2015) showed milk yield of cows with mastitis was 549.6 kg per 305 days of lactation which was significantly lower than healthy animals. Effect of clinical mastitis on the milk yield in Indian dairy cows was also reported by Bangar *et al.* (2018). They reported a reduction of 1.64 kg overall mean daily milk yield after the occurrence of mastitis and the

overall milk yield due to mastitis was reduced from 10.37 kg to 8.01 kg (Bangar *et al.*, 2018). The crossbred cows showed significantly higher reduction (2.37 kg/day) in milk yield due to mastitis than the indigenous (0.91 kg/day) lactating cows and the mean daily reduction in milk yield was observed as 1.64 ± 0.22 kg (Bangar *et al.*, 2018).

2.15 Genes associated with mastitis and udder type traits

Flury *et al.* (2014) reported that on bovine chromosome 6 (BTA 6), 11 SNPs were found between 88.44 and 88.99 Mb region which were significantly associated with fore udder length (FUL), 27 SNPs between 89.19 Mb and 91.25 Mb for teat diameter (TD). Also, 11 SNPs in the region of 117.28 Mb to 119.81 Mb on chromosome 3 had significant association with fore udder attachment (FUA). Out of 11 SNPs, two SNPs are positioned in the coding region of LRRFIP1 gene and two in the coding region of the MLPH gene. PRLH, RAB17 and COL6A3 genes were found in proximity of the SNPs in this region. There are 13 SNPs in the coding region of the SNX29 gene (BTA 25; 10,743,976-11,273,969 bp) which were found to be significantly associated with rear udder height (RUH). Total 19 SNPs spanning the region of 62.47 - 62.79 Mb on chromosome 17 were significantly associated with the trait RUW, RTP and FTP. TBX5 gene is located between the TBX3 gene (62.35 – 62.36 Mb) and the RBM19 gene (62.89 – 63.01 Mb) which had significant association with RUW. Beside the above mentioned SNPs on chromosome 17, three SNPs on chromosome 5 were significantly associated with FTP.

Tolleson (2016) reported 15 SNPs, exclusively within 26.4 Mb region on BTA 5 (21.6 to 48.1 Mb), having significant association for udder support. Of those 10 were located within a 5.1 Mb region (43.0 to 48.1 Mb), three were located within genes; VDR, IL22, and PTPRR. The other 12 associated SNPs were located at distances of 9,882 to 192,136 bp from the nearest known genes. Another gene, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2 (DYRK2), did not have any SNP located within its region, but 5 SNPs were located nearby (30,159 to 192,136 bp). A single SNP on BTA 5 was found to be associated with average teat diameter. This SNP was found 45,909 bp from LOC100848387 gene, which encodes 60S ribosomal protein L7. This SNP also had the strongest association with udder support. The list of genes associated with udder type traits, mastitis and milk production are given in Table 2.6.

Table 2.6 Genes associated with mastitis, udder type traits and milk production

Genes	BTA	Association	Reference
ETS2	1	Mastitis and milk production	Ogorevc <i>et al.</i> , 2009
ROBO1		Udder cleft, milk production	Strillacci <i>et al.</i> , 2014
SP5	2	Udder conformation	Pausch <i>et al.</i> , 2016
LRRFIP1	3	Fore udder attachment	Flury <i>et al.</i> , 2014
LOC104970680		Udder conformation	
MLPH		Fore udder attachment	
RAB17		Udder conformation	
COL6A3		Udder conformation	
PLXNA4	4	Clinical mastitis, Udder depth	Strillacci <i>et al.</i> , 2014
IGFBP-3		Milk traits and SCC, SCS	Zhang <i>et al.</i> , 2007
PD1A6		Teat length	Wu <i>et al.</i> , 2013
PTHLH	5	Milk traits and mastitis	Ogorevc <i>et al.</i> , 2009
SPCS3		Udder support	Tolleson, 2016
LOC100848544		Udder support	
VDR		Calcium related metabolism, Calcium Homeostasis	
PTPRR		Mammary epithelia proliferation, differentiation and apoptosis	
DYRK2		Proper mammary gland development	
DCK	6	Clinical mastitis, SCC	Wu <i>et al.</i> , 2015
GC		Clinical mastitis, SCC, Udder conformation	Pausch <i>et al.</i> , 2016; Wu <i>et al.</i> , 2015
NPFFR2		Clinical Mastitis, SCC and udder conformation	Pausch <i>et al.</i> , 2016
ABCG2		Mastitis and milk production	Ogorevc <i>et al.</i> , 2009

Table 2.6 **contd...**

Genes	BTA	Association	Reference
LETM1	6	Fore udder attachment, Rear Udder Height, Udder depth	Cole <i>et al.</i> , 2011
WHSC2		Udder Depth , Udder Cleft	
WDFY3		Fore Teat Placement, Rear Teat Placement, Teat Length	
RASSF6		Udder conformation	Pausch <i>et al.</i> , 2016
CRIM1	11	Udder conformation	Pausch <i>et al.</i> , 2016
RXFP2	12	Udder conformation	Strillacci <i>et al.</i> , 2014
KCNB1	13	Milk yield, Udder attachment , Udder Depth	
RBM19	17	Udder conformation	Flury <i>et al.</i> , 2014
TBX5		Rear Udder Width , Rear Teat Placement, Fore teat Placement	
ABHD2	21	Teat length	Cole <i>et al.</i> , 2011
FAM19	22	Protein yield, Udder Depth	Strillacci <i>et al.</i> , 2014
SNX29	25	Rear Udder Height	Flury <i>et al.</i> , 2014

2.15.1 Vitamin D Receptor (VDR) Gene

VDR regulates a variety of other metabolic pathways, such as those involved in the immune response and cancer (Haussler *et al.*, 1998). This gene mediates the action of vitamin D3 by controlling the expression of hormone sensitive genes (Akutsu *et al.*, 2001). VDR gene ranges from 32.5 to 32.6 Mb on BTA 5, encodes nuclear hormone receptor for vitamin D3 (1, 25-dihydroxyvitamin D3), which plays a crucial role in calcium related metabolism and calcium homeostasis. The expression of 1 α -hydroxylase is responsible for the conversion of 25 dihydroxy vitamin D3 to active hormone 1, 25-dihydroxyvitamin D3, which changes gene expression of macrophages isolated from milk of an infected gland (Nelson *et al.*, 2010b). Observations indicate that the vitamin D pathway influences innate and adaptive immune responses in cattle as it does in humans. Research examining the effects of vitamin D on the severity of experimentally induced mastitis in dairy cattle has provided in vivo evidence for

an intracrine mechanism of vitamin D signaling pathway in macrophages and has demonstrated the potential for vitamin D to reduce the severity of bacterial infection of the bovine mammary gland (Nelson *et al.*, 2012).

Cows receiving 25-dihydroxyvitamin D3 had significantly lower bacterial counts, reduced numbers of leukocytes in mammary secretions from infected glands, and lower body temperatures than control cows demonstrating that 25-dihydroxyvitamin D3 can limit the severity of bacterial-induced mastitis (Nelson *et al.*, 2012). It also has involvement in cell differentiation and proliferation, bone and cartilage development and regulation of osteoporosis, as well as a vital role in calcium homeostasis (Gao *et al.*, 2013). It has been suggested that maintaining vitamin D status above that needed for normal calcium homeostasis is required for optimal immune responses. It is known that intramammary infections activate bovine macrophages found in the milk through the Toll-like receptors pathways resulting in the up regulation of the expression of the 1 α -hydroxylase gene (Shahmohammadi *et al.*, 2014) (Figure 2.1).

2.15.2 Protein Tyrosine Phosphatase, Receptor Type, R (PTPRR) Gene

The possible contribution of PTPs to the development, involution, and remodeling of the mammary gland and their possible inhibitory action on maintaining high expression of milk genes during lactation, it is found that some PTPs were up regulated during gestation, suggesting that these enzymes are involved in development of the mammary gland. These PTPs then decreased during lactation. At the involution stage after weaning, most PTPs were up-regulated and their expression returned almost to the virgin level. Protein tyrosine phosphorylation has been shown to play critical roles in regulating fundamental cellular processes such as proliferation, differentiation, development and to be controlled by the balance of PTP (Aoki *et al.*, 1999). This gene ranges from 42.9 to 43.1 Mb on BTA 5. The protein encoded by PTPRR is a member of the protein tyrosine phosphatase (PTP) family. These proteins are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation (The NCBI handbook, 2002) (Figure 2.2).

2.15.3 High mobility group AT-hook 2 Gene (HMGA2) Gene

HMGA2 is found to be involved in fat cell proliferation and adipose tissue development (Anand and Chada, 2000). The let-7 family of miRNAs which act as a regulator of mammary gland development, lactation and involution is also involved in the regulation of

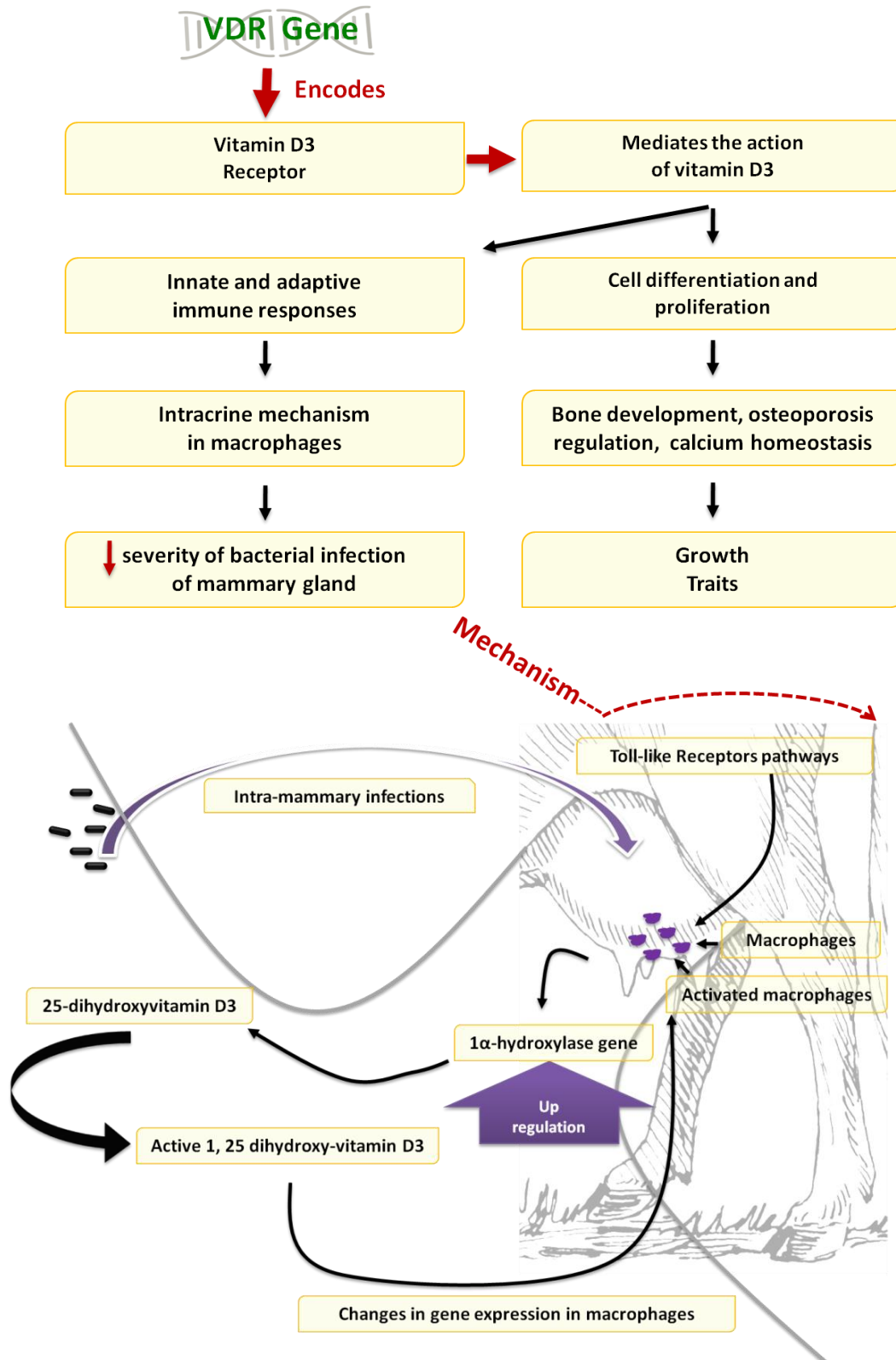
HMGA2 gene (Lee and Dutta, 2007). This gene ranges from 48.05 to 48.20 Mb on BTA 5. The ‘high mobility group’ HMGA protein family consists of four members: HMGA1a, HMGA1b and HMGA1c, which result from translation of alternative spliced forms of one gene and HMGA2, which is encoded by another gene. HMGA proteins are characterized by three DNA-binding domains, called AT-hooks, and an acidic carboxy-terminal tail. HMGA proteins are architectural transcription factors that both positively and negatively regulate the transcription of a variety of genes. They do not display direct transcriptional activation capacity, but regulate gene expression by changing the DNA conformation by binding to AT-rich regions in the DNA and/or direct interaction with several transcription factors. In this way, they influence a diverse array of normal biological processes including cell growth, proliferation, differentiation and death. Both HMGA1 and HMGA2 are hardly detectable in normal adult tissue but are abundantly and ubiquitously expressed during embryonic development. In malignant epithelial tumors as well as in leukemia, however, expression of HMGA1 is again strongly elevated to embryonic levels thus leading to ectopic expression of (fetal) target genes. HMGA2 over-expression also has a causal role in inducing neoplasia. Besides over-expression of full length HMGA proteins in different tumors, the HMGA genes are often involved in chromosomal rearrangements. Such translocations are mostly detected in benign tumors of mesenchymal origin and are believed to be one of the most common chromosomal rearrangements in human neoplasia (Cleynen and Ven, 2008).

The HMGA2 gene encodes a non-histone chromosomal protein that influences chromatin structure and regulates expression of several genes (Pfannkuche *et al.*, 2009). HMGA2 is found to be associated with age at puberty (Fortes *et al.*, 2011), birth weight (Saatchi *et al.*, 2014), stature (Bolormaa *et al.*, 2014), milk fat yield (Pegolo *et al.*, 2017) and navel length (Aguilar *et al.*, 2018) in cattle. High mobility AT-hook 2 gene (HMGA2) encodes a major transcription factor affecting the expression of IGF2. Regulation of IGF2 by HMGA2 has been demonstrated to occur directly or through increased expression of PLAG1 (Klemke *et al.*, 2014; Abi Habib *et al.*, 2018). PLAG1 is potential candidate gene and udder morphology (Pausch *et al.*, 2016) and for milk production traits (Fink *et al.*, 2017) while IGF2 is associated with milk production in cattle (Dux *et al.*, 2018) (Figure 2.3).

2.15.4 Interleukin 22 (IL22) Gene

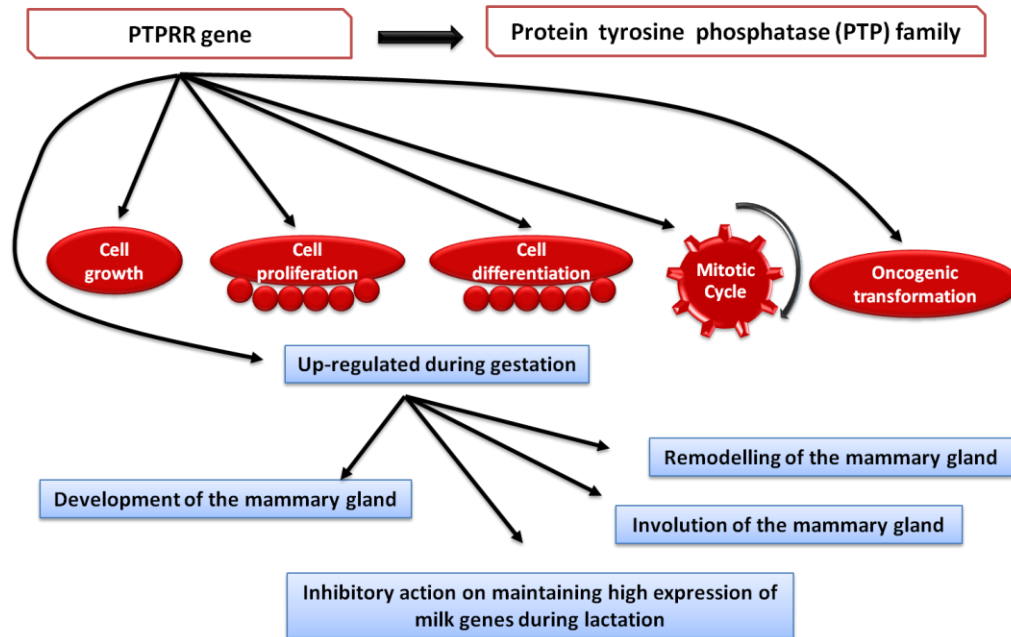
Genetic studies in mice have revealed roles for Stat proteins 3, 5a and b, and 6 in the adult mammary gland during a pregnancy cycle. According to Chapman *et al.* (1999), Stat3 is

Figure 2.1 Mechanism of action of VDR gene



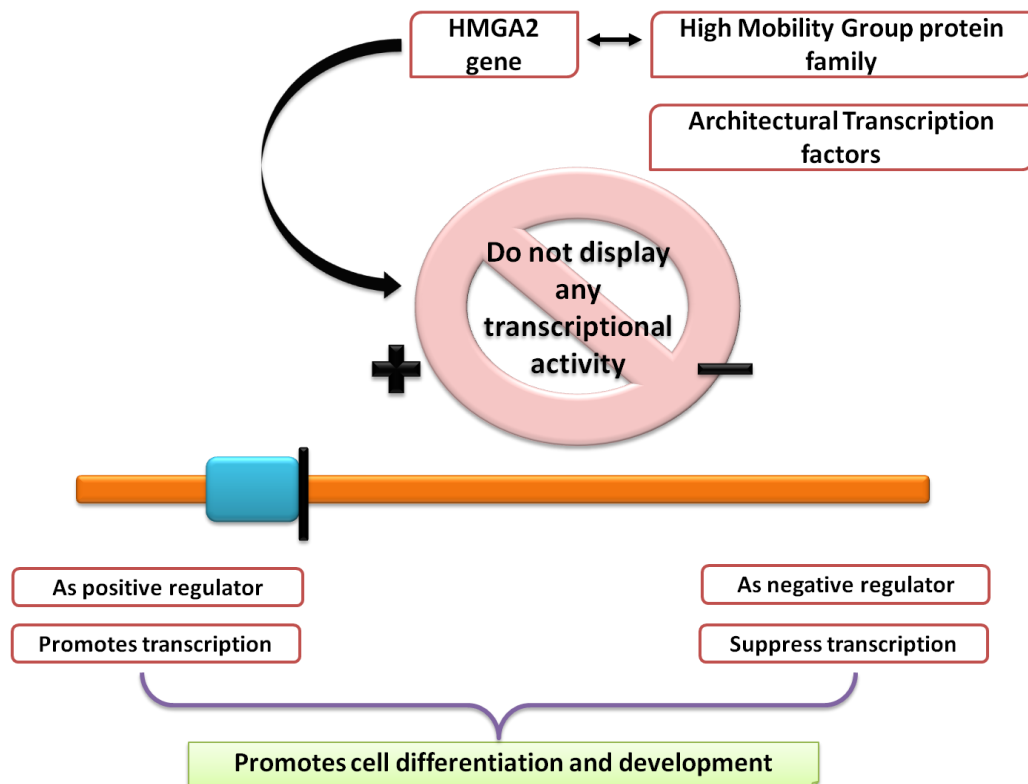
Akutsu *et al.* (2001); Nelson *et al.* (2012); Gao *et al.* (2013); Shahmohammadi *et al.* (2014)

Figure 2.2 Mechanism of action of PTPRR gene



Aoki et al. (1999); The NCBI Handbook (2002)

Figure 2.3 Mechanism of action of HMGA2 gene



Cleyen and Ven (2008)

essential for the initiation of apoptosis and remodeling following forced weaning. In the absence of Stat3, involution is dramatically delayed and the reversible phase can be extended for up to 6 day (Humphreys *et al.*, 2002). It promotes antimicrobial immunity, inflammation, and tissue repair, which could aid in mastitis prevention and resistance, thus creating a possible link to udder traits. IL22 mediates the action of Stat3 (Signal transducer and activator of transcription 3). Stat3 activity is highest on the day of parturition and during the first 6 days of mammary gland involution (Watson, 2006). Stat proteins have also been shown to play an important role in fertility and early embryonic development (Khatib *et al.*, 2009). Cytokines and their Stat targets have important roles in lineage commitment, survival, and death of mammary gland epithelium (Hynes and Watson, 2010). Interleukin-22 IL22 appears to play an important role in maintaining barrier function on exposed body surfaces (Tizard, 2012).

Interleukin 22 (IL22) ranges from 45.721 to 45.727 Mb on BTA 5. In a Genome-wide association study for udder composition traits in *Bos indicus* –*Bos taurus* cows, Tolleson (2016) has reported a genome-wide significant SNP (ARS-USMARC-614) within the IL22 gene at position 45.7 Mb of BTA 5 for udder support. IL22 is a member of a group of naturally occurring proteins that mediate communication between cells. Interleukins regulate cell growth, differentiation, and motility. They are particularly important in stimulating immune responses, such as inflammation. Interleukins are a subset of a larger group of cellular messenger molecules called cytokines, which are modulators of cellular behavior.

CHAPTER –3

Materials & Methods

3. Materials and methods

3.1 Source of data

3.1.1 Udder and teat type traits

Data on udder and teat type traits were collected from the herd of Sahiwal and Karan Fries cattle maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal and they were collected from all lactating cows within the period of research work.

3.1.2 Milk production data

The milk production data of the animals were collected from daily milk yield register kept in the Livestock Record Unit of Animal Breeding & Genetics Division.

3.1.3 Incidence of mastitis

Incidence of mastitis among the lactating animals of Sahiwal and Karan Fries cattle were recorded from Mastitis Register kept in the Animal Health Complex of ICAR-National Dairy Research Institute, Karnal.

3.2 Recording of data

The data on Sahiwal and Karan Fries cattle were collected from birth register, body weight register and history cum pedigree sheets maintained in the record room of Animal Genetics and Breeding Division and Livestock Research Centre. The following information was collected:

General information

- i. Breed
- ii. Animal Number
- iii. Date of measurement
- iv. Age at scoring in months
- v. Date of calving
- vi. Stage of lactation
- vii. Parity
- viii. Season of calving

Traits recorded

- i. Monthly test day milk yield (kg)
- ii. 305-Days or less milk yield (kg)
- iii. Incidence of Mastitis: Date of onset of symptoms, treatment history, date of cure/recovery were recorded.

3.3 Traits generated

Total 8 udder measurement traits, 6 teat measurement traits and 11 visual observational udder and teat type traits were generated for lactating animals of both Karan Fries and Sahiwal.

3.3.1 Procedure of measurement of Type traits:

Measuring Instrumentation and Precaution

Measuring Instruments:-

- Teat measurements were done by Vernier Caliper.
- Udder measurements were done by meter long rope with weight fixed in one end, Metal tape (200 cm long) or any other convenient instrument.

Time:-

- One to two hours before the evening milking

Precaution:-

- Animals were secured in a standing position on a leveled floor for accuracy.

3.3.2 Scoring of Type traits:

The range for each udder and teat type trait measurement was calculated by subtracting the minimum value from maximum. As the animals were evaluated into 9 subclasses for each trait, the range was divided by 9, to get the class interval/unit score point. This class interval/unit score was added to the minimum value to get the range for each subclass one. The subsequent subclasses were obtained by adding class interval/unit score to the highest value of pervious classes.

The scoring of various traits is as follows:

Following 25 udder and teat type traits were scored on possible score scale from one biological extreme to other (Plates 3.1-3.2):

Plate 3.1 Measurement of udder type traits



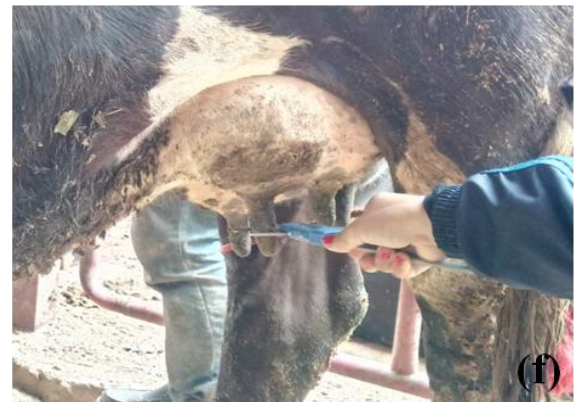
(a) Rear udder width

(b) Rear udder length

(c) Udder width

(d) Udder circumference

Plate 3.2 Measurement of teat type traits



- (a) Distance between front and rear teats
- (b) Teat circumference
- (c) Teat length
- (d) Shortest distance from rear teat ends to floor
- (e) Teat diameter
- (f) Teat diameter

Udder Measurements

1. Fore udder attachment (FUA) (degree)

It is the strength of attachment of fore udder to the abdominal wall. A completely even transition between udder and body and an udder attached over the whole width, was assigned score 9. A loose and narrow attachment between udder and body received score 1. In case of a significant difference in the quality of udder attachment of both sides by scoring, then the worse side must be scored. The scores were assigned as “score 1-3 for weak and loose condition”, “4-6 for intermediate acceptable” and “7-9 for extremely strong and tight” udder attachment (Figure 3.1a).

2. Rear udder height (RUH) (cm)

When assessing the rear udder height, the point of the milk secreting tissue has to be found. This point was assessed in relation to the distance between the pins and the hocks. If the point was right in the middle, the score 5 was given. If the point was much higher, then score 9 was given, while a very low one received score 1. The scoring was done as; “score 1-3 for very low”, “4-6 for intermediate” and “7-9 for high” classes (Figure 3.1b).

3. Udder depth (UD) (cm)

The udder depth was measured by subtracting two distances, (a) from the barn floor to the udder floor (b) from the barn floor to the base of the udder. It is the distance from the lowest part of the udder floor to the hock. As per the recommendation of ICAR (2001), if both were on the same level then score 3 was given. If the bottom of udder was 18 cm above the hocks, then score 9 was given, whereas, a distance of 6 cm above the hocks received score 5. The scale for the score was 1 unit per cm from the hocks upwards. If the bottom of the udder was 3 cm below the hocks, then score 2 was given. An udder reaching 6 cm below hocks received score 1. The scoring was done as; “score 1 for below hock”, “2 for level with hock”, “5 for intermediate” and “9 for shallow” classes (Figure 3.1c).

4. Udder Balance (UB) (cm)

The depth of the rear udder was assessed in relation to the depth of the front udder. The depth was assessed from the side where the deepest point of the rear udder was compared with the point where the front teats are located. Scores were given according to the classes made based on lowest and highest values of measurement, taking care of all

positive and negative values of the traits. The trait was classified according to the scores. The scoring was done as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long” classes (Figure 3.2a).

5. Rear udder width (RUW) (cm)

Rear udder width was evaluated at the point of attachment. Like rear udder height, rear udder width was an indicator of udder capacity. It was the width of the udder at the point where the milk secretion tissue is attached to the body. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores, which were given as; “score 1-3 for narrow”, “4-6 for intermediate” and “4-9 for wide” classes (Figure 3.2b).

6. Central ligament (CL) (cm)

The Central ligament or the depth of cleft was measured at the base of the rear udder. A 15 cm length scale was used which was inserted at the base of the rear udder and record the point where depth of scale coincides with base of rear udder was recorded. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1 for convex to flat floor (+1 cm)”, “2 for +0.5 cm”, “3 for +0 cm”, “4 for slight definition (-1 cm)”, “5 for -2 cm”, “6 for -3 cm”, “7 for deep definition (-4 cm)”, “8 for -5 cm” and “9 for -6 cm” (Figure 3.2c).

7. Udder length (UL) (cm)

The udder length was measured from the rear attachment of the udder, near the escutcheon, to the front of the udder where it blends smoothly with the body. The length of udder was measured separately from left and right sides and the average was calculated. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long” classes (Figure 3.3).

8. Udder width (UW) (cm)

The udder width was measured as a distance between two lateral lines of attachment of the udder to abdominal wall, beneath the flank. The measuring tape was kept in position on one side of the cow, under flank, near the stifle joint and it was pass over in between fore and rear teats to the other side. Scores were given according to the classes made

Figure 3.1 Udder type traits- Fore udder attachment, Rear udder height and Udder depth

a) Fore Udder Attachment - FUA

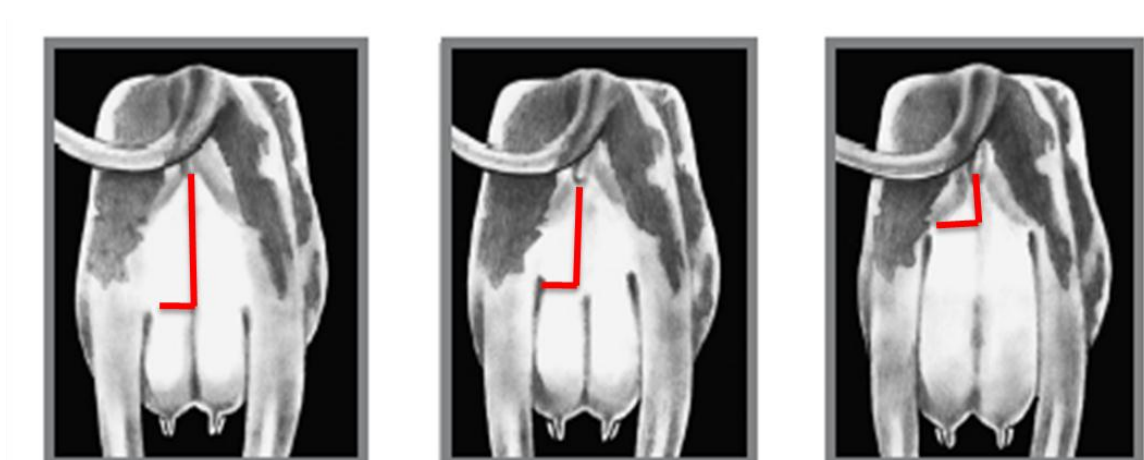


Extremely broken & Loose attachment

Intermediate strength

Extremely Strong, firm and tight attachment

b) Rear Udder Height -RUH



Low

Intermediate

High

c) Udder Depth - UD



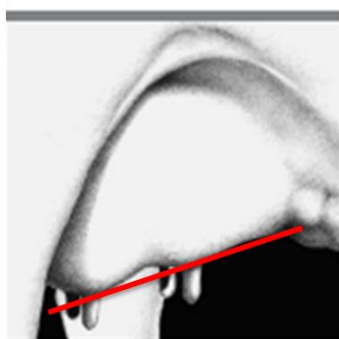
Extremely deep udder floor below hock

Udder floor above hocks

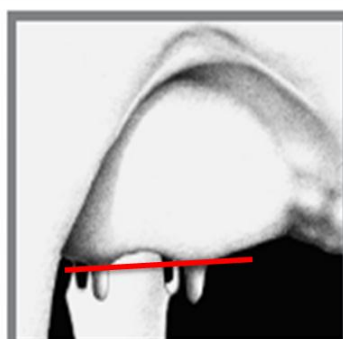
Extreme height of Udder floor above hock

Figure 3.2 Udder type teat traits- Udder balance, Rear udder width and Udder cleft

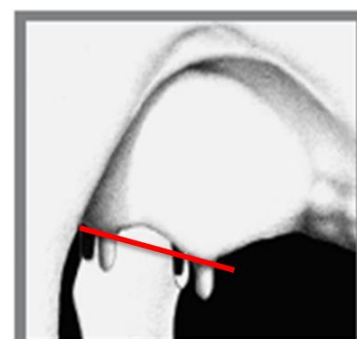
a) Udder Balance - UB



Rear quarters deep



Front and rear quarters
on same level



Front quarters deep

b) Rear Udder Width - RUW



Narrow

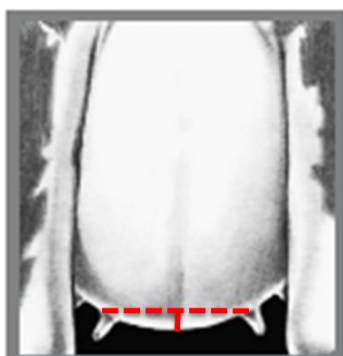


Intermediate wide

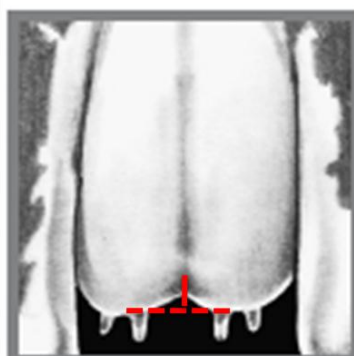


Wide

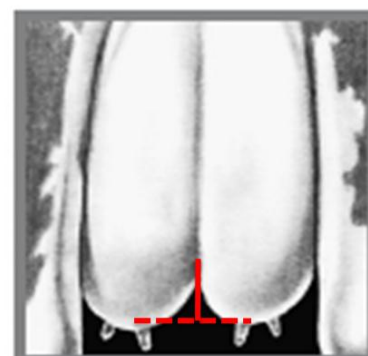
c) Udder Cleft - UC



Weak Udder cleft

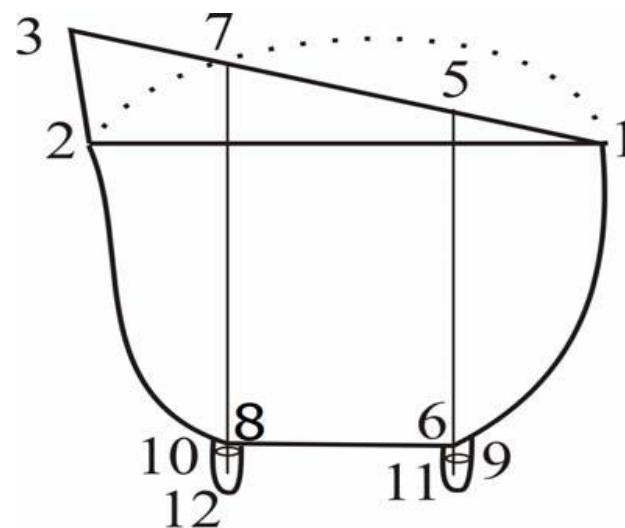
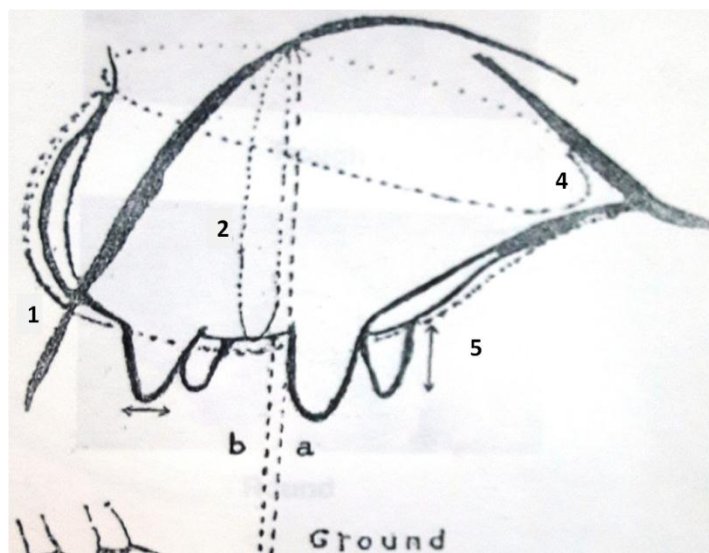


Intermediate halving



Extremely strong cleft

Figure 3.3 Diagrammatic Sketch of Udder and Teat measurements



- 1. Udder length
- 2. Udder width
- 3. Udder depth (a-b)
- 4. Udder Circumference
- 5. Teat Length (cm)

- 1 to 2 : Udder circumference
- 1 to 3 : Udder length
- 5 to 6 : Fore-quarter depth
- 7 to 8 : Hind-quarter depth
- 6 to 11 : Fore teat length
- 8 to 12 : Hind-teat length
- 9 & 10 : Fore & hind teat diameters

Saini and Gill (1989); Ahmed and Barbary (2000)

based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long” classes (Figure 3.3).

9. Udder circumference (UC) (cm)

Udder circumference was recorded as the average of two measurements a) Measuring tape was passed round the udder near the base of udder close to body, b) Measuring tape was passed round the udder near the emergence of teats. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short/small”, “4-6 for intermediate” and “7-9 for long/large” classes (Figure 3.3).

10. Udder shape (US)

Shape of udder was determined through visual appraisal method adopted by Rao (2006) and accordingly categorized into different types, viz., trough, round, and pendulous. The trait was classified according to the scores given as’ “score 1 for trough”, “2 for round” and “3 for pendulous” udder shape (Figure 3.4a).

11. Udder suspension (USus)

It was the subjective assessments of udder support. Placement and closeness of the udder towards the body cavity, position of the udder floor from the hocks, level of the quarters from the side and rear view, rear attachment and the median suspensory ligament was observed to determine type and scores of udder suspension. The trait was classified according to the scores given as; “score 1 for very tight”, “2 for tight”, “5 for intermediate/ medium”, “6 for pendulous” and “9 for very pendulous/ broken floor” (Figure 3.4b).

12. Teat end shape (TES)

It was visual observation of the shape of teat end /teat tip shape following Britt and Farnsworth (2011). Status of the teat tip shape for each animal was analysed and the following classes and scores were given as; “score 1 for flat”, “2 for round”, “5 for funnel shape (inverted)”, “6 for disk” and “9 for pointed/ hyperkeratosis” type teat end shape (Figure 3.4c).

13. Fore and Rear Teat placement (FTPI & RTPI)

Fore and rear teat placement was recorded as the position of the front teat from centre of quarter. Scores were given according to the classes made based on observed location of the teat on the udder quarter. The trait was classified according to the scores given as; “score 1-2 for outside of quarter”, “4-6 for middle of quarter” and “7-9 for inside of quarter” (Figure 3.5a & b).

14. Teat diameter (TD) (cm)

Teat diameter was measured at the mid-point length by Vernier Caliper to the nearest 0.01 cm. All four teats were measured individually and average width both of front and rear teats were taken. Teat thickness was measured as the thickness of the teat in the middle of the teat. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1 for thin”, “5 for intermediate” and “9 for thick” teat (Figure 3.5c).

15. Fore and Rear Teat length (FTL & RTL) (cm)

Teat length was measured from the upper part of the teat, where it hanged perpendicularly from the quarter to the tip of teat. The distance between the root and the tip of the teat was measured in cm. Teat length was measured to the nearest 0.01 cm using Vernier Caliper. All four teats were measured individually and average length both of front and rear teats was taken. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long” teats (Figure 3.6a).

16. Fore and Rear Teat position (FTP & RTP)

Front and rear teat positions were defined as the position of the teat end from centre of quarter as viewed from the rear. Teats which were placed at the inner side of the quarter and pointing inwards receives score 9. If they were placed right under the quarter, score 4 was given, while rear teats placed a far from each other and on the outside of the quarter, received score 1. The trait was classified according to the scores given as; “score

Figure 3.4 Udder and teat type traits- Udder shape, Udder suspension and Teat end shape

Udder Shape



Pendulous

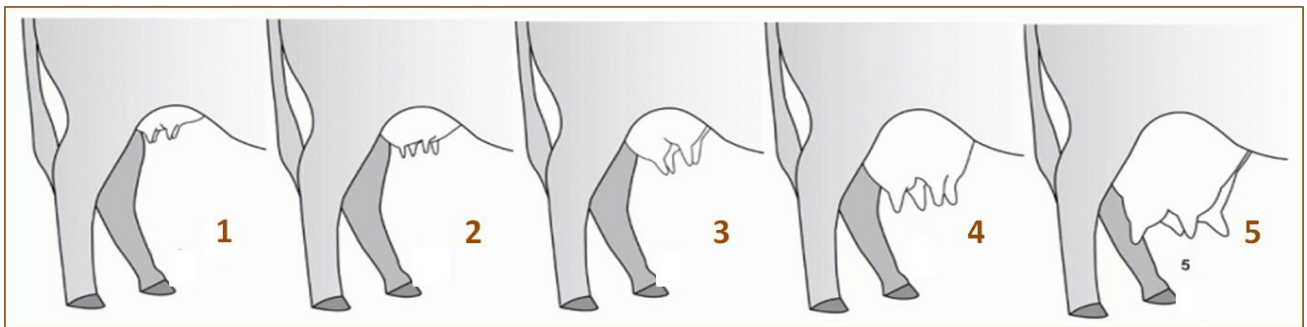


Trough



Round

Udder Suspension



Very tight attachment

Tight attachment

Intermediate attachment

Pendulous attachment

Very Pendulous/
Broken

Teat end shape



Flat/Platform



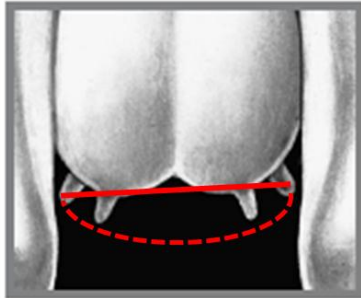
Round/Semi platform



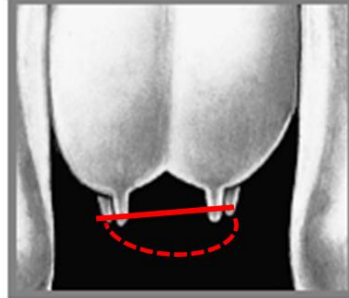
Pointed

Figure 3.5 Teat type traits- Fore teat placement, Rear teat placement and Teat Diameter

a) Fore Teat Placement – FTPI



Extremely wide placement on outside of quarter

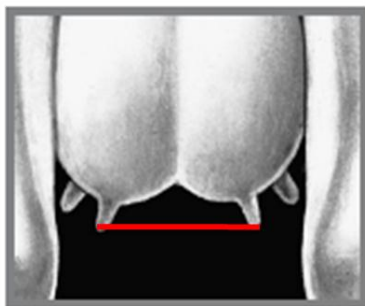


Centrally placed, with mid symmetry

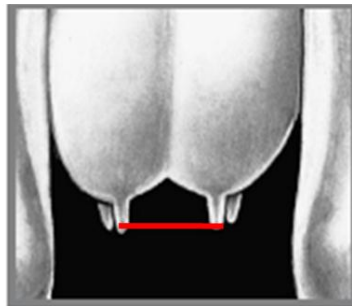


Base of teats on extreme inside of quarter

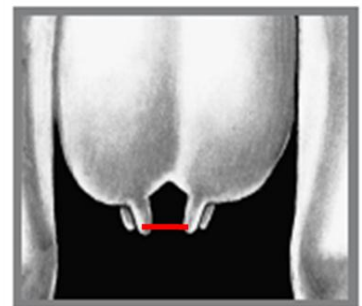
b) Rear Teat Placement - RTPI



Extremely wide placement on outside of quarter



Centrally placed, with mid symmetry



Base of teats on extreme inside of quarter

c) Teat Diameter - TD



Thin



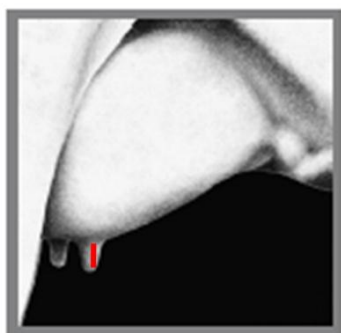
Intermediate



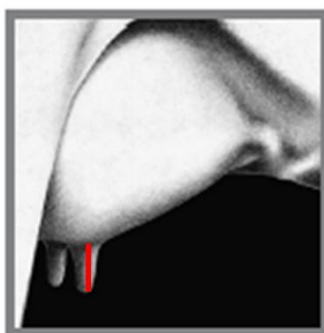
Thick

Figure 3.6 Teat type traits- Teat length, Fore teat position and Rear teat position

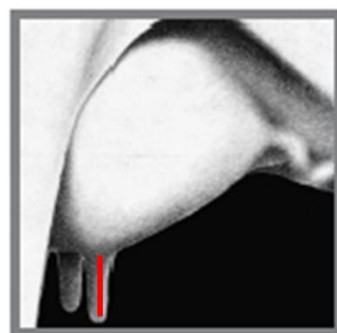
a) Teat Length - TL



Smaller

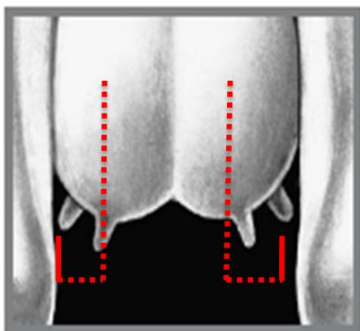


Intermediate

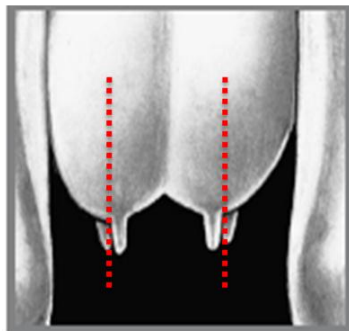


Longer

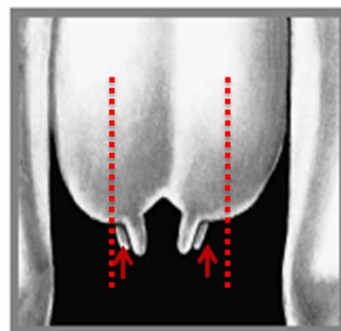
b) Fore Teat Position - FTP



Outside of quarter

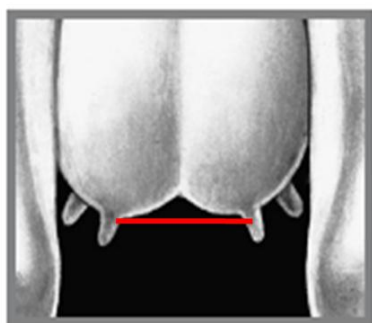


Middle of quarter



Inside of quarter

c) Rear Teat Position - RTP



Outside of quarter



Middle of quarter



Inside of quarter

1-3 for outside or quarter”, “4-6 for middle of quarter” and “7-9 for inside of quarter” (where 8 is for touching and 9 is for crossing) (Figure 3.6b & c).

17. Teat circumference (TC) (cm)

Teat circumference was measured with a measuring tap at the midpoint of teat length. The measurements were recorded on each animal when standing evenly on its feet. The measurements were taken to nearest centimeter (cm) and with utmost precision. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for small/short”, “4-6 for intermediate” and “7-9 for large/long” (Figure 3.7a).

18. Distance between teats (DFR & DLR) (cm)

It was measured as the distance between teats at midpoint of the teat length. The measurements were recorded on each animal when standing evenly on its feet. The measurements were taken to nearest centimeter (cm) and with utmost precision. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long” (Figure 3.7b).

19. Teat Size (TSi) (cm)

It is a visual observation and subjective assessments of teat length, teat thickness and symmetry among the teats. Scores were given according to the classes made based on observed category. The trait was classified according to the scores given as; “score 1 for very small”, “2 for small”, “5 for intermediate/medium”, “6 for large” and “9 for very large and balloon shaped” (Figure 3.7c).

20. Shortest distance from front and rear teat ends to floor (SDF & SDR) (cm)

The shortest distance from front and rear teat ends to floor were measured as distance from front and rear teat ends to floor. Average of distance from both the front teat ends to floor was taken as the value of shortest distance from front teat ends to floor (SDF), the same was done for rear teats. Scores were given according to the classes made based on lowest and highest values of measurement. The trait was classified according to the scores given as; “score 1-3 for short”, “4-6 for intermediate” and “7-9 for long”.

21. Teat shape (TS)

Shape of teat was observed grossly and categorized into different types, viz., cylindrical, funnel, bottle and pear shaped teats as per the visual appraisal method followed by Britt and Farnsworth (2011) and accordingly categorized into different types, viz., Funnel/Conical, Cylinder, Wide/Flat and Bottle. The scoring was done as; “score 1 for funnel/ conical”, “2 for cylinder”, “5 for wide/ flat”, “6 for bottle” and “9 for funnel/ conical” (Figure 3.8).

22. Skin Condition (SC)

It is visual observation of the skin on teat based on texture of the skin (dryness, roughness, cracking etc) following Mein *et al.* (2001). Status of the skin condition for each animal was analysed and the following classes and scores were given as; “score 1 for normal-smooth and healthy”, “2 for dry- no cracking” and “3 for rough-dryness + cracking”.

23. Long Term Changes in Teat-End Condition (LTC TEC)

It is visual appraisal of the degree of teat-end hyperkeratosis (roughness, cornification or callosity), which is a dynamic condition following Mein *et al.* (2001). Status of teat-ends for each animal was analysed and the following classes and scores were given as; “score 1 for smooth bottom, no or smooth callous, no lesions”, “2 for raised callous ring with slight roughness”, “5 for rough callous with hyperkeratosis”, “6 for very rough callous with hyperkeratosis and cracking” and “9 for open skin, haemorrhage, trauma/abnormal condition” (Figure 3.9).

3.4 Classification of data

The data on udder type trait scores, udder and teat measurements, milk production and incidence of clinical mastitis were classified for determining the effect of parity, season, stage of lactation and level of milk production as given in the following sections:

3.4.1 Classification of season

The climatic conditions throughout the year showed wide variation, differences being more between the seasons and less within the season. Hence, evaluation and correction of records for seasons is important for standardizing the variations on trait.

Figure 3.7 Teat type traits- Teat circumference, Distance between teats and Teat size

a) Teat circumference



Short



Intermediate

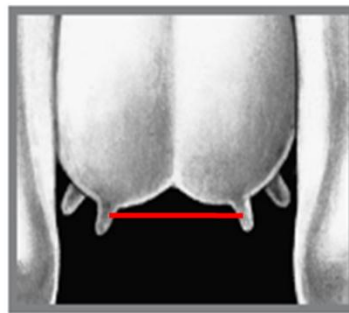


Long

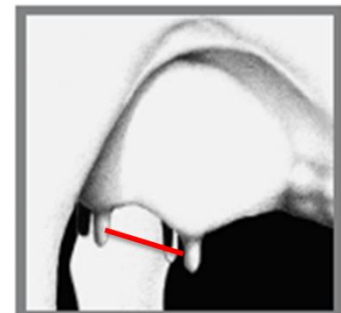
b) Distance between teats



Distance between
Fore Teats

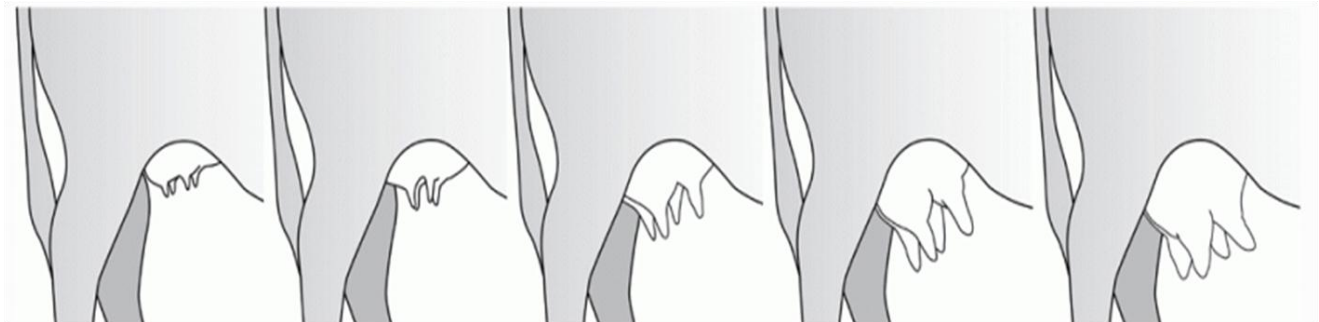


Distance between
Rear Teats



Distance between
Fore and Rear Teats

c) Teat size



Very Small
symmetrical

Small
symmetrical

Intermediate
symmetrical

Large
asymmetrical

Very Large
asymmetrical

Figure 3.8 Different teat shapes in cattle



Funnel shaped teat



Cylinder shaped teat



Wide shaped teat

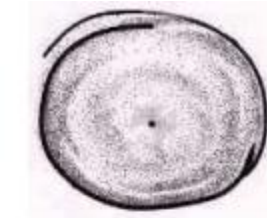


Bottle shaped teat

Figure 3.9 Long-Term Changes in Teat-End Condition

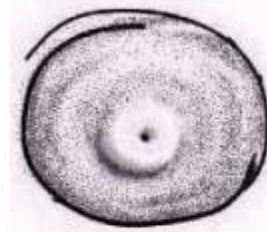
1. No ring

Teat end is smooth with small, even orifice



2. Smooth/slightly rough ring

A raised ring, smooth/slightly rough ring 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 upto ≥ 4 mm from the orifice – Flowered appearance



Chances of Mastitis

Min

Max

The year was divided in to four seasons on the basis of prevailing climatic conditions and fodder availability at farm (Table 3.1).

Table 3.1 Classification of season

Season	Duration	Codes
Summer	April to June	1
Rainy	July to August	2
Autumn	September to November	3
Winter	December to March	4

3.4.2 Classification of stage of lactation

Current lactation length was divided into four stages as follows (Table 3.2).

Table 3.2 Classification of stage of lactation

Stage Group	Days in Lactation	Codes
1	0 –90 days	1
2	90 days – 180 days	2
3	180 days– 270 days	3
4	> 270 days	4

Current lactation length was calculated from the difference of date of calving to the date of type scoring. Animals in stage of lactation 180 days–270 days (3rd stage) and above (4th stage) are grouped together in the third stage of lactation *i.e.* 180 days– 270 days as very few number of animals are present in the stage 4 (> 270 days) in both the breeds.

3.4.3 Parity of the animal

On the basis of parity order, the cows were divided into following five parity groups (Table 3.3).

Table 3.3 Classification of parity of the animal

Parity	Codes
First Parity	1
Second Parity	2
Third Parity	3
Fourth Parity	4
Fifth and above	5

Animals of parity fifth and above were grouped together as very few animals of above fifth parity are available in the herd for both Karan Fries and Sahiwal animals.

3.4.4 Level of milk production of the animal

On the basis of 305 day milk production (kg), the cows were divided into following three production level groups (Table 3.4).

Table 3.4 Classification of level of milk production of the animal

Level of milk production for Karan Fries cows	Level of milk production for Sahiwal cows	Code
Low (<1900 kg)	Low (< 1000 kg)	3
Medium (1900-4500 kg)	Medium (1000-1800 kg)	2
High (>4500 kg)	High (>1800 kg)	1

3.5 Isolation of genomic DNA

3.5.1 Blood collection

Blood samples were collected from 166 Karan Fries and 87 Sahiwal lactating animals. Around 10 ml of venous blood was collected aseptically from the jugular vein of the animals in a 15ml polypropylene centrifuge tube under sterile condition using 0.5 ml of EDTA as an anticoagulant. The tube was shaken gently to facilitate thorough mixing of blood with the anti-coagulant. The tubes containing blood samples were transported to the laboratory as soon as possible in an icebox containing ice packs and were stored in the refrigerator at -20°C temperature until further processing.

3.5.2 DNA extraction

Genomic DNA was isolated from blood samples by Phenol: Chloroform: Isoamyl alcohol extraction method as described by Sambrook and Russell (2001) with modifications to suit our laboratory condition. The protocol followed for isolation of genomic DNA was as follows:

1. The blood samples were thawed to room temperature if stored at -20°C.
2. The tubes were filled with chilled RBC lysis buffer (1x), mixed end to end, incubated in ice for 10 min and centrifuged at 4000 rpm for 15-20 min at room temperature.
3. The reddish tinged supernatant containing plasma and lysed RBC were discarded by pipetting.

4. Steps 2-3 were repeated, till the WBC pellet appeared nearly white in colour.
5. About 3 ml of DNA extraction buffer (at 3 ml/10ml of blood) was added in the tubes, tapped to disperse the WBC pellet in the extraction buffer and kept in incubator at 37°C for 30 minutes (Ensured that the buffy coat was completely suspended so that the cells were accessible to SDS and Proteinase K).
6. 10% SDS at 200 μ l per 10 ml of blood was added and was gently mixed in the tubes by inverting. The content of the tube became viscous indicating lysis of WBC. The samples were handled gently from that time in order to avoid shearing of DNA.
7. 20 μ l of Proteinase K (at 20 mg Proteinase K /ml of triple distilled H₂O for 10 ml blood) was added in two pulses to the tubes i.e. half the requirement was added to the tubes in 1st pulse, mixed gently end to end and kept in water bath at 50°C. After 3-4 hrs the second pulse of the remaining amount of Proteinase K was added and it was incubated at 50°C overnight.
8. After 24 hours, the contents were transferred into a clean, sterile, autoclaved polypropylene tubes, containing equal amount of equilibrated phenol (Tris saturated phenol pH >7.8), mixed the contents by inverting gently for 15 min. till a light coffee colored uniform solution was formed and centrifugation was done at 4000 rpm for 15-20 min.
9. The upper aqueous phase containing DNA was transferred into fresh 15 ml, clean, sterile, autoclaved polypropylene tubes using 1ml tip (the tip has been cut to widen the bore). Upper aqueous phase (containing DNA) was very viscous and care was taken during transfer of aqueous phase so that lower organic phase (containing phenol, cell lysate, proteins etc.) and white interface (containing proteins) were not be disturbed.
10. Similar extractions were repeated (as in steps 8 and 9) once with equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) and then with chloroform: isoamyl alcohol (24: 1).
11. To the final aqueous phase, 3M sodium acetate (pH-5.2) at 100 μ l/per ml of aqueous phase was added and was mixed gently.
12. Two volumes of ethanol (chilled)/ isopropanol were added, and the tubes were gently mixed by inversion and kept at room temperature to allow precipitation of DNA.

13. DNA along with 500 µl of isopropanol was transferred into fresh eppendorffs (1.5 ml) using wide bore tips and centrifuged in micro centrifuge at 10,000 rpm for 10 min.
14. The supernatant was discarded by gentle inversion where DNA pellet was intact, otherwise aspirated when DNA pellet was loose.
15. Washed the DNA pellet twice in 500µl of 70% ethanol in eppendorffs by centrifugation at 10000 rpm for 10 min at room temperature.
16. Finally, the DNA pellet was air dried after inverting on blotting paper so that last traces of ethanol were removed. However, the pellet should not be over dried since it becomes difficult to dissolve.
17. Approximately 200 µl of TE buffer was added and put in water bath at 60°C for 2 hr to inactivate DNase or other enzymes
18. Stored the eppendorffs at 4°C for a week so that DNA was dissolved properly and subsequently shifted to -20°C temperature for further use.

3.5.3 Quality and quantity checking of genomic DNA

Agarose gel electrophoresis was carried out for checking the quality of DNA. 2 µl DNA mixed with 2 µl of 6X gel-loading dye was loaded in 0.8% agarose gel [0.8gm agarose + 10 ml TBE (10X) + 90 ml double distilled water] in horizontal mini electrophoresis unit using 1X TBE as running buffer at 100 volts for 45 minutes. Gel was stained with 1% ethidium bromide solution and photograph was taken by gel documentation system (Bio-Rad). Sharp and intact bands indicated good quality DNA. Quality and quantity of DNA were also estimated by Biospec-nano spectrophotometer (Schimadzu Cooperation, Japan). DNA Samples with OD₂₆₀/OD₂₈₀ ranging between 1.7 and 1.9 were of good quality, while quantity of DNA was calculated using the following formula:

$$\text{Quantity of DNA (}\mu\text{g/ml)} = \text{OD}_{260} \times \text{dilution factor} \times 50$$

3.5.4 Preparation of template DNA for PCR

The stock DNA was diluted with nuclease free water to achieve the final concentration of 50 ng/µl and was used as working solution.

3.6 PCR amplification

3.6.1 Primer designing for Bovine VDR, PTPRR, IL-22 and HMGA2 gene

In silico primer designing for target regions of Bovine VDR, PTPRR, IL22 and HMGA2 gene in cattle chromosome BTA 5 available at NCBI (NCBI Ref Seq: AC_003099192.1:c39272-37783) were carried out using Primer3 software (<http://www.primer3.ut.ee>) (Untergrasser *et al.*, 2012) and gene specific sequence (NCBI Ref Seq: AC_000162.1:91771436-91820144) available at NCBI database (<http://www.ncbi.nlm.nih.gov>). Eight sets of forward and reverse region-specific oligonucleotide primers were designed.

Primers were designed to include intron-exon boundaries, in order to evaluate possible mutations in target regions in Sahiwal and Karan Fries cattle. Primers were procured from M/s. Eurofins Genomics India Pvt. Ltd (Bangalore). The sequence of forward and reverse (F & R) primers, their nucleotide numbers and corresponding region of targeted SNPs have been given in Table 3.5. PCR reactions were set up for a total volume of 25 µl for each sample and PCR reactions were done for all DNA samples in the studied breeds.

3.6.2 Polymerase chain reaction (PCR) for amplification of target regions

Targeted regions on VDR, PTPRR, IL22 and HMGA2 genes were amplified by standardizing PCR program using genomic DNA and gene specific set of forward and reverse primers in a fixed reaction mixture with the help of Thermal cycler. Targeted regions on VDR, PTPRR, IL22 and HMGA2 genes were amplified based on standardization of PCR. Only good quality genomic DNA was used for amplification of the genes by polymerase chain reaction under optimized conditions. The best amplification of the desired fragments of the genes, were taken for further analysis. The standard programme for PCR amplification is given in the Table 3.6-3.7.

3.6.2.1 Preparation of PCR reaction mixture

1. Working solutions of both forward and reverse primers were prepared from stock solutions by adding primer and milliQ water in 1:9 ratios to obtain final concentration of 10 pmol/µl for each primer.
2. PCR reaction mixture was prepared using PCR master mix (2X) (Fermentas). Final reaction mixture (25 µl) is given in Table 3.6.
3. All 0.2 ml tubes were marked with respective DNA sample number. The PCR amplification was performed in programmed thermal cycler (Bio-Rad T100 TM).

Table 3.5 Details of the designed primers for targeted SNPs

Genes	Amplified region	Primer Sequence (3'-5')		Target SNPs
VDR	Intron 1	F	AGGATCGAGATTAGGCGGCGTAGG	rs454303072
		P	TCCACTTAAAAGATCCAACTCCAT	
VDR	Exon 6	F	AGGGCTGGAAGCTGGTCT	rs470608846
		R	TGGGCAAGTCACACCTCTT	
PTPRR	Intron 5	F	AAAACCCATGGAACGTACAGC	rs382671389
		R	GGCCCTGTCCCTACTTTTT	
PTPRR	3' UTR	F	RAAGCCATGAAGCCAAGTCG	rs435289107
		R	TGTTAAAAATCCATCATCAATCAC	
HMGA2	Exon 4	F	GAGGTTCTGTGGGTGTTG	rs434871480
		P	TGTTAGCTTCATATTTCTGTTGTT	
IL 22	Intron 2	F	CTGCTGGTATACCTGTCATCAT	rs383429860
		P	ATAATAACCAATAAGGGGAAATAA	
IL 22	Intron 5	F	TTTCTGGAATGGTTTGATACTTA	rs136785910
		P	GAGCATTCTTGTTTCTGAGGT	
IL 22	Intron 5	F	TCTTCCTCTTTCCTTTTCCTTCTT	rs135884509
		P	GTCTCCCACACTCCACTTTTCTAA	

Table 3.6 Standard reaction mixture used for amplification of SNP loci in PCR thermal cycler

Components	Concentration	Volume (μL)
10X Buffer	10 x	2.5
15mM MgCl ₂	15 mM	1.5
dNTP's	200 μM	0.5
F-primer	30 pm	3.0
R-primer	30 pm	3.0
Taq (5 units/μl)	1 unit	0.2
Template	100 ng	1.0
Distilled Water	-	14.3 (to make 25 μl)

3.6.3 PCR programme for VDR, PTPRR, IL22 and HMGA2 genes in Sahiwal and Karan Fries cattle

Eight sets of primers were used to amplify targeted regions of the genes in Sahiwal and Karan Fries cows. Detailed PCR programme and annealing temperatures are given in Table 3.5.

3.6.4 Checking of PCR Product

3.6.4.1 Gel assembly

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

3.6.4.2 Agarose gel electrophoresis

Horizontal submarine agarose gel electrophoresis was carried out to check the amplified product as described by Sambrook and Russell (2001). A 2% w/v agarose (Low EEO) gel was prepared in 1X TBE. After cooling it to 60°C, ethidium bromide at 1.5 μl per 100 ml of agarose solution was added and was mixed gently. The agarose solution was poured in to the sealed casting tray. The gel was prepared to about 4 mm thickness.

**Table 3.7 Standard PCR programme for amplification of targeted SNPs in
Karan Fries and Sahiwal cattle**

S. No	Steps	Temperature		Time
1	Initial Denaturation	95°C		3 min.
2	Denaturation	94°C		30 sec.
3	Annealing	rs470608846	55.7 °C	30 sec.
		rs382671389	58.5 °C	
		rs435289107	58 °C	
		rs434871480	58 °C	
		rs454303072	59.8 °C	
		rs136785910	59 °C	
		rs135884509	56.8 °C	
		rs383429860	53.8 °C	
4	Extension	72°C		30 sec.
5	Go to step 2, (34 X)			
6	Final Extension	72°C		10 min.
7	Hold	10°C		Forever

The agarose gel was allowed to set completely for about half an hour and then the comb was gently removed. The solidified gel was submerged in the electrophoresis tank containing 1X TBE buffer. Subsequently 5 µl PCR product mixed with 2 µl of 6x gel loading dye (bromophenol blue and xylene cyanol) was loaded along with 100 bp ladder as a marker in a middle well of the gel. The electrophoresis was done at 80V for 45 min.

3.6.4.3 Visualization of gel

At the end of run, power supply was turned off and the gel was carefully removed from the electrophoresis chamber. The amplified products in the gel were observed

under UV transilluminator and documented by gel documentation system. A single sharp band indicated proper amplification of the target DNA.

3.7 PCR-RFLP analysis

The PCR amplified products were subjected to restriction digestion with suitable restriction enzymes (RE) for genotyping. Suitable restriction enzymes were selected for each SNP loci by the help of NEBcutter V2.0 online software using (www.tools.neb.com/NEBcutter2) website. The expected fragments were calculated for each SNP locus along with its genotypes (Table 3.8).

Table 3.8 Expected fragment size after restriction enzyme digestion and its genotypes for targeted SNPs

SNP	RE	Expected fragments of RE digested PCR products and genotypes		
		First H _o	H _t	Second H _o
rs454303072	BsrI	285 ,147 (GG)	106, 147, 179, 285, 147 (AG)	106, 147, 179 (AA)
rs135884509	HinfI	202 (AA)	202, 150, 52 (AG)	150, 52 (GG)
rs382671389	HinpII	171(TT)	171, 120, 51(TC)	120,51(CC)
rs136785910	HpaII	306,231(AA)	306,231,108,198 (AG)	231,198,108(GG)
rs383429860	Sau96I	493 (CC)	493, 433, 60 (CT)	433, 60 (TT)
rs435289107	TaqI	410 (TT)	272, 138, 410 (CT)	272, 138 (CC)
rs470608846	BamHI	412 (AA)	228, 184, 412 (AT)	228, 184 (TT)
rs434871480	HaeIII	350 (TT)	350, 135, 422 (CT)	135, 422 (CC)
		H _o = Homozygous; H _t = Heterozygous;		

3.7.1 Setting up of restriction enzyme digestion

Digestion was performed in the same 0.2 ml PCR tubes used for PCR amplification. Master mix (includes restriction enzyme, digestion buffer and nuclease free water) for digestion of amplified product was prepared on ice. Calculated amount of 10x assay buffer was first taken followed by required amount of the enzyme. The master mix was vortexed for proper mixing and spun for few seconds and then the master mix was distributed in 0.2 ml PCR tube containing 10 μ l PCR product. A negative control consisting of only master mix was also taken to check contamination in the reaction components.

3.7.2 Digestion conditions

The digestion of PCR products were carried out with required time and temperature as provided in kit of each restriction enzyme which is shown in Table 3.9. The digested products were then kept at -20 °C till further study.

Table 3.9 Conditions for restriction enzyme digestion of PCR products for all SNP loci under investigation

SNP	RE	Working Buffer	Digestion		Company
			Temp. (°C)	Time (hr)	
rs454303072	BsrI	1X NEB	65	16	M/s. BioLabs
rs135884509	HinfI	1X NEB	37	16	
rs382671389	HinpII	1X NEB	37	16	
rs136785910	HpaII	1X NEB	37	16	
rs383429860	Sau96I	1X NEB	37	16	
rs435289107	TaqI	1X NEB	65	16	
rs470608846	BamHI	1X NEB	37	16	
rs434871480	HaeIII	1X NEB	37	16	

3.7.3 Agarose gel electrophoresis of RE digested PCR products

The digested PCR products were subjected to electrophoresis in 3 to 3.5% w/v agarose gel (low EEO) for 1hour at 120Volts. The RE digested PCR product mixed with 6X gel loading dye was loaded into each well along with 100 bp or 50 bp DNA ladder in a separate lane for different digested PCR products. The banding patterns of

the gels were visualized using UV light and documented under gel documentation system. Each individual revealed a particular pattern or RFLP genotype depending on presence and absence of RE sites.

3.7.4 DNA sequencing for variant confirmation

The amplified PCR products from all sets of primers were sent to 1st Base sequencing INT (Singapore) for purification and custom sequencing from both ends (5' and 3' ends). The sequence were analysed using Bioedit software for confirmation of the variants

3.8 Genotype and allelic frequencies

Genotype and allelic frequencies were calculated using following formulas:

$$\text{Genotype frequency (\%)} = \frac{\text{Number of animals of a particular genotype}}{\text{Total number of animals}}$$

Allele frequency was calculated as follows:

$$\text{Allele frequency} = 1/N (D + 1/2 H)$$

where,

D	=	No. of homozygote
H	=	No. of heterozygote
N	=	Total no. of individuals

3.9 Statistical analysis

The statistical analysis was carried out using SAS Version 9.3, IBM SPSS and LSML (Harvey, 1990) softwares under the following headings:

3.9.1 Descriptive Analysis

The descriptive analysis for mean and standard error of all traits was carried out by using standard statistical procedures (Snedecor and Cochran, 1994).

3.9.2 Least squares analysis

Least squares analysis of variance for unequal and non-orthogonal data using the technique described by Harvey (1990) was used to study effect of non-genetic factors. The model was used with assumptions that different components being fitted into the

model are linear, independent and additive. The model for analysis of effects of non-genetic factors on udder and teat type traits and milk production traits of Sahiwal and Karan Fries cows is described as under:

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

where,

Y_{ijkl} = l^{th} Observation of cow in i^{th} stage of lactation, j^{th} parity and k^{th} season of calving

μ = Overall mean

L_i = Fixed effect of i^{th} stage of lactation ($i = 1$ to 4)

P_j = Fixed effect of j^{th} parity ($j = 1$ to 4)

S_k = Effect of k^{th} season of calving ($k = 1$ to 4)

e_{ijkl} = Random error NID ($0, \sigma_e^2$)

3.9.3 ANOVA of non-genetic factors for udder type traits and milk production traits in Sahiwal and Karan Fries cattle

The analysis of variance for stage of lactation, parity and season of calving affecting both udder and teat type traits and milk production traits under model was computed by the following method (Table 3.10).

Table 3.10 Standard ANOVA table for udder and teat type traits and milk production traits in Karan Fries and Sahiwal cattle

Source of variation	Df	S.S.	M.S.
Stage of lactation (l)	$l-1$	SS_l	MS_l
Parity (p)	$p-1$	SS_p	MS_p
Season of calving (s)	$s-1$	SS_s	MS_s
Error	By subtraction	SS_e	MS_e

where,

l , p and s are the total number of stages of lactation, parity groups and seasons on which the data for the respective character had been spread.

3.9.4 Duncan's Multiple Range Test (DMRT)

The difference of means between subclasses was tested for significance using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957) as given below.

$$PD = (Y_i - Y_j) \sqrt{2 / (C_{ii} + C_{jj} - C_{ij})} > \sigma_e Z_{pn2}$$

where,

$(Y_i - Y_j)$ = Mean difference between two subclasses

C_{ii} = Corresponding diagonal element of i^{th} subclass

C_{jj} = Corresponding diagonal element of j^{th} subclass

C_{ij} = Corresponding off-diagonal element of ij^{th} subclass

Z_{pne} = Significant ($p < 0.05$ and $p < 0.01$) Studentized Range

3.9.5 Effect of non genetic factors on incidence of clinical mastitis

The effect of non genetic factors on incidence of clinical mastitis was analysed using the maximum likelihood method of LOGISTIC procedure of SAS Version 9.3. The dichotomous logistic model is given below.

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

Marginal one unit increase in X_j brings about an increase in $\text{Log} [p_i / 1 - p_i]$ by β_1

P = Probability of mastitis resistance animals

β_0 = Intercept

$\beta_j X_j$ = Partial regression coefficients

3.10 Association analysis

Statistical analysis was performed on the adjusted data using the Least-squares analysis to find out any significance difference between genotypes. The significant effect of genotypes on udder and teat type traits and milk production traits were analyzed using the following general linear models (GLM) using IBM SPSS.

3.10.1 Association of genotype with udder and teat type traits and milk production

The association of genotypes with the corresponding udder and teat type scores/ traits of the animals was assessed using the following model:

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

where,

Y_{ij} = Adjusted value of udder and teat type traits/milk production of j^{th} animal of i^{th} genotype

μ = Overall mean

G_i = Effect of i^{th} Genotypes

e_{ij} = Residual error NID (0, σ_e^2)

3.10.2 Association of genotypes with incidence of clinical mastitis

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

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

3.10.3 Association of udder and teat type traits/scores with incidence of clinical mastitis

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

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

3.11 Estimation of phenotypic correlations (r_p) between milk production and udder and teat type scores/traits

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

CHAPTER –4

Results and Discussion

4. Results and Discussion

In the present investigation, an attempt has been made to investigate the association of genetic polymorphism in VDR, PTPRR, HMGA2 and IL-22 genes with udder type traits, incidence of clinical mastitis and milk production in Karan Fries and Sahiwal cattle. Hence, blood samples and data on udder type traits, milk production traits and incidence of clinical mastitis were collected from lactating Karan Fries (N = 166) and Sahiwal (N = 87) cows. Animals were selected randomly to minimize any type of biasness in the results.

Total 8 udder measurement traits, 6 teat measurement traits and 11 visual observational udder and teat type traits were generated for each animal. The single nucleotide polymorphisms (SNPs) in targeted genes were selected based on previous reports of genetic association between SNP loci and different udder type traits and incidence of clinical mastitis (Tolleson, 2016), these loci were subsequently tested on Karan Fries and Sahiwal cattle population. The PCR products were custom sequenced using automated sequencing. The raw sequences obtained were assembled and specific restriction enzymes targeting the desired SNP mutation were selected using NEB cutter v2.0 (<http://www.labtools.us/nebcutter-v2-0/>). After standardization of PCR and restriction digestion protocol, total 4 genes with 8 different SNP loci were targeted for identification of polymorphism and association study. These loci constituted two SNP loci from each VDR and PTPRR gene, one from HMGA2 gene and three from IL-22 gene. The results of the present study are described under the following headings:

4.1 Isolation of genomic DNA from blood

The genomic DNA was isolated from 10 ml frozen (-20°C) blood by using Phenol: Chloroform: Isoamyl alcohol extraction method as described by Sambrook and Russell (2001). The quality of genomic DNA samples was assessed with 0.8% submarine horizontal agarose gel electrophoresis. Most of the genomic DNA samples showed clear and distinct bands under UV transilluminator indicating good quality of DNA. However, a few samples showed certain degree of smearing and they were reprocessed following the same protocol to get good quality DNA. The representative genomic DNA of Karan Fries and Sahiwal have been shown in Plate 4.1 and 4.2

Purity of DNA samples were judged by UV spectrophotometer. The concentration of DNA samples were calculated using spectrophotometer (Nanodrop; ND-1000). The ratio of optical density at the two wavelengths (OD_{260}/OD_{280}) indicates the purity of DNA sample.

The OD₂₆₀/OD₂₈₀ ratio of samples ranged from 1.70-1.92 with a mean of 1.79 ± 0.02 indicating high purity of the extracted genomic DNA. The overall yield from blood samples ranged from 110-4114 µg (Average = 1240.7 ± 162) and 112-3756 µg (Average = 1248 ± 122) for Karan Fries (KF) and Sahiwal, respectively. Most of the DNA samples were found to lie within this range. Few samples with OD ratio varying from this range were reprocessed. The DNA samples were diluted in nuclease free water in such a way that 1 µl solution contains approximately 50-60 ng of dsDNA. The diluted DNA samples were used for PCR amplification. The results of the mean yield and purity of DNA samples are presented in the Table 4.1.

Table 4.1 Mean yield and purity of DNA isolated in Karan Fries and Sahiwal cattle

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

4.2. Standardization of Polymerase Chain Reaction (PCR)

4.2.1. Template DNA concentration

DNA template concentrations varied between 100 to 300 ng per 25 µl reaction volume with increments of 25 ng. There was no apparent difference in the yield and specificity of the DNA samples observable by visual appraisal in agarose gel in case of different samples. Hence, a concentration of 100 to 300 ng per 25 µl reaction volume was used.

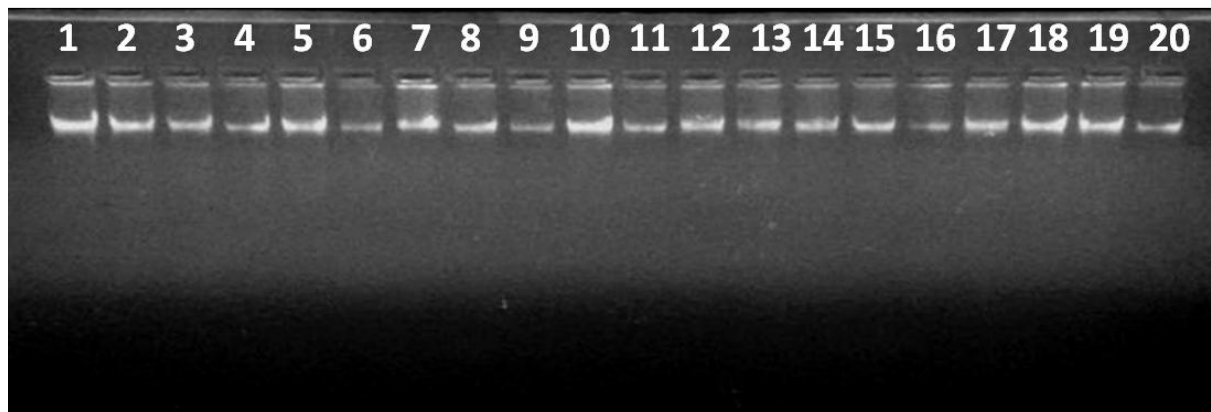
4.2.2. Primer concentration for PCR

The concentration of forward and reverse primer was optimized by setting up PCR trails. The optimum level of primer used was 10 pmol. 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).

4.2.3. Annealing temperature of primers

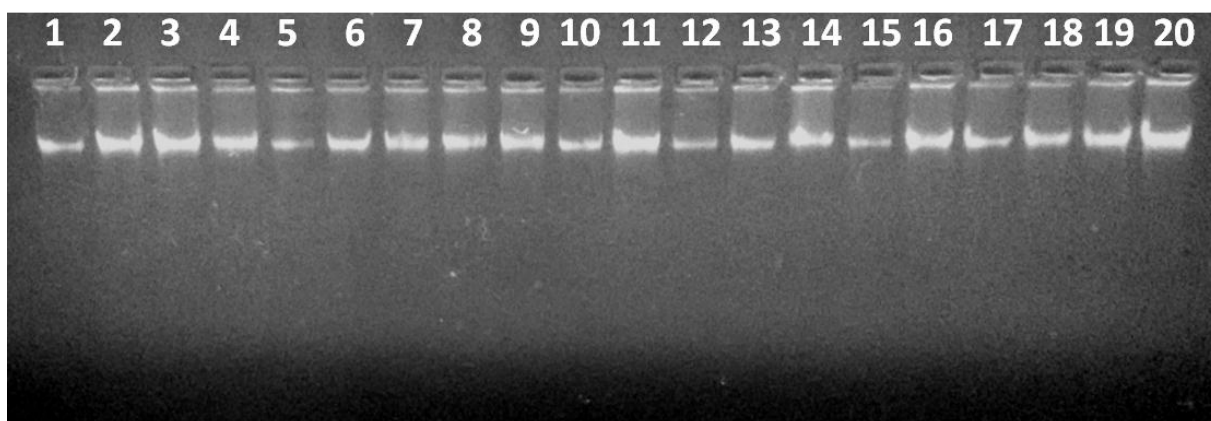
One of the most critical factors is the annealing temperature (Ta) which affects both specificity and yield of the PCR product. Two primer sets of bovine VDR and PTPRR gene,

Plate 4.1 Quality checking of genomic DNA isolated from blood of Karan Fries cattle



Lane 1-20: Genomic DNA samples of Karan Fries cattle

Plate 4.2 Quality checking of genomic DNA isolated from blood of Sahiwal cattle



Lane 1-20: Genomic DNA samples of Sahiwal cattle

one primer HMGA2 gene and three primers of IL22 gene were optimized using gradient PCR. At lower annealing temperatures, non-specific products were formed while at higher than optimal temperature, yield of the products were reduced. The annealing temperature of different primer sets and amplicon size have been presented in the Table 4.2.

Table 4.2 Primer sequence and annealing temperature of targeted SNP loci

Genes	Amplified region	Size (bp)	Primer Sequence (3'-5')		Ta (°C)	Target SNP
VDR	Intron 1	432	F	AGGATCGAGATTAGGCGGCGTAGG	59.8	rs454303072
			P	TCCACTTAAAAGATCCAACTCCAT		
VDR	Exon 6	412	F	AGGGCTGGAAGCTGGTCT	55.7	rs470608846
			R	TGGGCAAGTCACACCTCTT		
PTPRR	Intron 5	412	F	AAAACCCATGGAACGTACAGC	58.5	rs382671389
			R	GGCCCTGTCCCTACTTTTT		
PTPRR	3' UTR	410	F	RAAGCCATGAAGCCAAGTCG	58	rs435289107
			R	TGTTAAAAATCCATCATCAATCAC		
HMGA2	Exon 4	557	F	GAGGTTCTGTGGGTGTTG	58	rs434871480
			P	TGTTAGCTTCATATTTCTGTTGTT		
IL 22	Intron 2	581	F	CTGCTGGTATACCTGTCATCAT	53.8	rs383429860
			P	ATAATAACCAATAAGGGGAAATAA		
IL 22	Intron 5	441	F	TTTCTGGAATGGTTTGATACTTA	59	rs136785910
			P	GAGCATTCTTGTTTCTGAGGT		
IL 22	Intron 5	563	F	TCTTCCTCTTTCTTTTCCTTCTT	56.8	rs135884509
			P	GTCTCCCACACTCCACTTTTCTAA		

4.3. Size and quality of PCR product

Targeted genomic regions of all the primer sets were successfully amplified in all the animals of the present study. The size and quality of PCR products of all eight targeted regions were checked on 0.8% agarose gel electrophoresis (Plate 4.3 to 4.18).

4.4. DNA sequencing and SNP identification

PCR products of eight primer sets amplified using random DNA samples of both cattle breeds were custom sequenced from M/s. 1st Base sequencing INT (Malaysia). The raw sequences were first assembled using BioEdit and MEGA 6.0 (Molecular Evolutionary Genetics Analysis) software. The final sequences of each locus were aligned using Clustal W in MEGA7.0.26 and the targeted SNPs were identified using DNA Baser software. The targeted SNPs were then subjected to *in silico* restriction digestion using NEB cutter to identify the specific restriction enzyme.

4.5. Restriction enzyme digestion of PCR products

Once the specific restriction enzyme for the each targeted SNP was identified, the appropriate PCR- RFLP protocol was designed and all PCR amplified products of 8 different SNP loci were digested with specific RE with optimum conditions related to time and temperature as described in Materials and Methods in Table 3.9. One I.U. restriction enzyme was used for each reaction along with 1x buffer as working solution. After completion of RE digestion, 3-3.5% w/v agarose gel was used to visualize the digested fragments using UV light and documented under gel documentation system.

4.6. Genotyping of the animals

Genotyping of all animals was done according to the band pattern obtained after restriction digestion of the PCR products. Animals were identified as homozygote or heterozygote based on the band pattern obtained on agarose gel. PCR-RFLP is a biallelic and co-dominant marker system in which maximum three genotypes from two alleles can be identified at a time. The allelic and genotypic frequencies of all the loci in both Karan Fries and Sahiwal cattle population have been given in upcoming sections and Table 4.3 and 4.4.

4.6.1. Genotyping of Bovine VDR gene

The VDR gene was genotyped at two loci, the SNP rs454303072 was located at intron 1 and the SNP rs470608846 was located at exon 6. These SNPs have been reported to be associated with udder type traits in *Bos indicus* X *Bos taurus* cattle (Tolleson, 2016). The SNP rs454303072 was digested by BsrI restriction enzyme. The restriction digestion produced two genotypes in both KF and Sahiwal cattle, viz., AA (106 bp, 147 bp and 179 bp) and GG (285 bp and 147 bp) (Plate 4.3 - 4.4 and Figure 4.1). The A allele was found to be the

Figure 4.1: Chromatogram showing the nucleotide change in SNP rs454303072 of Karan Fries and Sahiwal cattle

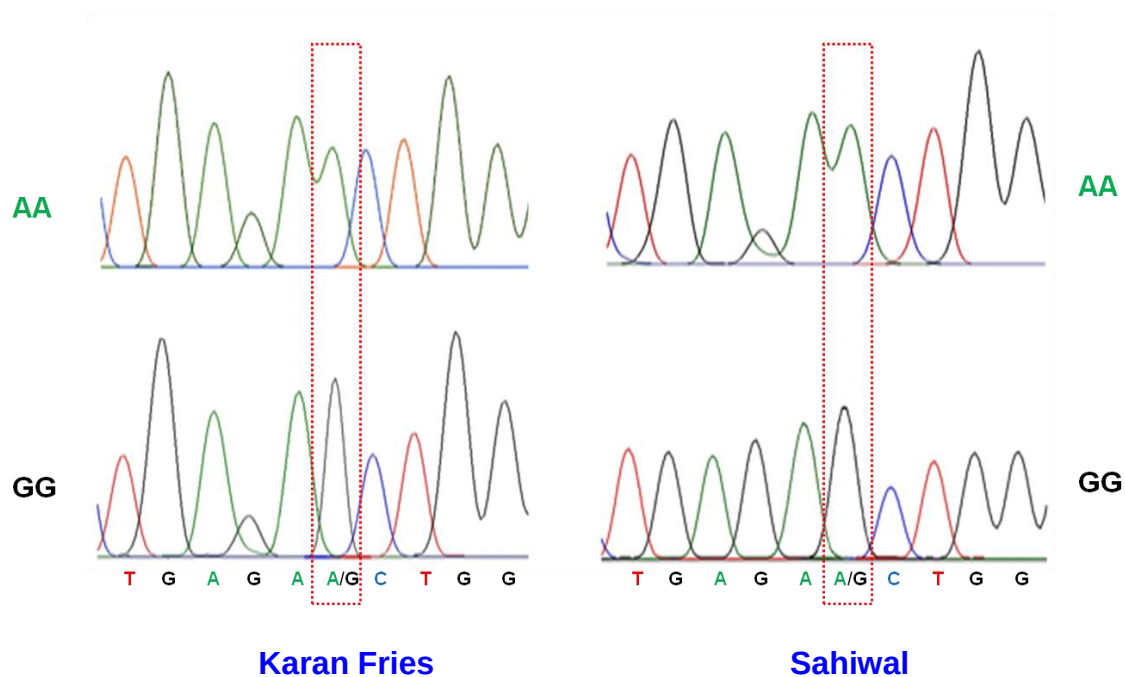


Figure 4.2: Chromatogram showing the nucleotide change in SNP rs470608846 of Karan Fries and Sahiwal cattle

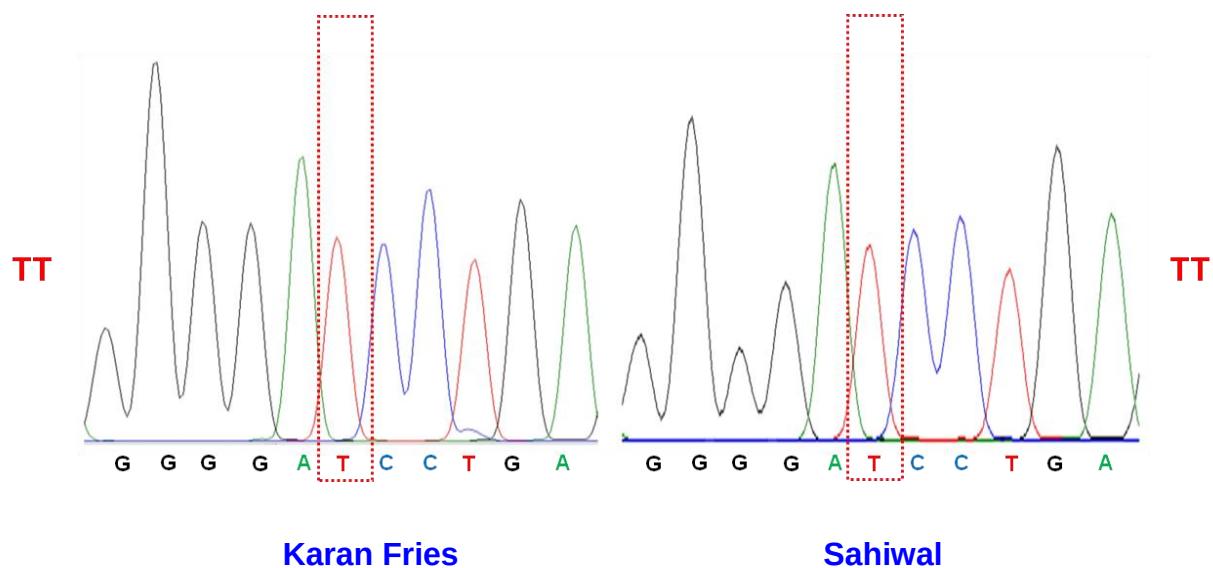


Figure 4.3: Chromatogram showing the nucleotide change in SNP rs382671389 of Karan Fries and Sahiwal cattle

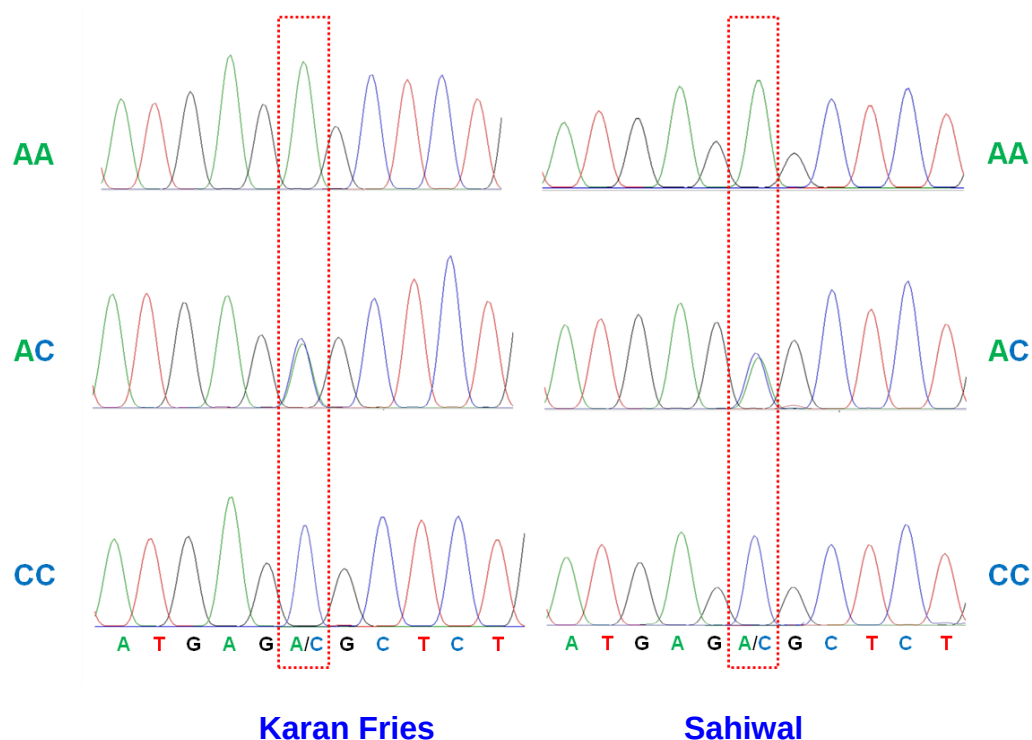


Figure 4.4: Chromatogram showing the nucleotide change in SNP rs435289107 of Karan Fries and Sahiwal cattle

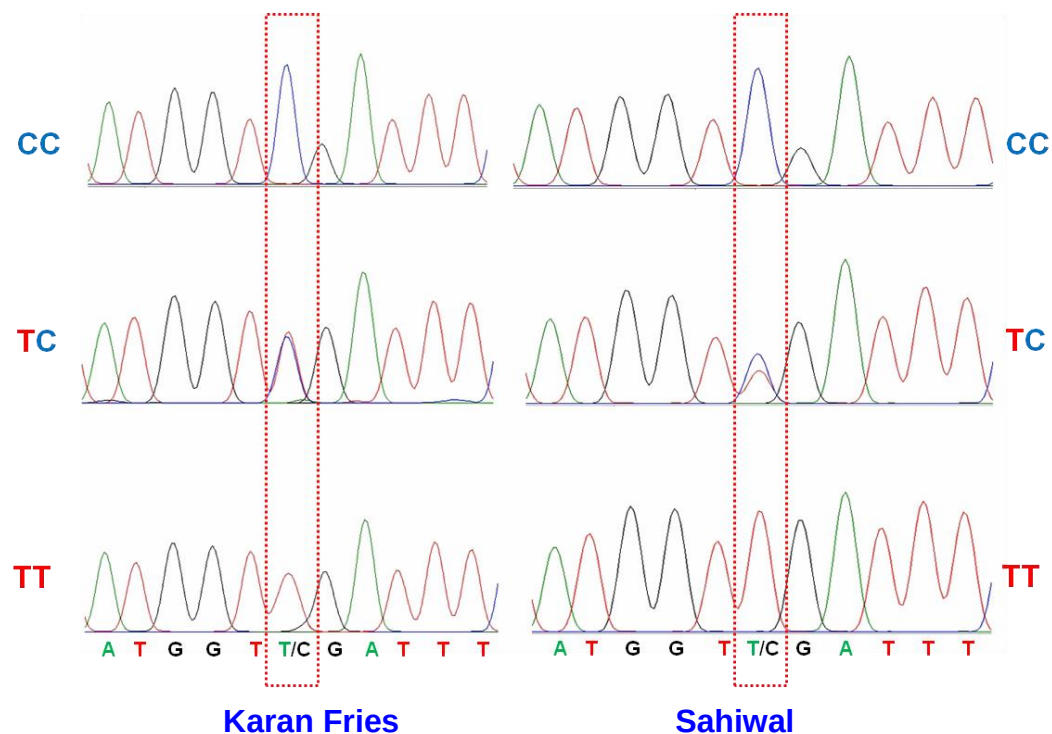
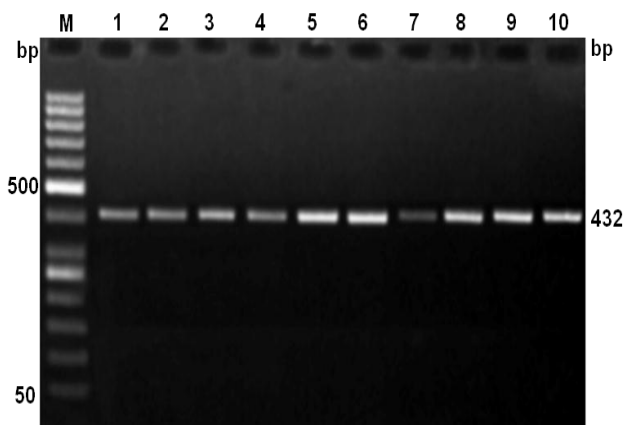


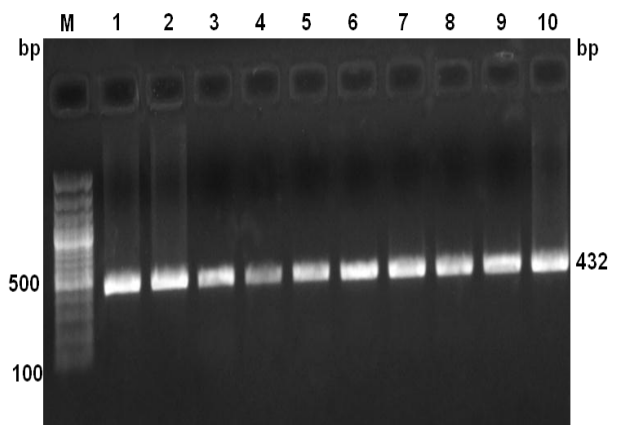
Plate 4.3: Resolution of PCR product of intron 1 of VDR gene (target SNP rs454303072) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 432 bp

Lane M: 100 bp ladder

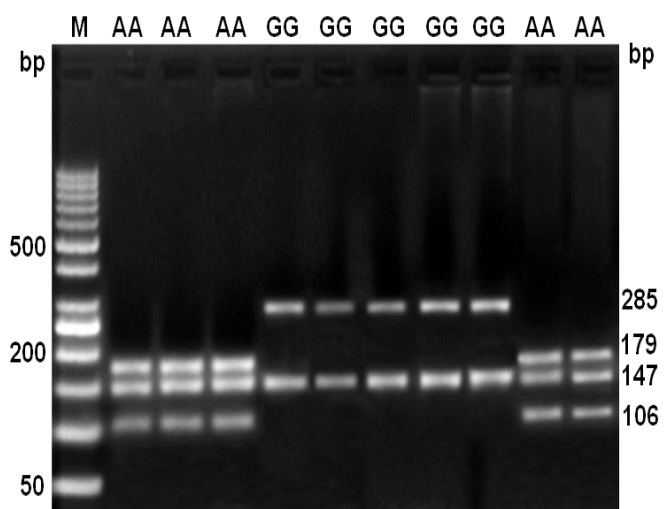


Sahiwal

Lane 1-10: PCR Products, 432 bp

Lane M: 100 bp ladder

Plate 4.4: Resolution of PCR-RFLP (*Bsr* I) for rs454303072 in Karan Fries and Sahiwal cattle

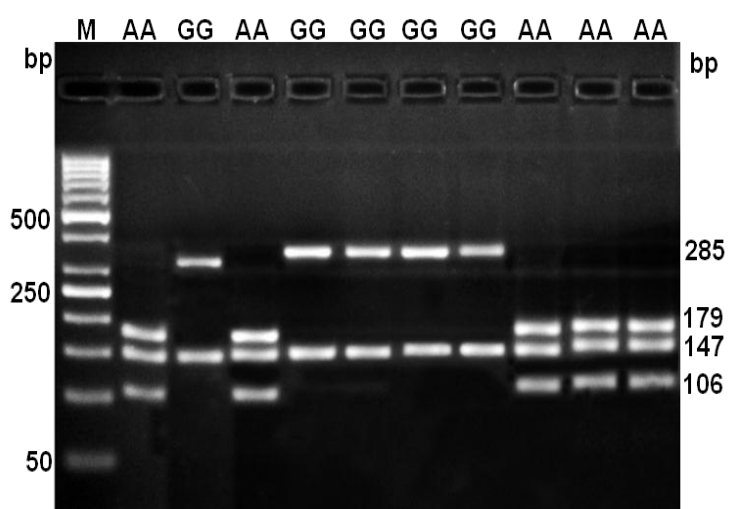


Karan Fries

Lane 1-3, 9-10 : AA Genotype (179, 147 & 106 bp)

Lane 4- 8 : GG Genotype (285 & 147 bp)

Lane M : 50 bp ladder



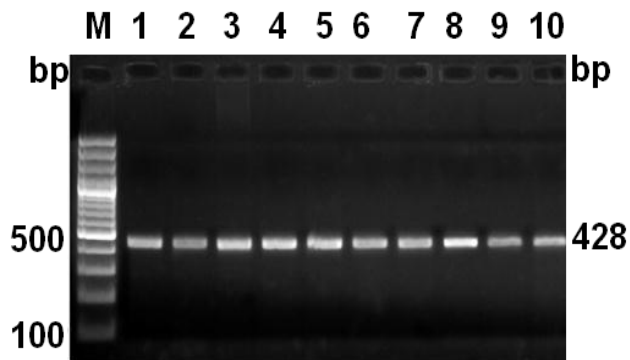
Sahiwal

Lane 1,3, 8-10 : AA Genotype (179, 147 & 106 bp)

Lane 2, 4- 7 : GG Genotype (285 & 147 bp)

Lane M : 50 bp ladder

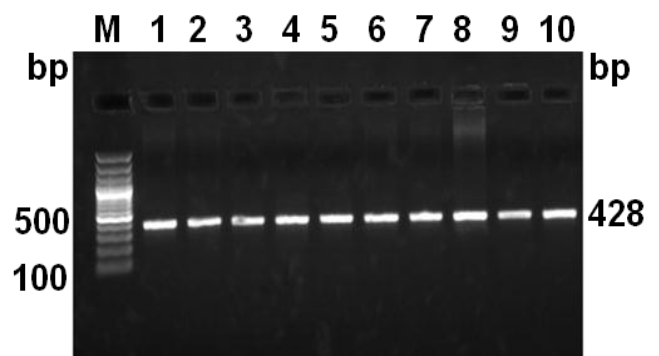
Plate 4.5: Resolution of PCR product of exon 6 of VDR gene (target SNP rs470608846) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 428 bp

Lane M: 100 bp ladder

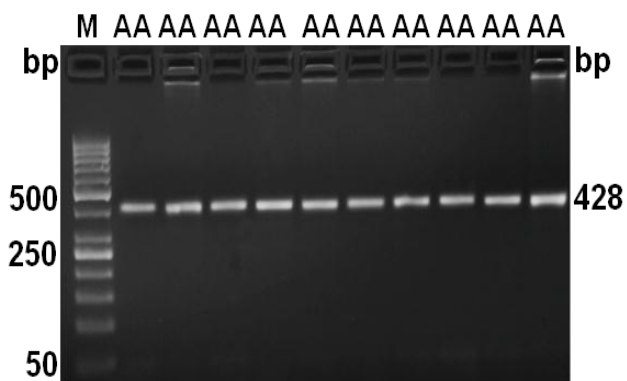


Sahiwal

Lane 1-10: PCR Products, 428 bp

Lane M: 100 bp ladder

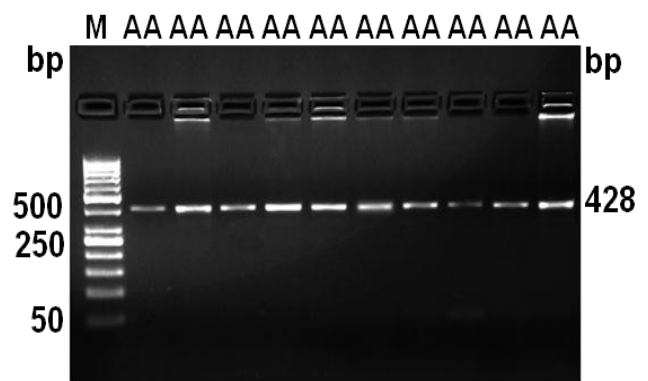
Plate 4.6: Resolution of PCR-RFLP (*Bam*HI) for rs470608846 in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1 - 10 : AA Genotype (428 bp)

Lane M : 50 bp ladder



Sahiwal

Lane 1 - 10 : AA Genotype (428 bp)

Lane M : 50 bp ladder

major allele in both breeds. The second SNP, rs470608846 was digested by BamHI restriction enzyme. Although the restriction site was detected at the targeted SNP, but, the site was found to be monomorphic in both KF and Sahiwal cattle (Plate 4.5- 4.6 and Figure 4.2).

4.6.2. Genotyping of Bovine PTPRR gene

The PTPRR gene was genotyped at two loci, the SNP rs382671389 which was situated in intron 5 and the SNP rs435289107 which was situated in 3' UTR. These SNPs have been reported to be associated with udder type traits in *Bos indicus* X *Bos taurus* cattle (Tolleson, 2016). The SNP rs382671389 was digested by restriction enzyme Hinp1I and produced AA (350 bp), CC (184 bp and 174 bp) and AC (350 bp, 184 bp and 174 bp) genotypes in both Karan Fries and Sahiwal cows (Plate no.4.7-4.8; Figure 4.3). A allele was found to be the major allele in both breeds. The homozygote AA genotype had the highest frequency in both breeds. Restriction digestion of SNP rs435289107, by restriction enzyme TaqI produced CC (272 bp and 138 bp), TC (410 bp, 272 bp and 138 bp) and TT (410 bp) genotypes in both KF (Karan Fries) and Sahiwal cow (Plate no. 4.9 - 4.10; Figure 4.4). CC genotype had the highest frequency in both breeds. The C allele was found to be the major allele in both KF and Sahiwal breed.

4.6.3. Genotyping of Bovine HMGA2 gene

The bovine HMGA2 gene was genotyped at one locus following the report from Tolleson (2016). The SNP rs434871480 is located in exon 4 and was reported to be associated with udder support in *Bos indicus* X *Bos Taurus* cattle (Tolleson, 2016). The SNP was digested by restriction enzyme HaeIII to target the specific SNP. Although, the recognition sequence was found in the sequence, the site was found to be monomorphic in both KF and Sahiwal cattle (Plate no. 4.11 - 4.12 and Figure 4.5).

4.6.4. Genotyping of Bovine IL-22 (Interleukin-22) gene

Bovine IL-22 gene has been proposed as a candidate gene affecting the udder type traits and udder health, in particular mastitis (Tolleson, 2016). As reported by Tolleson (2016), the gene was genotyped at three loci (rs135884509, rs136785910 and rs383429860). The SNP rs135884509 was situated in intron 5 region and it was digested by restriction enzyme HinfI. The digestion of the SNP produced three genotypes in KF (Karan Fries) cattle viz., AA (563 bp), AC (563 bp, 395 bp and 168 bp) and CC (395 bp and 168 bp), whereas, in Sahiwal cattle only two genotypes, AA and AC were detected (Plate no. 4.13 - 4.14 and Figure 4.6). AA genotype had the highest frequency in KF breed while the heterozygote AC was the genotype with highest frequency in Sahiwal cattle. The A allele was found to be the

major allele in both breeds. The SNP rs136785910 was situated in intron 5 region and was digested by HpaII restriction enzyme. The digestion produced two genotypes in both KF and Sahiwal viz., TT (441 bp) and TC (441 bp, 271 bp and 170 bp) (Plate no.4.15 - 4.16 and Figure 4.7) with TT as the most frequent genotype. The T allele was found to be the major allele in both breeds studied. The SNP rs383429860 was situated in intron 2 region and it was digested by Sau96I restriction enzyme. The digestion produced three genotypes, AA (581 bp) and AG (581 bp, 461 bp and 120 bp), GG (120 bp and 461 bp) in both KF and Sahiwal cattle (Plate no 4.17- 4.18 and Figure 4.8) with AA genotype having the highest frequency. The A allele was the major allele in both KF and Sahiwal cattle.

4.7. Population Genetic Analysis

Population genetic analysis was carried out using Popgen v1.32 software (Yeh *et al.*, 1999). Gene and genotypic frequencies for each locus were calculated using traditional formula given by Falconer and Mackay (1996). The analysis was performed in two steps, first the classical population genetic parameters were calculated that included the gene and genotypic frequencies and test for Hardy Weinberg Equilibrium, second step was estimation of PIC and other parameters used for estimation of informativeness of markers.

4.7.1. Population genetic parameters and Hardy Weinberg Equilibrium (HWE)

The genotypic frequencies were calculated using formula given in section 3.8 of materials and methods. Six nucleotide changes were found to be polymorphic both in Sahiwal and Karan Fries cows. Karan Fries cows had three genotypes (homozygous and heterozygous) at rs383429860, rs382671389, rs135884509 and rs435289107 loci and only two genotypes at remaining 2 loci i.e., rs136785910 and rs454303072. Sahiwal cows exhibited all the three genotypes (homozygous and heterozygous) at rs383429860, rs382671389 and rs435289107 loci, whereas, in the loci rs454303072, rs135884509 and rs136785910 only two genotypes were observed. The allelic frequencies were calculated using Popgen v1.32 (Yeh *et al.*, 1999). In Sahiwal cattle, allele C of loci rs136785910 was found in minimum frequency (0.081), whereas, allele T of the same loci was found in maximum frequency (0.920). In Karan Fries cows, allele C of loci rs136785910 was found in minimum frequency (0.187), whereas, allele T of the same loci was found in maximum frequency (0.813). Out of the six polymorphic loci, the allelic frequencies of mutated alleles at 4 loci i.e. allele 'A' at locus rs454303072, rs135884509 and rs382671389 and allele 'T' at locus rs136785910 were found to be higher in Sahiwal cows as compared to Karan Fries cows. The allelic frequency of mutated allele 'C' at polymorphic locus rs435289107 and allele 'A' at locus rs383429860 in Karan Fries cows was found to be higher (Table 4.3 - 4.4).

Figure 4.5: Chromatogram showing the nucleotide change in SNP rs434871480 of Karan Fries and Sahiwal cattle

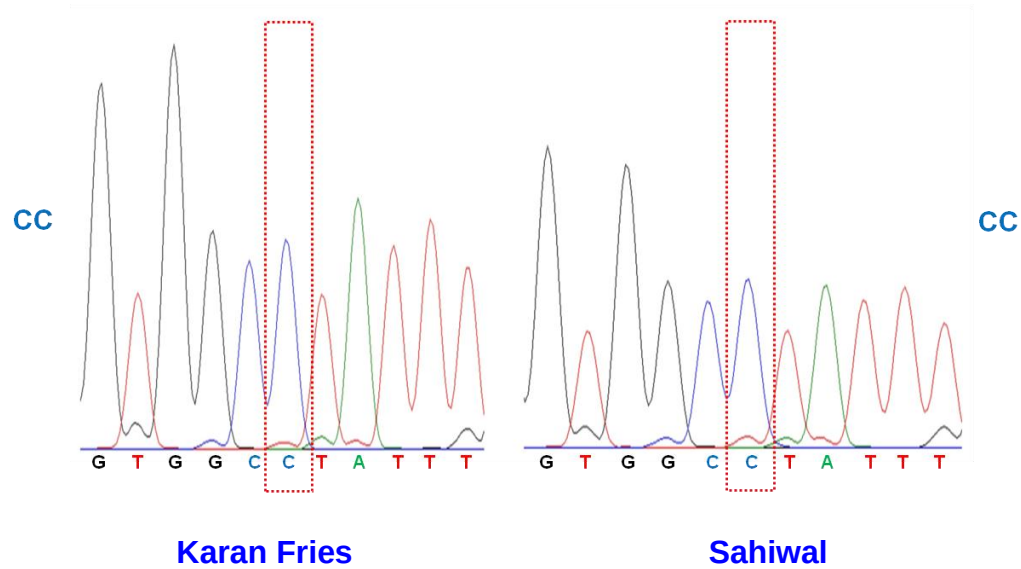


Figure 4.6: Chromatogram showing the nucleotide change in SNP rs135884509 of Karan Fries and Sahiwal cattle

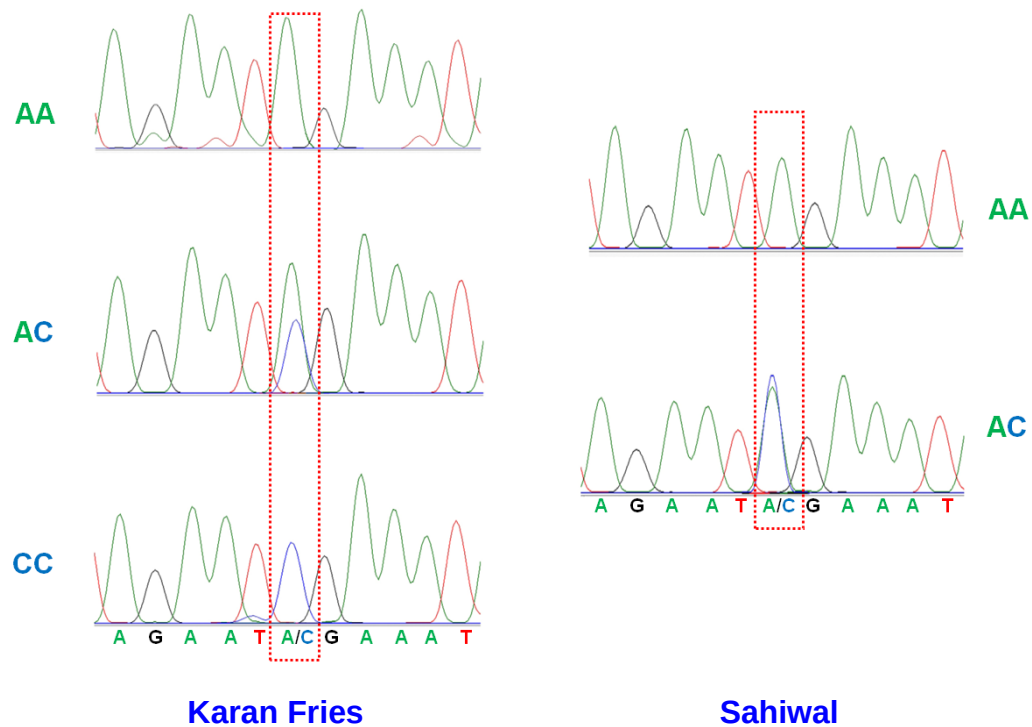


Figure 4.7: Chromatogram showing the nucleotide change in SNP rs136785910 of Karan Fries and Sahiwal cattle

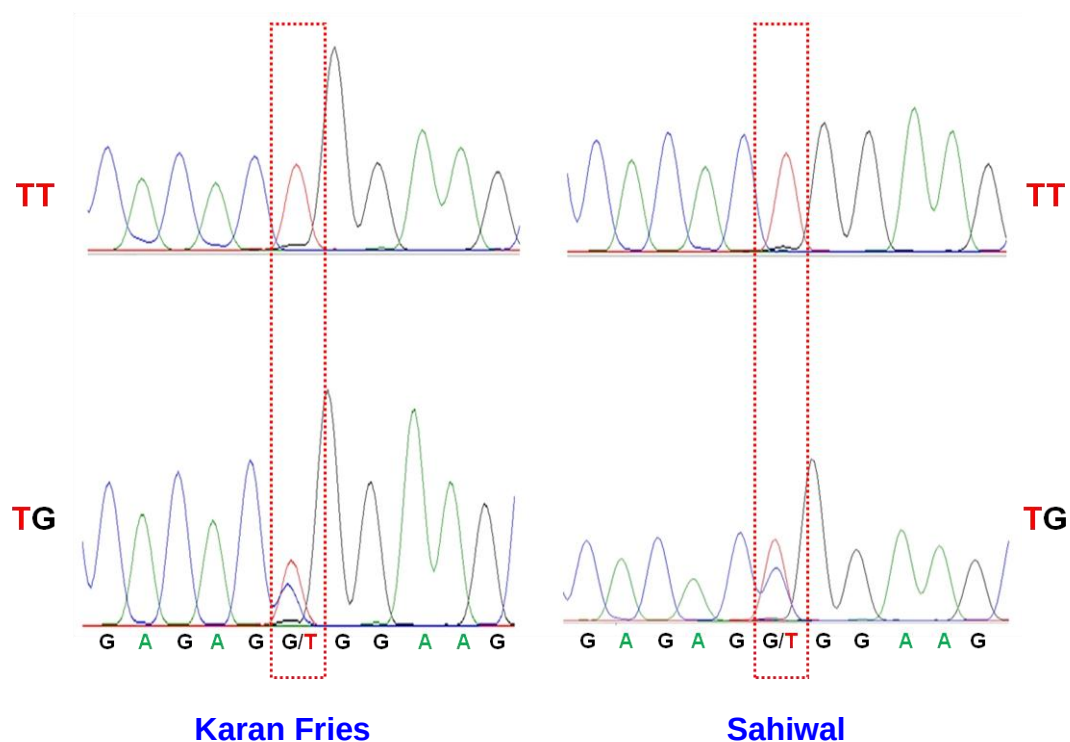


Figure 4.8: Chromatogram showing the nucleotide change in SNP rs383429860 of Karan Fries and Sahiwal cattle

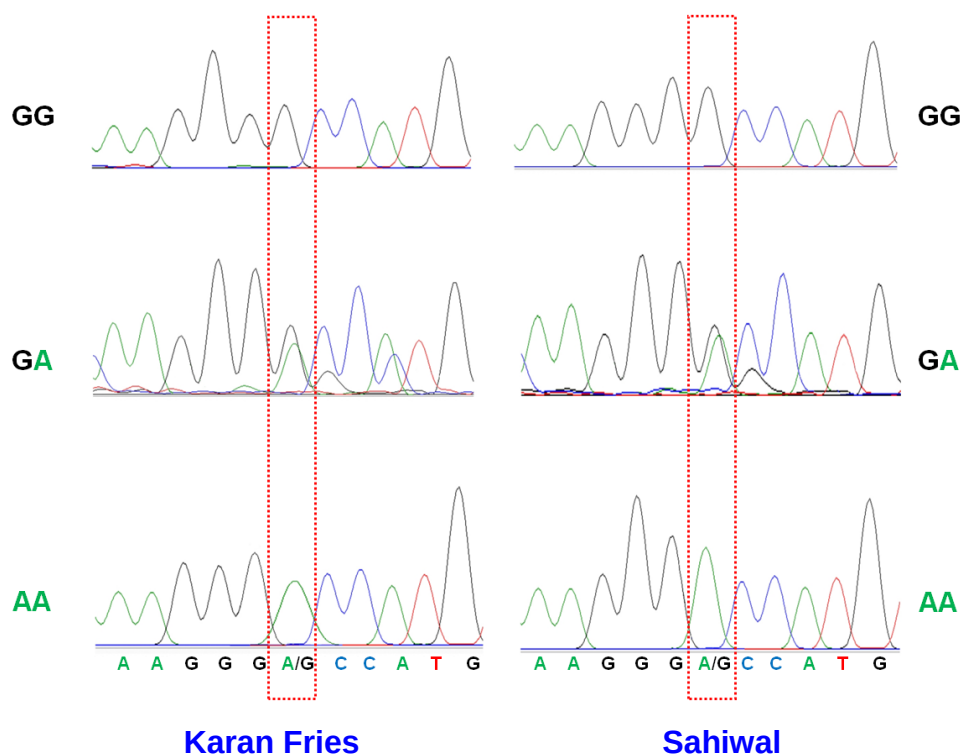
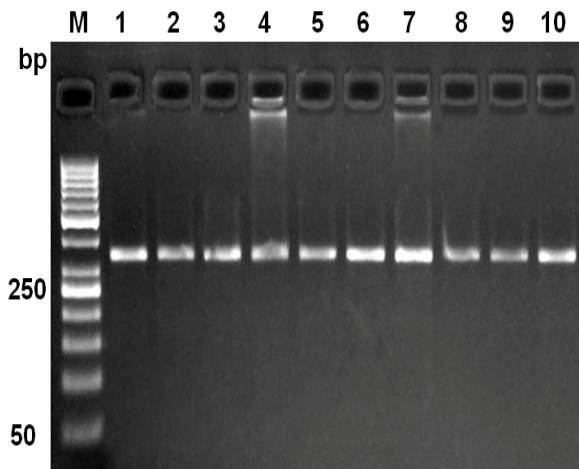


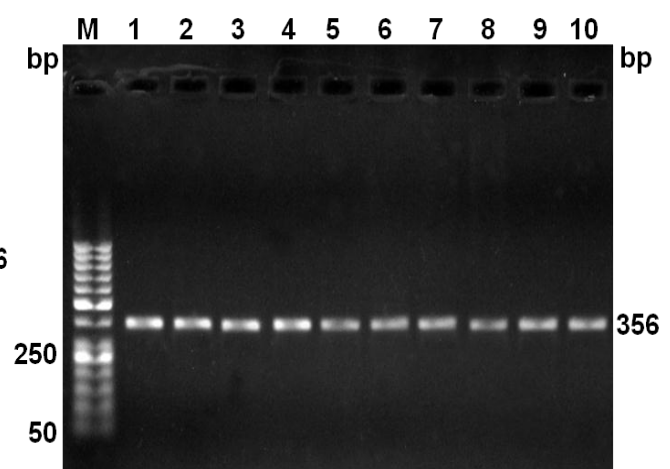
Plate 4.7: Resolution of PCR product of intron 5 of PTPRR gene (target SNP rs382671389) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 356 bp

Lane M: 100 bp ladder

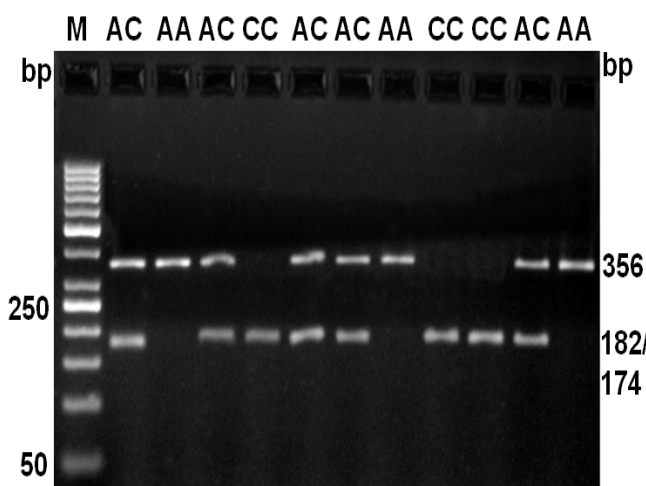


Sahiwal

Lane 1-10: PCR Products, 356 bp

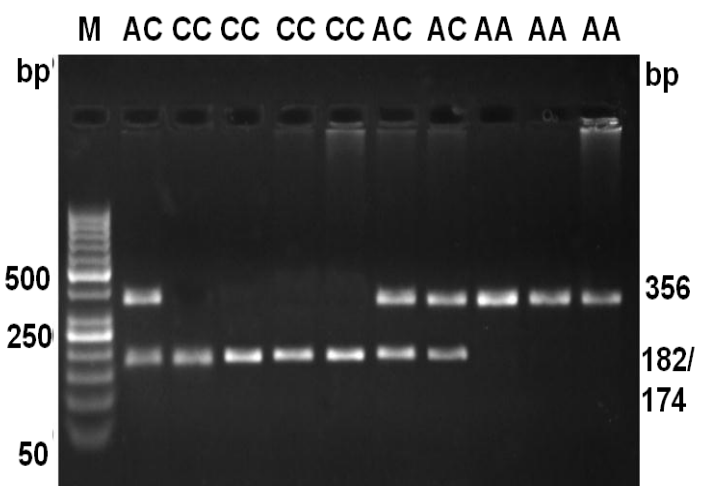
Lane M: 100 bp ladder

Plate 4.8: Resolution of PCR-RFLP (*Hinf*1I) for rs382671389 in Karan Fries and Sahiwal cattle



Karan Fries

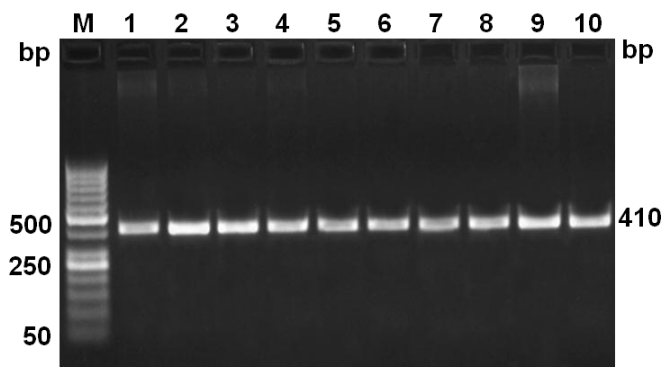
Lane 1, 3, 5, 6, 9 : AC Genotype (356, 182 & 174 bp)
 Lane 2, 7, 11 : AA Genotype (356 bp)
 Lane 4, 8, 9 : CC Genotype (182 & 174 bp)
 Lane M : 50 bp ladder



Sahiwal

Lane 1, 6-7 : AC Genotype (356, 182 & 174 bp)
 Lane 2, 7, 11 : AA Genotype (356 bp)
 Lane 8-10 : CC Genotype (182 & 174 bp)
 Lane M : 50 bp ladder

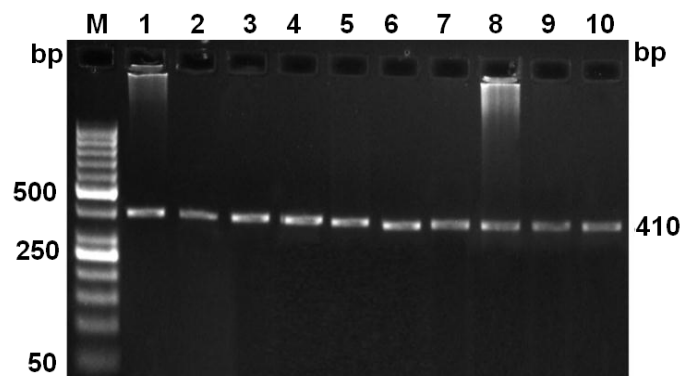
Plate 4.9: Resolution of PCR product of 3' UTR of PTPRR gene (target SNP rs435289107) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 410 bp

Lane M: 50 bp ladder

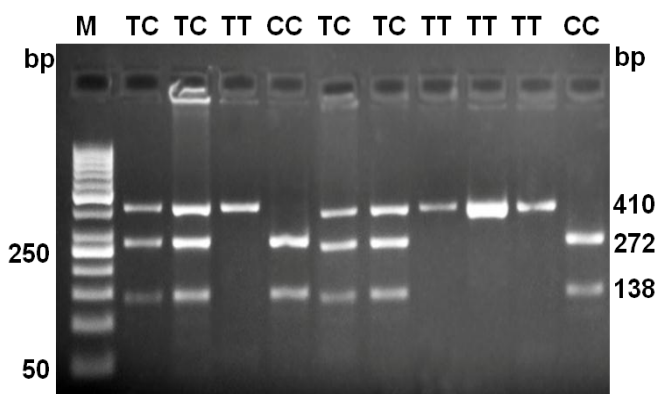


Sahiwal

Lane 1-10: PCR Products, 410 bp

Lane M: 50 bp ladder

Plate 4.10: Resolution of PCR-RFLP (*TaqI*) for rs435289107 in Karan Fries and Sahiwal cattle



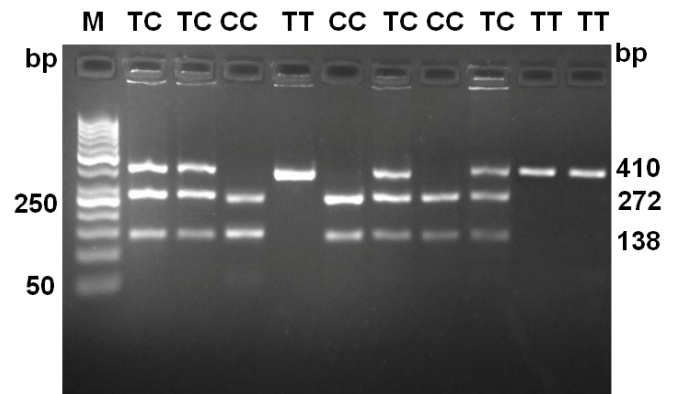
Karan Fries

Lane 1, 2, 5, 6 : TC Genotype (410, 272 & 138 bp)

Lane 3, 7-9 : TT Genotype (410 bp)

Lane 4, 10 : CC Genotype (272 & 138 bp)

Lane M : 50 bp ladder



Sahiwal

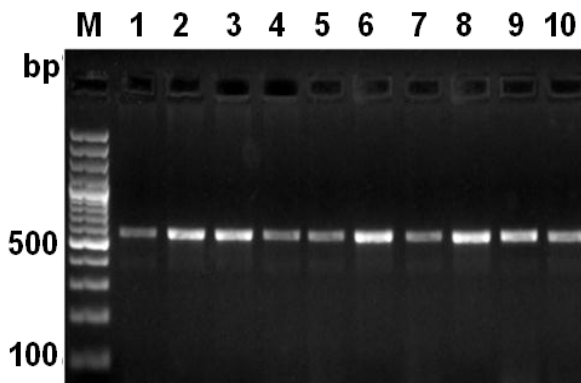
Lane 1-2, 6, 8 : TC Genotype (410, 272 & 138 bp)

Lane 4, 9-10 : TT Genotype (410 bp)

Lane 3,5,7 : CC Genotype (272 & 138 bp)

Lane M : 50 bp ladder

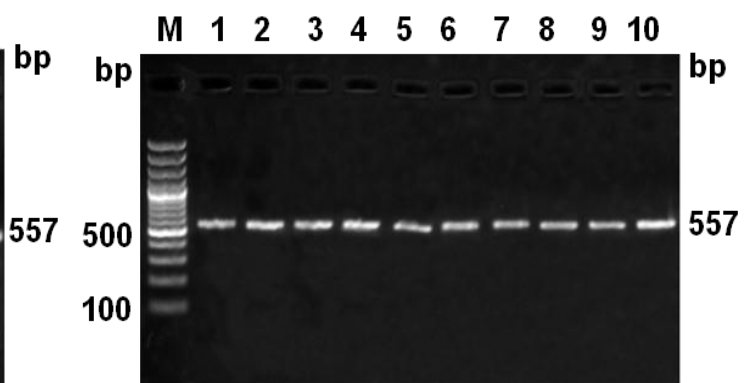
Plate 4.11: Resolution of PCR product of exon 4 of HMGA2 gene (target SNP rs434871480) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 557 bp

Lane M: 100 bp ladder

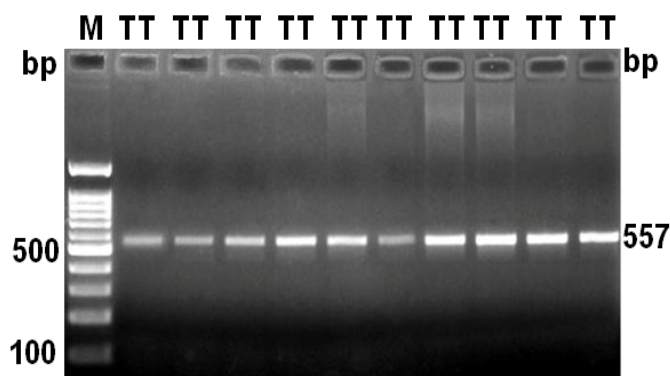


Sahiwal

Lane 1-10: PCR Products, 557 bp

Lane M: 100 bp ladder

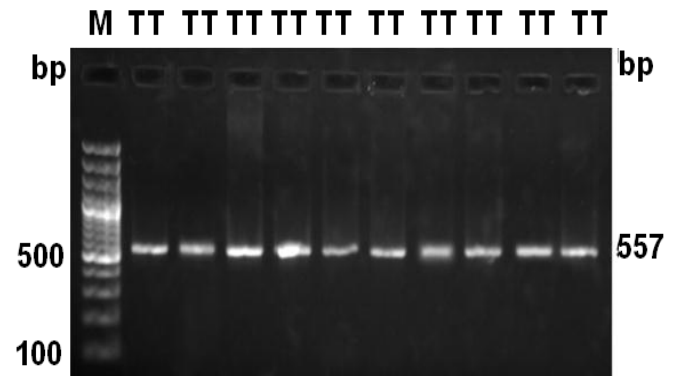
Plate 4.12: Resolution of PCR-RFLP (*HaeIII*) for rs434871480 in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1 - 10 : TT Genotype (557 bp)

Lane M : 100 bp ladder

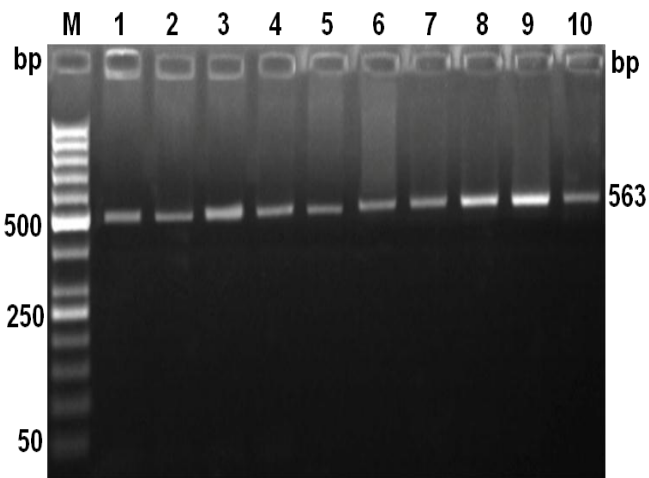


Sahiwal

Lane 1 - 10 : TT Genotype (557 bp)

Lane M : 100 bp ladder

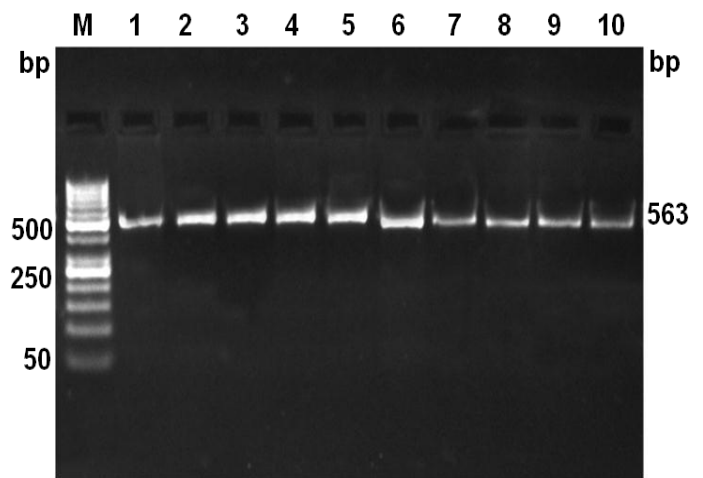
Plate 4.13: Resolution of PCR product of intron 5 of IL22 gene (target SNP rs135884509) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 563 bp

Lane M: 100 bp ladder

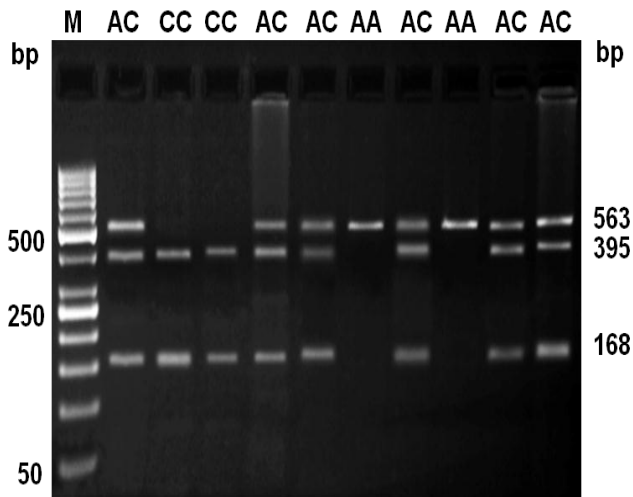


Sahiwal

Lane 1-10: PCR Products, 563 bp

Lane M: 100 bp ladder

Plate 4.14: Resolution of PCR-RFLP (*Hinf* I) for rs135884509 in Karan Fries and Sahiwal cattle



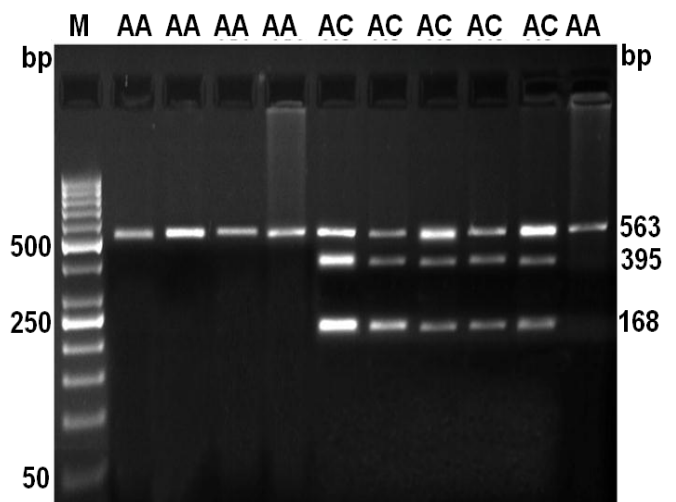
Sahiwal

Lane 1, 4, 5, 7, 9, 10 : AC Genotype (563, 395 & 168 bp)

Lane 2, 3 : CC Genotype (395 & 168 bp)

Lane 6, 8 : AA Genotype (563bp)

Lane M : 50 bp ladder



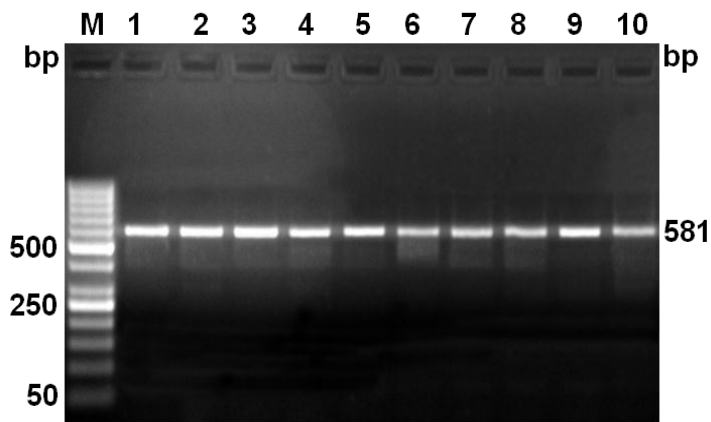
Karan Fries

Lane 5- 9 : AC Genotype (563, 395 & 168 bp)

Lane 1-4, 10 : AA Genotype (563bp)

Lane M : 50 bp ladder

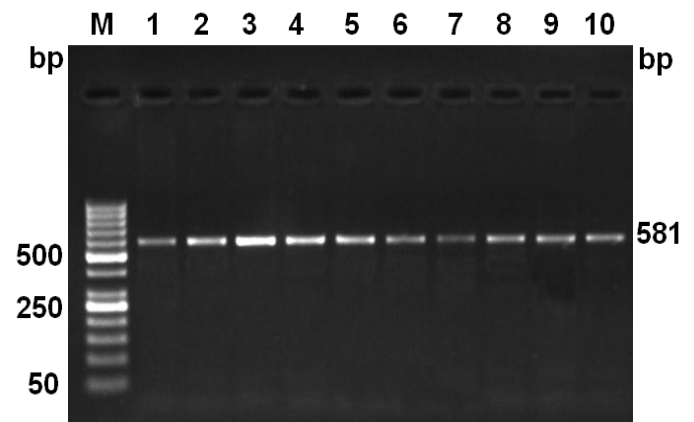
Plate 4.15: Resolution of PCR product of Intron 2 of IL22 gene (target SNP rs383429860) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 581 bp

Lane M: 50 bp ladder

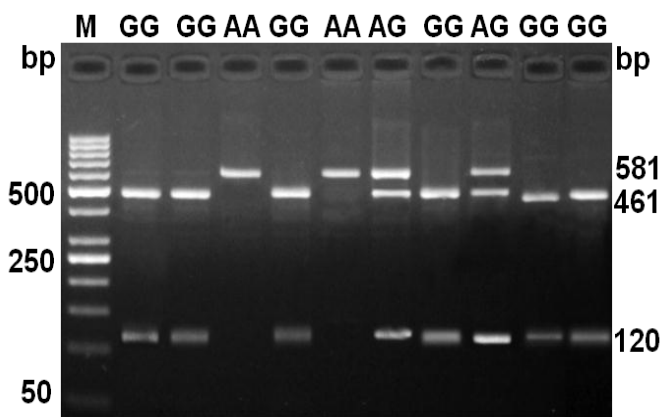


Sahiwal

Lane 1-10: PCR Products, 581 bp

Lane M: 50 bp ladder

Plate 4.16: Resolution of PCR-RFLP (*Sau96I*) for rs383429860 in Karan Fries and Sahiwal cattle



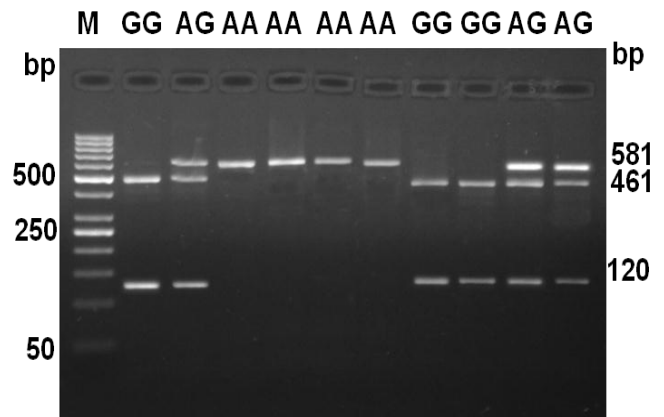
Karan Fries

Lane 1, 2, 4, 7, 9, 10 : GG Genotype (461 & 120 bp)

Lane 3, 5 : AA Genotype (581 bp)

Lane 6, 8 : AG Genotype (581, 461&120 bp)

Lane M : 50 bp ladder



Sahiwal

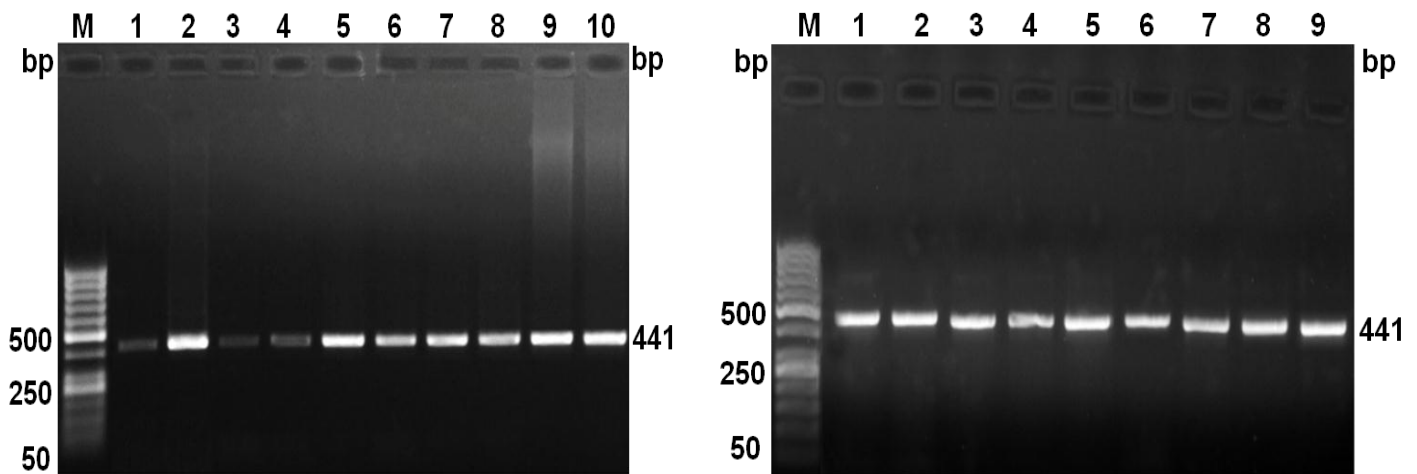
Lane 1, 7-8 : GG Genotype (461 & 120 bp)

Lane 3 - 6 : AA Genotype (581 bp)

Lane 2, 9-10 : AG Genotype (581, 461 & 120 bp)

Lane M : 50 bp ladder

Plate 4.17: Resolution of PCR product of Intron 5 of IL 22 gene (target SNP rs136785910) in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1-10: PCR Products, 441 bp

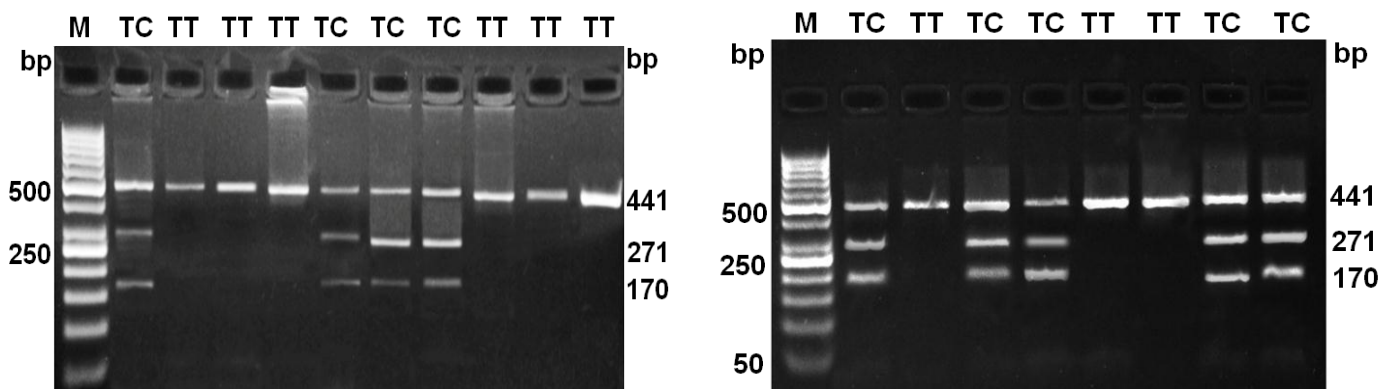
Lane M: 50 bp ladder

Sahiwal

Lane 1-10: PCR Products, 441bp

Lane M: 50 bp ladder

Plate 4.18: Resolution of PCR-RFLP (*HpaII*) for rs136785910 in Karan Fries and Sahiwal cattle



Karan Fries

Lane 1, 5 - 7 : TC Genotype (441, 271 & 170 bp

Lane 2 - 4, 8 - 9 : TT Genotype (441 bp)

Lane M : 50 bp ladder

Sahiwal

Lane 1, 3 – 4, 7-8 : TC Genotype (441, 271 & 170 bp)

Lane 2, 5-6 : TT Genotype (441 bp)

Lane M : 50 bp ladder

Hardy-Weinberg equilibrium (HWE) test was used to predict whether, the population is stable or not on the genotyped loci. Population can significantly deviate from HWE due selection pressure at particular locus, genetic drift and small sample size. In KF cattle all SNP loci were significantly deviating ($P < 0.05$) from Hardy Weinberg Equilibrium. However, in Sahiwal cattle, three SNPs viz., rs454303072, rs470608846 and rs382671389 were found to be significantly deviating ($P < 0.05$) from HWE. The SNPs rs136785910 and rs383429860 were found to be in Hardy Weinberg equilibrium ($P < 0.05$) (Table 4.3 - 4.4).

Table 4.3 Summary of population genetic parameters and test for Hardy Weinberg equilibrium for all loci in Karan Fries cattle

SNP ID	Alleles	Allele frequency	Genotypes	Genotype frequency	HWE		PIC#
					χ ²	P- value*	
rs454303072	A	0.663	AA	110 (0.663)	167.2	0.000	0.347
	G	0.337	GG	56 (0.337)			
rs470608846	Monomorphic		AA				
rs382671389	A	0.503	AA	58 (0.350)	25.06	0.000	0.375
	C	0.497	AC	51 (0.307)			
			CC	57 (0.343)			
rs435289107	C	0.708	CC	104 (0.626)	61.88	0.000	0.328
	T	0.292	TC	27 (0.163)			
			TT	35 (0.211)			
rs434871480	Monomorphic		TT				
rs135884509	A	0.536	AA	73 (0.439)	62.88	0.000	0.374
	C	0.464	AC	32 (0.193)			
			CC	61 (0.368)			
rs136785910	C	0.187	TC	62 (0.374)	8.59	0.000	0.258
	T	0.813	TT	104 (0.626)			
rs383429860	A	0.557	AA	66 (0.397)	21.04	0.000	0.372
	G	0.443	AG	53 (0.320)			
			GG	47 (0.283)			

*Significant at $P < 0.05$; [#]PIC – Polymorphic information content (Bostein *et al.*, 1980)

Table 4.4 Summary of population genetic parameters and test for HWE for all loci in Sahiwal cattle

SNP ID	Alleles	Allele frequency	Genotypes	Genotype frequency	HWE		PIC [#]
					χ ²	P-value*	
rs454303072	A	0.839	AA	73 (0.839)	89.8	0.00	0.234
	G	0.161	GG	14 (0.161)			
rs470608846	Monomorphic		AA				
rs382671389	A	0.649	AA	45 (0.518)	15.76	0.00	0.352
	C	0.351	AC	23 (0.264)			
	-	-	CC	19 (0.218)			
rs435289107	T	0.489	TT	33 (0.356)	19.77	0.00	0.375
	C	0.512	TC	23 (0.264)			
	-	-	CC	33 (0.370)			
rs434871480	Monomorphic		TT				
rs135884509	A	0.615	AA	20 (0.230)	33.53	0.00	0.361
	C	0.385	AC	67 (0.770)			
rs136785910	C	0.081	TT	73 (0.839)	0.61	0.43	0.138
	T	0.92	TC	14 (0.161)			
rs383429860	A	0.535	AA	29 (0.333)	3.38	0.06	0.374
	G	0.466	AG	35 (0.402)			
	-	-	GG	23 (0.265)			

*Significant at P<0.05; [#]PIC – Polymorphic information content (Bostein *et al.*, 1980)

4.7.2. Polymorphic information content

The statistical assessment of informativeness of a marker is denoted by PIC value. The PIC was calculated using allelic frequencies of each SNP loci with the help of formula given by Bostein *et al.* (1980). The PIC values varied from 0 to 1, a value of zero means that the marker has only one allele, whereas, PIC value 1 means that the marker has infinite number of alleles. PIC values above 0.77 are considered to be highly informative. But in case of biallelic markers it can reach maximum up to 0.375 (Bostein *et al.*, 1980).

In the present study, the PIC values for all loci were low to high and varied between 0.258 to 0.375 and 0.138 to 0.375 in Karan Fries and Sahiwal respectively. The SNP rs136785910 was having lowest PIC values in both KF (0.258) and Sahiwal (0.138), whereas, maximum PIC value (0.375) was obtained in rs382671389 (KF cattle) and in rs435289107 (Sahiwal cattle) (Table 4.3 - 4.4).

4.7.3. Heterozygosity and genic variation statistics

Heterozygosity and genic variation statistics were calculated using Popgen v1.32 (Yeh *et al.*, 1999). As with the case of biallelic markers, the observed number of alleles was two for all polymorphic markers, whereas, the effective number alleles (n_e) varied from 1.436 (rs136785910) to 2.000 (rs382671389) (Average = 1.819) in Karan Fries cattle. In Sahiwal cattle, the effective number of alleles varied from 1.174 (rs136785910) to 1.999 (rs435289107) (Average = 1.712) (Table 4.5). The effective number of alleles is described as the number of equally frequent alleles that will give rise to same expected heterozygosity as in the studied population. The average heterozygosity values (also calculated as Nei's genetic diversity) for all loci varied between 0.304 (rs136785910) to 0.500 (rs382671389) in KF cattle and between 0.148 (rs136785910) to 0.500 (rs435289107) in Sahiwal cattle (Table 4.5).

Shannon's Information index was calculated as a measure of within population diversity for both KF and Sahiwal cattle populations for all loci, it varied between 0.482 (rs136785910) to 0.693 (rs382671389) in KF cattle and from 0.280 (rs136785910) to 0.691 (rs383429860) in Sahiwal cattle (Table 4.5). The average observed homozygosity values for KF population was 0.774 and for Sahiwal cattle population was 0.690. Similarly, the values for observed heterozygosity were low; it was 0.226 for KF cattle and 0.310 for Sahiwal cattle (Table 4.5). The values of genic variation statistics as well as the genotypic array in both populations indicate condition of heterozygote deficiency in studied population.

Table 4.5 Summary of genic variation statistics for all SNP loci in Karan Fries and Sahiwal cattle

Karan Fries									
Locus	na¹	ne²	I³	O_Hom⁴	O_Het⁵	E_Hom⁶	E_Het⁷	Nei⁸	Ave_Het⁹
rs454303072	2	1.809	0.639	1.000	0.000	0.552	0.448	0.447	0.447
rs135884509	2	1.990	0.691	0.807	0.193	0.501	0.499	0.497	0.497
rs382671389	2	2.000	0.693	0.693	0.307	0.499	0.502	0.500	0.500
rs136785910	2	1.436	0.482	0.627	0.374	0.695	0.305	0.304	0.304
rs383429860	2	1.974	0.687	0.681	0.319	0.505	0.495	0.493	0.493
rs435289107	2	1.705	0.604	0.837	0.163	0.585	0.415	0.414	0.414
Mean	2	1.819	0.633	0.774	0.226	0.556	0.444	0.443	0.443
St. Dev	-	0.222	0.082	0.137	0.137	0.076	0.076	0.076	0.076
Sahiwal									
Locus	na¹	ne²	I³	O_Hom⁴	O_Het⁵	E_Hom⁶	E_Het⁷	Nei⁸	Ave_Het⁹
rs454303072	2	1.370	0.441	1.000	0.000	0.728	0.272	0.270	0.270
rs135884509	2	1.900	0.667	0.230	0.770	0.524	0.476	0.474	0.474
rs382671389	2	1.836	0.648	0.736	0.264	0.542	0.458	0.455	0.455
rs136785910	2	1.174	0.280	0.839	0.161	0.851	0.149	0.148	0.148
rs383429860	2	1.991	0.691	0.598	0.402	0.500	0.501	0.498	0.498
rs435289107	2	1.999	0.693	0.736	0.264	0.497	0.503	0.500	0.500
Mean	2	1.712	0.570	0.690	0.310	0.607	0.393	0.391	0.391
St. Dev	-	0.351	0.171	0.262	0.262	0.148	0.148	0.147	0.147

na¹ – Observed number of alleles

ne² – Effective number of alleles

I³ – Shannon's Information index

O_Hom⁴ – Observed homozygosity

O_Het⁵ – Observed heterozygosity

E_Hom⁶ – Expected homozygosity

E_Het⁷ – Expected heterozygosity

Nei⁸ – Nei's genetic diversity

Avg_Het⁹ – Average heterozygosity

[Popgen v1.32]

4.8. Linkage disequilibrium analysis of the SNPs in Karan Fries and Sahiwal cattle

The pairwise Linkage Disequilibrium (LD) analysis was carried out by estimating D' and r^2 statistics using SHEsis online software programme. In order to avoid effect of small sample size on D' values, D' values were compared with r^2 parameter. In KF cattle, it was found that SNP loci exhibited very weak pairwise LD, as the pairwise Lewontin's D' values were less than 0.50 and pairwise r^2 values were also less than 0.20. While in Sahiwal cows, SNPs loci exhibited weak to strong LD, as Lewontin's D' values ranged between 0.012 to 0.99. High D' values were seen among SNP rs135884509 and rs136785910; SNP rs454303072 and rs382671389; SNP rs454303072 and SNP rs435289107; SNP rs454303072 and SNP rs136785910; SNP rs136785910 and SNP rs435289107. However, the r^2 for all the pairs were low varying from 0.00 to 0.039, hence haplotype analysis could not be performed. The D' and r^2 values for the SNPs in both the breeds are presented in Table 4.6 and Figure 4.9 - 4.10.

Table 4.6 D' and r^2 statistics for all studied loci in Karan Fries and Sahiwal cattle

Karan Fries											
D'	M2	M3	M4	M5	M6	r^2	M2	M3	M4	M5	M6
M1	0.269	0.048	0.235	0.023	0.358	M1	0.032	0.001	0.006	0.000	0.027
M2	-	0.043	0.220	0.011	0.135	M2	-	0.002	0.013	0.000	0.009
M3	-	-	0.241	0.067	0.078	M3	-	-	0.013	0.004	0.002
M4	-	-	-	0.313	0.388	M4	-	-	-	0.018	0.084
M5	-	-	-	-	0.271	M5	-	-	-	-	0.024
Sahiwal											
D'	M2	M3	M4	M5	M6	r^2	M2	M3	M4	M5	M6
M1	0.129	0.615	0.556	0.198	0.860	M1	0.005	0.134	0.005	0.009	0.149
M2	-	0.049	0.998	0.179	0.200	M2	-	0.002	0.055	0.023	0.026
M3	-	-	0.012	0.066	0.233	M3	-	-	0.000	0.003	0.031
M4	-	-	-	0.405	0.686	M4	-	-	-	0.013	0.039
M5	-	-	-	-	0.138	M5	-	-	-	-	0.017

where,

M1 - rs454303072

M2 - rs135884509

M3 - rs382671389

M4 - rs136785910

M5 - rs383429860

M6 - rs435289107

4.9. Adjustment of data for non genetic factors

The non genetic factors that were assessed in present study included season, parity, stage of lactation and level of production. The significance of effects of non-genetic factors on milk production traits (Monthly milk yield, test day milk yield, 305-day milk yield and total milk yield), udder and teat type traits were analyzed using ANOVA and the least squares mean values in Sahiwal and Karan Fries cattle. The data was analyzed using model described in section 3.9.2 and the results are presented under this section.

4.9.1. Effect of non-genetic factors on milk production traits in Karan Fries and Sahiwal cattle

In Karan Fries cows, milk production traits i.e. 305-day milk yield (305MY) was significantly ($P \leq 0.05$) affected by parity of the cows, while, monthly milk yield (MMY) and test day milk yield (TDMY) were significantly ($P < 0.01$) affected by stage of lactation of the cows (Table 4.7a). The overall least squares mean of monthly milk yield (MMY), test day milk yield (TDMY) and 305-day milk yield (305MY) with respect to non genetic factors was found to be 375.69 ± 13.7 kg, 13.20 ± 0.45 kg and 3525.97 ± 103.2 kg, respectively (Table 4.7b). Monthly milk yield (MMY) and test day milk yield (TDMY) were reducing significantly with advancing of the lactation stages, while the first stage (0-90 days) is having maximum monthly and test day milk yield (TDMY). Result of the present study were in concordance with results of Singh and Kumar (2007), which reported that Karan Fries cattle generally attain peak yield during 6-9 weeks, then it proceed to a downfall after maintaining steady phase for a short time (Singh and Kumar, 2007). The 305-day milk yield (305MY) did not show any significant difference from parity, first to third, followed by a significant decrease from parity third to fourth, which showed a significant increase in fifth parity (Table 4.7b).

In Sahiwal cows, milk production traits i.e. monthly milk yield (MMY), test day milk yield (TDMY) and 305-day milk yield (305MY) were significantly ($P \leq 0.05$) affected by parity of the cows (Table 4.8a). Overall least squares means of monthly milk yield (MMY), test day milk yield (TDMY) and 305-day milk yield (305MY) for Sahiwal cows were found to be 213.92 ± 20.33 kg, 6.93 ± 0.67 kg and 2016.136 ± 144.97 kg (Table 4.8b). Monthly milk yield (MMY), test day milk yield (TDMY) and 305-day milk yield (305MY) were not showing any significant difference from parity first to third followed by a significant increase from parity third to fourth and a significant decrease in fifth parity (Table 4.8b). There was no consistent trend for variation in milk production with advance in parity/years which may be due to the environmental variation in different years. Whereas, there was an evident increase

Figure 4.9 Estimation of single population linkage disequilibrium of studied loci in Karan Fries cattle

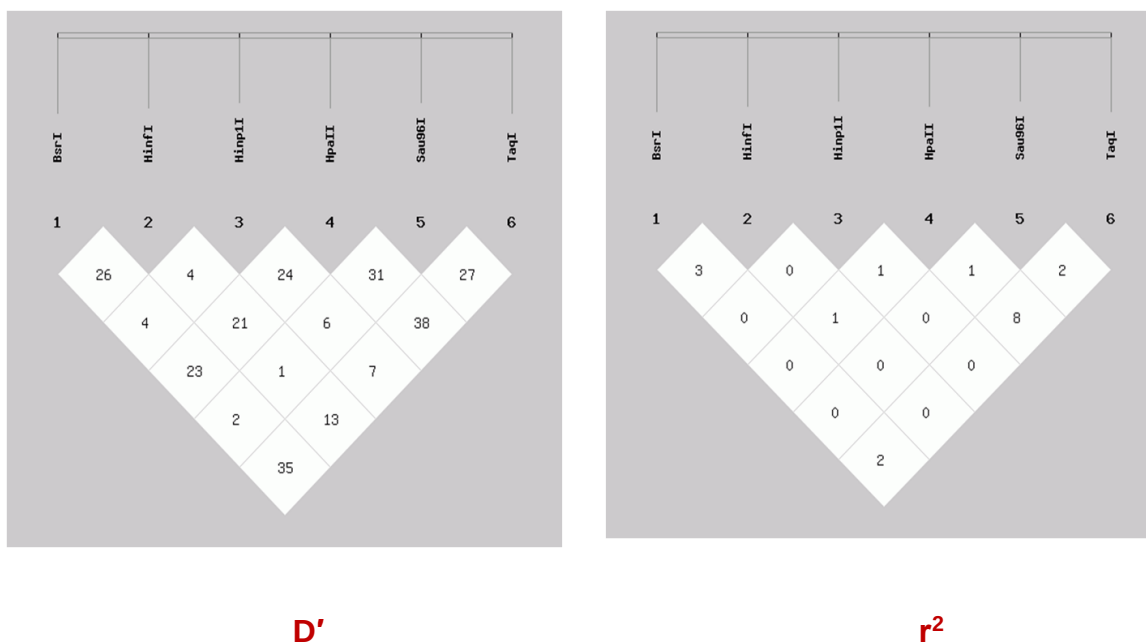
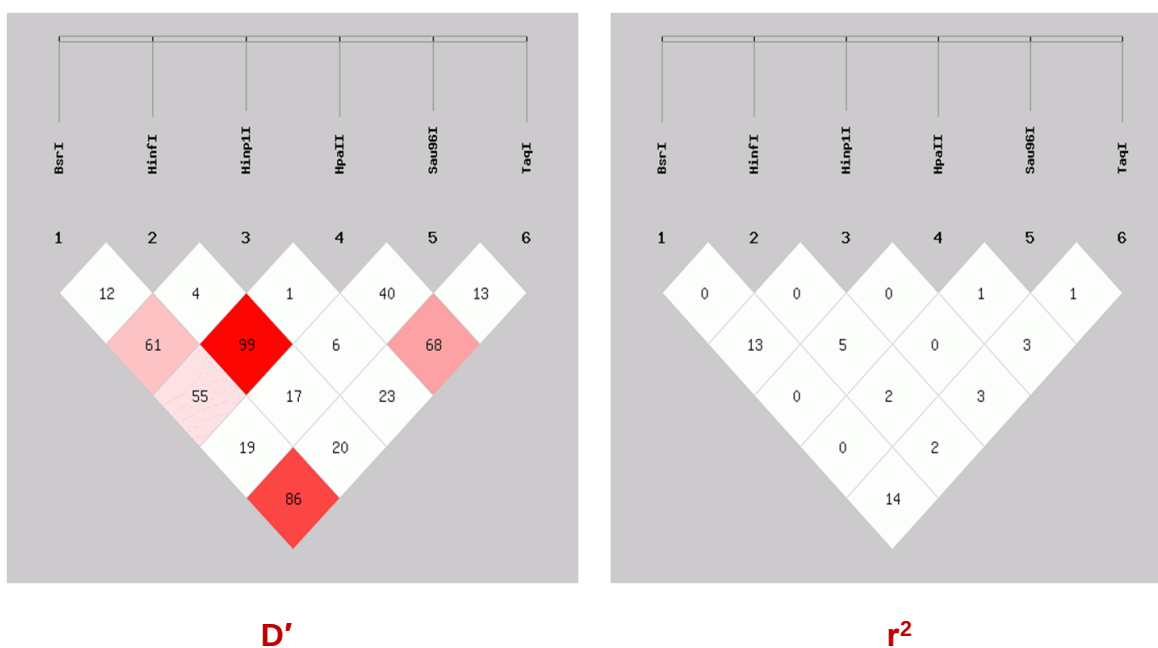


Figure 4.10 Estimation of single population linkage disequilibrium of studied loci in Sahiwal cattle



of milk production from third to fifth parity (Table 4.7b, Table 4.8b). This is in agreement with Anderson (1985) and Pawar *et al.* (2012) who reported that production of cow reached the peak around fifth parity, when animal was seven to eight years old and gained the adult body size. Therefore, increase in production, body weight and mammary gland was caused by maturation. Mammary gland grows and develops parallel to growing, maturation and recurring pregnancies and lactations as reported by Anderson (1985). Earlier report (Bagnato and Oltenacu, 1994) showed that cows of the same age but different parity have different production, and that differences were particularly evident for the first and second parity. The highest 305d milk yield was observed in animals in fifth parity with no consistent increase or decrease over the advancement of the parities (Ahmad *et al.*, 1993; Javed *et al.*, 2001).

Table 4.7a Effect of non-genetic factors on milk production traits in Karan Fries cattle

Source		MMY	TDMY	MY	TMY
Season	df	3	3	3	3
	SS	50960.22	86.76	2492870.36	12910232.78
	MSS	16986.74	28.92	830956.78	4303410.92
Parity	df	4	4	4	4
	SS	171159.74	155.08	13639147.80	25569216.26
	MSS	42789.93	38.77	3409786.95*	6392304.06
Stage of lactation	df	2	2		
	SS	572293.89	717.06		
	MSS	286146.94**	358.53**		

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

Table 4.7 (b) Least squares means for milk production traits in Karan Fries cattle

Effects	MMY (kg)	TDMY (kg)	305MY (kg)	TMY (kg)
Overall (μ)	375.69 $\pm$ 13.7	13.20 $\pm$ 0.45	3525.97 $\pm$ 103.2	4180.01 $\pm$ 166.8
SEASON				
Summer (52)	365.49 $\pm$ 23.33	13.09 $\pm$ 0.76	3510.61 $\pm$ 151.73	3889.17 $\pm$ 245.05
Rainy (38)	391.24 $\pm$ 31.41	14.14 $\pm$ 1.02	3599.45 $\pm$ 216.28	4278.51 $\pm$ 349.31
Autumn (32)	398.03 $\pm$ 28.99	13.67 $\pm$ 0.94	3669.54 $\pm$ 208.82	4616.49 $\pm$ 337.26
Winter (44)	348.01 $\pm$ 25.33	11.91 $\pm$ 0.82	3324.29 $\pm$ 184.66	3935.87 $\pm$ 298.24
PARITY				
1(57)	379.80 $\pm$ 20.79	12.99 $\pm$ 0.68	3510.17 ^b $\pm$ 1 55.65	3946.21 $\pm$ 251.38
2(48)	401.46 $\pm$ 22.15	14.25 $\pm$ 0.72	3521.06 ^b $\pm$ 166.34	4172.15 $\pm$ 268.66
3(22)	335.77 $\pm$ 33.06	11.73 $\pm$ 1.08	3418.46 ^b $\pm$ 241.22	3928.51 $\pm$ 389.59
4(26)	325.40 $\pm$ 29.62	12.20 $\pm$ 0.96	2962.18 ^a $\pm$ 223.12	3424.62 $\pm$ 360.35
>5(13)	436.03 $\pm$ 41.98	14.84 $\pm$ 1.37	4217.99 ^c $\pm$ 318.59	5028.55 $\pm$ 514.55
STAGE OF LACTATION				
1 (53)	442.54 ^c $\pm$ 27.29	15.82 ^c $\pm$ 0.89	-	
2 (60)	401.54 ^b $\pm$ 24.24	13.81 ^b $\pm$ 0.79		
3 (53)	283.00 ^a $\pm$ 20.71	9.98 ^a $\pm$ 0.67		

Means with different letter (a, b, c) indicates significant difference (P<0.05).

Table 4.8a Effect of non-genetic factors on milk production traits in Sahiwal cattle

Source		MMY	TDMY	MY	TMY
Season	df	3	3	3	3
	SS	33457.58	44.14	4405762.10	6661813.80
	MSS	11152.52	14.71	1468587.36	2220604.60
Parity	Df	4	4	4	4
	SS	227151.10	201.02	13250433.02	11471435.95
	MSS	56787.77**	50.25*	3312608.25*	2867858.98
Stage of lactation	Df	2	2		
	SS	25851.05	43.49		
	MSS	12925.52	21.74		

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

Table 4.8b Least squares means for milk production traits in Sahiwal cattle

Effects	MMY (kg)	TDMY (kg)	305MY (kg)	TMY (kg)
Overall (μ)	213.92 $\pm$ 20.33	6.93 $\pm$ 0.67	2016.136 $\pm$ 144.97	2124.650 $\pm$ 166.93
SEASON				
Summer (52)	242.89 $\pm$ 24.33	8.55 $\pm$ 0.80	1771.45 $\pm$ 153.21	1813.25 $\pm$ 176.42
Rainy (13)	199.99 $\pm$ 44.30	6.19 $\pm$ 1.46	2414.91 $\pm$ 278.64	2616.86 $\pm$ 320.84
Autumn (6)	229.33 $\pm$ 60.15	6.06 $\pm$ 1.99	1850.07 $\pm$ 411.09	2041.20 $\pm$ 473.35
Winter (16)	183.45 $\pm$ 35.65	6.93 $\pm$ 1.18	2028.10 $\pm$ 250.60	2027.27 $\pm$ 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	1984.63 ± 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		

Means with different letter (a, b, c) indicates significant difference (P<0.05).

4.9.2. Effect of non-genetic factors on udder and teat type traits in Karan Fries and Sahiwal cattle

In Karan Fries cows, udder and teat type traits viz. fore udder attachment (FUA), rear udder height (RUH), udder depth (UD), udder length (UL), udder width (UW), teat circumference (TC), fore and rear teat length (FTL and RTL), shortest distance from front and rear teat ends to floor (SDF and SDR) and teat diameter (TD) were significantly (P<0.05) affected by parity. Whereas, stage of lactation had significant (P<0.05) effect on udder circumference, distance between fore and rear teats (DFR) and distance between left and right teats (DLR) (Table 4.9a & b).

The overall least squares means for FUA, RUH, UD, UL, UW, TC, FTL, RTL, SDF, SDR, TD, UC, DFR and DLR were 131 ± 2.02 degree, 23.67 ± 0.49 cm, 53.90 ± 0.56 cm, 60.34 ± 0.68 cm, 71.47 ± 0.83 cm, 7.50 ± 0.14 cm, 5.33 ± 0.14 cm, 4.62 ± 0.10 cm, 46.99 ± 0.52 cm, 47.00 ± 0.51cm, 2.22 ± 0.03 cm, 142.58 ± 2.28 cm, 6.56 ± 0.36 cm and 8.86 ± 0.44 cm respectively, with respect to non genetic factors (Table 4.10a, b & c). Height of the rear udder (RUH) increased significantly with increasing parity number of the animals, Parity (>5) was found to have the highest RUH (27.08 ± 1.49 cm). Depth of the udder (UD) reduced significantly from the first parity (60.44±1.70 cm) to the next onwards, Parity (>5) had the

Table 4.9a Effect of non-genetic factors on udder and teat type traits in Karan Fries cattle

Source		FUA	RUH	RUW	UB	UD	UL	UW	UC	CL
Season	df	3	3	3	3	3	3	3	3	3
	SS	457.12	94.72	1.13	129.79	177.02	244.42	109.42	1237.47	5.42
	MSS	152.37	31.57	0.377	43.26	59.00	81.47	36.47	412.49	1.8
Parity	df	4	4	4	4	4	4	4	4	4
	SS	4694.74	562.40	59.72	93.16	2423.99	2117.14	4484.03	2323.34	1.10
	MSS	1173.68**	140.6***	14.93	23.29	605.99***	529.28***	1121.0***	580.83	0.27
Stage of lactation	df	2	2	2	2	2	2	2	2	2
	SS	511.60	35.09	6.27	1.95	13.98	299.37	365.65	3620.91	0.0975
	MSS	255.80	17.54	3.13	0.97	6.99	149.68	182.82	1810.45*	0.04

*p≤0.05, **p≤0.01, ***p≤0.001

Table 4.9b Effect of non-genetic factors on udder and teat type traits in Karan Fries cattle

Source		TC	FTL	RTL	DFR	DLR	SDF	TD	SDR
Season	df	3	3	3	3	3	3	3	3
	SS	3.50	5.05	3.27	108.91	95.58	150.70	0.0160	123.81
	MSS	1.16	1.68	1.09	36.30	31.86	50.23	0.005	41.27
Parity	df	4	4	4	4	4	4	4	4
	SS	53.27	38.55	29.73	50.24	29.09	2325.02	2.73	2430.85
	MSS	13.31***	9.63**	7.43***	12.56	7.27	581.25**	0.68**	607.71**
Stage of lactation	df	2	2	2	2	2	2	2	2
	SS	3.62	2.37	4.29	46.12	87.96	101.25	.1751	71.70
	MSS	1.81	1.18	2.14	23.06*	43.98*	50.62	0.08	35.85

*p≤0.05, **p≤0.01, ***p≤0.001

lowest udder depth *i.e.* 48.95±0.84 cm (Table 4.10a). Udder length (UL) increased significantly from first parity (54.08±1.01cm) to the next (60.58 ±1.08 cm) with almost similar length/no change to the next parities (Table 4.10a). Udder width (UW) also increased significantly from first parity (62.54±1.23 cm) to the next parities; Parity (>5) had the highest udder width (80.19±2.49 cm). Teat circumference (TC) increased significantly from first parity (6.52±0.22 cm) to third parity (7.85±0.35 cm) with almost similar circumference to the next parities (Table 4.10b). Fore teat length (FTL), rear teat length (RTL) and teat diameter (TD) increased significantly from first parity to third parity with almost no change to the next parity followed by a significant increase in the fifth parity (Table 4.10b). Shortest distance from front and rear teat ends to floor (SDF and SDR) decreased significantly with increasing parity number of the animals, Parity (>5) was found to have the lowest distance from teat ends to floor (Table 4.10c).

Table 4.10a Least square means for udder and teat type traits in Karan Fries cattle

Effects	FUA (degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)
Overall (μ)	131 ± 2.02	23.67 ± 0.49	12.87 ± 0.24	53.90 ± 0.56	0.75 ± 0.53	60.34 ± 0.68
SEASON						
Summer (52)	120.98 ± 1.30	22.66 ± 0.83	12.98 ± 0.41	53.38 ± 0.94	2.31 ± 0.89	62.07 ± 1.14
Rainy (38)	118.26 ± 2.37	25.20 ± 1.12	12.96 ± 0.55	55.63 ± 1.27	-0.70 ± 1.20	59.92 ± 1.53
Autumn (32)	123.54 ± 3.23	23.70 ± 1.03	12.73 ± 0.50	54.38 ± 1.17	0.59 ± 1.10	60.78 ± 1.41
Winter (44)	119.02 ± 6.17	23.13 ± 0.90	12.82 ± 0.44	52.23 ± 1.02	0.82 ± 0.96	58.59 ± 1.23
PARITY						
1(57)	148.02 ± 1.62	20.99 ^a ± 0.74	11.82 ± 0.36	60.44 ^d ± 1.70	0.01 ± 0.79	54.08 ^a ± 1.01
2(48)	132.45 ± 1.84	21.75 ^a ± 0.78	12.89 ± 0.38	55.84 ^c ± 1.20	1.11 ± 0.84	60.58 ^b ± 1.08
3(22)	119.54 ± 1.81	24.05 ^b ± 1.17	12.94 ± 0.58	52.61 ^b ± 1.34	0.21 ± 1.26	62.00 ^{bc} ± 1.61
4(26)	105.36 ± 2.38	24.49 ^b ± 1.05	13.02 ± 0.52	51.67 ^b ± 1.20	2.16 ± 1.13	62.13 ^{bc} ± 1.44
>5(13)	96.89 ± 2.08	27.08 ^c ± 1.49	13.69 ± 0.73	48.95 ^a ± 0.84	0.25 ± 1.60	62.92 ^c ± 2.05
STAGE OF LACTATION						
1 (53)	119.36 ± 1.83	22.87 ± 0.97	13.15 ± 0.47	54.41 ± 1.10	0.94 ± 1.04	62.50 ± 1.33
2 (60)	118.84 ± 2.17	23.93 ± 0.86	12.88 ± 0.42	53.49 ± 0.98	0.61 ± 0.92	59.99 ± 1.18
3 (53)	123.15 ± 1.58	24.22 ± 0.73	12.59 ± 0.36	53.81 ± 0.83	0.69 ± 0.79	58.53 ± 1.01

Means with different letter (a, b, c) indicates significant difference (P<0.05)

Table 4.10b Least squares means for udder and teat type traits in Karan Fries cattle

Effects	UW (cm)	UC (cm)	CL (cm)	TC (cm)	FTL (cm)
Overall (μ)	71.47 $\pm$ 0.83	142.58 $\pm$ 2.28	3.18 $\pm$ 0.13	7.50 $\pm$ 0.14	5.33 $\pm$ 0.14
SEASON					
Summer (52)	72.01 $\pm$ 1.38	140.62 $\pm$ 3.8	3.01 $\pm$ 0.22	7.75 $\pm$ 0.24	5.06 $\pm$ 0.23
Rainy (38)	71.00 $\pm$ 1.86	146.49 $\pm$ 5.1	3.13 $\pm$ 0.30	7.29 $\pm$ 0.33	5.35 $\pm$ 0.31
Autumn (32)	72.54 $\pm$ 1.72	137.92 $\pm$ 4.7	3.07 $\pm$ 28	7.52 $\pm$ 0.30	5.33 $\pm$ 0.29
Winter (44)	70.34 $\pm$ 1.50	143.71 $\pm$ 4.1	3.49 $\pm$ 0.24	7.42 $\pm$ 0.26	5.58 $\pm$ 0.25
PARITY					
1(57)	62.54 ^a $\pm$ 1.23	139.93 $\pm$ 3.40	3.04 $\pm$ 0.41	6.52 ^a $\pm$ 0.22	4.47 ^a $\pm$ 0.21
2(48)	70.01 ^b $\pm$ 1.31	148.25 $\pm$ 3.62	3.20 $\pm$ 0.29	7.20 ^b $\pm$ 0.23	5.25 ^b $\pm$ 0.22
3(22)	73.44 ^c $\pm$ 1.96	141.17 $\pm$ 5.40	3.23 $\pm$ 0.32	7.85 ^c $\pm$ 0.35	5.59 ^{cd} $\pm$ 0.33
4(26)	71.18 ^b $\pm$ 1.76	144.81 $\pm$ 4.8	3.25 $\pm$ 0.21	7.78 ^c $\pm$ 0.31	5.48 ^{bc} $\pm$ 0.29
>5(13)	80.19 ^d $\pm$ 2.49	136.76 $\pm$ 6.8	3.17 $\pm$ 0.20	8.13 ^c $\pm$ 0.44	5.86 ^d $\pm$ 0.42
STAGE OF LACTATION					
1 (53)	73.82 $\pm$ 1.62	150.71 ^b $\pm$ 4.46	3.21 $\pm$ 0.26	7.29 $\pm$ 0.28	5.54 $\pm$ 0.27
2 (60)	71.17 $\pm$ 1.44	137.51 ^a $\pm$ 3.96	3.18 $\pm$ 0.23	7.75 $\pm$ 0.25	5.16 $\pm$ 0.24
3 (53)	69.43 $\pm$ 1.23	138.34 ^a $\pm$ 3.38	3.14 $\pm$ 0.20	7.45 $\pm$ 0.21	5.29 $\pm$ 0.20

Means with different letter (a, b, c) indicates significant difference (P<0.05).

Table 4.10c Least squares means for udder and teat type traits in Karan Fries cattle

Effects	RTL (cm)	DFR (cm)	DLR (cm)	SDR (cm)	TD (cm)	SDF (cm)
Overall (μ)	4.62 $\pm$ 0.10	6.56 $\pm$ 0.36	8.86 $\pm$ 0.44	47.00 $\pm$ 0.51	2.22 $\pm$ 0.03	46.99 $\pm$ 0.52
SEASON						
Summer (52)	5.94 $\pm$ 1.34	7.02 $\pm$ 0.97	8.69 $\pm$ 1.06	41.25 $\pm$ 1.19	2.61 $\pm$ 0.09	40.37 $\pm$ 1.24
Rainy (38)	6.35 $\pm$ 2.45	3.00 $\pm$ 1.77	4.40 $\pm$ 1.93	44.38 $\pm$ 2.18	2.75 $\pm$ 0.17	42.80 $\pm$ 2.26
Autumn (32)	6.31 $\pm$ 3.32	1.31 $\pm$ 2.41	2.19 $\pm$ 2.62	41.42 $\pm$ 2.96	2.89 $\pm$ 0.23	40.63 $\pm$ 3.07
Winter(44)	6.00 $\pm$ 1.97	7.04 $\pm$ 1.43	7.94 $\pm$ 1.55	42.78 $\pm$ 1.75	2.51 $\pm$ 0.14	41.31 $\pm$ 1.82
PARITY						
1(57)	3.89 ^a $\pm$ 0.15	5.44 $\pm$ 1.21	6.22 $\pm$ 1.31	53.53 ^c $\pm$ 0.78	1.99 ^a $\pm$ 0.04	53.39 ^c $\pm$ 0.77
2(48)	4.44 ^b $\pm$ 0.16	3.64 $\pm$ 1.37	4.14 $\pm$ 1.49	48.48 ^d $\pm$ 0.84	2.20 ^b $\pm$ 0.05	48.33 ^d $\pm$ 0.82
3(22)	4.99 ^c $\pm$ 0.25	4.70 $\pm$ 1.35	6.37 $\pm$ 1.47	46.69 ^c $\pm$ 1.25	2.30 ^{cd} $\pm$ 0.07	46.88 ^c $\pm$ 1.22
4(26)	4.83 ^c $\pm$ 0.22	4.14 $\pm$ 1.78	5.87 $\pm$ 1.93	44.65 ^b $\pm$ 1.12	2.24 ^{bc} $\pm$ 0.06	45.52 ^b $\pm$ 1.09
>5(13)	4.95 ^c $\pm$ 0.32	5.06 $\pm$ 1.56	6.43 $\pm$ 1.69	41.60 ^a $\pm$ 1.59	2.36 ^d $\pm$ 0.09	40.89 ^a $\pm$ 1.55
STAGE OF LACTATION						
1 (53)	7.11 $\pm$ 1.89	3.04 $\pm$ 1.37	3.99 $\pm$ 1.48	45.07 $\pm$ 1.68	2.74 $\pm$ 0.13	43.65 $\pm$ 1.74
2 (60)	5.99 $\pm$ 2.23	2.90 $\pm$ 1.62	3.43 $\pm$ 1.76	42.77 $\pm$ 1.99	2.70 $\pm$ 0.16	41.13 $\pm$ 2.07
3 (53)	5.35 $\pm$ 1.63	7.83 $\pm$ 1.18	9.99 $\pm$ 1.28	39.54 $\pm$ 1.45	2.68 $\pm$ 0.10	39.05 $\pm$ 1.50

Means with different letter (a, b, c) indicates significant difference ($P \leq 0.05$)

In Sahiwal cows udder and teat type traits *viz.* fore udder attachment (FUA), rear udder width (at attachment) (RUW), udder depth (UD), udder width (UW) and teat diameter (TD) were significantly affected by parity. Teat type trait *i.e.* teat circumference (TC) was significantly affected by season and stage of lactation. Stage of lactation had significant effect on distance between fore and rear teats (DFR), distance between left and right teats (DLR), shortest distance from rear teat ends to floor (SDR) and udder circumference (UC) (Table 4.11a & b). Overall least square means for fore udder attachment, rear udder width (at attachment), udder depth, udder width, udder circumference, teat diameter, distance between fore and rear teats, distance between left and right teats, shortest distance from rear teat ends to floor and teat circumference were 107 $\pm$ 2.10 cm, 8.99 $\pm$ 0.32 cm, 49.37 $\pm$ 0.88 cm, 63.45 $\pm$ 1.20 cm, 142.64 $\pm$ 1.80 cm, 2.71 $\pm$ 0.08 cm, 4.59 $\pm$ 0.81cm, 5.81 $\pm$ 0.88 cm, 41.28 $\pm$ 1.04

cm and 8.64 ± 0.88 cm respectively, with respect to season, parity and stage of lactation on udder and teat type traits (Table 4.12a, b & c).

Fore udder attachment and udder depth was decreasing significantly from 1st parity to 5th parity. Rear udder width and udder width were increasing significantly from 1st to 4th parity, udder width then significantly reduced from 4th to 5th parity but Rear udder width continued to increase significantly. Udder circumference continued to decrease significantly as stage of lactation increases. Teat circumference decreased significantly from 1st to 2nd stage of lactation, afterwards continued to increase significantly. Circumference of teat again reduced significantly from summer to rainy season which did not change till autumn, however a significant increase in circumference was there after autumn to winter. Distance between fore and rear teats (DFR) and distance between left and right teats (DLR) were almost same in the first two stages of lactation which increased significantly from second to third stage of lactation (Table 4.12a, b & c).

These results are in accordance with previous studies by Singh *et al.* (2010), who reported that in Vrindavani crossbred cattle, the mean udder length, width, depth, teat length and teat diameter was significantly affected by parity. Dubey (2010) also reported significant effect of parity on teat length in Sahiwal cows. Parity had significant effect on fore udder attachment, rear udder height, udder width, udder depth and balance, teat length and thickness in Holstein cattle (Marinov *et al.*, 2015). The parity differences were justified because of development of udder from lactation first onward. A significant effect of parity was observed for udder length, width, depth and circumference in Sahiwal cows (Khan and Khan, 2016). The significant effect of parity on TC, SDF and SDR might be due to the increase in teat diameter and teat length as the parity increases (Singh *et al.*, 2010; Prasad *et al.*, 2010b).

Effect of stage of lactation was seen on UC, TC, DFR, DLR and SDR in both the breeds. These findings are in agreement with Khan and Khan (2016), who revealed stage of lactation was a significant source of variation for udder circumference. The stage of lactation might influence all the udder and teat measurements because of effects of proceeding lactation on udder measurements and on milk yield. The increasing value of SDR as parity increases, might be due to a significant effect of the stage of lactation on rear udder width and height in dairy cattle (Krastanov, 1995) and a significantly decreasing value of udder depth and height at third lactation stage reported in Holstein cows (Zwertvaegher *et al.*, 2012). The decreasing value of DLR and DFR as parity increases might be due to the effect

Table 4.11a Effect of non-genetic factors on udder type traits in Sahiwal cattle

Source		FUA	RUH	RUW	UB	UD	UL	UW	UC	CL
Season	df	3	3	3	3	3	3	3	3	03
	SS	141.35	5.96	9.39	12.37	222.04	63.53	136.99	217.21	6.35
	MSS	47.11	1.98	3.13	4.12	74.01	21.17	45.66	72.40	2.11
Parity	df	4	4	4	4	4	4	4	4	4
	SS	25247.64	339.79	46.58	51.54	627.10	276.17	871.13	561.33	4.80
	MSS	6311.91***	84.94	11.64**	12.88	156.77**	69.04	217.78**	140.33	1.20
Stage of lactation	df	2	2	2	2	2	2	2	2	2
	SS	108.87	14.42	2.54	1.15	132.48	80.79	56.80	825.62	3.45
	MSS	54.43	7.21	1.27	0.57	66.24	40.39	28.40	412.81*	1.72

*p≤0.05, **p≤0.01, ***p≤0.001

Table 4.11b

Effect of non-genetic factors on teat type traits in Sahiwal cattle

Source		TC	FTL	RTL	DFR	DLR	SDR	TD	SDF
Season	df	3	3	3	3	3	3	3	3
	SS	434.42	10.98	0.9427	132.12	156.16	73.90	0.2835	40.15
	MSS	144.80*	3.66	0.31	44.04	52.05	24.63	0.09	13.38
Parity	df	4	4	4	4	4	4	4	4
	SS	109.61	144.75	117.78	43.66	66.91	322.02	2.402	158.29
	MSS	27.40	36.18	29.44	10.91	16.72	80.50	0.60*	39.57
Stage of lactation	df	2	2	2	2	2	2	2	2
	SS	366.57	51.81	26.28	161.53	264.26	217.34	0.0263	165.26
	MSS	183.28*	25.90	13.14	80.76*	132.13**	108.67*	0.01	82.63

*p≤0.05, **p≤0.01

of stage of lactation on teat position, central ligament, udder length, width and circumference (Angelova, 2006; Khan and Khan, 2016).

Table 4.12a Least squares means for udder and teat type traits in Sahiwal cattle

Effects	FUA (degree)	RUH (cm)	RUW (cm)	UD (cm)	UB (cm)	UL (cm)
Overall (μ)	107 $\pm$ 2.10	22.69 $\pm$ 0.99	8.99 $\pm$ 0.32	49.37 $\pm$ 0.88	-1.83 $\pm$ 0.48	51.30 $\pm$ 0.94
SEASON						
Summer (52)	120.98 $\pm$ 1.30	22.80 $\pm$ 1.18	9.55 $\pm$ 0.39	47.87 $\pm$ 1.05	-1.88 $\pm$ 0.57	51.71 $\pm$ 1.13
Rainy (13)	118.26 $\pm$ 2.37	22.15 $\pm$ 2.15	8.28 $\pm$ 0.71	53.58 $\pm$ 1.05	-2.73 $\pm$ 1.05	49.47 $\pm$ 2.06
Autumn (6)	123.54 $\pm$ 3.23	22.44 $\pm$ 2.93	8.49 $\pm$ 0.97	48.11 $\pm$ 1.92	-1.10 $\pm$ 1.42	53.14 $\pm$ 2.80
Winter(16)	119.02 $\pm$ 6.17	23.36 $\pm$ 1.73	9.64 $\pm$ 0.57	47.90 $\pm$ 2.61	-1.63 $\pm$ 0.84	50.91 $\pm$ 1.66
PARITY						
1(29)	138.02 ^c $\pm$ 1.62	22.24 $\pm$ 1.47	7.85 ^a $\pm$ 0.49	53.80 ^d $\pm$ 1.31	-2.94 $\pm$ 0.71	48.33 $\pm$ 1.41
2(22)	132.45 ^d $\pm$ 1.84	21.39 $\pm$ 1.67	8.45 ^{ab} $\pm$ 0.55	49.54 ^b $\pm$ 1.49	-2.41 $\pm$ 0.81	51.40 $\pm$ 1.60
3(16)	119.54 ^c $\pm$ 1.81	23.80 $\pm$ 1.64	8.92 ^b $\pm$ 0.54	50.28 ^b $\pm$ 1.47	-1.87 $\pm$ 0.80	51.18 $\pm$ 1.57
4(9)	105.36 ^b $\pm$ 2.38	27.04 $\pm$ 2.16	9.68 ^c $\pm$ 0.71	47.38 ^a $\pm$ 1.93	-1.53 $\pm$ 1.05	51.77 $\pm$ 2.07
>5(11)	96.69 ^a $\pm$ 2.08	18.97 $\pm$ 1.89	10.05 ^c $\pm$ 0.62	45.82 ^a $\pm$ 1.69	-0.42 $\pm$ 0.92	53.83 $\pm$ 1.81
STAGE OF LACTATION						
1 (40)	119.36 $\pm$ 1.84	22.59 $\pm$ 1.66	8.72 $\pm$ 0.55	49.90 $\pm$ 1.48	-2.04 $\pm$ 0.81	52.43 $\pm$ 1.59
2 (21)	118.84 $\pm$ 2.17	21.86 $\pm$ 1.97	8.92 $\pm$ 0.65	51.72 $\pm$ 1.76	-1.75 $\pm$ 0.96	52.46 $\pm$ 1.88
3 (26)	123.15 $\pm$ 1.58	23.60 $\pm$ 1.43	9.33 $\pm$ 0.47	46.48 $\pm$ 1.28	-1.71 $\pm$ 0.69	49.02 $\pm$ 1.37

Means with different letter (a, b, c) indicates significant difference (P<0.05).

Table 4.12b Least squares means for udder and teat type traits in Sahiwal cattle

Effects	UW (cm)	UC (cm)	CL (cm)	TC (cm)	FTL (cm)
Overall (μ)	63.45 $\pm$ 1.20	142.64 $\pm$ 1.80	3.40 $\pm$ 0.19	8.64 $\pm$ 0.88	7.14 $\pm$ 1.00
SEASON					
Summer (52)	65.21 $\pm$ 1.43	143.94 $\pm$ 2.15	3.81 $\pm$ 0.23	10.02 ^b $\pm$ 1.05	6.79 $\pm$ 1.20
Rainy (13)	60.76 $\pm$ 2.61	138.65 $\pm$ 3.93	3.22 $\pm$ 0.43	4.75 ^a $\pm$ 1.92	7.39 $\pm$ 2.19
Autumn (6)	64.60 $\pm$ 3.55	144.71 $\pm$ 5.33	2.53 $\pm$ 0.58	4.99 ^a $\pm$ 2.61	8.18 $\pm$ 2.98
Winter(16)	63.23 $\pm$ 2.10	143.28 $\pm$ 3.16	4.02 $\pm$ 0.34	14.81 ^c $\pm$ 1.55	6.19 $\pm$ 1.76
PARITY					
1(29)	58.21 ^a $\pm$ 1.78	138.14 $\pm$ 2.68	3.10 $\pm$ 0.29	9.12 $\pm$ 1.31	5.13 $\pm$ 1.50
2(22)	61.28 ^b $\pm$ 2.02	142.08 $\pm$ 3.04	3.06 $\pm$ 0.33	6.97 $\pm$ 1.49	8.24 $\pm$ 1.70
3(16)	64.97 ^c $\pm$ 1.99	145.01 $\pm$ 2.99	3.63 $\pm$ 0.32	9.94 $\pm$ 1.46	7.82 $\pm$ 1.67
4(9)	68.69 ^c $\pm$ 2.62	143.91 $\pm$ 3.94	3.73 $\pm$ 0.43	7.45 $\pm$ 1.93	6.65 $\pm$ 2.20
>5(11)	64.10 ^d $\pm$ 2.29	144.08 $\pm$ 3.45	3.46 $\pm$ 0.37	9.73 $\pm$ 1.69	7.85 $\pm$ 1.92
STAGE OF LACTATION					
1 (40)	64.76 $\pm$ 2.01	147.99 ^a $\pm$ 3.03	3.22 $\pm$ 0.33	8.23 ^b $\pm$ 1.48	8.50 $\pm$ 1.69
2 (21)	62.34 $\pm$ 2.39	142.03 ^b $\pm$ 3.59	3.09 $\pm$ 0.39	4.46 ^a $\pm$ 1.76	6.76 $\pm$ 2.00
3 (26)	63.26 $\pm$ 1.74	137.91 ^c $\pm$ 2.61	3.88 $\pm$ 0.28	13.23 ^c $\pm$ 1.28	6.15 $\pm$ 1.46

Means with different letter (a, b, c) indicates significant difference (P<0.05).

Table 4.12c Least squares means for udder and teat type traits in Sahiwal cattle

Effects	RTL (cm)	DFR (cm)	DLR (cm)	SDR (cm)	TD (cm)	SDF (cm)
Overall (μ)	6.15 ± 1.12	4.59 ± 0.81	5.81 ± 0.88	41.28 ± 1.04	2.71 ± 0.08	42.46 ± 1.00
SEASON						
Summer (52)	4.66 ± 0.17	6.01 ± 0.60	8.11 ± 0.74	47.31 ± 0.86	2.21 ± 0.05	46.43 ± 0.88
Rainy (13)	4.54 ± 0.23	5.25 ± 0.81	8.05 ± 1.00	48.76 ± 1.16	2.20 ± 0.07	48.84 ± 1.19
Autumn (6)	4.43 ± 0.22	7.18 ± 0.74	9.13 ± 0.92	46.37 ± 1.07	2.22 ± 0.06	46.76 ± 1.10
Winter(16)	4.85 ± 0.19	7.80 ± 0.65	10.16 ± 0.81	45.56 ± 0.94	2.23 ± 0.05	45.93 ± 0.96
PARITY						
1 (29)	3.89 ± 0.15	7.41 ± 0.53	9.35 ± 0.66	53.39 ± 0.77	1.99 ^a ± 0.04	53.53 ± 0.78
2 (22)	4.44 ± 0.16	6.63 ± 0.57	9.33 ± 0.71	48.33 ± 0.82	2.20 ^b ± 0.05	48.48 ± 0.84
3 (16)	4.99 ± 0.25	6.01 ± 0.85	8.14 ± 1.06	46.88 ± 1.22	2.30 ^b ± 0.07	46.69 ± 1.25
4 (9)	4.83 ± 0.22	5.96 ± 0.76	8.67 ± 0.94	45.52 ± 1.09	2.24 ^b ± 0.06	44.65 ± 1.12
>5 (11)	4.95 ± 0.32	6.81 ± 1.08	8.83 ± 1.35	40.89 ± 1.55	2.36 ^b ± 0.09	41.60 ± 1.59
STAGE OF LACTATION						
1 (40)	7.11 ± 1.89	3.04 ^a ± 1.37	3.99 ^a ± 1.48	45.07 ± 1.68	2.74 ± 0.13	43.65 ± 1.74
2 (21)	5.99 ± 2.23	2.90 ^a ± 1.62	3.43 ^a ± 1.76	42.77 ± 1.99	2.70 ± 0.16	41.13 ± 2.07
3 (26)	5.35 ± 1.63	7.83 ^b ± 1.18	9.99 ^b ± 1.28	39.54 ± 1.45	2.68 ± 0.1	39.05 ± 1.50

Means with different letter (a, b, c) indicates significant difference (P<0.05).

4.9.3. Effect of non-genetic factors on incidence of clinical mastitis in Karan Fries and Sahiwal cattle

For the analysis of dichotomous or binary trait like incidence of clinical mastitis, logistic regression analysis was used to evaluate the effect of season, parity and level of production on the incidence of clinical mastitis. A multiple logistic regression model was fitted incorporating all the non-genetic factors. The probability is modeled to the event of incidence of non -mastitis. Incidence of clinical mastitis in both Karan Fries and Sahiwal cattle breeds in different parity, season and level of lactation has been presented in Table 4.13. In Karan Fries cattle, the percentage of incidence of mastitis was maximum in fourth parity (41.1%) similarly rainy season and high level of milk production showed maximum percentage of incidence of clinical mastitis viz., 30.5% and 31.0%, respectively (Table 4.13).

In Karan Fries cattle, Wald Chi square analysis revealed that parity had significant effect for incidence of clinical mastitis ($P \leq 0.05$). The incidence of clinical mastitis was lesser in the Parity (1), Parity (2) and Parity (>5) in comparison to the Parity (3) and Parity (4). The odds ratio of Parity (3) for the non-incidence of mastitis was highest (7.799) indicating that third parity was having the maximum odds of being associated with mastitis (Table 4.14). This indicated that for Parity (3) the incidence of clinical mastitis was 779.9% more as compared to Parity (>5) with confidence interval 2.204-27.598 (Table 4.14).

In Sahiwal cattle, the percentage of incidence of mastitis was maximum in fourth parity (52.8%), similarly, rainy season and high level of milk production showed maximum percentage of incidence of clinical mastitis viz., 30.4% and 31.3%, respectively (Table 4.13). Wald Chi square analysis revealed that parity and level of production had significant effect on incidence of clinical mastitis in Sahiwal cows. The incidence of clinical mastitis was lesser in the Parity(1), Parity(2), Parity(3) and Parity(>5) in comparison to the Parity(4), as odds ratio of Parity(4) against Parity(>5) for the incidence of non-mastitis was higher i.e. 5.167. This indicated that the odds of incidence of clinical mastitis were 516.7% (CI: 0.632-42.227) more in Parity(4) as compared to other parity classes (Table 4.15).

These results support the previous studies of Jingar *et al.* (2014) who reported that parity significantly influenced mastitis incidence in indigenous ($P < 0.05$) and crossbred cows ($P < 0.01$). The prevalence of mastitis for indigenous and crossbred cows of India was significantly higher in multiparous cows (ranged between 30.34% to 40.16%) than in primiparous cows (16.36 to 22.00%) (Bangar *et al.*, 2018). The increased incidence of mastitis with increased parity suggests that multiparous cows consistently suffer severe yield

losses than primiparous cows due to their higher milk yield and also effectiveness of streak canal as barrier to infection decreases (Wilson *et al.*, 2004, Khate and Yadav, 2010).

Table 4.13 Incidence of clinical mastitis under different categories of non genetic factors in Karan Fries and Sahiwal cattle

Non-Genetic	Karan Fries		Sahiwal	
	N = 166	Incidence (%)	N = 87	Incidence (%)
Factors				
Parity(1)	57	8.0%	24	3.0%
Parity(2)	48	15.70%	20	19.62%
Parity(3)	22	34.30%	18	17.30%
Parity(4)	26	41.10%	14	52.80%
Parity(>5)	13	15.40%	11	27.20%
Season(1)	62	19.30%	52	24.90%
Season(2)	28	30.50%	13	30.40%
Season(3)	32	23.40%	6	21.00%
Season(4)	44	19.30%	16	17.10%
High (1)	58	31%	27	31.30%
Medium (2)	82	23%	34	22.20%
Low (3)	26	14%	26	16.54%
Over all incidence	20.40%		17.20%	

Table 4.14 Effect of non-genetic factors on incidence of clinical mastitis in Karan Fries cattle

Effect	Mastitis			
	Estimate $\pm$ SE	Wald Chi sq	Odds ratio	95% CI
Intercept		11.831***		
Parity				
Parity (1)	0.694 $\pm$ 0.629	1.217	2.001	0.584 - 6.860
Parity (2)	1.734 $\pm$ 0.662	6.857**	5.661	1.546 - 20.723
Parity (3)	2.054 $\pm$ 0.645	10.147***	7.799	2.204 - 27.598
Parity (4)	0.579 $\pm$ 0.938	0.381	1.785	0.284 - 11.226
Parity (>5)		13.518**		
Season				
Summer (1)	0.360 $\pm$ 0.608	0.352	1.434	0.436 - 4.716
Rainy (2)	0.623 $\pm$ 0.568	1.203	1.865	0.612 - 5.682
Autumn (3)	-0.052 $\pm$ 0.583	0.008	0.950	0.303 - 2.974
Winter (4)		1.770		
Level of production				
High (1)	-0.537 $\pm$ 0.460	1.364	0.585	0.238 - 1.439
Medium (2)	-1.254 $\pm$ 0.722	3.020	0.285	0.069 - 1.174
Low (3)		3.453		

*Significant at $P < 0.05$; ** Significant at $P < 0.01$

Sahiwal cattle with medium and low milk production were having lesser incidence of clinical mastitis in comparison to high milk producing animals as odds ratio of high and medium milk producing animals against Low (Level 3) producing animals for the non-incidence of mastitis was higher i.e. 8.216 and 7.681, respectively. The odds ratio revealed the incidence of clinical mastitis was 721.6% more in high milk producing animals as compared to low milk producing animals with confidence interval 1.596-42.296. The odds ratio of medium milk producing animals revealed the incidence of clinical mastitis was 668.1% more in medium milk producing animals as compared to low milk producing animals with confidence interval 1.174 - 50.244 (Table 4.15).

Table 4.15 Effect of non-genetic factors on incidence of clinical mastitis in Sahiwal cattle

Effect	Mastitis			
	Estimate ± SE	Wald Chi sq	Odds ratio	95% CI
Intercept		6.072		
Parity				
Parity (1)	-20.258±	0.000	0.000	0.000
Parity (2)	-0.120±0.946	0.016	0.887	0.139-5.668
Parity (3)	-0.661±1.011	0.428	0.516	0.071-3.743
Parity (4)	1.642±1.072	2.348*	5.167	0.632-42.227
Parity (>5)		6.072		
Season				
Summer (1)	0.325-0.860	0.143	1.384	0.257-7.468
Rainy (2)	0.691±1.066	0.420	1.996	0.247-16.116
Autumn (3)	0.401±1.517	0.070	1.494	0.076-29.182
Winter (4)		0.425		
Level of production				
High (1)	2.106±0.836	6.346**	8.216	1.596-42.296
Medium (2)	2.039±0.958	4.526*	7.681	1.174-50.244
Low (3)		6.954		

*Significant at P<0.05; ** Significant at P<0.01

These results are in accordance of Jingar *et al.* (2014), who reported that the level of milk production significantly affected the incidence of clinical mastitis in SW and KF cows. The animals having higher or lower milk yield suffered more with incidence of mastitis in

comparison to medium. A decreasing mastitis incidence in order of early, mid and late lactation in indigenous (SW and TP) cows was reported by Singh *et al.* (2001). The incidence of mastitis was highest in early lactation stage in SW cattle may be due to non-adaptation of animals to the milking methods, more udder pressure and weaning associated milking behaviour (Chishty *et al.*, 2007 and Kavitha *et al.*, 2009). Highest incidence of mastitis was reported in many breeds of cattle during early lactation (Grohn *et al.*, 2004; Wilson *et al.*, 2004; Biffa *et al.*, 2005; Kocak, 2006; Zwald *et al.*, 2006; Steeneveld *et al.*, 2008; Fadlemula *et al.*, 2009; Corbett, 2009; Sharma, *et al.*, 2011). In contrast the effect of level of milk production was not found to be significant in Karan Fries cattle. This might be due to less variability among the production levels of the studied Karan Fries cows.

4.10. Correlation between udder and teat type traits (measurements) and milk production traits

The Pearson correlation among the milk production traits and udder type traits were calculated using SPSS. Results of the correlation study have been listed in Table 4.16 and Table 4.17 for Karan Fries and Sahiwal cows, respectively.

In Karan Fries cows, moderate to high correlation was observed among udder length and udder width; rear teat length and fore teat length; distance between left and right teats (DLR) and distance between fore and rear teats (DFR); shortest distance from rear teat ends to floor (SDR) and fore teat ends to floor (SDF); shortest distance from fore teat ends to floor (SDF) and udder depth; shortest distance from rear teat ends to floor and udder depth. In zebu cows, highly positive genetic correlations (0.84 ± 0.02) were found among udder length and udder width (Mingoas *et al.*, 2017) which supports the correlation among udder length and udder width in the present study. A positive and highly significant correlation ($r_p = 0.60$) was found between udder depth and height in zebu cattle (Mingoas *et al.*, 2017). Dubey (2010) studied linear type traits in Sahiwal and revealed udder depth was positively correlated with rear udder height, fore udder attachment, front and rear teat placement. Udder height, rear udder height, fore udder attachment and teat placement contribute to distance from teat ends to floor which might be the reason for moderate to high correlation among shortest distance from teat ends to floor (SDF and SDR) and udder depth. There was moderate to high correlation among all the milk production traits viz. total milk yield, test day milk yield, monthly milk yield and 305-day milk yield. Low to moderate correlation was observed among distance between left and right teats (DLR) and udder width; teat diameter and teat circumference; teat diameter and fore teat length; udder circumference and udder width, monthly milk yield and test day milk yield (Table 4.16 and Figure 4.11). Udder

Table 4.16 Correlation matrix of udder and teat type traits and milk production traits in Karan Fries cattle

	RUH	RUW	FUA	UD	UB	UL	UW	UC	CL	DLR	DFR	SDF	SDR	FTL	RTL	TC	TD	T1	T2	T3	T4
RUH	1.00																				
RUW	0.12	1.00																			
FUA	-0.17	-0.02	1.00																		
UD	0.05	0.01	0.13	1.00																	
UB	-0.04	0.12	0.06	0.12	1.00																
UL	-0.16	0.17	0.00	-0.24	0.02	1.00															
UW	-0.16	0.08	-0.08	-0.38	0.04	0.54	1.00														
UC	-0.31	0.20	0.17	-0.13	0.02	0.27	0.29	1.00													
CL	-0.02	-0.13	0.00	-0.14	-0.10	-0.05	0.05	0.14	1.00												
DLR	-0.05	-0.02	-0.05	-0.23	0.02	0.31	0.46	0.13	0.03	1.00											
DFR	-0.15	0.08	-0.03	-0.15	0.07	0.22	0.27	0.14	-0.01	0.69	1.00										
SDF	0.00	-0.05	0.10	0.62	0.03	-0.28	-0.38	-0.18	-0.29	-0.21	-0.17	1.00									
SDR	0.07	0.04	0.12	0.65	0.02	-0.46	-0.53	-0.22	-0.21	-0.35	-0.24	0.86	1.00								
FTL	-0.01	0.07	0.10	0.01	0.05	0.00	0.13	0.01	0.18	0.12	0.12	-0.16	-0.06	1.00							
RTL	0.03	0.11	0.13	0.02	0.07	0.11	0.10	0.00	0.07	0.04	0.02	-0.04	-0.08	0.67	1.00						
TC	0.09	-0.04	0.02	-0.10	0.19	-0.01	0.11	0.07	0.04	0.12	-0.02	-0.14	-0.16	0.16	0.17	1.00					
TD	-0.05	0.11	-0.03	-0.02	0.14	0.18	0.21	0.08	-0.03	0.12	0.09	-0.10	-0.08	0.34	0.27	0.48	1.00				
T1	0.03	0.01	0.04	0.04	0.07	0.04	0.07	0.18	0.13	0.12	0.15	-0.09	-0.07	-0.04	0.06	0.08	0.03	1.00			
T2	-0.25	-0.04	0.14	0.00	0.04	0.10	0.16	0.34	0.19	0.00	0.00	-0.10	-0.09	-0.07	-0.02	0.15	0.01	0.54	1.00		
T3	-0.24	0.07	0.15	0.01	-0.01	0.09	0.14	0.34	0.12	-0.03	-0.02	-0.05	-0.04	-0.11	-0.05	0.09	-0.03	0.51	0.85	1.00	
T4	0.03	0.02	0.03	0.04	0.05	0.05	0.07	0.18	0.13	0.12	0.15	-0.09	-0.07	-0.04	0.06	0.08	0.03	0.99	0.56	0.53	1.00

T1 – Milk yield (305d), T2 – Monthly milk yield, T3- Test day milk yield, T4 – Total milk yield

Table 4.17 Correlation matrix of udder and teat type traits and milk production traits in Sahiwal cattle

	RUH	RUW	UW	FUA	UC	CL	RTL	UD	UL	TC	UB	FTL	DFR	DLR	SDF	SDR	TD	T1	T2	T3	T4
RUH	1.00																				
RUW	0.99	1.00																			
UW	0.17	0.17	1.00																		
FUA	0.15	0.13	0.06	1.00																	
UC	-0.08	-0.11	0.16	0.08	1.00																
CL	0.02	0.00	0.18	0.09	0.15	1.00															
RTL	-0.06	-0.07	0.23	-0.15	-0.01	-0.03	1.00														
UD	0.14	0.13	0.15	0.21	-0.14	0.24	0.11	1.00													
UL	-0.14	-0.18	-0.02	0.04	0.43	0.01	-0.09	-0.12	1.00												
TC	-0.06	-0.06	0.15	0.23	-0.11	0.12	0.01	-0.05	-0.04	1.00											
UB	-0.10	-0.14	0.02	0.06	0.08	0.20	0.15	0.25	0.06	0.12	1.00										
FTL	0.00	-0.02	0.22	-0.07	0.02	-0.05	0.96	0.08	-0.01	0.08	0.17	1.00									
DFR	0.03	0.04	0.05	-0.13	0.00	0.06	-0.01	0.02	0.23	-0.12	-0.06	-0.02	1.00								
DLR	-0.02	-0.01	0.06	-0.05	-0.05	0.18	-0.06	-0.01	0.08	-0.12	0.04	-0.07	0.73	1.00							
SDF	-0.07	-0.03	-0.14	0.04	0.10	-0.01	0.03	0.04	-0.10	0.08	0.03	0.02	-0.56	-0.65	1.00						
SDR	0.00	0.04	-0.11	-0.02	0.02	0.01	-0.01	0.10	-0.19	0.05	0.00	-0.03	-0.51	-0.67	0.91	1.00					
TD	-0.17	-0.20	0.04	-0.04	0.02	0.16	0.04	0.02	0.08	0.02	0.33	0.04	-0.13	0.04	-0.24	-0.34	1.00				
T1	0.18	0.17	1.00	0.06	0.16	0.18	0.23	0.15	-0.01	0.15	0.03	0.22	0.05	0.06	-0.14	-0.11	0.04	1.00			
T2	0.34	0.30	0.41	-0.06	0.30	0.10	0.04	0.05	0.27	0.05	-0.02	0.06	0.13	0.04	-0.07	-0.11	0.01	0.41	1.00		
T3	0.10	0.09	0.39	-0.11	0.42	0.02	0.13	0.00	0.33	0.07	0.01	0.14	0.19	-0.09	0.06	0.03	0.05	0.39	0.59	1.00	
T4	0.20	0.16	0.89	0.17	0.18	0.19	0.19	0.13	0.01	0.16	0.09	0.19	-0.01	0.02	-0.16	-0.13	0.02	0.89	0.40	0.29	1.00

T1 – Milk yield (305d), T2 – Monthly milk yield, T3- Test day milk yield, T4 – Total milk yield

circumference (contributing to udder width and udder length) had strong, positive phenotypic correlation with teat angle [contributing to distance between left and right teats (DLR)] (Sezenler *et al.*, 2016). This might be the reason for low to moderate correlation among distance between left and right teats (DLR) and udder width. A strong positive correlation ($r_p=0.60$) between the length (39.51 ± 6.44 mm) and diameter (19.85 ± 3.08 mm) of teats ($r_p=0.78$) were found in the zebu cows (Mingoas *et al.*, 2017) which corroborates with the correlation among teat diameter and fore teat length. Udder circumference had strong, positive phenotypic correlation with udder width (Sezenler *et al.*, 2016) which was in support of low to moderate correlation of udder circumference and udder width of present investigation. Low to moderate correlation was observed among teat diameter and fore teat length which is in concurrence with highly significant ($P<0.01$) correlations observed between teat length and teat diameter by Khatri *et al.* (2017).

In Sahiwal cows, moderate to high correlation was observed among udder width and total milk yield, test day milk yield, monthly milk yield and 305 day milk yield; fore teat length and rear teat length; udder length and udder circumference; rear udder width and rear udder height; distance between left and right teats (DLR) and distance between fore and rear teats (DFR); shortest distance from rear teat ends to floor (SDR) and fore teat ends to floor (SDF); total milk yield and 305 day milk yield; test day milk yield and monthly milk yield. Low to moderate correlation was observed among monthly milk yield and rear udder width, rear udder height, udder circumference; test day milk yield and udder circumference; rear teat length and 305 day milk yield; udder length and monthly milk yield, test day milk yield; udder balance and udder depth, teat diameter; rear teat length and udder width; 305 day milk yield and monthly milk yield, test day milk yield; monthly milk yield and total milk yield (Table 4.17 and Figure 4.12). The correlation observed among udder width and milk production in the present study is in accordance with the previous findings by Modh *et al.* (2017), Khatri *et al.* (2017) and Khan and Khan (2016) who reported positive and significant ($p<0.05$) correlation between milk yield and udder width in cattle and buffaloes. 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 (Khatri *et al.*, 2017). In zebu cows, there are reports of significant correlations between udder diameter and height with milk yield and also between the udder size (depth and height) and milk yield (Mingoas *et al.*, 2017). All these findings from previous studies are supporting the correlation observed among monthly milk yield and rear udder width, rear udder height, udder circumference; test day milk yield and udder circumference; udder length and milk yield. Dubey (2010) reported

positive correlation between teat length with first lactation milk yield which is in accordance with the positive correlation found among rear teat length and 305-day milk yield in the present study. Highly significant ($P<0.01$) correlations were observed between various teat measurements, viz., fore teat length, rear teat length, fore teat diameter and rear teat diameter (Khatri *et al.*, 2017). These reports support the correlation observed among fore teat length and rear teat length, distance between left and right teats (DLR) and distance between fore and rear teats (DFR) ; shortest distance from rear teat ends to floor (SDR) and fore teat ends to floor (SDF) in the present study. A strong positive correlation ($r_p=0.60$) between udder diameter (contributing to circumference) (240.21 ± 28.58 mm) and height (131.12 ± 23.64 mm) of udders ($p<0.001$) was found in the zebu cows (Mingoas *et al.*, 2017). Udder circumference had strong, positive estimates of phenotypic correlation with udder width (Sezenler *et al.*, 2016). These studies explain the moderate to high correlation among rear udder width and rear udder height in present investigation. Udder circumference had strong, positive phenotypic correlation with udder length (Sezenler *et al.*, 2016) which corroborates with the correlation among udder length and udder circumference in the present study. Dubey (2010) studied linear type traits in Sahiwal and found udder balance to be positively correlated with teat diameter and udder height. A positive and highly significant correlation was found between udder depth and height in zebu cattle (Mingoas *et al.*, 2017). These studies support the correlation found among udder balance with teat diameter and udder depth in the present study. Positive and significant association of udder width with average rear teat length and overall teat length was observed in cattle (Ahlawat *et al.*, 2008, Singhai *et al.*, 2013). Significant positive correlation was recorded among teat length and udder height in cattle (Dubey, 2010). Strong positive correlation was reported between the diameter (contributing to udder width) and height of udders in zebu cows (Mingoas *et al.*, 2017). These findings are in concordance with correlation among teat length and udder width revealed in the present study.

4.11. Classification of udder and teat type traits in Sahiwal and Karan Fries cattle

International Committee for Animal Recording (ICAR) has recommended use of type traits in describing the dairy cattle. In present investigation, Sahiwal cows and Karan Fries cows were studied using the system as recommended by ICAR (2001).

4.11.1. Rear udder height (RUH)

Rear udder height indicates the distance from top of the milk secreting tissue to bottom of the vulva (as shown in Figure 3.1 of Materials and Methods). In present investigation, the mean value for this trait was 23.44 ± 0.41 cm, ranging from 11.43 to 41.91

Figure 4.11 Correlation between continuous udder type traits and milk production traits in Karan Fries cattle

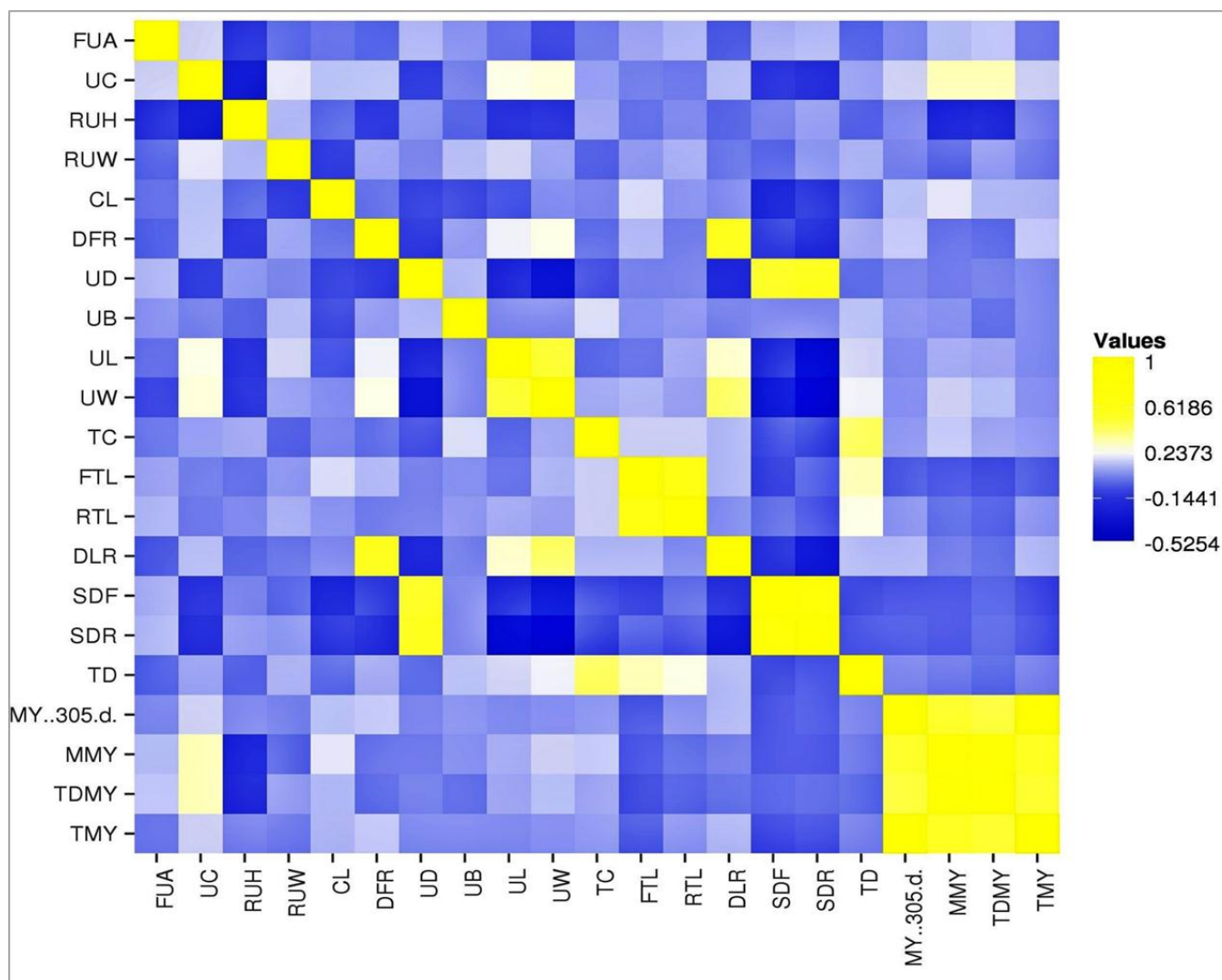
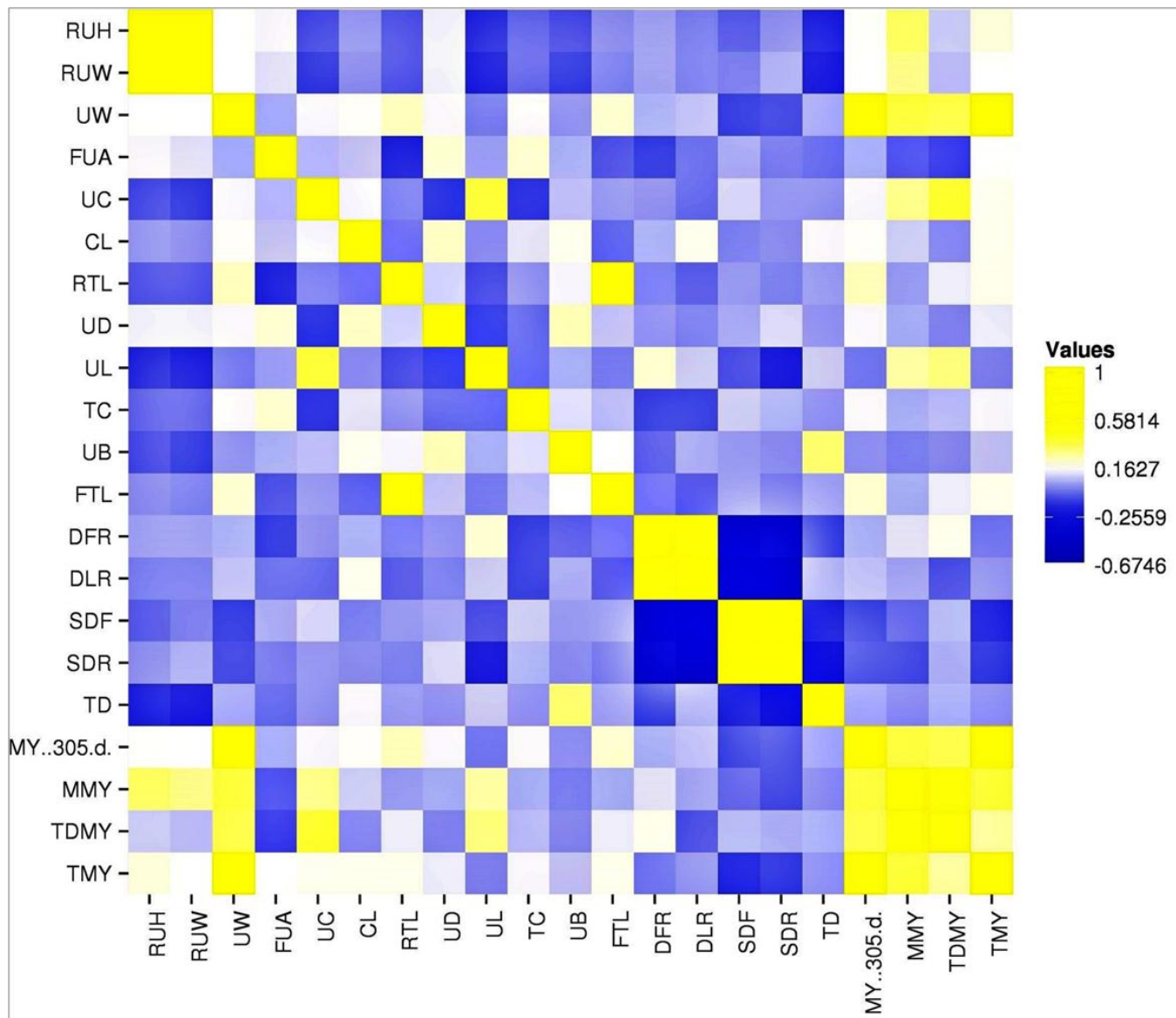


Figure 4.12 Correlation between continuous udder type traits and milk production traits in Sahiwal cattle



cm (Table 4.18) in Karan Fries cattle, while, in Sahiwal, the mean value for this trait was 22.52 ± 0.65 cm ranging from 8.89 cm to 36.87 cm (Table 4.19). In 1-9 point scale scoring system, the rear udder height was classified as low, intermediate and high groups having score range of 1-3, 4-6 and 7-9 score point respectively for each group. The maximum frequency was observed in intermediate group in both breeds which was followed by high and low rear udder height groups in Sahiwal cattle, but in Karan Fries cattle it was followed by low and high rear udder height groups. The number of animals against each class and their frequency are given in the Table 4.20 for Karan Fries and Sahiwal cattle.

As per the recommendation of ICAR, the high rear udder height is considered better than other groups as this will prevent sagging of the udder, causes a smaller udder distance from the floor and the udder base placed below the hock joint in the animal. In Sahiwal cows, the average score point was found to be in intermediate height for rear udder. This finding is in accordance with Wall *et al.* (2005) and Berry (2007) in Holstein-Friesian cows as they obtained intermediate rear udder height with average score point of 5.7 ± 1.4 cm and 5.8 ± 0.59 cm respectively, under 1-9 point scale scoring system. The average score point in Sahiwal and Holstein-Friesian cows were in intermediate group. In spite of this, a variation in average score point was clearly seen, which made differences of udder capacity in both the breed leading to production less milk in Sahiwal cows in comparison to Holstein-Friesian. However, Mitra *et al.* (1998) obtained the high rear udder height in Karan Fries with average score point of 82.75 ± 0.55 cm under 50-99 score point scale which support the present findings in Karan Fries cattle. In the aforesaid score point scale, the score range of 50-59, 60-69, 70-79, 80-89 and 90-99 denoted extremely low, low, intermediate, high and extremely high rear udder height. The level of udder depth and rear height of udder depends upon the ligament (commonly known as udder's suspensory apparatus) which is responsible for the attachment of the udder to the ventral abdominal wall and the pelvic floor as reported by Atkins (2007). Thus, difference in the arrangement of this ligament caused variation in depth and height of udder.

4.11.2 Rear udder width (RUW)

Rear udder width is evaluated at the point of attachment. Like rear udder height, rear udder width is an indicator of udder capacity (as shown in Figure 3.2 of Materials and Methods). In present investigation, the mean value for this trait was 12.62 ± 0.2 cm, ranging from 7.23 to 20.35 cm (Table 4.18) in Karan Fries cattle, while, in Sahiwal, the mean value for this trait was 22.85 ± 0.66 cm (Table 4.19) ranging from 4.82 cm to 15.24 cm. In 1-9

point scale scoring system, the rear udder width was classified as low, intermediate and high groups having score range of 1-3, 4-6 and 7-9 score point respectively for each group. The maximum frequency was observed in intermediate group which was followed by low and high rear udder width groups in both the breeds. The number of animals against each class and their frequency is given in the Table 4.20 for Karan Fries and Sahiwal, respectively.

The present results are in accordance with Lande (2018) who reported maximum number of medium size rear udder (68.50 %) followed by small size rear udder (27.75 %) in indigenous Red Kandhari cattle. Rear udder width is evaluated at the point of attachment and it is an indicator of udder capacity so a wider rear udder is more preferable for milk production. But a maximum frequency of intermediate udder is desirable for less susceptibility to mastitis as well as higher milk production (Stamschror, 2000). Singhai *et al.* (2013) reported that the mean udder width in Gir cows was 20.51 ± 0.99 cm. Patel *et al.* (2016) found the average width of udder were 65.45 ± 0.70 cm in the milking herd of crossbred cows. Modh *et al.* (2017) studied the width of udder in Gir cows and recorded an average value of 62.99 ± 1.17 cm.

4.11.3. Udder length (UL)

Udder length is measured as the distance from the rear attachment of udder to the point where fore udder blends smoothly with the body passing cloth tape in between right and left teats (as shown in Figure 3.3 of Materials and Methods). In present investigation, the mean value for this trait was 60.47 ± 0.58 cm, ranging from 46.57 to 94.01 cm (Table 4.18) in Karan Fries cattle. In Sahiwal cattle, the mean value for this trait was 50.82 ± 0.66 cm ranging from 39.37 cm to 68.61 cm (Table 4.19). In 1-9 point scale scoring system, the udder length was classified as low, intermediate and high groups having score range of 1-3, 4-6 and 7-9 score point respectively for each group. The maximum frequency was observed in low and intermediate group in Karan Fries and Sahiwal, respectively; which was followed by intermediate and high udder length groups in Karan Fries and low and high Udder length group in Sahiwal. The number of animals against each class and there frequency is given in the Table 4.20 for Karan Fries and Sahiwal.

The present result is in accordance with Patel *et al.* (2016) found the average length of udder was 58.24 ± 0.68 cm in the milking herd of crossbred cows. Prasad *et al.* (2010a) worked on dairy cows of Bangladesh and recorded the range for UL as 46-82 cm and found the measurements to increase with increase in lactation number. Modh *et al.* (2017) studied the average length of udder was 61.95 ± 1.20 cm in cows.

Table 4.18 Descriptive statistics of udder and teat type traits (measurements) and milk production traits in Karan Fries cattle

Trait name	Mean $\pm$ SE	Standard deviation	Sample variance	Range	Minimum	Maximum
Rear udder height (cm)	23.44 $\pm$ 0.41	5.30	28.06	28.96	9.54	38.50
Rear udder width (cm)	12.62 $\pm$ 0.2	2.62	6.85	16.41	5.59	22.00
Udder width (cm)	71.42 $\pm$ 0.68	8.82	77.85	54.35	45.00	99.35
Fore udder attachment (degree)	131.46 $\pm$ 1.23	15.85	251.32	92.00	82.00	174.00
Udder circumference (cm)	142.74 $\pm$ 1.96	25.28	638.96	176.86	39.92	216.78
Central ligament (cm)	3.15 $\pm$ 0.11	1.43	2.06	8.13	0.00	8.13
Rear teat length (cm)	4.66 $\pm$ 0.09	1.12	1.25	4.83	2.55	7.39
Udder depth (cm)	53.65 $\pm$ 0.46	5.96	35.51	42.16	29.55	71.72
Udder length (cm)	60.47 $\pm$ 0.58	7.47	55.79	53.34	38.85	92.19
Teat circumference (cm)	7.54 $\pm$ 0.12	1.54	2.38	10.75	0.30	11.05
Udder balance (cm)	1.04 $\pm$ 0.44	5.68	32.31	40.11	-23.11	17.00
Fore teat length (cm)	5.30 $\pm$ 0.11	1.47	2.16	8.81	2.75	11.56
Distance between front and rear teat (cm)	6.75 $\pm$ 0.30	3.86	14.92	46.10	1.78	47.88
Distance between right and left teat (cm)	8.99 $\pm$ 0.37	4.77	22.76	46.23	0.89	47.12
Shortest distance from front teat end to floor (cm)	46.93 $\pm$ 0.43	5.51	30.39	28.45	30.82	59.27
Shortest distance from rear teat end to floor (cm)	46.79 $\pm$ 0.44	5.64	31.86	28.17	29.90	58.07
Teat diameter (cm)	2.22 $\pm$ 0.03	0.33	0.11	2.06	1.50	3.56
Milk yield (305d) (kg)	4132.04 $\pm$ 139.94	1802.96	3250682.13	9509.49	145.30	9654.80
Monthly milk yield (kg)	371.66 $\pm$ 11.56	148.89	22169.20	660.54	10.15	670.69
Test day milk yield (kg)	13.08 $\pm$ 0.38	4.88	23.86	22.84	1.39	24.22
Total milk yield (kg)	4076.84 $\pm$ 142.69	1838.44	3379872.47	9289.00	129.50	9418.50

Table 4.19 Descriptive statistics of udder and teat type traits (measurements) and milk production trait in Sahiwal cattle

Trait name	Mean $\pm$ SE	Standard deviation	Sample variance	Range	Minimum	Maximum
Rear udder height (cm)	22.52 $\pm$ 0.65	6.08	37.00	29.21	7.62	36.83
Rear udder width (cm)	22.85 $\pm$ 0.66	6.13	37.61	30.34	6.56	36.90
Udder width (cm)	1921.34 $\pm$ 104.14	971.36	943546.56	3872.00	73.62	3945.62
Fore udder attachment (degree)	107.10 $\pm$ 2.07	19.30	372.56	117.00	33.00	150.00
Udder circumference (cm)	142.19 $\pm$ 1.17	10.94	119.67	48.60	114.97	163.56
Central ligament (cm)	3.57 $\pm$ 0.13	1.20	1.43	6.35	0.09	6.35
Rear teat length (cm)	6.13 $\pm$ 0.71	6.62	43.76	64.77	2.27	64.77
Udder depth (cm)	48.7 $\pm$ 0.58	5.45	29.70	36.36	24.33	60.69
Udder length (cm)	50.82 $\pm$ 0.66	6.15	37.85	29.27	39.31	68.58
Teat circumference (cm)	8.76 $\pm$ 0.55	5.10	26.03	48.17	1.40	49.57
Udder balance (cm)	-2.26 $\pm$ 0.32	2.95	8.69	16.51	-11.43	5.08
Fore teat length (cm)	6.88 $\pm$ 0.65	6.02	36.25	56.52	3.18	59.69
Distance between front and rear teat (cm)	6.09 $\pm$ 0.55	5.14	26.44	46.86	-1.71	45.15
Distance between right and left teat (cm)	7.19 $\pm$ 0.59	5.46	29.81	49.15	2.29	51.44
Shortest distance from front teat end to floor (cm)	42.23 $\pm$ 0.67	6.24	38.94	51.18	4.70	55.88
Shortest distance from rear teat end to floor (cm)	43.11 $\pm$ 0.67	6.29	39.63	46.74	6.60	53.34
Teat diameter (cm)	2.60 $\pm$ 0.54	0.50	2.52	2.65	1.80	4.45
Milk yield (305d) (kg)	1919.94 $\pm$ 104.15	971.45	943722.21	3872.00	71.45	3943.45
Monthly milk yield (kg)	204.06 $\pm$ 14.25	132.94	17673.36	492.50	9.00	501.50
Test day milk yield (kg)	8.04 $\pm$ 0.45	4.15	17.25	20.17	0.17	20.34
Total milk yield (kg)	1848.97 $\pm$ 127.45	1188.74	1413095.52	5385.00	28.00	5413.00

4.11.4. Udder depth (UD)

The difference between two measurements a) vertical distance from floor to the base of udder near flank, b) vertical distance from floor to the udder floor at lowest point is taken as udder depth (as shown in Figure 3.1 of Materials and Methods). In KF cattle, the mean value for UD was 53.65 ± 0.46 cm ranging from 36.17 to 73.66 cm (Table 4.18). While, in Sahiwal, the mean value for UD was 48.7 ± 0.58 cm, ranging from 24.63 cm to 59.99 cm (Table 4.19). In 1-9 point scale scoring system, the rear udder height was classified as low, intermediate and high groups having score range of 1-3, 4-6 and 7-9 score point respectively for each group. The maximum frequency was observed in intermediate and high group in Karan Fries and Sahiwal, respectively; which was followed by high and low udder depth groups in Karan Fries and intermediate and low udder depth groups in Sahiwal. The number of animals against each class and their frequency has been given in the Table 4.20.

The depth of udder is distance from lowest part of the udder floor to the hock. 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 the type traits to functional longevity (Larroque and Ducrocq, 2001). The present findings are in accordance with Perez-Cabal and Alenda (2002) and Berry (2007), where they obtained the intermediate udder depth with average score point of 5.2 ± 1.4 and 5.5 under 1-9 score point scale. However, Mitra *et al.* (1988) had obtained udder floor clearly above the hock in Karan Fries cattle with average score point of 82.15 ± 0.89 under 50-99 score point scale. In the aforesaid 50-99 score point scale, the score range of 50-59, 60-69, 70-79, 80-89 and 90-99 denotes extremely deep, udder floor clearly below hock, udder floor at hock, udder floor clearly above hock and extremely shallow udder, respectively.

In the present study udder depth was found to be intermediate in Karan Fries with lesser frequency of animals in shallow group. Thus, it shows that there is still much scope for development of this important udder trait in Sahiwal cattle to make it a better milch breed.

4.11.5. Udder circumference (UC)

Average of two measurements taken as (a) measuring tape was passed round the udder near the base of udder close to body, (b) measuring tape was passed round the udder near the emergence of teats measures the udder circumference (as shown in Figure 3.3 of Materials and Methods). In KF cattle, the mean value for UC was 142.74 ± 1.96 cm ranging from 90.95 cm to 225.29 cm (Table 4.18). In Sahiwal cattle, the mean value for UC was 142.19 ± 1.17 cm, ranging from 110.23 cm to 168.99 cm (Table 4.19). In 1-9 point scale

scoring system, the udder circumference was classified as small/short, intermediate and large groups having score range of 1-3, 4-6 and 7-9 score point respectively for each group. The maximum frequency was observed in intermediate udder circumference group; which was followed by small and large circumference groups in Karan Fries and large and small udder circumference group in Sahiwal. The number of animals against each class and there frequency, for both KF and Sahiwal cattle, have been mentioned in the Table 4.20.

As per the recommendation of ICAR, the large udder circumference is considered as better than other groups. This might be due to the reason that there would be more space available for secretory tissues, which are known to synthesize milk. Thus, more will be the surface area and volume of the udder better will be the milk production. All these reports are similar to results of the present investigation, which indicates that there is enough scope for improving udder volume of the animals which consecutively will increase the milk production of the animals.

4.11.6. Udder balance (UB)

It is the depth of rear udder when assessed in relation to the depth of the front udder (as shown in Figure 3.2 of Materials and Methods). In present investigation, the mean value for this trait was 1.04 ± 0.44 cm, ranging from -12.95 cm to 23.114 cm in Karan Fries cattle (Table 4.18), while, in Sahiwal, the mean value for this trait was -2.26 ± 0.32 cm ranging from -11.43 cm to 5.12 cm (Table 4.19) indicating same level of front and rear udder for Sahiwal cows. In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz. deep rear udder, both front and rear udder on same level and deep front udder with score point range of 1-3, 4-6 and 7-9 respectively. The maximum frequency for this trait in percentage was found to be in deep front udder group followed by same level rear udder group and deep rear udder group in Karan Fries cattle, while, in Sahiwal cattle the maximum frequency was found to be in same level rear udder group followed by deep rear udder group and deep front udder group. The frequencies in percentage for deep rear, same level and deep front udder groups are mentioned in the Table 4.20 for Karan Fries and Sahiwal cattle.

In spite of the intermediate value, a high frequency for this trait was observed in deep rear udder group. The balance between front and rear udder and the overall udder's exterior form and location largely depends upon the attachment of central ligament with the udder (Atkins, 2007). A weak udder cleft may be the reason for finding maximum frequency of deep front udder group in Karan Fries cattle. In Sahiwal cows, the udder cleft was found to be in the intermediate group, this might be one of the reason that maximum frequency of animals were observed in same level rear udder group.

Table 4.20 Frequency distribution of udder and teat type traits (measurements) under different classes in Karan Fries and Sahiwal cattle

Karan Fries				Sahiwal		
Trait	Score	N	Frequency	Score	N	Frequency
Rear Udder Height	1	7	0.039	1	5	0.057
	2	11	0.065	2	4	0.046
	3	35	0.209	3	5	0.057
	4	54	0.327	4	13	0.149
	5	27	0.163	5	27	0.310
	6	24	0.144	6	15	0.172
	7	3	0.020	7	9	0.103
	8	4	0.026	8	4	0.046
	9	1	0.007	9	5	0.057
Rear Udder Width	1	2	0.013	1	3	0.034
	2	8	0.046	2	11	0.126
	3	26	0.157	3	23	0.264
	4	43	0.261	4	17	0.195
	5	43	0.261	5	17	0.195
	6	25	0.150	6	7	0.080
	7	12	0.072	7	5	0.057
	8	4	0.026	8	4	0.046
	9	2	0.013	-	-	-
Udder length	1	9	0.052	1	5	0.057
	2	27	0.163	2	17	0.195
	3	51	0.307	3	11	0.126
	4	38	0.229	4	23	0.264
	5	30	0.183	5	13	0.149
	6	7	0.039	6	5	0.057
	7	2	0.013	7	5	0.057
	8	1	0.007	8	4	0.046
	9	1	0.007	9	4	0.046
Udder Depth	1	1	0.007	1	5	0.057
	2	2	0.013	2	2	0.023
	3	9	0.052	3	7	0.080
	4	23	0.137	4	16	0.184
	5	47	0.281	5	25	0.287
	6	44	0.268	6	22	0.253
	7	25	0.150	7	10	0.115
	8	10	0.059	8	-	-
	9	5	0.033	9	-	-

Table 4.20 contd...

Karan Fries				Sahiwal		
Trait	Score	N	Frequency	Score	N	Frequency
Udder Circumference	1	2	0.013	1	5	0.057
	2	7	0.039	2	5	0.057
	3	22	0.131	3	5	0.057
	4	47	0.281	4	11	0.126
	5	55	0.333	5	22	0.253
	6	23	0.137	6	13	0.149
	7	8	0.046	7	17	0.195
	8	2	0.013	8	4	0.046
	9	1	0.007	9	5	0.057
Udder Balance	1	8	0.046	1	4	0.046
	2	29	0.176	2	6	0.069
	3	48	0.288	3	10	0.115
	4	61	0.366	4	21	0.241
	5	14	0.085	5	28	0.322
	6	3	0.020	6	4	0.046
	7	1	0.007	7	10	0.115
	8	0	0.000	8	4	0.046
	9	2	0.013	-	-	-
Udder Width	1	1	0.007	1	8	0.092
	2	2	0.013	2	11	0.126
	3	13	0.078	3	17	0.195
	4	39	0.235	4	16	0.184
	5	47	0.281	5	15	0.172
	6	36	0.216	6	7	0.080
	7	15	0.092	7	5	0.057
	8	7	0.039	8	3	0.034
	9	7	0.039	9	5	0.057
Udder Cleft or Central Ligament	1	5	0.033	1	2	0.023
	2	20	0.118	2	3	0.034
	3	54	0.327	3	3	0.034
	4	30	0.183	4	19	0.218
	5	27	0.163	5	12	0.138
	6	17	0.105	6	18	0.207
	7	5	0.033	7	13	0.149
	8	3	0.020	8	14	0.161
	9	3	0.020	9	3	0.034

Table 4.20contd...

Karan Fries				Sahiwal		
Trait	Score	N	Frequency	Score	N	Frequency
Fore Teat Length	1	9	0.052	1	76	0.874
	2	41	0.248	2	7	0.080
	3	44	0.268	3	4	0.046
	4	44	0.268	-	-	-
	5	18	0.111	-	-	-
	6	5	0.033	-	-	-
	7	1	0.007	-	-	-
	8	1	0.007	-	-	-
	9	1	0.007	-	-	-
Rear Teat Length	1	18	0.111	1	70	0.805
	2	21	0.124	2	13	0.149
	3	30	0.183	3	4	0.046
	4	29	0.176	-	-	-
	5	26	0.157	-	-	-
	6	17	0.105	-	-	-
	7	13	0.078	-	-	-
	8	4	0.026	-	-	-
	9	7	0.039	-	-	-
Teat Diameter	1	17	0.105	1	5	0.057
	2	29	0.176	2	27	0.310
	3	55	0.333	3	23	0.264
	4	43	0.261	4	13	0.149
	5	14	0.085	5	6	0.069
	6	1	0.007	6	4	0.046
	7	4	0.026	7	3	0.034
	8	0	0.000	8	4	0.046
	9	1	0.007	9	2	0.023
Teat Circumference	1	22	0.131	1	78	0.897
	2	40	0.242	2	6	0.069
	3	41	0.248	3	3	0.034
	4	29	0.176	-	-	-
	5	20	0.118	-	-	-
	6	2	0.013	-	-	-
	7	7	0.039	-	-	-
	8	3	0.020	-	-	-
	9	2	0.013	-	-	-

Table 4.20contd...

Trait	Karan Fries			Sahiwal		
	Score	N	Frequency	Score	N	Frequency
DFR	1	7	0.039	1	60	0.690
	2	23	0.137	2	23	0.264
	3	42	0.255	3	4	0.046
	4	46	0.275	-	-	-
	5	28	0.170	-	-	-
	6	10	0.059	-	-	-
	7	9	0.052	-	-	-
	8	0	0.000	-	-	-
	9	2	0.013	-	-	-
DLR	1	2	0.013	1	57	0.655
	2	15	0.092	2	24	0.276
	3	36	0.216	3	4	0.046
	4	49	0.294	4	2	0.023
	5	31	0.190	-	-	-
	6	21	0.124	-	-	-
	7	7	0.039	-	-	-
	8	4	0.026	-	-	-
	9	1	0.007	-	-	-
SDF	1	14	0.085	1	3	0.034
	2	11	0.065	2	4	0.046
	3	26	0.157	3	15	0.172
	4	21	0.124	4	36	0.414
	5	31	0.190	5	26	0.299
	6	25	0.150	6	3	0.034
	7	23	0.137	-	-	-
	8	11	0.065	-	-	-
	9	4	0.026	-	-	-
SDR	1	1	0.007	1	3	0.034
	2	3	0.020	2	2	0.023
	3	10	0.059	3	2	0.023
	4	24	0.144	4	10	0.115
	5	23	0.137	5	23	0.264
	6	34	0.203	6	35	0.402
	7	36	0.216	7	12	0.138
	8	25	0.150	-	-	-
	9	11	0.065	-	-	-
Fore Udder Attachment	1	2	0.012	1	2	0.023
	2	2	0.012	2	6	0.069
	3	17	0.102	3	19	0.218
	4	20	0.120	4	23	0.264
	5	51	0.307	5	21	0.241
	6	42	0.253	6	10	0.115
	7	17	0.102	7	6	0.069
	8	10	0.060	-	-	-
	9	5	0.030	-	-	-

4.11.7. Udder width (UW)

Average of two distances (a) between two lateral lines at the base of udder near flank/stifle joint were measured by passing measuring tape in between fore and hind teats and (b) distance between two lateral lines at the base of udder near flank/stifle joint by passing measuring tape front of fore teats, was taken as udder width (as shown in Figure 3.3 of Materials and Methods). In KF cattle, the mean value for this trait was found to be 71.42 ± 0.68 cm ranging from 43.06 to 99.06 cm (Table 4.18). In Sahiwal, the mean value for this trait was recorded as 71.47 ± 0.83 cm, ranging from 48.76 to 85.65 cm (Table 4.19). In 1-9 point scale scoring system of ICAR (2001), the udder width was classified as small, intermediate and large groups having score range of 1-3, 4-6 and 7-9 respectively for each group. The maximum frequency was observed in intermediate group which was followed by high and low udder width groups in Karan Fries cattle but in Sahiwal cattle it was followed by low and high udder width groups. The number of animals against each class and there frequency are given in the Table 4.20.

These results are in concordance with the recommendation of ICAR, which consider the intermediate udder width as better than other groups. This might be due to the reason that there would be optimum room for glandular tissues while large udder width may result in pendulous udder and a loose udder suspension. In Sahiwal cows, the average udder width was found to be in intermediate group.

4.11.8. Udder cleft (Central ligament) (CL)

Udder cleft is the depth of cleft measured at the base of the udder, which is caused by central suspensory ligament (as shown in Figure 3.2 of Materials and Methods). In Karan Fries cattle, the mean value for this trait indicating intermediate definition of udder cleft was 3.15 ± 0.11 cm, ranging from 0.90 cm and 8.20 cm (Table 4.18). In Sahiwal, the mean value of CL was 3.57 ± 0.13 cm, ranging from 0.0 to 6.38 cm (Table 4.19). In 1-9 point scale scoring system of ICAR (2001), the udder cleft was classified as weak (broken), intermediate and strong with score points of 1-3, 4-6 and 7-9, respectively. The maximum frequency for udder cleft was observed in weak and intermediate group in Karan Fries and Sahiwal cattle which was followed by intermediate and strong in Karan Fries cattle; strong and weak in Sahiwal cattle. The number of animals against each class and there frequency have been given in the Table 4.20.

As per the recommendation of ICAR (2001), central ligament with deep definition is considered better in cattle. This might be due to the fact that such ligament can more easily bear the weight of udder in a better way, while loose definition makes the udder susceptible

for damage. It is one of the fundamental traits related to udder health. Moreover, the udder cleft or udder support had the maximum influence on functional longevity (Vollema *et al.*, 2000; Larroque and Ducrocq, 2001). The udder cleft is the trait which involves the assessment of strength of ligaments which is responsible for the attachment of the udder to the pelvic floor. Without adequate strength in this area, the productive life of a cow would be seriously compromised (Atkins, 2007).

Wall *et al.* (2005) and Berry (2007) observed average score point of 5.8 ± 1.4 and 6 ± 0.42 respectively under 1-9 score point scale for Holstein-Friesian cattle. All these reports are in contrary to results of the present investigation, which might be due to less number of animals under the sub-classes (11 animals in KF & 30 in SW for strong udder cleft) of the trait. It indicates that there is enough scope for improving udder support by selecting the animals for strong suspensory ligament of the animals which consecutively increase the milk production of the animals.

4.11.9. Teat length (TL) (FTL & RTL)

The length of the front/ rear teat from the base to the end of the teat and is represented separately as fore teat length (FTL) and rear teat length (RTL) (as shown in Figure 3.6 of Materials and Methods). In Karan Fries cattle, the value for fore teat length varied from 3.10 cm to 11.68 cm with a mean value of 5.30 ± 0.11 cm (Table 4.18). For rear teat length the values varied from 2.97 cm to 7.49 cm with mean value of 4.66 ± 0.09 (Table 4.18). The mean value for fore and rear teat length in Sahiwal cattle was found to be 6.88 ± 0.65 cm, ranging from 3.17 cm to 11.68 cm and 6.13 ± 0.71 cm ranging from 2.79 cm to 11.04 cm (Table 4.19), respectively.

In 1-9 point scale scoring system, the teat length were classified as short, intermediate and long with score points of 1-3, 4-6 and 7-9 score point, respectively. The maximum frequency for fore and rear teat length in Karan Fries and Sahiwal cattle was observed in short and intermediate group, respectively, which was followed by intermediate and long group for fore teat length; short and long group for rear teat length. The number of animals against each class and there frequency have given in the Table 4.20.

As per the recommendation of ICAR (2001), teat with intermediate length is preferred in cattle because long teats are more easily damaged due to increased susceptibility to injury, thus, increasing the chances of infection and are genetically predisposed to a higher incidence of mastitis (Van Dorp *et al.*, 1998), while short teats cause problem for milking machines. An average score point of 3.54 indicating intermediate length of teat was found to be more common in Sahiwal breed (Dubey, 2010), which supports present finding of intermediate teat

length in Sahiwal. Berry (2007) in Holstein-Friesian obtained intermediate teat length with average score point of 4.7 ± 0.70 under 1-9 point scale scoring system, however, Solkner and Petschina (1999) obtained long teats with average score point of 6.53 ± 1.04 under 1-9 point scale scoring system in Simmental breed of cattle.

The presence of intermediate teat in Sahiwal cows showed that the teat length might not be a factor for udder infection, like mastitis, in this breed. Further improvement of this trait could be carried in Karan Fries cattle so that teat with uniform length could be attained to avoid problem associated with short teats.

4.11.10. Teat diameter (TD)

Teat diameter is the thickness of teat when assessed from the middle of front teat (as shown in Figure 3.5 of Materials and Methods). In KF cattle, the mean value for this trait was found to be 2.22 ± 0.03 cm ranging from 1.5 cm to 3.7 cm (Table 4.18). In Sahiwal, the mean value for this trait was found to be 2.60 ± 0.54 cm ranging from 1.8 to 4.45 cm (Table 4.19), thus indicating presence of thin teats in both the breeds. In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz., thin, intermediate and thick with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for this trait was observed in thin group followed by intermediate and thick group respectively, in both Karan Fries and Sahiwal cattle. The frequency in percentage for thin, intermediate and thick groups was 80.3, 18.8 and 0.9, respectively in Sahiwal cows studied by Dubey (2010). The average score point for this trait was found to be 2.76, thus indicating presence of thin teats in Sahiwal cows (Dubey, 2010). The frequencies for thin, intermediate and thick groups have been given in the Table 4.20.

4.11.11. Teat circumference (TC)

Teat circumference is measured at the midpoint of teat length (as shown in Figure 3.7 of Materials and Methods). In KF cattle, the mean value for this trait was found to be 7.54 ± 0.12 cm, ranging from 6.21 cm to 11.684 cm (Table 4.18). In Sahiwal, the mean value for this trait was found to be 8.76 ± 0.55 cm, ranging from 6.32 to 14.78 cm (Table 4.19), thus indicating presence of thin teats in both the breeds. In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz. thin, intermediate and thick with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for this trait was observed in thin group followed by intermediate and thick group in Karan Fries; thin teat group in Sahiwal cattle as intermediate group was absent among the Sahiwal animals. The values in frequencies for thin, intermediate and thick groups have been given in the Table 4.20 for both KF and Sahiwal cattle.

4.11.12. Distance between teats (DFR & DLR)

Distance between teats is measured at the midpoint of teat length (as shown in Figure 3.7 of Materials and Methods). In KF cattle, mean for the distance between fore and rear teats (DFR) was found to be 6.75 ± 0.30 cm ranging from 3.12 cm to 13.97 cm (Table 4.18). In Sahiwal, the mean value for this trait was found to be 7.19 ± 0.59 cm ranging from 1.39 to 11.38 cm (Table 4.19). The mean distance between left and right teats (DLR) was found to be 8.99 ± 0.37 cm ranging from 0.88 cm to 20.34 cm (Table 4.18) in Karan Fries cattle, while, in Sahiwal, the average value for this trait was found to be 7.19 ± 0.59 cm, ranging from 1.39 to 11.38 cm (Table 4.19). In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz. close, mid and wide distance between teat group with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for the distance between fore and rear teats was observed in mid distance group followed by close and wide group in Karan Fries; while, in Sahiwal the maximum frequency is observed in close distance group followed by wide group. For the distance between left and right teats (DLR) maximum frequency was observed in mid distance group followed by close and wide group in Karan Fries; while in Sahiwal maximum frequency was observed in close distance group followed by mid and wide group. The values in frequencies for close, mid and wide groups are given in the Table 4.20 for both Karan Fries and Sahiwal cattle.

4.11.13. Shortest distance from front and rear teat ends to floor (SDF & SDR)

Distance from front and rear teat ends to floor are measured as the distance between teat tip to the ground. In present investigation, mean value for the shortest distance from front teat ends to floor (SDF) was found to be 46.93 ± 0.43 cm ranging from 35.43 cm to 64.00 cm in Karan Fries cattle (Table 4.18), while, in Sahiwal, the mean value for this trait was found to be 42.23 ± 0.67 cm, ranging from 28.44 to 55.88 (Table 4.19). The shortest distance from rear teat ends to floor (SDR) was found to be 46.79 ± 0.44 cm ranging from 31.49 cm to 62.48 cm (Table 4.18) in Karan Fries cattle, while, in Sahiwal, the average value for this trait was found to be 43.11 ± 0.67 cm, ranging from 8.89 to 36.87 cm (Table 4.19). In 1-9 point scale scoring system of ICAR (2001) this trait was categorized into three groups viz. short, mid and long distance between teat ends to floor with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for the shortest distance from front teat ends to floor (SDF) was observed in mid distant group followed by short and long distant group in Karan Fries; while, in Sahiwal the maximum frequency was observed in long distant group followed by mid and wide distant group. For the shortest distance from rear teat ends to floor (SDR) maximum frequency was observed in mid distant group followed by long and short

distant group in Karan Fries; while in Sahiwal maximum frequency was observed in long distant group followed by mid and short distant group. The values in frequencies for short, mid and long distance between teat ends to floor groups are given in the Table 4.20 for both Karan Fries and Sahiwal.

4.11.14. Fore udder attachment (FUA)

Fore udder attachment was assessed as an angle between the abdominal wall and front part of udder (as shown in Figure 3.1 of Materials and Methods). In the present investigation, the mean value for this trait was found to be 131.46 ± 1.23^0 , ranging from 82^0 and 174^0 (Table 4.18) for Karan Fries cattle indicating intermediate fore udder attachment for Karan Fries cattle. In Sahiwal, the mean value for this trait was found to be 107.10 ± 2.07^0 ranging from 33^0 and 150^0 (Table 4.19), indicating the intermediate fore udder attachment. In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three group viz. loose, intermediate and strong groups. The maximum frequency for this trait was observed in intermediate group which was followed by strong and weak groups in both the breeds. The values in frequencies for weak, intermediate and strong groups are given in the Table 4.20 for both Karan Fries and Sahiwal cattle.

This finding is in accordance with Perez-Cabal and Alenda (2002) in Holstein cattle where intermediate fore udder attachment with average score points of 4.9 ± 1.2 was observed under 1-9 point scale score system. In Sahiwal cows, Dubey, (2010) reported maximum frequency (67.3 per cent) in intermediate group, which probably keeps this breed under moderate level of milk production. Moreover, Dahiya and Rathi (2002) in Tharparkar, Norman *et al.* (1988) in Ayrshire, Brown Swiss, Guernsey, Jersey and Shorthorn breed of cattle also showed the presence of intermediate type of fore udder attachment with corresponding scores of 75.07 ± 1.08 , 79.9 ± 5.1 , 28.6 ± 5.6 , 78.4 ± 5.5 , 78.3 ± 6.0 and 29.4 ± 5.0 , respectively under 50-99 score point scale with exception of Brown Swiss and Shorthorn breed in which 1-50 score point range was used. However, Mitra *et al.* (1998) had obtained the strong and firm attachment in Karan Fries cattle with average score point of 80.22 ± 0.96 under 50-99 score point scale.

Generally strong attachment is preferred over others, as it indicates a strong median suspensory ligament essential for udder conformation. The tighter to the body cavity that the udder is placed, the more desirable it is for milk production. So, there is scope of further improvement in this trait to achieve maximum milk production by selecting animals for strong fore udder attachment.

4.11.15. Teat Size (TSi)

Teat size is the subjective assessment of teat length and circumference. According to Rasby (2011) teat Size can be classified as large and long (1), large (2), large & asymmetrical (3), small & asymmetrical (4), small (5) and small and short (6) (as shown in Figure 3.7 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle was large and long (1) and small and short (6) teat size; large and long (1) and small (5) teat size group, respectively (Table 4.21). In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz. large, intermediate and small with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for this trait was observed in large & asymmetrical (3) teat size group followed by small & asymmetrical (4), large (2), large and long (1), small and short (6) and small (5) in Karan Fries cattle and small & asymmetrical (4) followed by small (5), large and long (1) and large & asymmetrical (3) in Sahiwal cattle (Table 4.21).

Teat size can vary considerably. As teat becomes smaller, more symmetrical, and more central in placement on the quarter, more it is desirable. As teat size becomes larger they tend to be thicker, less symmetrical, and suspend below the hock which makes it difficult for a new-born calf to find, attach, and suckle. When engorged with milk, this kind of teats will not be perpendicular to the ground requiring interventions for machine milking and mastitis prevention. In cattle the small, intermediate, and large teat size were common (Rasby, 2011).

4.11.16. Fore teat placement (FTPI)

Fore teat placement is the measurement of the placement of front teat on the quarter which can be observed and classified as wide (1), outside (2), out (3), mid (4), inside (5) and inward (6) teat placement (as shown in Figure 3.5 of Materials and Methods). In present investigation, the minimum and maximum scores for this trait were 1 and 6, respectively in both the breeds. In 1-9 point scale scoring system of ICAR (2001), this trait were categorized into three group viz. wide, intermediate and close groups with corresponding score point range of 1-3, 4-6 and 7-9. The maximum frequency for this trait was observed in out (3) teat placement group followed by inside (5), outside (2), wide (1), mid (4) and inward (6) teat placement group in Karan Fries cattle, while, in Sahiwal the maximum frequency for this trait was observed in Inside (5) teat placement group followed by mid (4), wide (1), out (3), outside (2), and inward (6) teat placement group. The values in frequencies for wide (1), outside (2), out (3), mid (4), inside (5) and inward (6) teat placement have been given in the Table 4.21 for Karan Fries and Sahiwal cattle.

Dubey (2010) reported dairy cattle with close fore teat placement were desirable, and recorded a frequency of 0.282 in Karan Fries and 0.528 in Sahiwal. Average intermediate score point of 0.054 and 0.207 was observed in Karan Fries and Sahiwal cattle, respectively. The present findings indicate the scope of further improvement in this trait to achieve the mid placement. In 1-9 point scale scoring system of ICAR (2001), the most desirable front teat placement (FTPI) on udder quarter was found to be mid placement followed by outward (7) and inward (5) teat position groups. Intermediate placement of the teats on udder quarter of the teats on udder quarter, is more desirable. Front teat placement was found better in Sahiwal cows than in Karan Fries cows.

4.11.17. Rear teat placement (RTPI)

Rear teat placement is the visual observation of the placement of rear teat on the quarter which can be observed and classified as complete outside (1), outside (2), outward (3), mid (4), inward (5), inside (6) and complete inside (7) teat placement (as shown in Figure 3.5 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle was complete outside (1) and complete inside (7) teat placement group; outside (2) and inward (5) teat placement group, respectively in both the breeds. In 1-9 point scale scoring system of ICAR (2001), this trait was categorized into three groups viz. wide, intermediate and close with corresponding score point range of 1-3, 4-6 and 7-9 points. The maximum frequency for this trait was observed in outward (3) teat placement group followed by inward (5), inside (7), complete outside (1), inside (6) and mid (4) in Karan Fries cattle and outside (2) teat placement group followed by outward (3), mid (4) and inward (5) teat placement group in Sahiwal cattle. The values in frequencies for complete outside (1), outside (2), outward (3), mid (4), inward (5), inside (6) and complete inside (7) teat placement groups have been given in the Table 4.21 for Karan Fries and Sahiwal.

Berry (2007) in Holstein-Friesian obtained intermediate placement for rear teats with average score point of 5.7 ± 0.65 under 1-9 point score scale system. The front and rear teat placement in Sahiwal breed was found in intermediate group, which was of desirable type (Dubey, 2010). Extreme values of measurements of this trait in cows increased the chances of being culled (Berry, 2007). The rear teat placement along with udder depth and texture were the udder traits that had a significant influence on functional survival of dairy cows (Sewalem *et al.*, 2004). Rear teat placement was found better in Sahiwal cows than in Karan Fries cows.

**Table 4.21 Frequency distribution of udder and teat type traits (visual observations)
in Karan Fries and Sahiwal cattle**

Karan Fries				Sahiwal			
Trait	Sub cat	N	Freq	Trait	Sub cat	N	freq
Fore teat Placement	1	25	0.150	Fore teat placement	1	10	0.115
	2	27	0.162		2	5	0.057
	3	58	0.347		3	8	0.092
	4	9	0.054		4	18	0.207
	5	39	0.234		5	43	0.494
	6	8	0.048		6	3	0.034
Front teat Position	1	17	0.102	Front teat position	1	13	0.149
	2	22	0.132		2	3	0.034
	3	39	0.234		3	8	0.092
	4	20	0.120		4	12	0.138
	5	45	0.269		5	42	0.483
	6	23	0.138		6	3	0.034
LTCTEC	1	48	0.287		7	3	0.034
	2	76	0.455		8	1	0.011
	3	31	0.186		9	2	0.023
	4	11	0.066	LTCTEC	1	22	0.253
Rear teat placement	1	21	0.126		2	13	0.149
	3	52	0.311		3	52	0.598
	4	9	0.054	Rear teat placement	2	57	0.655
	5	43	0.257		3	16	0.184
	7	17	0.102		4	7	0.080
	8	24	0.144		5	7	0.080

Table 4.21 contd...

Karan Fries				Sahiwal			
Trait	Sub cat	N	Freq	Trait	Sub cat	N	freq
Rear teat Position	1	22	0.132	Rear teat position	1	2	0.023
	2	25	0.150		2	2	0.023
	3	51	0.305		3	48	0.552
	4	38	0.228		4	1	0.011
	5	30	0.180		5	18	0.207
Skin condition	1	73	0.437		6	8	0.092
	2	27	0.162		7	8	0.092
	3	66	0.395	Skin condition	1	71	0.816
Teat end shape	1	35	0.210		2	16	0.184
	2	71	0.425	Teat end shape	1	8	0.092
	5	60	0.359		2	45	0.517
Teat shape	1	83	0.497		3	34	0.391
	2	56	0.335		1	37	0.425
	3	27	0.162	Teat shape	2	44	0.506
Teat size	1	23	0.138		3	6	0.069
	2	24	0.144	Teat size	1	15	0.172
	3	43	0.257		3	12	0.138
	4	33	0.198		4	32	0.368
	5	20	0.120		5	28	0.322
	6	23	0.138	Udder shape	1	13	0.149
Udder shape	1	50	0.299		2	25	0.287
	2	39	0.234	Udder suspension	3	49	0.563
	3	77	0.461		1	1	0.011
Udder suspension	1	14	0.084		2	18	0.207
	2	43	0.257		3	56	0.644
	3	85	0.509		4	12	0.138
	4	24	0.144				

4.11.18. Front teat position (FTP)

Front teat position is the visual appraisal of Position of the centre of front teat placement at the point of the udder as viewed from rear which can be observed and classified as clubbed inside and inwards (1), inside with inward inclination (2), inside (3), inward (4), mid (5), outward (6), outside (7), outside with outward inclination (8) and expanded outwards (9) teat position (as shown in Figure 3.6 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait are 1 and 6 in Karan Fries and 1 and 9 in Sahiwal cattle. The maximum frequency for this trait was observed in mid (5) teat Position in both the breeds followed by clubbed inside and inwards (1), inward (4), inside with inward inclination (2), outward (6), inside (3) teat position in Karan Fries cattle; outside with outward inclination (8), expanded outwards (9), inside with inward inclination (2), outward (6), outside (7), inside (3), inward (4), and teat position clubbed inside and inwards (1) in Sahiwal cattle. The frequencies for clubbed inside and inwards (1), inside with inward inclination (2), inside (3), inward (4), mid (5), outward (6), outside (7), outside with outward inclination (8) and expanded outwards (9) teat position groups have been given in the Table 4.21 for Karan Fries and Sahiwal cattle.

In 1-9 point scale scoring system of ICAR (2001), the Front teat Position (FTP) was classified as clubbed inside and inwards (1), inside with inward inclination (2), inside (3), inward (4), mid (5), outward (6), outside (7), outside with outward inclination (8) and expanded outwards (9) teat position. The most preferable position among all these classes was mid (5) rear teat position which was followed by Outward (7) and Inward (5) teat position groups. Intermediate position of the teats on udder quarter, is more desirable. This recommendation is in favour of the results observed in the present study.

4.11.19. Rear teat Position (RTP)

Rear teat Position is the visual appraisal of position of the centre of rear teat placement at the point of the udder as viewed from rear which can be observed and classified as inside with inward inclination (1), inside (2), inward (5), mid (6), outward (7), outside (8) and outside with outward inclination (9) teat position (as shown in Figure 3.6 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait were 1 and 7 in Karan Fries and 1 and 9 in Sahiwal cattle. The maximum frequency for this trait was observed in inward (5) teat position in both the breeds followed by inside with inward inclination (1), inside (2), outward (7) and mid (6) in Karan Fries cattle; mid (6), inside with inward inclination (1), inside (2), outside (8), outside with outward inclination (9) and outward (7) teat position in Sahiwal cattle. The values in frequencies for Inside with inward

inclination (1), inside (2), inward (5), mid (6), outward (7), outside (8) and outside with outward inclination (9) teat position groups are given in the Table 4.21.

In 1-9 point scale scoring system of ICAR (2001), the rear teat position (RTP) was classified as inside with inward inclination (1), inside (2), inward (5), mid (6), outward (7), outside (8) and outside with outward inclination (9) teat position. The most preferable position among all these classes is mid (6) rear teat position which was followed by outward (7) and Inward (5) teat position groups. Intermediate position of the teats on udder quarter, was more desirable.

4.11.20. Udder shape (US)

Udder shape is the visual appraisal of shape of the udder which can be observed and classified as pendulous (1), trough (2) and round (3) udder shapes (as shown in Figure 3.4 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle were 1 and 3. The maximum frequency for this trait was observed in trough (2) udder shape groups followed by pendulous (1) and round (3) udder shapes in Karan Fries cattle; but in Sahiwal cattle the maximum frequency for this trait was observed in round (3) udder shape groups followed by trough (2) and pendulous (1) udder shapes. The values in frequencies for pendulous (1), trough (2) and round (3) udder shape groups have been given in the Table 4.21.

Singh (2005) studied different udder types of Karan Fries cows and reported that trough shaped (45.94%) udder was most common with maximum milk production followed by round (11.94%), goat (15.09%), pendulous (19.47%) and unbalanced (7.51%) shaped udder. Singh (2006) reported that in Sahiwal trough shaped (50.0%) udder was most common followed by round (42.8%) and pendulous (7.14%) shaped udder. Kamboj *et al.* (2007) studied in Karan Fries cows trough shape udder were most frequent (45.94%), followed by pendulous (19.47%), goaty (15.09%), round (11.94%) and unbalanced udders (07.51%). Similarly, Ahlawat (2007) also studied the same in Sahiwal and reported that pendulous shaped (22.2 %) udder was most common followed by trough (44.4 %) and round (33.3 %) shaped udder. Rao (2006) reported that trough shaped udder was related to more milk yield, milk protein and less lactose. All these reports are in accordance with the results of Karan Fries cattle in the present finding but in contrary to the results of Sahiwal cattle. These might be due to lesser number of animals available under the subclasses of the trait (13 animals for pendulous udder shape and 25 animals for Trough udder shape in Sahiwal). The maximum frequency of round udder shape groups also suggests ample chance of improving milk production in the herd through selection of the more desirable trough udder shape group.

4.11.21. Udder suspension (USus)

Subjective assessments of udder support which can be observed and classified as very tight (1), tight (2), intermediate/medium (3), pendulous (4) and very pendulous/broken floor (5) udder suspension (as shown in Figure 3.4 of Materials and Methods). A strong median suspensory ligament leads to a tight udder suspension, is essential for udder conformation. A weak median suspensory ligament results in a lowering of the floor of the udder sometimes below the hock which leads to pendulous and very pendulous udder suspension, which makes it more difficult for the calf to nurse and the teats might drag in the mud when the cow walks, and the teats might be suspended inward or outward when filled with milk instead of straight down. The tighter to the body cavity that the udder is placed, the more desirable. This allows the calf to locate the teats easily and it is less likely for the teats to drag in the mud as reported by Rasby, 2011. So a tight to intermediate udder suspension is desirable in both indigenous and crossbred cattle.

In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle are 1 and 4. The maximum frequency for this trait was observed in intermediate/medium (3) udder suspension groups, which is followed by very tight (1), pendulous (4) and tight (2) udder suspension groups. Though the most frequent intermediate udder suspension group in the present study was preferable for both milk production and susceptibility to mastitis, hence, there is ample chance of increasing milk production of the animals by selection the superior ‘tight’ sub-category of udder suspension. The values in frequencies for very tight (1), tight (2), intermediate/medium (3), pendulous (4) and very pendulous/broken floor (5) udder suspension groups have been given in the Table 4.21.

4.11.22. Long Term Changes in Teat-End Condition (LTC TEC)

Long Term Changes in Teat-End Condition is the visual appraisal of the status of teat-ends for an individual cow or herd which can be observed and classified as Smooth bottom, no or smooth callous, no lesions (1), Raised callous ring with slight roughness (2), Rough callous (3) and Rough callous with hyperkeratosis and visible lesions (4) (Mein *et al.*, 2001) (as shown in Figure 3.9 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle were 1 and 4; 1 and 3 (Table 4.21), respectively. The maximum frequency for this trait was observed in raised callous ring with slight roughness (2) teat groups for Karan Fries cattle and rough callous (3) teat groups for Sahiwal cattle, followed by smooth bottom, no or smooth callous, no lesions (1), rough callous (3) and rough callous with hyperkeratosis and visible lesions (4) in Karan Fries cattle; smooth bottom, no or smooth callous, no lesions (1) and raised callous ring with

slight roughness (2) teat groups in Sahiwal cattle. The values in frequencies for smooth bottom, no or smooth callous, no lesions (1), raised callous ring with slight roughness (2), rough callous (3) and rough callous with hyperkeratosis and visible lesions (4) teat groups have been given in the Table 4.21.

According to Mein *et al.* (2001), changes in teat-end hyperkeratosis appeared to be long term effect (typically 2-8 weeks) in the absence of unusually harsh weather conditions. The degree of teat-end hyperkeratosis (roughness, cornification or callosity) is a dynamic condition. Status of teat-ends for an individual cow or herd can change within days, especially in regions subject to harsh weather conditions or sudden weather changes. Seasonal conditions might affect dryness and hardness of keratin. In the absence of unusually harsh weather conditions, however, changes in teat-end status occur over a period of 2-8 weeks. The major factors affecting this include teat-end shape, production level and stage of lactation and interactions between milking management and machine factors. According to Juozaitiene *et al.* (2019) only 11.8% of the teats were found as rough and 2.9% as very rough for teat hyperkeratosis, while 44.3% of the teats were classified as no ring, 41.1% were classified as smooth ring in *Bos taurus* cattle. There were significant differences between front and rear teats for the prevalence of hyperkeratosis in first, second and third (≥ 3 lactations) lactation cows ($P < 0.001$) (Juozaitiene *et al.*, 2019). The development of hyperkeratosis varied based on a number of lactation. It was found that multiparous cows had increased degrees of hyperkeratosis compared to younger ones ($P < 0.01$) (Juozaitiene *et al.*, 2019). Some studies have indicated that a number of lactations had no effect on hyperkeratosis development (Chrystal *et al.*, 1999). Juozaitiene *et al.* (2019) detected significantly ($P < 0.05$) higher teat hyperkeratosis in cows for rear quarters than for front quarters of the udder and showed that rear udder quarters had 1.28-1.40 times higher ($P < 0.01$) frequency of subclinical and clinical mastitis cases.

The maximum frequency for this trait was observed in raised callous ring with slight roughness (2) teat groups for Karan Fries cattle and rough callous (3) teat groups for Sahiwal cattle. This might be due to harsh tropical weather condition, medium to high level of milk production in the animals and interactions between milking management and machine factors.

4.11.23. Teat shape (TS)

Teat shape is the visual appraisal of the teat shape which can be observed and classified as funnel/conical (1), cylinder (2) and wide/flat (3) (Britt and Farnsworth, 2011) (as shown in Figure 3.8 of Materials and Methods). In present investigation, the minimum and

maximum scores for the trait in Karan Fries and Sahiwal cattle were 1 and 3. The maximum frequency for this trait was observed in funnel/conical (1) groups followed by cylinder (2) and wide/flat (3) in both Karan Fries and Sahiwal cattle. The frequencies for funnel/conical (1), cylinder (2) and wide/flat (3) groups have been given in the Table 4.21.

Results of the present finding are in accordance with Singh (2005) who studied different teat types in Karan Fries cow and reported that conical/funnel shaped (78.1%) teats were most common followed by cylindrical (11.9%) and bottle (10.0%) shaped teats. Rao (2006) studied different teat types in Karan Fries cows and reported conical shaped (25.0%) teats were most common followed by cylindrical (75.0%), supernumerary (18.7%) shaped teats. Singh (2006) studied the same in Sahiwal cows and found that conical (78.6%) shaped teats were most common followed by cylindrical (21.5%) shaped teats. Ahlawat (2007) also reported that conical (44.4%) shaped teats were most common followed by cylindrical (38.8%) and bottle (16.7%) shaped teats in Sahiwal cows.

4.11.24. Skin Condition (SC)

Skin Condition is the visual appraisal of the skin on teat which can be observed and classified as normal – smooth and healthy (1), dry – no cracking (2) and rough-dryness + cracking (3) (Britt and Farnsworth, 2011). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle are 1 and 3; 1 and 2 (Table 4.21), respectively. The maximum frequency for this trait was observed in normal – smooth and healthy groups followed by dry – no cracking (2) and rough-dryness + cracking (3) in Karan Fries cattle; dry – no cracking (2) in Sahiwal cattle. The values in frequencies for normal – smooth and healthy (1), dry – no cracking (2) and rough-dryness + cracking (3) groups are given in the Table 4.21.

According to Britt and Farnsworth (2011) teat condition had little effect on somatic cell count or clinical mastitis unless skin cracks or ulcers were present. Teat skin protects the deep teat tissues from infections. When damaged, the skin might allow microorganisms to invade the teat tissue, resulting in necrosis, fibrosis, scarring and sloughing. These conditions reduce milkability and increase culling. Changes in teat skin often are related to cold weather, wind chill or incorrect use of chemicals. As the maximum number of animals were observed in normal – smooth and healthy group followed by dry – no cracking and rough-dryness + cracking group, so most of the animals were not in risk of mastitis having good milkability.

4.11.25 Teat end shape (TES)

Teat end shape is the visual observation of the teat tip shape which can be observed as pointed (hyperkeratosis) (1), flat (2), round (3), (Britt and Farnsworth, 2011) (as shown in

Figure 3.4 of Materials and Methods). In present investigation, the minimum and maximum scores for the trait in Karan Fries and Sahiwal cattle were 1 and 3. The maximum frequency for this trait was observed in flat (2) teat groups followed by round (3) and pointed (1) teat group. The values in frequencies for pointed (hyperkeratosis) (1), flat (2) and round (3) teat groups are given in the Table 4.21.

According to Britt and Farnsworth (2011) teat ends were scored for changes which may occur, including callous formation of the annular ring, roughness, hyperkeratosis and radial cracking. The four teats on an individual cow might have different evaluations. Teat length and shape affect hyperkeratosis formation. Pointed teats have more hyperkeratosis than rounded ones, flat teats have the least hyperkeratosis. The hyperkeratosis condition reduce milkability and increase culling. Studies showed that high-yielding cows had significantly worse teat ends than low yielders.

As the maximum frequency for this trait was observed in flat teat groups followed by round and pointed teat group. So most of the animals were having least hyperkeratosis, in turn least risk of mastitis and having good milkability.

In conclusion, it could be possible to recommend that most of the scores for linear type traits in Holstein-Friesian and Holstein breed ranged near the midpoint of the intermediate group, however, with Karan Fries and Sahiwal, in present investigation, only fewer traits fell near the mid-point of the intermediate group. This might be one of the reasons that inspite of having good genetic potential for milk production, Sahiwal and Karan Fries cows of the herd were unable to produce milk at par with high producing milch breeds. So in order to improve production of these milch breeds of cattle, the traits needs to be improved as depicted.

4.12. Association between genotypes, udder and teat type traits, clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

The association between various genotypes and udder and teat type traits, mastitis & milk production traits in Karan Fries and Sahiwal cattle was estimated using suitable models in SPSS/ SAS as described earlier in Section 3.10.

4.12.1. Association analysis of VDR gene with udder and teat type traits in Karan Fries and Sahiwal cattle

VDR gene has been reported to be involved in udder development and associated with type traits and mastitis (Tolleson, 2016). As per the previous reports, two significant SNPs namely rs454303072 and rs470608846 were selected for testing of association between genotypes at these loci and type traits, production traits and mastitis in KF and Sahiwal cattle.

In present study, although the sequencing data showed presence of recognition sequence, the SNP rs470608846 was found to be monomorphic in studied population. Hence, further statistical analysis could only be performed for SNP rs454303072, which will be described and discussed under subsequent subsections.

4.12.1.1 (a) Association of SNP rs454303072 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs454303072 situated in intron 1 of Bovine VDR gene (located in BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits.

In Karan Fries cattle, SNP rs454303072 found to be significantly ($P \leq 0.05$) associated with RUH, RUW, UC, DFR and DLR [Table 4.22 (a)]. AA genotype was found to be superior with highest RUH i.e., 24.273 ± 0.494 cm, as compared to GG genotypes. GG genotype was found to have superior RUW (13.199 ± 0.346 cm), UC (150.034 ± 3.315 cm), DFR (7.605 ± 0.511 cm) and DLR (10.088 ± 0.631 cm), as compared to AA genotypes [Table 4.22 (b)]. However, as only two genotypes were detected on this locus, multiple comparisons of genotypes could not be performed. Multivariate regression was used to test association between observational udder and teat type traits and genotypes. The association study revealed significant ($P \leq 0.05$) association of SNP rs454303072 genotypes with visual observational traits viz., front teat position, rear teat position, teat shape, teat size, udder shape and udder suspension (Table 4.23). Pair-wise estimation showed AA genotype having higher odds (expressed as exponentiation of B coefficient; Exp B) of being associated with trait sub category 2 of fore teat position, sub category 4 rear teat position, sub category 2 of teat size, sub category 2 of udder shape and sub category 3 of udder suspension, as compared to other genotype (Table 4.23).

In Sahiwal cattle, SNP rs454303072 was found to be significantly ($P \leq 0.05$) associated with RUH, RUW, UW and UC [Table 4.24 (a)]. AA genotype was found to be superior with highest RUH i.e., 24.019 ± 0.591 cm, highest RUW i.e., 24.251 ± 0.614 cm, highest UW i.e., 2079.097 ± 106.103 cm and highest UC i.e., 143.255 ± 1.255 cm as compared to GG genotype [Table 4.24 (b)]. However, as only two genotypes were detected multiple comparison procedure could not be performed. Among the visual observational trait, the genotypes had significant ($P \leq 0.05$) effect on fore teat placement, rear teat placement and teat shape (Table 4.25). Pair-wise estimation showed that genotype AA was having higher odds of being associated with trait subcategory 4 of fore teat placement, trait subcategory 3 of rear teat placement and trait subcategory 1 of teat shape (Table 4.25).

Table 4.22(a) Association of SNP rs454303072 (RE: BsrI) with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P – value
1	Rear udder height	Contrast	225.420	225.420	8.394*	0.004
		Error	4404.452	26.856		
2	Rear udder width	Contrast	28.318	28.318	4.217*	0.042
		Error	1101.245	6.715		
3	Udder width	Contrast	136.716	136.716	1.764	0.186
		Error	12707.725	77.486		
4	Fore udder attachment	Contrast	3.699	3.699	2.195	0.179
		Error	167.756	1.509		
5	Udder circumference	Contrast	4495.330	4495.330	7.304*	0.008
		Error	100932.810	615.444		
6	Udder balance	Contrast	7.250	7.250	0.223	0.637
		Error	5324.246	32.465		
7	Central ligament	Contrast	1.423	1.423	0.691	0.407
		Error	337.835	2.060		
8	Udder depth	Contrast	1.438	1.438	0.040	0.841
		Error	5858.456	35.722		
9	Udder length	Contrast	80.004	80.004	1.438	0.232
		Error	9125.174	55.641		
10	Fore teat length	Contrast	0.078	0.078	0.036	0.849
		Error	355.802	2.170		
11	Rear teat length	Contrast	0.951	0.951	0.757	0.386
		Error	206.118	1.257		
12	Teat circumference	Contrast	1.159	1.159	0.486	0.487
		Error	391.336	2.386		
13	Teat diameter	Contrast	0.002	0.002	0.015	0.903
		Error	17.961	0.110		
14	Distance between front and rear teat	Contrast	61.250	61.250	4.185*	0.042
		Error	2400.509	14.637		
15	Distance between right and left teat	Contrast	101.491	101.491	4.555*	0.034
		Error	3654.458	22.283		
16	Shortest distance from front teat end to floor	Contrast	6.378	6.378	0.209	0.648
		Error	5008.369	30.539		
17	Shortest distance from rear teat end to floor	Contrast	46.707	46.707	1.470	0.227
		Error	5210.254	31.770		

* Significant at $P \leq 0.05$

Table 4.22(b) Pair-wise comparison of SNP rs454303072 (RE: BsrI) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ Std. Error	P – value
1	Rear udder height	AA	24.273 $\pm$ 0.494	0.004*
		GG	21.808 $\pm$ 0.693	
2	Rear udder width	AA	12.326 $\pm$ 0.247	0.042*
		GG	13.199 $\pm$ 0.346	
3	Udder width	AA	70.770 $\pm$ 0.839	0.186
		GG	72.689 $\pm$ 1.176	
4	Fore udder attachment	AA	130.282 $\pm$ 1.508	0.179
		GG	133.786 $\pm$ 2.113	
5	Udder circumference	AA	139.027 $\pm$ 2.365	0.008*
		GG	150.034 $\pm$ 3.315	
6	Udder balance	AA	0.886 $\pm$ 0.543	0.637
		GG	1.328 $\pm$ 0.761	
7	Central ligament	AA	3.217 $\pm$ 0.137	0.407
		GG	3.022 $\pm$ 0.192	
8	Udder depth	AA	53.580 $\pm$ 0.570	0.841
		GG	53.777 $\pm$ 0.799	
9	Udder length	AA	59.970 $\pm$ 0.711	0.232
		GG	61.439 $\pm$ 0.997	
10	Fore teat length	AA	5.287 $\pm$ 0.140	0.849
		GG	5.333 $\pm$ 0.197	
11	Rear teat length	AA	4.605 $\pm$ 0.107	0.386
		GG	4.765 $\pm$ 0.150	
12	Teat circumference	AA	7.483 $\pm$ 0.147	0.487
		GG	7.660 $\pm$ 0.206	
13	Teat diameter	AA	2.224 $\pm$ 0.032	0.903
		GG	2.217 $\pm$ 0.044	
14	Distance between front and rear teat	AA	6.320 $\pm$ 0.365	0.042*
		GG	7.605 $\pm$ 0.511	
15	Distance between right and left teat	AA	8.435 $\pm$ 0.450	0.034*
		GG	10.088 $\pm$ 0.631	
16	Shortest distance from front teat end to floor	AA	47.069 $\pm$ 0.527	0.648
		GG	46.654 $\pm$ 0.738	
17	Shortest distance from rear teat end to floor	AA	47.166 $\pm$ 0.537	0.227
		GG	46.044 $\pm$ 0.753	

* Significant at $P \leq 0.05$

Table 4.23 Association of SNP rs454303072 (RE:BsrI) with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	6.998	5	-	-	-
2	Front teat position	14.744*	5	AA	2	0.950
3	Long term changes in teat-end condition	0.311	3	-	-	-
4	Rear teat placement	4.676	5	-	-	-
5	Rear teat position	10.389*	4	AA	4	1.364
6	Skin condition	0.327	2	-	-	-
7	Teat end shape	0.104	2	-	-	-
8	Teat shape	12.307*	2	-	-	-
9	Teat size	12.858*	5	AA	2	6.417
10	Udder shape	6.463*	2	AA	2	1.16
11	Udder suspension	10.597*	3	AA	3	2.481

*Significant at $P \leq 0.05$

4.12.1.1(b) Association of SNP rs454303072 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

To establish association between genotypes and mastitis, logistic regression was used. The incidence of mastitis was assigned as 1 for mastitis and 0 for healthy animal. Healthy condition [Mastitis = 0] was taken as event of interest and probability was modeled against mastitis = 0. In Karan Fries cattle, genotypes of SNP rs454303072 had significant effect ($P \leq 0.05$) on incidence of clinical mastitis (Table 4.26). The odds ratio revealed that AA genotype had 12.6% more chance of occurring with healthy animals as compared to genotype GG with confidence interval 0.029 – 0.553. In Sahiwal cattle, the genotypes had significant effect ($P \leq 0.05$) on incidence of clinical mastitis. The odds ratio revealed AA genotype had 356.2% more chance of occurring with healthy animals as compared to genotype GG with confidence interval 1.089-11.656 (Table 4.27).

Table 4.24 (a) Association of SNP rs454303072 (RE: BsrI) with udder and teat type traits (measurements) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P-value
1	Rear udder health	Contrast	1015.435	1015.435	39.841*	0.000
		Error	2166.419	25.487		
2	Rear udder width	Contrast	893.416	893.416	32.442*	0.000
		Error	2340.771	27.538		
3	Udder width	Contrast	11290338.238	11290338.238	13.738*	0.000
		Error	69854666.333	821819.604		
4	Fore udder attachment	Contrast	101.019	101.019	0.269	0.605
		Error	31939.050	375.754		
5	Udder circumference	Contrast	516.418	516.418	4.491*	0.037
		Error	9774.928	114.999		
6	Central ligament	Contrast	3.070	3.070	2.179	0.144
		Error	119.756	1.409		
7	Rear teat length	Contrast	29.083	29.083	0.662	0.418
		Error	3734.336	43.933		
8	Udder diameter	Contrast	23.187	23.187	0.779	0.380
		Error	2530.751	29.774		
9	Udder length	Contrast	22.838	22.838	0.601	0.441
		Error	3232.550	38.030		
10	Teat circumference	Contrast	8.096	8.096	0.309	0.580
		Error	2230.296	26.239		
11	Udder balance	Contrast	1.167	1.167	0.133	0.716
		Error	746.058	8.777		
12	Fore teat length	Contrast	45.428	45.428	1.257	0.265
		Error	3071.989	36.141		
13	Distance between front and rear teat	Contrast	12.979	12.979	0.488	0.487
		Error	2261.020	26.600		
14	Distance between right and left teat	Contrast	0.463	0.463	0.015	0.902
		Error	2563.207	30.155		
15	Shortest distance from front teat end to floor	Contrast	33.372	33.372	0.856	0.358
		Error	3315.165	39.002		
16	Shortest distance from rear teat end to floor	Contrast	47.248	47.248	1.195	0.277
		Error	3360.600	39.536		
17	Teat diameter	Contrast	0.003	0.003	0.000	0.991
		Error	2174.621	25.584		

*Significant at $P \leq 0.05$

Table 4.24 (b) Pair-wise comparison of SNP rs454303072 (RE: BsrI) genotypes with udder and teat type traits (measurements) in Sahiwal cattle

Trait		Genotype	Mean $\pm$ SE	P – value
1	Rear udder height	AA	24.019 $\pm$ 0.591	0.000*
		GG	14.721 $\pm$ 1.349	
2	Rear udder width	AA	24.251 $\pm$ 0.614	0.000*
		GG	15.530 $\pm$ 1.403	
3	Udder width	AA	2079.097 $\pm$ 106.103	0.000*
		GG	1098.732 $\pm$ 242.284	
4	Fore udder attachment	AA	107.575 $\pm$ 2.269	0.605
		GG	104.643 $\pm$ 5.181	
5	Udder circumference	AA	143.255 $\pm$ 1.255	0.037*
		GG	136.624 $\pm$ 2.866	
6	Central ligament	AA	3.650 $\pm$ 0.139	0.144
		GG	3.139 $\pm$ 0.317	
7	Rear teat length	AA	6.381 $\pm$ 0.776	0.418
		GG	4.808 $\pm$ 1.771	
8	Udder diameter	AA	48.929 $\pm$ 0.639	0.38
		GG	47.524 $\pm$ 1.458	
9	Udder ligament	AA	51.047 $\pm$ 0.722	0.441
		GG	49.653 $\pm$ 1.648	
10	Teat circumference	AA	8.897 $\pm$ 0.600	0.58
		GG	8.067 $\pm$ 1.369	
11	Udder balance	AA	-2.206 $\pm$ 0.347	0.716
		GG	-2.521 $\pm$ 0.792	
12	Fore teat length	AA	7.201 $\pm$ 0.704	0.265
		GG	5.234 $\pm$ 1.607	
13	Distance between front and rear teat	AA	6.260 $\pm$ 0.604	0.487
		GG	5.209 $\pm$ 1.378	
14	Distance between right and left teat	AA	7.223 $\pm$ 0.643	0.902
		GG	7.025 $\pm$ 1.468	
15	Shortest distance from front teat end to floor	AA	41.962 $\pm$ 0.731	0.358
		GG	43.648 $\pm$ 1.669	
16	Shortest distance from rear teat end to floor	AA	42.783 $\pm$ 0.736	0.277
		GG	44.788 $\pm$ 1.680	
17	Teat diameter	AA	26.078 $\pm$ 0.592	0.991
		GG	26.094 $\pm$ 1.352	

*Significant at $P \leq 0.05$

Table 4.25 Association of SNP rs454303072 (RE: BsrI) with udder and teat type traits (visual observations) in Sahiwal cattle

Trait		Chi-Square	df	Pairwise estimation		
				Significant genotype*	Trait sub category	Exp (B)
1	Fore teat placement	16.341*	5	AA	4	5.66
2	Front teat position	9.763	8	-	-	-
3	Long term changes in teat-end condition	0.936	2	-	-	-
4	Rear teat placement	9.721*	3	AA	3	1
5	Rear teat position	4.944	6	-	-	-
6	Skin condition	1.05	1	-	-	-
7	Teat end shape	2.959	2	-	-	-
8	Teat shape	6.314*	2	AA	1	3.5
9	Teat size	4.599	3	-	-	-
10	Udder shape	1.781	2	-	-	-
11	Udder suspension	5.061	3	-	-	-

*Significant at $P \leq 0.05$

Table 4.26 Association of SNP rs454303072 (RE: BsrI) with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	31 (0.911)	81 (0.613)	0.006*	0.126 (0.029 – 0.553)
GG	3 (0.089)	51 (0.387)		-
N	34	132		

* Significant at $P \leq 0.05$

Table 4.27 Association of SNP rs454303072 (RE: BsrI) with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	13 (0.722)	61 (0.884)	0.035*	3.562 (1.089-11.656)
GG	5 (0.278)	8 (0.116)		-
N	18	69		

*Significant at $P < 0.05$

In Karan Fries cows, SNP rs454303072 genotypes had significant ($P \leq 0.05$) effect on monthly milk yield, test day milk yield, 305-day milk yield and total milk yield [Table 4.28 (a)]. GG genotype was found to be superior with higher 305-day milk yield (5136.771 ± 221.614 kg), monthly milk yield (423.537 ± 19.327 kg), test day milk yield (14.772 ± 0.634 kg) and total milk yield (5104.625 ± 225.838 kg) as compared to genotype AA [Table 4.28 (b)]. However, as only two genotypes were detected, multiple comparison procedure could not be performed. In Sahiwal cattle, SNP rs454303072 genotypes had significant ($P \leq 0.05$) effect on monthly milk yield, test day milk yield, 305-day milk yield and total milk yield [Table 4.29 (a)]. The genotype AA was found to be superior with higher 305-day milk yield (2078.098 ± 106.071 kg), monthly milk yield (223.034 ± 14.786 kg), test day milk yield (8.381 ± 0.480 kg) and total milk yield (2048.459 ± 129.134 kg) as compared to genotype GG [Table 4.29 (b)]. However, as only two genotypes were detected multiple comparison procedure could not be performed.

Table 4.28(a) Association of SNP rs454303072 (RE: BsrI) with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P – value*
Milk yield (305 d)	Contrast	85310204.319	85310204.319	31.018*	0.000
	Error	451052346.414	2750319.185		
Monthly milk yield	Contrast	227422.337	227422.337	10.872*	0.001
	Error	3430496.132	20917.659		
Test day milk yield	Contrast	242.939	242.939	10.787*	0.001
	Error	3693.646	22.522		
Total milk yield	Contrast	89270963.083	89270963.083	31.256*	0.000
	Error	468407995.025	2856146.311		

* Significant at $P < 0.05$

Table 4.28 (b) Pair-wise comparison of SNP rs454303072 (RE: BsrI) genotypes with milk production traits in Karan Fries cattle

Trait	Genotype	Mean $\pm$ Std. Error	P – value
Milk yield (305 d)	AA	3620.545 $\pm$ 158.123	0.000*
	GG	5136.771 $\pm$ 221.614	
Monthly milk yield	AA	345.252 $\pm$ 13.790	0.001*
	GG	423.537 $\pm$ 19.327	
Test day milk yield	AA	12.214 $\pm$ 0.452	0.001*
	GG	14.772 $\pm$ 0.634	
Total milk yield	AA	3553.600 $\pm$ 161.136	0.000*
	GG	5104.625 $\pm$ 225.838	

* Significant at P<0.05

Table 4.29 (a) Association of SNP rs454303072 (RE: BsrI) with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P – value
Milk yield (305 d)	Contrast	11346696.778	11346696.778	13.815*	0.000
	Error	69813413.501	821334.276		
Monthly milk yield	Contrast	163266.024	163266.024	10.229*	0.002
	Error	1356642.879	15960.504		
Test day milk yield	Contrast	53.353	53.353	3.172*	0.049
	Error	1429.884	16.822		
Total milk yield	Contrast	18053902.145	18053902.145	14.831*	0.000
	Error	103472312.752	1217321.326		

*Significant at P<0.05

The association of udder and teat type traits with VDR gene in the present study might be due to the association of bovine VDR gene with growth traits and it's crucial role in cell differentiation and proliferation, bone and cartilage development and regulation of osteoporosis which in turn plays a role in mammary gland development (Gao *et al.*, 2013). In a Genome-wide association study for udder composition traits in *Bos indicus* x *Bos taurus*

cows, Tolleson (2016) has reported a significant SNP within the VDR gene for udder support. VDR mediates the action of vitamin D3 by controlling the expression of hormone sensitive genes (Akutsu *et al.*, 2001) and has major role in calcium related metabolism and calcium homeostasis (Gao *et al.*, 2013), which effect milk production of the animal.

Table 4.29 (b) Pair-wise comparison of SNP rs454303072 (RE: BsrI) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ Std. Error	P – value
Milk yield (305 d)	AA	2078.098 $\pm$ 106.071	0.000*
	GG	1095.289 $\pm$ 242.212	
Monthly milk yield	AA	223.034 $\pm$ 14.786	0.002*
	GG	105.143 $\pm$ 33.764	
Test day milk yield	AA	8.381 $\pm$ 0.480	0.049*
	GG	6.250 $\pm$ 1.096	
Total milk yield	AA	2048.459 $\pm$ 129.134	0.000*
	GG	808.750 $\pm$ 294.875	

*Significant at $P < 0.05$

This may be the reason for association of milk production traits with SNPs of this gene. Metabolic pathways involved in the immune response are also regulated by VDR gene (Haussler *et al.*, 1998). The vitamin D pathway influences innate and adaptive immune responses in cattle. It has potential to reduce the severity of bacterial infection of the bovine mammary gland (Nelson *et al.*, 2012). There is correlation between linear type traits and somatic cell score: – 0.28 for udder depth, – 0.21 for front teat placement and – 0.16 for udder cleft (Schutz *et al.*, 1993). Most studies have focused on somatic cell score as a measure of mastitis (Heyen *et al.*, 1999; Schrooten *et al.*, 2000; Kuhn *et al.*, 2003; Schrooten *et al.*, 2004; Ashwell *et al.*, 2005). Heyen *et al.* (1999) and Lund *et al.* (2008) found that QTIs at BTA 5 were found to have significant affect on somatic cell score and clinical mastitis as well, it appears that presence of VDR gene might be one of the reasons for this association, however, no reports could be found pertaining to association of VDR gene variants with somatic cell score and clinical mastitis. These findings are in close agreement with our results

underscoring the usefulness of SNP at VDR gene for use as marker for udder type and mastitis resistant trait in cattle.

4.12.2. Association analysis of PTPRR gene with udder and teat type traits in Karan Fries and Sahiwal cattle

The PTPRR gene plays role in cellular differentiation and proliferation. As per the previous reports (Tolleson, 2016), two significant SNPs namely rs382671389 and rs435289107 were selected for testing of association between genotypes at these loci and type traits, production traits and mastitis in KF and Sahiwal cattle. Both the loci were polymorphic and the results of their association analysis will be described and discussed under subsequent subsections.

4.12.2.1(a) Association of SNP rs382671389 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs382671389 situated in intron 5 of bovine PTPRR gene (located in BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits. In Karan Fries cattle, SNP rs382671389 genotypes were found to be significantly ($P \leq 0.05$) associated with RUH, RUW, CL and FTL [Table 4.30 (a)]. For RUH and CL, the genotype CC was having the highest mean value, $24.544 \pm 0.694\text{cm}$ and $3.518 \pm 0.188\text{cm}$, respectively. Similarly, for RUW the genotype AA was found to have highest mean value ($13.039 \pm 0.340\text{cm}$). However, all these differences were found to be statistically non significant. For FTL, genotype AA was having the highest mean value ($5.665 \pm 0.189\text{cm}$) which was significantly ($P \leq 0.05$) different from AC. Also, the genotype CC was having mean value of 5.306 ± 0.191 which was not different from both AA and AC genotypes ($P \leq 0.05$) [Table 4.30 (b)]. Association analysis between genotypes and observational udder type traits showed significant of genotypes on front teat position, rear teat position and teat size (Table 4.31). Odds ratio estimation showed that odds of finding genotype AA with trait subcategory 5 of front teat position (15.167), trait subcategory 3 of rear teat position (12.007) and trait subcategory 1 of teat size (0.996) were significantly higher than other genotypes (Table 4.31). In Sahiwal cattle, SNP rs382671389 genotypes were found to be significantly ($P \leq 0.05$) associated with RUH, RUW, FUA, UC and TC [Table 4.32 (a)]. For RUH and RUW, the genotype AA was having the highest mean value of $25.022 \pm 0.815\text{ cm}$ and $25.390 \pm 0.821\text{ cm}$, respectively, which was significantly different from other genotypes ($P \leq 0.05$) [Table 4.32 (b)]. For FUA the genotype CC ($117.105 \pm 4.269\text{ cm}$) was having highest mean that was significantly different from other genotypes,

similarly, for UC the genotype CC was having significantly lowest mean value (137.220 ± 2.442 cm) ($P \leq 0.05$) [Table 4.32 (b)].

Table 4.30(a) Association of SNP rs382671389 (RE: Hinp1I) with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P – value
1	Rear udder height	Contrast	150.440	75.220	2.737*	0.048
		Error	4479.433	27.481		
2	Rear udder width	Contrast	38.060	19.030	2.842*	0.041
		Error	1091.503	6.696		
3	Udder width	Contrast	3.250	1.625	0.021	0.980
		Error	12841.190	78.780		
4	Fore udder attachment	Contrast	2051.383	1345.691	0.215	0.736
		Error	19006.879	255.270		
5	Udder circumference	Contrast	120.883	60.442	0.094	0.911
		Error	105307.257	646.057		
6	Udder balance	Contrast	18.614	9.307	0.286	0.752
		Error	5312.883	32.594		
7	Central ligament	Contrast	11.707	5.853	2.913*	0.047
		Error	327.550	2.010		
8	Udder depth	Contrast	2.688	1.344	0.037	0.963
		Error	5857.207	35.934		
9	Udder length	Contrast	176.733	88.367	1.595	0.206
		Error	9028.444	55.389		
10	Fore teat length	Contrast	16.505	8.253	3.964*	0.021
		Error	339.375	2.082		
11	Rear teat length	Contrast	5.245	2.622	2.118	0.124
		Error	201.824	1.238		
12	Teat circumference	Contrast	0.244	0.122	0.051	0.951
		Error	392.252	2.406		
13	Teat diameter	Contrast	0.005	0.003	0.024	0.976
		Error	17.957	0.110		
14	Distance between front and rear teat	Contrast	59.629	29.814	2.023	0.136
		Error	2402.130	14.737		
15	Distance between right and left teat	Contrast	61.281	30.641	1.352	0.262
		Error	3694.667	22.667		
16	Shortest distance from front teat end to floor	Contrast	28.434	14.217	0.465	0.629
		Error	4986.313	30.591		
17	Shortest distance from rear teat end to floor	Contrast	19.693	9.846	0.306	0.736
		Error	5237.268	32.130		

* Significant at $P < 0.05$

Table 4.30(b) Pair-wise comparison of SNP rs382671389 (RE: Hinp1I) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ Std. Error	P – value
1	Rear udder height	AA ^a	22.263 $\pm$ 0.688	0.048*
		AC ^a	23.550 $\pm$ 0.734	
		CC ^a	24.544 $\pm$ 0.694	
2	Rear udder width	AA ^a	13.039 $\pm$ 0.340	0.041*
		AC ^a	11.914 $\pm$ 0.362	
		CC ^a	12.827 $\pm$ 0.343	
3	Udder width	AA	71.593 $\pm$ 1.165	0.98
		AC	71.251 $\pm$ 1.243	
		CC	71.386 $\pm$ 1.176	
4	Fore udder attachment	AA	132.517 $\pm$ 2.090	0.736
		AC	131.667 $\pm$ 2.229	
		CC	130.211 $\pm$ 2.109	
5	Udder circumference	AA	141.738 $\pm$ 3.338	0.911
		AC	142.710 $\pm$ 3.559	
		CC	143.788 $\pm$ 3.367	
6	Udder balance	AA	1.312 $\pm$ 0.750	0.752
		AC	1.235 $\pm$ 0.799	
		CC	0.574 $\pm$ 0.756	
7	Central ligament	AA ^a	2.945 $\pm$ 0.186	0.047*
		AC ^a	2.976 $\pm$ 0.198	
		CC ^a	3.518 $\pm$ 0.188	
8	Udder depth	AA	53.640 $\pm$ 0.787	0.963
		AC	53.483 $\pm$ 0.839	
		CC	53.798 $\pm$ 0.794	
9	Udder length	AA	59.719 $\pm$ 0.977	0.206
		AC	59.720 $\pm$ 1.042	
		CC	61.892 $\pm$ 0.986	

Table 4.30(b) contd...

Trait		Genotype	Mean $\pm$ Std. Error	P – value
10	Fore teat length	AA ^b	5.665 $\pm$ 0.189	0.021*
		AC ^a	4.885 $\pm$ 0.202	
		CC ^{a,b}	5.306 $\pm$ 0.191	
11	Rear teat length	AA	4.859 $\pm$ 0.146	0.124
		AC	4.420 $\pm$ 0.156	
		CC	4.670 $\pm$ 0.147	
12	Teat circumference	AA	7.527 $\pm$ 0.204	0.951
		AC	7.599 $\pm$ 0.217	
		CC	7.508 $\pm$ 0.205	
13	Teat diameter	AA	2.225 $\pm$ 0.044	0.976
		AC	2.213 $\pm$ 0.046	
		CC	2.226 $\pm$ 0.044	
14	Distance between front and rear teat	AA	7.569 $\pm$ 0.504	0.136
		AC	6.255 $\pm$ 0.538	
		CC	6.370 $\pm$ 0.508	
15	Distance between right and left teat	AA	9.779 $\pm$ 0.625	0.262
		AC	8.318 $\pm$ 0.667	
		CC	8.796 $\pm$ 0.631	
16	Shortest distance from front teat end to floor	AA	47.034 $\pm$ 0.726	0.629
		AC	47.405 $\pm$ 0.774	
		CC	46.395 $\pm$ 0.733	
17	Shortest distance from rear teat end to floor	AA	46.711 $\pm$ 0.744	0.736
		AC	47.274 $\pm$ 0.794	
		CC	46.430 $\pm$ 0.751	

* Significant at $P \leq 0.05$ ^{a,b,c} Means with different letter superscript differ significantly ($P \leq 0.05$)

Table 4.31 Association of SNP rs382671389 with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	3.854	10	-	-	-
2	Front teat position	25.105*	10	AA	5	15.167
3	Long term changes in teat-end condition	1.130	6	-	-	-
4	Rear teat placement	10.221	10	-	-	-
5	Rear teat position	32.266*	8	AA	3	12.007
6	Skin condition	5.753	4	-	-	-
7	Teat end shape	5.886	4	-	-	-
8	Teat shape	0.716	4	-	-	-
9	Teat size	17.067*	10	-	-	-
10	Udder shape	4.595	4	-	-	-
11	Udder suspension	4.262	6	-	-	-

* Significant at $P < 0.05$

For TC, the genotype CC was having highest mean value (11.743 ± 1.125 cm) which was significantly different from AC but not from AA genotype [Table 4.32(b)]. For visual observational traits such as udder and teat type, significant association was seen between genotypes and long term changes in teat end condition (LTCTEC), rear teat position (RTP), skin condition (SC) and teat shape (TS) (Table 4.33). Estimation of odds ratio as Exp (B) values indicated that genotype AC was having higher odds of being associated with trait subcategory 2 of LTCTEC and trait subcategory 4 of RTP (Table 4.33). Similarly, the genotype AA was having higher odds of being associated with trait subcategory 1 of skin condition, whereas, for teat shape odds ratio could not associate any single genotype with trait subcategory (Table 4.33).

4.12.2.1 (b) Association of SNP rs382671389 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

Logistic regression analysis revealed genotypes of SNP rs382671389 had significant effect ($P \leq 0.05$) on incidence of clinical mastitis in Karan Fries cattle (Table 4.34). The odds ratio revealed genotype AA had 16.8% (compared to genotype CC and AC) and AC had

69.5% (compared to genotype CC and AA) more chance of occurring with healthy animals with confidence interval 0.053 – 0.534 and 0.176 – 2.741, respectively (Table 4.34).

Table 4.32 (a) Association of SNP rs382671389 (RE: Hinp1I) with udder and teat type traits (measurements) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P – value
1	Rear udder height	Contrast	670.728	335.364	11.218	0.000*
		Error	2511.125	29.894		
2	Rear udder width	Contrast	689.157	344.579	11.373	0.000*
		Error	2545.030	30.298		
3	Udder width	Contrast	3555699.650	1777849.825	1.925	0.152
		Error	77589304.921	923682.201		
4	Fore udder attachment	Contrast	2953.383	1476.691	4.265	0.017*
		Error	29086.686	346.270		
5	Udder circumference	Contrast	773.501	386.750	3.413	0.038*
		Error	9517.845	113.308		
6	Central ligament	Contrast	6.118	3.059	2.202	0.117
		Error	116.708	1.389		
7	Rear teat length	Contrast	28.881	14.440	0.325	0.724
		Error	3734.538	44.459		
8	Udder diameter	Contrast	34.200	17.100	0.570	0.568
		Error	2519.738	29.997		
9	Udder length	Contrast	4.263	2.132	0.055	0.946
		Error	3251.125	38.704		
10	Teat circumference	Contrast	217.431	108.715	4.519	0.014*
		Error	2020.961	24.059		
11	Udder balance	Contrast	1.291	0.645	0.073	0.930
		Error	745.934	8.880		
12	Fore teat length	Contrast	52.060	26.030	0.713	0.493
		Error	3065.357	36.492		
13	Distance between front and rear teat	Contrast	140.458	70.229	2.765	0.049*
		Error	2133.541	25.399		
14	Distance between right and left teat	Contrast	4.805	2.402	0.079	0.924
		Error	2558.866	30.463		
15	Shortest distance from front teat end to floor	Contrast	27.012	13.506	0.342	0.712
		Error	3321.524	39.542		
16	Shortest distance from rear teat end to floor	Contrast	14.867	7.434	0.184	0.832
		Error	3392.981	40.393		
17	Teat diameter	Contrast	8.406	4.203	0.163	0.850
		Error	2166.218	25.788		

* Significant at P<0.05

Table 4.32 (b) Pair-wise comparison of SNP rs382671389 (RE: Hinp1I) genotypes with udder and teat type traits (measurements) in Sahiwal cattle

Trait		Genotype	Mean $\pm$ Std. Error	P – value
1	Rear udder height	AA ^a	25.022 $\pm$ 0.815	0.000*
		AC ^b	21.164 $\pm$ 1.140	
		CC ^b	18.248 $\pm$ 1.254	
2	Rear udder width	AA ^a	25.390 $\pm$ 0.821	0.000*
		AC ^b	21.430 $\pm$ 1.148	
		CC ^b	18.542 $\pm$ 1.263	
3	Udder width	AA	2105.786 $\pm$ 143.270	0.152
		AC	1636.760 $\pm$ 200.400	
		CC	1828.971 $\pm$ 220.488	
4	Fore udder attachment	AA ^a	106.289 $\pm$ 2.774	0.017*
		AC ^a	100.435 $\pm$ 3.880	
		CC ^b	117.105 $\pm$ 4.269	
5	Udder circumference	AA ^b	144.718 $\pm$ 1.587	0.038*
		AC ^b	141.341 $\pm$ 2.220	
		CC ^a	137.220 $\pm$ 2.442	
6	Central ligament	AA	3.821 $\pm$ 0.176	0.117
		AC	3.247 $\pm$ 0.246	
		CC	3.355 $\pm$ 0.270	
7	Rear teat length	AA	6.672 $\pm$ 0.994	0.724
		AC	5.384 $\pm$ 1.390	
		CC	5.742 $\pm$ 1.530	
8	Udder diameter	AA	49.221 $\pm$ 0.816	0.568
		AC	47.723 $\pm$ 1.142	
		CC	48.662 $\pm$ 1.256	

Table 4.32 (b) contd...

Trait		Genotype	Mean $\pm$ Std. Error	P – value
9	Udder length	AA	50.907 $\pm$ 0.927	0.946
		AC	50.466 $\pm$ 1.297	
		CC	51.054 $\pm$ 1.427	
10	Teat circumference	AA ^{a,b}	8.041 $\pm$ 0.731	0.014*
		AC ^a	7.715 $\pm$ 1.023	
		CC ^b	11.743 $\pm$ 1.125	
11	Udder balance	AA	-2.246 $\pm$ 0.444	0.930
		AC	-2.109 $\pm$ 0.621	
		CC	-2.460 $\pm$ 0.684	
12	Fore teat length	AA	7.589 $\pm$ 0.901	0.493
		AC	5.792 $\pm$ 1.260	
		CC	6.537 $\pm$ 1.386	
13	Distance between front and rear teat	AA	5.136 $\pm$ 0.751	0.069
		AC	8.158 $\pm$ 1.051	
		CC	5.851 $\pm$ 1.156	
14	Distance between right and left teat	AA	7.031 $\pm$ 0.823	0.924
		AC	7.581 $\pm$ 1.151	
		CC	7.099 $\pm$ 1.266	
15	Shortest distance from front teat end to floor	AA	42.664 $\pm$ 0.937	0.712
		AC	41.335 $\pm$ 1.311	
		CC	42.303 $\pm$ 1.443	
16	Shortest distance from rear teat end to floor	AA	43.464 $\pm$ 0.947	0.832
		AC	42.483 $\pm$ 1.325	
		CC	43.010 $\pm$ 1.458	
17	Teat diameter	AA	26.055 $\pm$ 0.757	0.850
		AC	26.514 $\pm$ 1.059	
		CC	25.618 $\pm$ 1.165	

* Significant at $P \leq 0.05$ ^{a,b,c} Means with different letter superscript differ significantly ($P \leq 0.05$)

Table 4.33 Association of SNP rs382671389 (RE: Hinp1I) with udder and teat type traits (visual observations) in Sahiwal cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	15.906	10	-	-	-
2	Front teat position	13.866	16	-	-	-
3	Long term changes in teat-end condition	16.143*	4	AC	2	11.023
4	Rear teat placement	9.631	6	-	-	-
5	Rear teat position	20.993*	12	AC	4	8
6	Skin condition	15.632*	2	AA	1	5.733
7	Teat end shape	0.970	4	-	-	-
8	Teat shape	9.534*	4	-	-	-
9	Teat size	3.700	6	-	-	-
10	Udder shape	5.495	4	-	-	-
11	Udder suspension	2.754	6	-	-	-

* Significant at $P < 0.05$

Table 4.34 Association of SNP rs382671389 (RE: Hinp1I) with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value	ODDS ratio estimation
AA	23 (0.676)	38 (0.287)	0.001*	0.168 (0.053 – 0.534)
AC	06 (0.176)	44 (0.333)		0.695 (0.176 – 2.741)
CC	05 (0.147)	50 (0.379)		-
N	34	132		

* Significant at $P < 0.05$

In Sahiwal cattle the rs382671389 genotypes had no significant effect on incidence of clinical mastitis (Table 4.35). Though the odds ratio revealed genotype AA had 184.6% (compared to genotype CC and AC) and AC had 86.5% (compared to genotype CC and AA) more chance of occurring with healthy animals as compared to genotype CC with confidence interval 0.549–6.204 and 0.237– 3.153 , respectively, the overall effect was non significant ($P \leq 0.05$) (Table 4.35).

Table 4.35 Association of SNP rs382671389 (RE: Hinp1I) with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	p- value	ODDS ratio estimation
AA	7 (0.389)	39 (0.565)	0.367	1.846 (0.549 – 6.204)
AC	6 (0.333)	16 (0.232)		0.865 (0.237 – 3.153)
CC	5 (0.278)	14 (0.203)		-
N	18	69	.	

* Significant at P<0.05

In Karan Fries, SNP rs382671389 genotypes had significant (P<0.05) effect on monthly milk yield, test day milk yield, 305-day milk yield and total milk yield [Table 4.36 (a)]. CC genotype was found to be superior with higher monthly milk yield (403.596 ± 19.487 kg), test day milk yield (13.863 ± 0.639 kg), 305-day milk yield (4613.043 ± 233.920 kg) and total milk yield (4572.605 ± 238.339 kg) as compared to genotype AC and AA.

Table 4.36 (a) Association of SNP rs382671389 (RE: Hinp1I) genotypes with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P – value
Milk yield (305 d)	Contrast	27971934.719	13985967.360	4.484*	0.013
	Error	508390616.014	3118960.834		
Monthly milk yield	Contrast	129631.760	64815.880	2.994*	0.043
	Error	3528286.708	21645.931		
Test day milk yield	Contrast	140.578	70.289	3.018*	0.042
	Error	3796.007	23.288		
Total milk yield	Contrast	29897096.557	14948548.278	4.617*	0.011
	Error	527781861.552	3237925.531		

* Significant at P<0.05

However, this difference was statistically non significant for test day milk yield, not different from genotype AC for total milk yield, monthly milk yield and 305-day milk yield [Table 4.36 (b)].

Table 4.36(b) Pair-wise comparison of SNP rs382671389 (RE: Hinp1I) genotypes with milk production traits in Karan Fries cattle

Trait	Genotype	Mean $\pm$ Std. Error	P - value]
Milk yield (305 d)	AA ^a	3628.254 $\pm$ 231.895	0.013*
	AC ^{a,b}	4167.391 $\pm$ 247.298	
	CC ^b	4613.043 $\pm$ 233.920	
Monthly milk yield	AA ^a	336.752 $\pm$ 19.319	0.043*
	AC ^{a,b}	375.672 $\pm$ 20.602	
	CC ^b	403.596 $\pm$ 19.487	
Test day milk yield	AA ^a	11.828 $\pm$ 0.634	0.042*
	AC ^a	13.619 $\pm$ 0.676	
	CC ^a	13.863 $\pm$ 0.639	
Total milk yield	AA ^a	3554.784 $\pm$ 236.276	0.011*
	AC ^{a,b}	4116.451 $\pm$ 251.970	
	CC ^b	4572.605 $\pm$ 238.339	

* Significant at P<0.05

^{a,b,c} Means with different letter superscript differ significantly (P $\leq$ 0.05)

In Sahiwal cattle, SNP rs382671389 genotypes had significant (P $\leq$ 0.05) effect only on monthly milk yield [Table 4.37 (a)]. The genotype AA was having maximum mean value of monthly milk yield (238.456 $\pm$ 19.245 kg), and it was significantly different from genotype CC but was not significantly different from AC (P $\leq$ 0.05) [Table 4.37 (b)].

Table 4.37(a) Association of SNP rs382671389 (RE: Hinp1I) with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P - value
Milk yield (305 d)	Contrast	3565468.898	1782734.449	1.930	0.152
	Error	77594641.382	923745.731		
Monthly milk yield	Contrast	119858.403	59929.202	3.596*	0.032
	Error	1400050.499	16667.268		
Test day milk yield	Contrast	74.368	37.184	2.217	0.115
	Error	1408.868	16.772		
Total milk yield	Contrast	5256112.092	2628056.046	1.899	0.156
	Error	116270102.805	1384167.891		

* Significant at P $\leq$ 0.05

Table 4.37(b) Pair-wise comparison of SNP rs382671389 (RE: Hinp1I) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ Std. Error	Significant
Milk yield (305 d)	AA	2104.691 $\pm$ 143.275	0.152
	AC	1635.099 $\pm$ 200.407	
	CC	1827.202 $\pm$ 220.495	
Monthly milk yield	AA ^{a,c}	238.456 $\pm$ 19.245	0.032*
	AC ^{b,c}	180.957 $\pm$ 26.920	
	CC ^b	150.579 $\pm$ 29.618	
Test day milk yield	AA	8.586 $\pm$ 0.611	0.115
	AC	8.405 $\pm$ 0.854	
	CC	6.295 $\pm$ 0.940	
Total milk yield	AA	2063.567 $\pm$ 175.383	0.156
	AC	1481.391 $\pm$ 245.319	
	CC	1785.658 $\pm$ 269.909	

* Significant at $P \leq 0.05$

^{a,b,c} Means with different letter superscript differ significantly ($P \leq 0.05$)

4.12.2.2 (a) Association of SNP rs435289107 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs435289107 situated in 3' UTR of bovine PTPRR gene (located in BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits.

In Karan Fries cattle, SNP rs435289107 was found to be significantly ($P \leq 0.05$) associated with rear udder height (RUH), udder width (UW), udder circumference (UC), udder length (UL), distance between right and left teat (DLR) and shortest distance from rear teat end to floor (SDR) [Table 4.38 (a)]. For RUH there was significant difference among genotypes TT (26.396 $\pm$ 0.858 cm) and CC (22.368 $\pm$ 0.498 cm), however they were not different from TC (23.744 $\pm$ 0.977 cm) [Table 4.38 (b)]. A similar situation was observed for UW, where, the genotype CC (73.209 $\pm$ 0.839 cm) was having highest mean value which was significantly ($P \leq 0.05$) different from TT (68.202 $\pm$ 1.447 cm) [Table 4.38 (b)]. However, both the genotypes CC and TT were not different from TC genotype for UW [Table 4.38 (b)].

Table 4.38 (a) Association of SNP rs435289107 (RE: TaqI) with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P - value
1	Rear udder height	Contrast	427.726	213.863	8.296*	0.000
		Error	4202.147	25.780		
2	Rear udder width	Contrast	13.218	6.609	0.965	0.383
		Error	1116.344	6.849		
3	Udder width	Contrast	897.870	448.935	6.125*	0.003
		Error	11946.571	73.292		
4	Fore udder attachment	Constant	7731.890	3368.099	1.045	0.273
		Error	20811.844	577.077		
5	Udder circumference	Contrast	63380.179	31690.090	122.847*	0.000
		Error	42047.961	257.963		
6	Central ligament	Contrast	6.520	3.260	1.597	0.206
		Error	332.737	2.041		
7	Teat circumference	Contrast	1.053	0.526	0.219	0.803
		Error	391.443	2.401		
8	Udder balance	Contrast	18.409	9.204	0.282	0.754
		Error	5313.087	32.596		
9	Fore teat length	Contrast	7.323	3.661	1.712	0.184
		Error	348.558	2.138		
10	Rear teat length	Contrast	2.974	1.487	1.188	0.308
		Error	204.094	1.252		
11	Udder diameter	Contrast	114.654	57.327	1.626	0.200
		Error	5745.241	35.247		
12	Udder length	Contrast	695.069	347.534	6.657*	0.002
		Error	8510.109	52.209		
13	Distance between front and rear teat	Contrast	67.872	33.936	2.311	0.102
		Error	2393.887	14.686		
14	Distance between right and left teat	Contrast	150.593	75.297	3.404*	0.036
		Error	3605.355	22.119		
15	Shortest distance from front teat end to floor	Contrast	114.557	57.279	1.905	0.152
		Error	4900.190	30.063		
16	Shortest distance from rear teat end to floor	Contrast	243.580	121.790	3.960*	0.021
		Error	5013.381	30.757		
17	Teat diameter	Contrast	0.030	0.015	0.137	0.872
		Error	17.933	0.110		

* Significant at $P \leq 0.05$

Table 4.38(b) Pair-wise comparison of SNP rs435289107 (RE: TaqI) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ SE	P –value
1	Rear udder height	CC ^a	22.368 $\pm$ 0.498	0.000*
		TC ^{a,b}	23.744 $\pm$ 0.977	
		TT ^b	26.396 $\pm$ 0.858	
2	Rear udder width	CC	12.837 $\pm$ 0.257	0.383
		TC	12.202 $\pm$ 0.504	
		TT	12.299 $\pm$ 0.442	
3	Udder width	CC ^b	73.209 $\pm$ 0.839	0.003*
		TC ^{a,b}	68.682 $\pm$ 1.648	
		TT ^a	68.202 $\pm$ 1.447	
4	Fore udder attachment	CC	132.635 $\pm$ 1.552	0.273
		TC	131.889 $\pm$ 3.045	
		TT	127.657 $\pm$ 2.675	
5	Udder circumference	CC ^c	157.186 $\pm$ 1.575	0.000*
		TC ^b	129.011 $\pm$ 3.091	
		TT ^a	110.408 $\pm$ 2.715	
6	Central ligament	CC	3.299 $\pm$ 0.140	0.206
		TC	3.001 $\pm$ 0.275	
		TT	2.829 $\pm$ 0.242	
7	Rear teat length	CC	4.625 $\pm$ 0.110	0.308
		TC	4.483 $\pm$ 0.215	
		TT	4.899 $\pm$ 0.189	
8	Udder diameter	CC	53.116 $\pm$ 0.582	0.200
		TC	53.663 $\pm$ 1.143	
		TT	55.208 $\pm$ 1.004	

Table 4.38(b) contd...

Trait		Genotype	Mean $\pm$ SE	P –value
9	Udder ligament	CC ^b	62.037 $\pm$ 0.709	0.002*
		TC ^a	58.234 $\pm$ 1.391	
		TT ^a	57.519 $\pm$ 1.221	
10	Teat circumference	CC	7.587 $\pm$ 0.152	0.803
		TC	7.570 $\pm$ 0.298	
		TT	7.389 $\pm$ 0.262	
11	Udder balance	CC	0.923 $\pm$ 0.560	0.754
		TC	1.782 $\pm$ 1.099	
		TT	0.794 $\pm$ 0.965	
12	Fore teat length	CC	5.253 $\pm$ 0.143	0.184
		TC	5.013 $\pm$ 0.281	
		TT	5.673 $\pm$ 0.247	
13	Distance between front and rear teat	CC	7.246 $\pm$ 0.376	0.102
		TC	6.022 $\pm$ 0.738	
		TT	5.855 $\pm$ 0.648	
14	Distance between right and left teat	CC ^a	9.726 $\pm$ 0.461	0.036*
		TC ^a	7.885 $\pm$ 0.905	
		TT ^a	7.667 $\pm$ 0.795	
15	Shortest distance from front teat end to floor	CC	46.370 $\pm$ 0.538	0.152
		TC	47.109 $\pm$ 1.055	
		TT	48.452 $\pm$ 0.927	
16	Shortest distance from rear teat end to floor	CC ^a	45.952 $\pm$ 0.544	0.021*
		TC ^a	47.179 $\pm$ 1.067	
		TT ^b	48.971 $\pm$ 0.937	
17	Teat diameter	CC	2.220 $\pm$ 0.033	0.872
		TC	2.249 $\pm$ 0.064	
		TT	2.205 $\pm$ 0.056	

* Significant at $P \leq 0.05$

^{a,b,c} Means with different letter superscript differ significantly ($P \leq 0.05$)

For UC, all the three genotypes viz., CC (157.186 ± 1.575 cm), TC (129.011 ± 3.091 cm) and TT (110.408 ± 2.715 cm) were differing significantly from each other [Table 4.38 (b)]. For UL, the genotype CC (62.037 ± 0.709 cm) was having highest mean which was significantly ($P \leq 0.05$) different from TC and TT genotypes [Table 4.38 (b)]. For SDR, genotype TT (48.971 ± 0.937 cm) was having significantly higher mean as compared to other genotypes [Table 4.38 (b)]. However for DLR, there was difference in mean values of all genotypes but it was found to be non significant [Table 4.38 (b)].

Among the observational udder type traits, genotypes had significant effect on front teat position (FTP), long term changes in teat-end condition (LTC TEC) and teat shape (TS). CC genotype of the SNP was found to be having higher odds of being associated with trait sub category 4, 2 and 1 of front teat position, long term changes in teat-end condition and teat shape, respectively, compared to TT and TC genotype of the same gene (Table 4.39).

Table 4.39 Association of SNP rs435289107 (RE: TaqI) with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	12.242	10	-	-	-
2	Front teat position	22.151*	10	CC	4	1.8
3	Long-term changes in teat-end condition	46.116*	6	CC	2	5.583
4	Rear teat placement	14.912	10	-	-	-
5	Rear teat position	4.323	8	-	-	-
6	Skin condition	1.039	4	-	-	-
7	Teat end shape	5.478	4	-	-	-
8	Teat shape	53.245*	4	CC	1	12
9	Teat size	12.022	10	-	-	-
10	Udder shape	7.114	4	-	-	-
11	Udder suspension	7.321	6	-	-	-

* Significant at $P \leq 0.05$

In Sahiwal cattle, SNP rs435289107 was found to be significantly associated ($P \leq 0.05$) with rear udder height (RUH), rear udder width (RUW), udder width (UW), fore udder attachment (FUA), central ligament (CL), shortest distance from front teat end to floor (SDF) and shortest distance from rear teat end to floor (SDR) [Table 4.40 (a)].

Table 4.40(a) Association of SNP rs435289107 (RE:TaqI) with udder and teat type traits (measurements) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P - value
1	Rear udder height	Contrast	864.126	432.063	15.659*	0.000
		Error	2317.728	27.592		
2	Rear udder width	Contrast	840.059	420.030	14.737*	0.000
		Error	2394.128	28.502		
3	Udder width	Contrast	6606725.943	3303362.972	3.723*	0.028
		Error	74538278.627	887360.460		
4	Fore udder attachment	Contrast	8732.182	4366.091	15.735*	0.000
		Error	23307.887	277.475		
5	Udder circumference	Contrast	199.502	99.751	0.830	0.439
		Error	10091.843	120.141		
6	Udder balance	Contrast	1.852	0.926	0.104	0.901
		Error	745.373	8.873		
7	Central ligament	Contrast	12.863	6.432	4.913*	0.010
		Error	109.963	1.309		
8	Udder diameter	Contrast	64.349	32.175	1.086	0.342
		Error	2489.589	29.638		
9	Udder length	Contrast	1.697	0.848	0.022	0.978
		Error	3253.692	38.734		
10	Fore teat length	Contrast	114.677	57.338	1.604	0.207
		Error	3002.741	35.747		

Table 4.40(a) contd...

Dependent Variable			Sum of Squares	Mean Square	F	P - value
11	Rear teat length	Contrast	139.985	69.992	1.623	0.204
		Error	3623.434	43.136		
12	Teat circumference	Contrast	34.912	17.456	0.665	0.517
		Error	2203.480	26.232		
13	Teat diameter	Contrast	46.025	23.013	0.908	0.407
		Error	2128.599	25.340		
14	Distance between front and rear teat	Contrast	9.691	4.846	0.180	0.836
		Error	2264.308	26.956		
15	Distance between right and left teat	Contrast	15.410	7.705	0.254	0.776
		Error	2548.260	30.336		
16	Shortest distance from front teat end to floor	Contrast	213.175	106.587	2.856*	0.043
		Error	3135.362	37.326		
17	Shortest distance from rear teat end to floor	Contrast	210.182	105.091	2.761*	0.049
		Error	3197.666	38.067		

* Significant at $P \leq 0.05$

For trait RUH and RUW, multiple comparison procedure showed that genotype TT was having lowest mean value of 18.390 ± 0.943 cm and 18.836 ± 0.959 cm respectively, which was significantly lower than other genotypes ($P \leq 0.05$) [Table 4.40 (b)]. For UW, the genotype TC was having highest mean value (2290.580 ± 196.420 cm) which was significantly different from genotype TT (1591.188 ± 169.188 cm) but not from genotype CC (1974.126 ± 163.981 cm) [Table 4.40 (b)]. For FUA, the genotype CC was having highest value ($119.576 \pm 2.900^\circ$) which was significantly different from other genotypes [Table 4.40 (b)]. Similarly for CL, genotype CC was having highest value (4.002 ± 0.199 cm) which was significantly different from genotype TT (3.105 ± 0.205 cm) [Table 4.40 (b)]. For SDF and SDR, the genotype TT was having maximum mean value of 43.704 ± 1.097 cm and

44.544 ± 1.108 cm respectively, but it was not significantly different from other genotypes [Table 4.40 (b)].

Table 4.40(b) Pair-wise comparison of SNP rs435289107 (RE: TaqI) genotypes with udder and teat type traits (measurements) in Sahiwal cattle

Trait		Genotype	Mean ± Std. Error	P – value
1	Rear udder height	CC ^a	24.092 ± 0.914	0.000*
		TC ^a	25.842 ± 1.095	
		TT ^b	18.390 ± 0.943	
2	Rear udder width	CC ^a	24.168 ± 0.929	0.000*
		TC ^a	26.360 ± 1.113	
		TT ^b	18.836 ± 0.959	
3	Udder width	CC ^{a,b}	1974.126 ± 163.981	0.028*
		TC ^b	2290.580 ± 196.420	
		TT ^a	1591.188 ± 169.188	
4	Fore udder attachment	CC ^b	119.576 ± 2.900	0.000*
		TC ^a	96.087 ± 3.473	
		TT ^a	102.000 ± 2.992	
5	Udder circumference	CC	143.893 ± 1.908	0.439
		TC	142.204 ± 2.286	
		TT	140.360 ± 1.969	
6	Udder balance	CC	-2.070 ± 0.519	0.901
		TC	-2.385 ± 0.621	
		TT	-2.359 ± 0.535	
7	Central ligament	CC ^b	4.002 ± 0.199	0.010*
		TC ^{a,b}	3.567 ± 0.239	
		TT ^a	3.105 ± 0.205	
8	Udder diameter	CC	49.614 ± 0.948	0.342
		TC	48.858 ± 1.135	
		TT	47.619 ± 0.978	
9	Udder length	CC	50.661 ± 1.083	0.978
		TC	51.010 ± 1.298	
		TT	50.855 ± 1.118	

Table 4.40(b) contd...

Trait		Genotype	Mean $\pm$ Std. Error	P – value
10	Fore teat length	CC	6.758 $\pm$ 1.041	0.207
		TC	8.647 $\pm$ 1.247	
		TT	5.711 $\pm$ 1.074	
11	Rear teat length	CC	5.519 $\pm$ 1.143	0.204
		TC	8.233 $\pm$ 1.369	
		TT	5.215 $\pm$ 1.180	
12	Teat circumference	CC	9.569 $\pm$ 0.892	0.517
		TC	8.374 $\pm$ 1.068	
		TT	8.195 $\pm$ 0.920	
13	Teat diameter	CC	26.916 $\pm$ 0.876	0.407
		TC	26.043 $\pm$ 1.050	
		TT	25.220 $\pm$ 0.904	
14	Distance between front and rear teat	CC	6.470 $\pm$ 0.904	0.836
		TC	6.085 $\pm$ 1.083	
		TT	5.692 $\pm$ 0.932	
15	Distance between right and left teat	CC	7.713 $\pm$ 0.959	0.776
		TC	6.720 $\pm$ 1.148	
		TT	6.986 $\pm$ 0.989	
16	Shortest distance from front teat end to floor	CC ^a	40.255 $\pm$ 1.064	0.043*
		TC ^a	43.092 $\pm$ 1.274	
		TT ^a	43.704 $\pm$ 1.097	
17	Shortest distance from rear teat end to floor	CC ^a	41.136 $\pm$ 1.074	0.049*
		TC ^a	43.992 $\pm$ 1.287	
		TT ^a	44.544 $\pm$ 1.108	

* Significant at $P \leq 0.05$ ^{a,b,c} Means with different letter superscript differ significantly

Among the observational udder and teat type traits, the association study revealed significant ($P \leq 0.05$) association of SNP rs435289107 genotypes with teat end shape (TES) and teat shape (TS) (Table 4.41). The genotype TC had higher odds of being associated with

trait subcategory 1 of teat end shape and genotype CC was having higher odds of being associated with trait subcategory 1 of teat shape (Table 4.41).

Table 4.41 Association of SNP rs435289107 (RE: TaqI) with udder and teat type traits (visual observations) in Sahiwal cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	15.768	10	-	-	-
2	Front teat position	22.68	16	-	-	-
3	Long term changes in teat-end condition	5.373	4	-	-	-
4	Rear teat placement	4.891	6	-	-	-
5	Rear teat position	15.458	12	-	-	-
6	Skin condition	0.821	2	-	-	-
7	Teat end shape	9.418*	4	TC	1	>999.999
8	Teat shape	18.424*	4	CC	1	2.162
9	Teat size	5.464	6	-	-	-
10	Udder shape	4.234	4	-	-	-
11	Udder suspension	3.207	6	-	-	-

* Significant at $P \leq 0.05$

4.12.2.2 (b) Association of SNP rs435289107 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

Logistic regression analysis revealed genotypes of SNP rs435289107 had significant effect ($P \leq 0.05$) on incidence of clinical mastitis in Karan Fries cows (Table 4.42). The odds

Table 4.42 Association of SNP rs435289107 (RE: TaqI) genotypes with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value	ODDS ratio estimation
CC	19 (0.559)	85 (0.644)	0.031*	0.556 (0.151 – 2.049)
TC	11 (0.324)	17 (0.129)		0.188 (0.045 – 0.782)
TT	04 (0.118)	30 (0.227)		-
N	34	132		

*Significant at $P \leq 0.05$

ratio revealed genotype CC had 55.6 % more chance of occurring with healthy animals as compared to genotype CT and TT with confidence interval 0.151 - 2.049, whereas, genotype CT had 18.8 % more chance of occurring with healthy animals as compared to genotype CC and TT with confidence interval 0.045 - 0.782. In Sahiwal cattle, SNP rs435289107 had no significant effect ($P \leq 0.05$) on incidence of clinical mastitis (Table 4.43). Though the odds ratio revealed genotype CC had 81.8 % and CT had 272.7 % more chance of occurring with healthy animals as compared to genotype TT, CT and CC, TT with confidence interval 0.283 – 2.363 and 0.646 – 11.512, respectively (Table 4.43).

Table 4.43 Association of SNP rs435289107 (RE: TaqI) with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	p- value*	ODDS ratio estimation
CC	9 (0.500)	24 (0.348)	0.241	0.818 (0.283 – 2.363)
CT	2 (0.111)	22 (0.319)		2.727 (0.646 – 11.512)
TT	7 (0.389)	23 (0.333)		-
N	18	69		

* Significant at $P \leq 0.05$

In Karan Fries cows, SNP rs435289107 genotypes had significant effect ($P \leq 0.05$) only on monthly milk yield and test day milk yield [Table 4.44 (a)]. The genotype CC was

Table 4.44(a) Association of SNP rs435289107 (RE: TaqI) genotypes with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P – value
Milk yield (305 d)	Contrast	14203424.175	7101712.088	2.217	0.112
	Error	522159126.558	3203430.224		
Monthly milk yield	Contrast	274663.055	137331.527	6.616*	0.002
	Error	3383255.414	20756.168		
Test day milk yield	Contrast	302.238	151.119	6.778*	0.001
	Error	3634.348	22.297		
Total milk yield	Contrast	11564838.086	5782419.043	1.726	0.181
	Error	546114120.022	3350393.374		

*Significant at $P \leq 0.05$

found to be having higher monthly milk yield (400.800 ± 14.127 kg) and test day milk yield (14.056 ± 0.463 kg), which was significantly ($P \leq 0.05$) higher than genotype TT and TC [Table 4.44 (b)].

Table 4.44 (b) Pair-wise comparison of SNP rs435289107 (RE: TaqI) genotypes with milk production traits in Karan Fries cattle

Trait	Genotype	Mean $\pm$ Std. Error	P - value
Milk yield (305 d)	CC	4356.277 ± 175.506	0.112
	TC	3820.996 ± 344.450	
	TT	3705.698 ± 302.534	
Monthly milk yield	CC ^b	400.800 ± 14.127	0.002*
	TC ^a	351.060 ± 27.726	
	TT ^a	300.971 ± 24.352	
Test day milk yield	CC ^b	14.056 ± 0.463	0.001*
	TC ^a	12.297 ± 0.909	
	TT ^a	10.770 ± 0.798	
Total milk yield	CC	4278.894 ± 179.486	0.181
	TC	3802.000 ± 352.262	
	TT	3688.457 ± 309.395	

*Significant at $P \leq 0.05$

In Sahiwal cattle, SNP rs435289107 genotypes had significant ($P \leq 0.05$) effect on 305-day milk yield and monthly milk yield [Table 4.45 (a)]. The genotype TC was found to be having higher 305-day milk yield (2288.616 ± 196.428 kg) and monthly milk yield (254.804 ± 27.120 kg). This difference was significant as compared to genotype TT, but non-significant as compared to genotype TC ($P \leq 0.05$) [Table 4.45 (b)].

The association of udder and teat type traits with PTPRR gene in the present study might be due to the role of the proteins encoded by this gene as signaling molecules to regulate cell growth, differentiation and mitotic cycle (Tolleson, 2016). It also plays critical roles in regulating fundamental cellular processes such as cell proliferation, differentiation and development. These proteins get up-regulated during gestation (Aoki *et al.*, 1999).

Table 4.45(a) Association of SNP rs435289107 (RE: TaqI) genotypes with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P - value
Milk yield (305 d)	Contrast	6616230.124	3308115.062	3.728*	0.028
	Error	74543880.156	887427.145		
Monthly milk yield	Contrast	98956.179	49478.089	2.925*	0.049
	Error	1420952.723	16916.104		
Test day milk yield	Contrast	41.248	20.624	1.201	0.306
	Error	1441.989	17.167		
Total milk yield	Contrast	5979158.953	2989579.476	2.173	0.120
	Error	115547055.944	1375560.190		

* Significant at $P \leq 0.05$

Table 4.45 (b) Pair-wise comparison of SNP rs435289107 (RE: TaqI) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ SE	Significant
Milk yield (305 d)	CC ^{a,b}	1973.835 $\pm$ 163.987	0.028*
	TC ^b	2288.616 $\pm$ 196.428	
	TT ^a	1589.048 $\pm$ 169.194	
Monthly milk yield	CC ^{a,b}	202.288 $\pm$ 22.641	0.049*
	TC ^b	254.804 $\pm$ 27.120	
	TT ^a	168.306 $\pm$ 23.360	
Test day milk yield	CC	7.921 $\pm$ 0.721	0.306
	TC	9.115 $\pm$ 0.864	
	TT	7.363 $\pm$ 0.744	
Total milk yield	CC	2046.348 $\pm$ 204.166	0.12
	TC	2040.652 $\pm$ 244.555	
	TT	1496.629 $\pm$ 210.649	

* Significant at $P \leq 0.05$

Tolleson, (2016) has reported a Genome-wide significant SNP (Hapmap58370-rs29014396) within the PTPRR gene at position 43 Mb of BTA 5 for udder support. A study on mice by Aoki *et al.* (1999) suggested contribution of the proteins encoded by PTPRR gene to the development, involution and remodeling of mammary gland tissue for the next parturition and their inhibitory action on maintaining high expression of milk genes during lactation, which influence both the milk production and occurrence of mastitis. This finding suggests involvement of the gene in development and growth of the mammary gland system and justifies the association of PTPRR gene with milk production traits and clinical mastitis.

4.12.3. Association analysis of IL-22 gene with udder and teat type traits in Karan Fries and Sahiwal cattle

The IL-22 gene plays role in innate immune response and udder development. As per the previous reports (Tolleson, 2016), three significant SNPs namely rs135884509, rs136785910 and rs383429860 were selected for testing of association between genotypes at these loci with type traits, production traits and mastitis in KF and Sahiwal cattle. All the loci were polymorphic and the results of their association analysis will be described and discussed under subsequent subsections.

4.12.3.1(a) Association of SNP rs135884509 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs135884509, situated in intron 5 of bovine IL-22 gene (located on BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits.

In Karan Fries cattle, SNP rs135884509 was found to be significantly ($P \leq 0.05$) associated with RUH, RUW and UC [Table 4.46 (a)]. The genotype AA was having highest mean value of RUH (24.249 ± 0.610 cm) and RUW (13.164 ± 0.302 cm) respectively, which was significantly different from genotype AC but non-significantly different from CC [Table 4.46 (b)]. The AC genotype was having highest mean value of UC *i.e.*, 151.110 ± 4.425 cm, which was significantly different from genotype CC but not from genotype AA [Table 4.46 (b)].

Table 4.46 (a) Association of SNP rs135884509 (RE: HinfI) with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P - value
1	Rear udder height	Contrast	207.143	103.571	3.817*	0.024
		Error	4422.730	27.133		
2	Rear udder width	Contrast	45.119	22.559	3.391*	0.036
		Error	1084.444	6.653		
3	Udder width	Contrast	299.804	149.902	1.948	0.146
		Error	12544.637	76.961		
4	Fore udder attachment	Contrast	528.543	528.543	0.027	0.846
		Error	31121.516	467.391		
5	Udder circumference	Contrast	3273.500	1636.750	2.612*	0.046
		Error	102154.640	626.716		
6	Udder balance	Contrast	118.962	59.481	1.860	0.159
		Error	5212.534	31.979		
7	Central ligament	Contrast	8.589	4.294	2.117	0.124
		Error	330.669	2.029		
8	Udder depth	Contrast	81.444	40.722	1.149	0.320
		Error	5778.450	35.451		
9	Udder length	Contrast	112.623	56.312	1.009	0.367
		Error	9092.554	55.783		
10	Fore teat length	Contrast	0.388	0.194	0.089	0.915
		Error	355.492	2.181		
11	Rear teat length	Contrast	1.858	0.929	0.738	0.480
		Error	205.211	1.259		
12	Teat circumference	Contrast	2.672	1.336	0.559	0.573
		Error	389.823	2.392		
13	Teat diameter	Contrast	0.022	0.011	0.102	0.903
		Error	17.940	0.110		
14	Distance between front and rear teat	Contrast	16.522	8.261	0.551	0.578
		Error	2445.237	15.001		
15	Distance between right and left teat	Contrast	49.502	24.751	1.088	0.339
		Error	3706.446	22.739		
16	Shortest distance from front teat end to floor	Contrast	102.131	51.066	1.694	0.187
		Error	4912.616	30.139		
17	Shortest distance from rear teat end to floor	Contrast	79.452	39.726	1.251	0.289
		Error	5177.509	31.764		

* Significant at $P \leq 0.05$

Table 4.46(b) Pair-wise comparison of SNP rs135884509 (RE: HinfI) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ SE	P - value
1	Rear udder height	AA ^b	24.249 $\pm$ 0.610	0.024*
		AC ^a	21.225 $\pm$ 0.921	
		CC ^{a,b}	23.637 $\pm$ 0.667	
2	Rear udder width	AA ^b	13.164 $\pm$ 0.302	0.036*
		AC ^a	11.824 $\pm$ 0.456	
		CC ^{a,b}	12.388 $\pm$ 0.330	
3	Udder width	AA	71.610 $\pm$ 1.027	0.146
		AC	73.724 $\pm$ 1.551	
		CC	69.976 $\pm$ 1.123	
4	Fore udder attachment	AA	131.068 $\pm$ 1.865	0.846
		AC	130.625 $\pm$ 2.817	
		CC	132.377 $\pm$ 2.040	
5	Udder circumference	AA ^{a,b}	142.501 $\pm$ 2.930	0.046*
		AC ^b	151.110 $\pm$ 4.425	
		CC ^a	138.636 $\pm$ 3.205	
6	Udder balance	AA	1.915 $\pm$ 0.662	0.159
		AC	-0.264 $\pm$ 1.000	
		CC	0.663 $\pm$ 0.724	
7	Central ligament	AA	3.183 $\pm$ 0.167	0.124
		AC	3.543 $\pm$ 0.252	
		CC	2.908 $\pm$ 0.182	
8	Udder depth	AA	54.429 $\pm$ 0.697	0.320
		AC	53.216 $\pm$ 1.053	
		CC	52.935 $\pm$ 0.762	
9	Udder length	AA	60.950 $\pm$ 0.874	0.367
		AC	61.383 $\pm$ 1.320	
		CC	59.405 $\pm$ 0.956	
10	Fore teat length	AA	5.248 $\pm$ 0.173	0.915
		AC	5.348 $\pm$ 0.261	
		CC	5.344 $\pm$ 0.189	

Table 4.46(b) contd...

Trait		Genotype	Mean $\pm$ SE	P - value
11	Rear teat length	AA	4.643 $\pm$ 0.131	0.480
		AC	4.479 $\pm$ 0.198	
		CC	4.774 $\pm$ 0.144	
12	Teat circumference	AA	7.686 $\pm$ 0.181	0.573
		AC	7.417 $\pm$ 0.273	
		CC	7.437 $\pm$ 0.198	
13	Teat diameter	AA	2.233 $\pm$ 0.039	0.903
		AC	2.224 $\pm$ 0.059	
		CC	2.207 $\pm$ 0.042	
14	Distance between front and rear teat	AA	7.040 $\pm$ 0.453	0.578
		AC	6.874 $\pm$ 0.685	
		CC	6.347 $\pm$ 0.496	
15	Distance between right and left teat	AA	9.090 $\pm$ 0.558	0.339
		AC	9.910 $\pm$ 0.843	
		CC	8.394 $\pm$ 0.611	
16	Shortest distance from front teat end to floor	AA	46.409 $\pm$ 0.643	0.187
		AC	46.167 $\pm$ 0.970	
		CC	47.951 $\pm$ 0.703	
17	Shortest distance from rear teat end to floor	AA	46.326 $\pm$ 0.660	0.289
		AC	46.121 $\pm$ 0.996	
		CC	47.690 $\pm$ 0.722	

* Significant at $P \leq 0.05$; ^{a,b,c}Means with different letter superscript differ significantly

Among the observational udder and teat type traits the association study further revealed significant ($P \leq 0.05$) association of SNP rs135884509 with visual observational trait fore teat placement (FTP) and rear teat placement (RTP) (Table 4.47). The genotype AA was found to be significantly ($P \leq 0.05$) associated with trait sub category 3 of fore teat placement and rear teat placement as compared to AC and CC genotypes. AC genotype of the SNP was found to be significantly ($P \leq 0.05$) associated with trait sub category 4 of fore teat placement (Table 4.47).

Table 4.47 Association of SNP rs135884509 (RE: HinfI) with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	59.756*	10	AA	3	8.571
				AC	4	6.667
2	Front teat position	13.207	10	-	-	-
3	Long term changes in teat-end condition	3.955	6	-	-	-
4	Rear teat placement	56.708*	10	AA	3	43.000
5	Rear teat position	6.813	8	-	-	-
6	Skin condition	2.069	4	-	-	-
7	Teat end shape	2.682	4	-	-	-
8	Teat shape	2.202	4	-	-	-
9	Teat size	9.193	10	-	-	-
10	Udder shape	1.133	4	-	-	-
11	Udder suspension	3.195	6	-	-	-

* Significant at $P \leq 0.05$

In Sahiwal cattle, SNP rs135884509 was found to be significantly ($P \leq 0.05$) associated with udder width (UW), central ligament (CL), rear teat length (RTL), fore teat length (FTL) and distance between front and rear teat (DFR) [Table 4.48 (a)]. The AA genotype was found to be having maximum values of, UW (2288.646 ± 213.708 cm), CL (4.051 ± 0.262 cm), RTL (8.725 ± 1.453 cm), (9.188 $\pm$ 1.324 cm) and DFR (7.914 ± 1.134 cm) as compared to AC genotype, however as only two genotypes were detected, multiple comparison could not be performed [Table 4.48 (b)].

Table 4.48(a) Association of SNP rs135884509 (RE: HinfI) with udder and teat type traits (measurements) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P - value
1	Rear udder height	Contrast	0.755	0.755	0.020	0.887
		Error	3181.098	37.425		
2	Rear udder width	Contrast	2.480	2.480	0.065	0.799
		Error	3231.707	38.020		
3	Udder width	Contrast	3503777.289	3503777.289	3.836*	0.043
		Error	77641227.281	913426.203		
4	Fore udder attachment	Contrast	728.553	728.553	1.978	0.163
		Error	31311.516	368.371		
5	Udder circumference	Contrast	178.729	178.729	1.502	0.224
		Error	10112.616	118.972		
6	Central ligament	Contrast	6.074	6.074	4.422*	0.038
		Error	116.752	1.374		
7	Rear teat length	Contrast	175.124	175.124	4.148*	0.045
		Error	3588.295	42.215		
8	Udder diameter	Contrast	31.634	31.634	1.066	0.305
		Error	2522.304	29.674		
9	Udder length	Contrast	54.642	54.642	1.451	0.232
		Error	3200.746	37.656		
10	Teat circumference	Contrast	38.856	38.856	1.502	0.224
		Error	2199.535	25.877		
11	Udder balance	Contrast	6.599	6.599	0.757	0.387
		Error	740.626	8.713		
12	Fore teat length	Contrast	137.881	137.881	3.933*	0.047
		Error	2979.536	35.053		
13	Distance between front and rear teat	Contrast	86.295	86.295	3.353*	0.049
		Error	2187.705	25.738		
14	Distance between right and left teat	Contrast	47.722	47.722	1.612	0.208
		Error	2515.948	29.599		
15	Shortest distance from front teat end to floor	Contrast	57.320	57.320	1.480	0.227
		Error	3291.217	38.720		
16	Shortest distance from rear teat end to floor	Contrast	100.785	100.785	2.590	0.111
		Error	3307.064	38.907		
17	Teat diameter	Contrast	0.294	0.294	0.012	0.915
		Error	2174.330	25.580		

* Significant at $P \leq 0.05$

Table 4.48 (b) Pair-wise comparison of SNP rs135884509 (RE: HinfI) genotypes with udder and teat type traits (measurements) in Sahiwal cattle

Trait		Genotype	Mean $\pm$ SE	P - value
1	Rear udder height	AA	22.352 $\pm$ 1.368	0.887
		AC	22.573 $\pm$ 0.747	
2	Rear udder width	AA	22.538 $\pm$ 1.379	0.799
		AC	22.940 $\pm$ 0.753	
3	Udder width	AA	2288.646 $\pm$ 213.708	0.043*
		AC	1811.693 $\pm$ 116.761	
4	Fore udder attachment	AA	112.400 $\pm$ 4.292	0.163
		AC	105.522 $\pm$ 2.345	
5	Udder circumference	AA	144.811 $\pm$ 2.439	0.224
		AC	141.405 $\pm$ 1.333	
6	Central ligament	AA	4.051 $\pm$ 0.262	0.038*
		AC	3.423 $\pm$ 0.143	
7	Rear teat length	AA	8.725 $\pm$ 1.453	0.045*
		AC	5.353 $\pm$ 0.794	
8	Udder diameter	AA	49.807 $\pm$ 1.218	0.305
		AC	48.374 $\pm$ 0.666	
9	Udder length	AA	52.273 $\pm$ 1.372	0.232
		AC	50.390 $\pm$ 0.750	
10	Teat circumference	AA	9.987 $\pm$ 1.137	0.224
		AC	8.398 $\pm$ 0.621	
11	Udder balance	AA	-1.753 $\pm$ 0.660	0.387
		AC	-2.407 $\pm$ 0.361	
12	Fore teat length	AA	9.188 $\pm$ 1.324	0.047*
		AC	6.196 $\pm$ 0.723	
13	Distance between front and rear teat	AA	7.914 $\pm$ 1.134	0.049*
		AC	5.547 $\pm$ 0.620	
14	Distance between right and left teat	AA	8.547 $\pm$ 1.217	0.208
		AC	6.787 $\pm$ 0.665	
15	Shortest distance from front teat end to floor	AA	40.748 $\pm$ 1.391	0.227
		AC	42.677 $\pm$ 0.760	
16	Shortest distance from rear teat end to floor	AA	41.135 $\pm$ 1.395	0.111
		AC	43.693 $\pm$ 0.762	
17	Teat diameter	AA	26.188 $\pm$ 1.131	0.915
		AC	26.049 $\pm$ 0.618	

* Significant at $P \leq 0.05$

Among the visual observational trait, genotypes had significant effect on front teat position (FTP), rear teat position (RTP), teat size (TSi) and udder suspension (USus) (Table 4.49). The genotype AA was having higher odds of being associated with trait sub-category 5, 2, 4 and 1 of the trait FTP, RTP, TSi and USus, respectively (Table 4.49).

Table 4.49 Association of SNP rs135884509 (RE: HinfI) with udder and teat type traits (visual observations) in Sahiwal cattle

Trait		Chi-Square	df	Pairwise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	8.148	5	-	-	-
2	Front teat position	20.235*	8	AA	5	>999.999
3	Long-term changes in teat-end condition	8.785*	2	-	-	-
4	Rear teat placement	5.324	3	-	-	-
5	Rear teat position	13.4*	6	AA	2	>999.999
6	Skin condition	0.044	1	-	-	-
7	Teat end shape	3.109	2	-	-	-
8	Teat shape	0.374	2	-	-	-
9	Teat size	10.593*	3	AA	4	2.2
10	Udder shape	3.479	2	-	-	-
11	Udder suspension	8.89*	3	AA	1	>999.999

* Significant at $P \leq 0.05$

4.12.3.1 (b) Association of SNP rs135884509 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

In Karan Fries cattle, SNP rs135884509 genotypes had significant effect ($P \leq 0.05$) on incidence of clinical mastitis in Karan Fries cows (Table 4.50). The odds ratio revealed genotype AA had 613.3% (compared to genotype CC and AC) and AC had 127.0% (compared to genotype CC and AA) more chance of occurring with healthy animals with confidence interval 1.926 – 19.530 and 0.461 – 3.500, respectively (Table 4.50).

Table 4.50 Association of SNP rs135884509 (RE: HinfI) with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	5 (0.148)	65 (0.493)	0.008	6.133 (1.926 – 19.530)
AC	8 (0.235)	24 (0.181)		1.27 (0.461 – 3.500)
CC	21 (0.617)	43 (0.326)		-
N	34	132		

* Significant at $P \leq 0.05$

In Sahiwal cattle, the genotypes of SNP rs135884509 had no significant effect ($P \leq 0.05$) on incidence of clinical mastitis in Sahiwal cows (Table 4.51). The odds ratio revealed genotype AA had 110.2% more chance of occurring with healthy animals as compared to genotype AC with confidence interval 0.350-3.471 (Table 4.51).

Table 4.51 Association of SNP rs135884509 (RE: HinfI) with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	4 (0.222)	16 (0.232)	0.862	1.102 (0.350-3.471)
AC	1 (0.778)	53 (0.768)		-
N	18	69	-	

* Significant at $P \leq 0.05$

In Karan Fries cattle, SNP rs135884509 genotypes had significant effect ($P \leq 0.05$) on monthly milk yield, test day milk yield, 305- day milk yield and total milk yield [Table 4.52 (a)]. The genotype AA was found to having significantly higher values of 305-day milk yield (5004.517 ± 190.637 kg) and total milk yield (4999.432 ± 192.359 kg) as compared to other genotypes [Table 4.52 (b)]. Whereas, for monthly milk yield and test day milk yield the genotype CC was having significantly lower value of 309.544 ± 18.176 kg and 11.245 ± 0.603 kg, than the other genotypes [Table 4.52 (b)].

Table 4.52 (a) Association of SNP rs135884509 (RE: HinfI) genotypes with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P - value
Milk yield (305d)	Contrast	27971934.719	13985967.360	4.484*	0.013
	Error	508390616.014	3118960.834		
Monthly milk yield	Contrast	129631.760	64815.880	2.994*	0.053
	Error	3528286.708	21645.931		
Test day milk yield	Contrast	140.578	70.289	3.018*	0.052
	Error	3796.007	23.288		
Total milk yield	Contrast	29897096.557	14948548.278	4.617*	0.011
	Error	527781861.552	3237925.531		

* Significant at $P \leq 0.05$

Table 4.52 (b) Pair-wise comparison of SNP rs135884509 (RE: HinfI) genotypes with milk production traits

Trait	Genotype	Mean $\pm$ SE	P – value*
Milk yield (305d)	AA ^b	5004.517 $\pm$ 190.637	0.000*
	AC ^a	3758.867 $\pm$ 287.934	
	CC ^a	3283.699 $\pm$ 208.546	
Monthly milk yield	AA ^b	409.807 $\pm$ 16.615	0.000*
	AC ^b	403.053 $\pm$ 25.095	
	CC ^a	309.544 $\pm$ 18.176	
Test day milk yield	AA ^b	14.111 $\pm$ 0.551	0.001*
	AC ^b	14.211 $\pm$ 0.832	
	CC ^a	11.245 $\pm$ 0.603	
Total milk yield	AA ^b	4999.432 $\pm$ 192.359	0.000*
	AC ^a	3717.188 $\pm$ 290.535	
	CC ^a	3161.418 $\pm$ 210.431	

* Significant at $P \leq 0.05$

In Sahiwal cows, genotypes had significant ($P \leq 0.05$) effect on 305-day milk yield [Table 4.53 (a)]. The genotype AA was found to be having higher 305-day milk yield (2287.979 ± 213.710 kg) as compared to genotype AC, however as only two genotypes were detected multiple comparison procedure could not be performed [Table 4.53 (b)].

Table 4.53 (a) Association of SNP rs135884509 (RE: HinfI) with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P- value*
Milk yield (305 d)	Contrast	3517630.966	3517630.966	3.851*	0.050
	Error	77642479.313	913440.933		
Monthly milk yield	Contrast	1332.038	1332.038	0.075	0.785
	Error	1518576.864	17865.610		
Test day milk yield	Contrast	11.165	11.165	0.645	0.424
	Error	1472.072	17.318		
Total milk yield	Contrast	2614400.460	2614400.460	1.869	0.175
	Error	118911814.437	1398962.523		

* Significant at $P \leq 0.05$

Table 4.53 (b) Pair-wise comparison of SNP rs135884509 (RE: HinfI) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ SE	P - value
Milk yield (305 d)	AA	2287.979 $\pm$ 213.710	0.050*
	AC	1810.084 $\pm$ 116.762	
Monthly milk yield	AA	211.225 $\pm$ 29.888	0.785
	AC	201.925 $\pm$ 16.329	
Test day milk yield	AA	8.694 $\pm$ 0.931	0.424
	AC	7.842 $\pm$ 0.508	
Total milk yield	AA	2166.250 $\pm$ 264.477	0.175
	AC	1754.254 $\pm$ 144.499	

* Significant at $P \leq 0.05$

4.12.3.2 (a) Association of SNP rs136785910 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs136785910, situated in intron 5 of bovine IL-22 (at BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits. In Karan Fries cattle, SNP rs136785910 was found to be significantly ($P \leq 0.05$) associated with rear udder width (RUW), udder circumference (UC), teat circumference (TC), udder balance (UB), distance between right and left teat (DLR) and teat diameter (TD) [Table 4.54 (a)].

Table 4.54(a) Association of SNP rs136785910 (RE: HpaII) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P - value
1	Rear udder height	Contrast	7.321	7.321	0.260	0.611
		Error	4622.551	28.186		
2	Rear udder width	Contrast	31.35	31.35	4.682*	0.032
		Error	1098.213	6.696		
3	Udder width	Contrast	148.705	148.705	1.921	0.168
		Error	12695.74	77.413		
4	Fore udder attachment	Contrast	1349.331	584.665	1.798	0.393
		Error	29850.738	267.271		
5	Udder circumference	Contrast	11822.3	11822.298	20.713*	0.000
		Error	93605.84	570.767		

Table 4.54(a) contd...

Dependent Variable			Sum of Squares	Mean Square	F	P - value
6	Central ligament	Contrast	3.485	3.485	1.702	0.194
		Error	335.773	2.047		
7	Rear teat length	Contrast	0.179	0.179	0.142	0.707
		Error	206.89	1.262		
8	Udder diameter	Contrast	25.469	25.469	0.716	0.399
		Error	5834.426	35.576		
9	Udder length	Contrast	64.756	64.756	1.162	0.283
		Error	9140.422	55.734		
10	Teat circumference	Contrast	7.412	7.412	3.157*	0.047
		Error	385.083	2.348		
11	Udder balance	Contrast	88.27	88.27	2.761*	0.049
		Error	5243.226	31.971		
12	Fore teat length	Contrast	1.666	1.666	0.771	0.381
		Error	354.215	2.16		
13	Distance between front and rear teat	Contrast	18.302	18.302	1.228	0.269
		Error	2443.457	14.899		
14	Distance between right and left teat	Contrast	97.119	97.119	4.353*	0.038
		Error	3658.829	22.31		
15	Shortest distance from front teat end to floor	Contrast	43.724	43.724	1.443	0.231
		Error	4971.023	30.311		
16	Shortest distance from rear teat end to floor	Contrast	15.865	15.865	0.496	0.482
		Error	5241.095	31.958		
17	Teat diameter	Contrast	0.622	0.622	5.878*	0.016
		Error	17.341	0.106		

* Significant at $P \leq 0.05$

The genotype TT was found to be superior to be having maximum value of RUW (12.956 ± 0.254 cm), UC (149.256 ± 2.343 cm), TC (7.706 ± 0.150 cm), DLR (9.583 ± 0.463) and TD (2.269 ± 0.032) as compared to CT genotype [Table 4.54 (b)]. The genotype TC was found to be having higher value of UB (1.980 ± 0.718 cm) as compared to TT [Table 4.54 (b)]. However, as only two genotypes were observed so multiple comparisons analysis could not be performed.

Table 4.54 (b) Pair-wise comparison of SNP rs136785910 (RE: HpaII) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ SE	P – value
1	Rear udder height	TC	23.169 $\pm$ 0.674	0.611
		TT	23.603 $\pm$ 0.521	
2	Rear udder width	TC	12.058 $\pm$ 0.329	0.032*
		TT	12.956 $\pm$ 0.254	
3	Udder width	TC	70.191 $\pm$ 1.117	0.168
		TT	72.148 $\pm$ 0.863	
4	Fore udder attachment	TC	130.097 $\pm$ 2.015	0.393
		TT	132.279 $\pm$ 1.556	
5	Udder circumference	TC	131.810 $\pm$ 3.034	0.000*
		TT	149.256 $\pm$ 2.343	
6	Central ligament	TC	2.964 $\pm$ 0.182	0.194
		TT	3.263 $\pm$ 0.140	
7	Rear teat length	TC	4.617 $\pm$ 0.143	0.707
		TT	4.685 $\pm$ 0.110	
8	Udder diameter	TC	54.153 $\pm$ 0.757	0.399
		TT	53.344 $\pm$ 0.585	
9	Udder length	TC	59.657 $\pm$ 0.948	0.283
		TT	60.948 $\pm$ 0.732	
10	Teat circumference	TC	7.269 $\pm$ 0.195	0.047*
		TT	7.706 $\pm$ 0.150	
11	Udder balance	TC	1.980 $\pm$ 0.718	0.049*
		TT	0.472 $\pm$ 0.554	
12	Fore teat length	TC	5.173 $\pm$ 0.187	0.381
		TT	5.380 $\pm$ 0.144	
13	Distance between front and rear teat	TC	6.323 $\pm$ 0.490	0.269
		TT	7.010 $\pm$ 0.378	
14	Distance between right and left teat	TC	8.002 $\pm$ 0.600	0.038*
		TT	9.583 $\pm$ 0.463	
15	Shortest distance from front teat end to floor	TC	47.594 $\pm$ 0.699	0.231
		TT	46.533 $\pm$ 0.540	
16	Shortest distance from rear teat end to floor	TC	47.188 $\pm$ 0.718	0.482
		TT	46.549 $\pm$ 0.554	
17	Teat diameter	TC	2.142 $\pm$ 0.041	0.016*
		TT	2.269 $\pm$ 0.032	

* Significant at $P \leq 0.05$

Among the visual udder and teat type traits, the association study further revealed significant ($P \leq 0.05$) association of SNP rs136785910 with visual observational traits front teat position (FTP), long term changes in teat-end condition (LTCTEC), rear teat placement (RTPl), teat end shape (TES) and teat size (TSi) (Table 4.55). The genotype TC of the SNP was found to be significantly ($P \leq 0.05$) associated with trait sub category 5, 1, 5 and 1 of FTP, LTCTEC, RTPl and TSi, respectively (Table 4.55).

Table 4.55 Association of SNP rs136785910 (RE: HpaII) with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	6.749	5	-	-	-
2	Front teat position	9.906*	5	TC	5	1.137
3	Long term changes in teat-end condition	9.522*	3	TC	1	1.2
4	Rear teat placement	11.19*	5	TC	5	4.37
5	Rear teat position	7.273	4	-	-	-
6	Skin condition	0.228	2	-	-	-
7	Teat end shape	23.826*	2	-	-	-
8	Teat shape	8.01*	2	-	-	-
9	Teat size	10.849*	5	TC	1	1.19
10	Udder shape	3.404	2	-	-	-
11	Udder suspension	3.034	3	-	-	-

* Significant at $P \leq 0.05$

In Sahiwal cattle, SNP rs136785910 was found to be significantly ($P \leq 0.05$) associated with UW, FUA, UC and TC [Table 4.56 (a)]. The genotype TC was found to be having more value of UW (2518.909 ± 251.361 cm), FUA (119.214 ± 4.987 cm), UC (147.215 ± 2.880 cm) and TC (11.872 ± 1.321 cm) as compared to TT genotype [Table 4.56 (b)].

Table 4.56 (a) Association of SNP rs136785910 (RE: HpaII) with udder and teat type traits (measurement) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P- value
1	Rear udder height	Contrast	1.663	1.663	0.044	0.834
		Error	3180.191	37.414		
2	Rear udder width	Contrast	5.831	5.831	0.154	0.696
		Error	3228.356	37.981		
3	Udder width	Contrast	5958057.343	5958057.343	6.736	0.011*
		Error	75186947.228	884552.320		
4	Fore udder attachment	Contrast	2447.219	2447.219	7.029	0.010*
		Error	29592.850	348.151		
5	Udder circumference	Contrast	421.733	421.733	3.632	0.050*
		Error	9869.612	116.113		
6	Central ligament	Contrast	0.297	0.297	0.206	0.651
		Error	122.529	1.442		
7	Rear teat length	Contrast	1.545	1.545	0.035	0.852
		Error	3761.874	44.257		
8	Udder diameter	Contrast	14.678	14.678	0.491	0.485
		Error	2539.260	29.874		
9	Udder length	Contrast	59.118	59.118	1.572	0.213
		Error	3196.271	37.603		
10	Teat circumference	Contrast	161.184	161.184	6.596	0.012*
		Error	2077.208	24.438		
11	Udder balance	Contrast	0.386	0.386	0.044	0.834
		Error	746.839	8.786		
12	Fore teat length	Contrast	0.228	0.228	0.006	0.937
		Error	3117.189	36.673		

Table 4.56 (a) contd...

Dependent Variable			Sum of Squares	Mean Square	F	P- value
13	Distance between front and rear teat	Contrast	0.006	0.006	0.000	0.988
		Error	2273.993	26.753		
14	Distance between right and left teat	Contrast	34.675	34.675	1.165	0.283
		Error	2528.996	29.753		
15	Shortest distance from front teat end to floor	Contrast	37.754	37.754	0.969	0.328
		Error	3310.783	38.950		
16	Shortest distance from rear teat end to floor	Contrast	30.831	30.831	0.776	0.381
		Error	3377.018	39.730		
17	Teat diameter	Contrast	1.207	1.207	0.047	0.829
		Error	2173.417	25.570		

* Significant at $P \leq 0.05$ **Table 4.56(b) Pair-wise comparison of SNP rs136785910 (RE: HpaII) genotypes with udder and teat type traits (measurements) in Sahiwal cattle**

Trait		Genotype	Mean $\pm$ SE	P- value
1	Rear udder height	TC	22.207 $\pm$ 1.635	0.834
		TT	22.583 $\pm$ 0.716	
2	Rear udder weight	TC	22.256 $\pm$ 1.647	0.696
		TT	22.961 $\pm$ 0.721	
3	Udder width	TC	2518.909 $\pm$ 251.361	0.011*
		TT	1806.734 $\pm$ 110.078	
4	Fore udder attachment	TC	119.214 $\pm$ 4.987	0.010*
		TT	104.781 $\pm$ 2.184	
5	Udder circumference	TC	147.215 $\pm$ 2.880	0.050*
		TT	141.224 $\pm$ 1.261	

Table 4.56(b) contd...

Trait		Genotype	Mean $\pm$ SE	P- value
6	Central ligament	TC	3.701 $\pm$ 0.321	0.651
		TT	3.542 $\pm$ 0.141	
7	Rear teat length	TC	5.824 $\pm$ 1.778	0.852
		TT	6.186 $\pm$ 0.779	
8	Udder diameter	TC	49.641 $\pm$ 1.461	0.485
		TT	48.523 $\pm$ 0.640	
9	Udder length	TC	52.705 $\pm$ 1.639	0.213
		TT	50.462 $\pm$ 0.718	
10	Teat circumference	TC	11.872 $\pm$ 1.321	0.012*
		TT	8.167 $\pm$ 0.579	
11	Udder balance	TC	-2.105 $\pm$ 0.792	0.834
		TT	-2.286 $\pm$ 0.347	
12	Fore teat length	TC	6.767 $\pm$ 1.618	0.937
		TT	6.907 $\pm$ 0.709	
13	Distance between front and rear teat	TC	6.072 $\pm$ 1.382	0.988
		TT	6.095 $\pm$ 0.605	
14	Distance between right and left teat	TC	5.750 $\pm$ 1.458	0.283
		TT	7.468 $\pm$ 0.638	
15	Shortest distance from front teat end to floor	TC	40.729 $\pm$ 1.668	0.328
		TT	42.522 $\pm$ 0.730	
16	Shortest distance from rear teat end to floor	TC	41.746 $\pm$ 1.685	0.381
		TT	43.366 $\pm$ 0.738	
17	Teat diameter	TC	26.350 $\pm$ 1.351	0.829
		TT	26.029 $\pm$ 0.592	

* Significant at $P \leq 0.05$

However, as only two genotypes were found, hence, multiple comparison procedure could not be performed. Among the observational udder and teat type traits, the association study revealed significant ($P \leq 0.05$) association of SNP rs136785910 with rear teat position (RTP), udder shape (US) and udder suspension (USus) (Table 4.57). The genotype TC was having higher odds of being associated with associated with trait sub category 2 of RTP, US and USus (Table 4.57).

Table 4.57 Association of SNP rs136785910 (RE: HpaII) with udder and teat type traits (visual observations) in Sahiwal cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait subcategory	Exp (B)
1	Fore teat placement	3.85	5	-	-	-
2	Front teat position	6.322	8	-	-	-
3	Long term changes in teat-end condition	1.287	2	-	-	-
4	Rear teat placement	3.392	3	-	-	-
5	Rear teat position	11.67*	6	TC	2	>999.999
6	Skin condition	1.05	1	-	-	-
7	Teat end shape	2.446	2	-	-	-
8	Teat shape	2.056	2	-	-	-
9	Teat size	6.031	3	-	-	-
10	Udder shape	19.308*	2	TC	2	32
11	Udder suspension	19.503*	3	TC	2	1.6

* Significant at $P \leq 0.05$

4.12.3.2 (b) Association of SNP rs136785910 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

Logistic regression analysis revealed genotypes of SNP rs136785910 had no significant effect ($P \leq 0.05$) on incidence of clinical mastitis in Karan Fries cows (Table 4.58). Though the odds ratio revealed genotype TC had 187.1% more chance of occurring with healthy animals as compared to genotype TT with confidence interval 0.741 - 4.720, the overall effect of genotypes was non-significant (Table 4.58).

In Sahiwal cattle, SNP rs136785910 had no significant effect ($P \leq 0.05$) on incidence of clinical mastitis (Table 4.59). Though the odds ratio revealed genotype TC had 138.4% more chance of occurring with healthy animals as compared to genotype TT with confidence interval 0.349 – 5.479, the overall effect of genotypes was non-significant ($P \leq 0.05$) (Table 4.59).

Table 4.58 Association of SNP rs136785910 (RE: HpaII) genotypes with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value	ODDS ratio estimation
TC	9 (0.264)	52 (0.394)	0.184	1.871 (0.741 - 4.720)
TT	25(0.735)	80 (0.606)		-
N	34	132		

* Significant at $P \leq 0.05$

Table 4.59 Association of SNP rs136785910 (RE: HpaII) genotypes with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	P- value	ODDS ratio estimation
TC	2 (0.111)	12 (0.174)	0.064	1.384 (0.349 – 5.479)
TT	16 (0.889)	57 (0.826)		-
N	18	69		

* Significant at $P \leq 0.05$

In Karan Fries cows, SNP rs136785910 genotypes had significant ($P \leq 0.05$) effect on monthly milk yield, test day milk yield, 305-day milk yield and total milk yield [Table 4.60 (a)]. The genotype TT was found to be having higher values of 305-day milk yield (4330.307 ± 175.515 kg), monthly milk yield (386.682 ± 14.518 kg), test day milk yield (13.721 ± 0.473 kg) and total milk yield (4288.356 ± 178.792 kg) as compared to genotype TC, however, as only two genotypes were found multiple comparison procedure could not be performed [Table 4.60 (b)].

Table 4.60 (a) Association of SNP rs136785910 (RE: HpaII) genotypes with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P-value
Milk yield (305d)	Contrast	10945613.150	10945613.150	3.416*	0.046
	Error	525416937.583	3203761.815		
Monthly milk yield	Contrast	62820.795	62820.795	2.866*	0.042
	Error	3595097.674	21921.327		
Test day milk yield	Contrast	115.568	115.568	4.960*	0.027
	Error	3821.018	23.299		
Total milk yield	Contrast	12457936.836	12457936.836	3.747*	0.044
	Error	545221021.272	3324518.422		

* Significant at $P \leq 0.05$

Table 4.60 (b) Pair-wise comparison of SNP rs136785910 (RE: HpaII) genotypes with milk production traits in Karan Fries cattle

Trait	Genotype	Mean $\pm$ SE	P-value*
Milk yield (305 d)	TC	3799.470 $\pm$ 227.318	0.046*
	TT	4330.307 $\pm$ 175.515	
Monthly milk yield	TC	346.466 $\pm$ 18.803	0.042*
	TT	386.682 $\pm$ 14.518	
Test day milk yield	TC	11.996 $\pm$ 0.613	0.027*
	TT	13.721 $\pm$ 0.473	
Total milk yield	TC	3722.032 $\pm$ 231.563	0.044*
	TT	4288.356 $\pm$ 178.792	

* Significant at $P \leq 0.05$

In Sahiwal cattle, the SNP rs136785910 genotypes had significant effect ($P \leq 0.05$) on test day milk yield, 305-day milk yield and total milk yield [Table 4.61 (a)]. The genotype TC was found to be having higher values of 305-day milk yield (2518.535 ± 251.352 kg), test day milk yield (10.605 ± 1.074 kg) and total milk yield (2673.107 ± 304.302 kg) as compared to genotype TT, however, as only two genotypes were found multiple comparison procedure could not be performed [Table 4.61 (b)].

Table 4.61(a) Association of SNP rs136785910 (RE: HpaII) genotypes with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P-value
Milk yield (305 d)	Contrast	5,978,377.079	5,978,377.079	6.759*	0.011
	Error	75,181,733.200	884,490.979		
Monthly milk yield	Contrast	21,676.471	21,676.471	1.230	0.271
	Error	1,498,232.432	17,626.264		
Test day milk yield	Contrast	109.913	109.913	6.803*	0.011
	Error	1,373.323	16.157		
Total milk yield	Contrast	11,332,562.636	11,332,562.636	8.742*	0.004
	Error	110,193,652.261	1,296,395.909		

* Significant at $P \leq 0.05$

Table 4.61 (b) Pair-wise comparison of SNP rs136785910 (RE: HpaII) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ SE	P-value
Milk yield (305 d)	TC	2518.535 $\pm$ 251.352	0.011*
	TT	1805.147 $\pm$ 110.074	
Monthly milk yield	TC	240.107 $\pm$ 35.483	0.271
	TT	197.151 $\pm$ 15.539	
Test day milk yield	TC	10.605 $\pm$ 1.074	0.011*
	TT	7.546 $\pm$ 0.470	
Total milk yield	TC	2673.107 $\pm$ 304.302	0.004*
	TT	1690.911 $\pm$ 133.262	

* Significant at $P \leq 0.05$

4.12.3.3 (a) Association of SNP rs383429860 with udder and teat type traits in Karan Fries and Sahiwal cattle

Association analysis of SNP rs383429860, situated in intron 2 of bovine IL-22 (BTA 5) with udder and teat type traits (continuous traits and visual observations) revealed significant association with the different udder and teat type traits.

In Karan Fries cattle, SNP rs383429860 was found to be significantly ($P \leq 0.05$) associated with rear udder height (RUH), udder circumference (UC), central ligament (CL), udder balance (UB) and shortest distance from rear teat end to floor (SDR) ($P \leq 0.05$) [Table 4.62 (a)]. The genotype AG was found to be having highest mean values of RUH (24.130 $\pm$ 0.722 cm) and CL (3.647 $\pm$ 0.192 cm) as compared to AA and GG genotypes [Table 4.62 (b)]. This difference was found to be statistically non significant for RUH, however, for CL it was significantly higher than other genotypes [Table 4.62 (b)]. The GG genotype was found to be having highest UC (152.715 $\pm$ 3.593 cm) mean value, as compared to AA and GG genotypes, which was also significantly higher than other genotypes [Table 4.62 (b)]. The AA genotype was found to be superior with highest UB (1.795 $\pm$ 0.692 cm) and SDR (47.988 $\pm$ 0.688 cm) as compared to AG and GG genotypes, however this difference was statistically non-significant ($P \leq 0.05$) [Table 4.62 (b)].

Table 4.62 (a) Association of SNP rs383429860 (RE: Sau96I) with udder and teat type traits (measurements) in Karan Fries cattle

Dependent Variable			Sum of Squares	Mean Square	F	P-value
1	Rear udder height	Contrast	131.096	65.548	2.375*	0.046
		Error	4498.777	27.600		
2	Rear udder width	Contrast	4.903	2.451	0.355	0.702
		Error	1124.660	6.900		
3	Udder width	Contrast	303.789	151.894	1.974	0.142
		Error	12540.652	76.937		
4	Fore udder attachment	Contrast	728.553	728.553	0.078	0.813
		Error	31311.516	368.371		
5	Udder circumference	Contrast	6525.221	3262.610	5.377*	0.005
		Error	98902.919	606.766		
6	Central ligament	Contrast	19.550	9.775	4.984*	0.008
		Error	319.707	1.961		
7	Rear teat length	Contrast	3.665	1.832	1.468	0.233
		Error	203.404	1.248		
8	Udder diameter	Contrast	42.557	21.279	0.596	0.552
		Error	5817.338	35.689		
9	Udder length	Contrast	192.308	96.154	1.739	0.179
		Error	9012.869	55.294		
10	Teat circumference	Contrast	9.315	4.657	1.981	0.141
		Error	383.181	2.351		
11	Udder balance	Contrast	179.165	89.583	2.834*	0.042
		Error	5152.331	31.609		
12	Fore teat length	Contrast	0.478	0.239	0.110	0.896
		Error	355.402	2.180		
13	Distance between front and rear teat	Contrast	28.982	14.491	0.971	0.381
		Error	2432.777	14.925		
14	Distance between right and left teat	Contrast	43.564	21.782	0.956	0.386
		Error	3712.384	22.775		
15	Shortest distance from front teat end to floor	Contrast	136.914	68.457	2.288	0.105
		Error	4877.833	29.925		
16	Shortest distance from rear teat end to floor	Contrast	157.827	78.914	2.523*	0.043
		Error	5099.134	31.283		
17	Teat diameter	Contrast	0.122	0.061	0.556	0.575
		Error	17.841	0.109		

* Significant at $P \leq 0.05$

Table 4.62 (b) Pair-wise comparison of SNP rs383429860 (RE: Sau96I) genotypes with udder and teat type traits (measurements) in Karan Fries cattle

Trait		Genotype	Mean $\pm$ SE	P value
1	Rear udder height	AA ^a	23.888 $\pm$ 0.647	0.046*
		AG ^a	24.130 $\pm$ 0.722	
		GG ^a	22.036 $\pm$ 0.766	
2	Rear udder width	AA	12.828 $\pm$ 0.323	0.702
		AG	12.521 $\pm$ 0.361	
		GG	12.441 $\pm$ 0.383	
3	Udder width	AA	69.772 $\pm$ 1.080	0.142
		AG	72.247 $\pm$ 1.205	
		GG	72.792 $\pm$ 1.279	
4	Fore udder attachment	AA	130.879 $\pm$ 1.961	0.813
		AG	131.075 $\pm$ 2.188	
		GG	132.723 $\pm$ 2.324	
5	Udder circumference	AA ^a	138.676 $\pm$ 3.032	0.005*
		AG ^a	138.956 $\pm$ 3.384	
		GG ^b	152.715 $\pm$ 3.593	
6	Central ligament	AA ^a	2.866 $\pm$ 0.172	0.008*
		AG ^b	3.647 $\pm$ 0.192	
		GG ^a	2.994 $\pm$ 0.204	
7	Rear teat length	AA	4.490 $\pm$ 0.138	0.233
		AG	4.703 $\pm$ 0.153	
		GG	4.848 $\pm$ 0.163	
8	Udder diameter	AA	54.167 $\pm$ 0.735	0.552
		AG	53.639 $\pm$ 0.821	
		GG	52.922 $\pm$ 0.871	
9	Udder length	AA	59.428 $\pm$ 0.915	0.179
		AG	60.339 $\pm$ 1.021	
		GG	62.066 $\pm$ 1.085	
10	Teat circumference	AA	7.604 $\pm$ 0.189	0.141
		AG	7.783 $\pm$ 0.211	
		GG	7.185 $\pm$ 0.224	

Table 4.62 (b) contd...

Trait		Genotype	Mean $\pm$ SE	P value
11	Udder balance	AA ^a	1.795 $\pm$ 0.692	0.042*
		AG ^a	1.547 $\pm$ 0.772	
		GG ^a	-0.609 $\pm$ 0.820	
12	Fore teat length	AA	5.249 $\pm$ 0.182	0.896
		AG	5.299 $\pm$ 0.203	
		GG	5.381 $\pm$ 0.215	
13	Distance between front and rear teat	AA	6.461 $\pm$ 0.476	0.381
		AG	6.530 $\pm$ 0.531	
		GG	7.417 $\pm$ 0.564	
14	Distance between right and left teat	AA	8.378 $\pm$ 0.587	0.386
		AG	9.260 $\pm$ 0.656	
		GG	9.554 $\pm$ 0.696	
15	Shortest distance from front teat end to floor	AA	47.937 $\pm$ 0.673	0.105
		AG	45.787 $\pm$ 0.751	
		GG	46.801 $\pm$ 0.798	
16	Shortest distance from rear teat end to floor	AA ^a	47.988 $\pm$ 0.688	0.043*
		AG ^a	45.973 $\pm$ 0.768	
		GG ^a	46.021 $\pm$ 0.816	
17	Teat diameter	AA	2.220 $\pm$ 0.041	0.575
		AG	2.255 $\pm$ 0.045	
		GG	2.185 $\pm$ 0.048	

* Significant at $P \leq 0.05$;

^{a,b,c}Means with different letter superscript differ significantly at $P \leq 0.05$

Among the observational udder and teat type traits, the association study revealed significant ($P \leq 0.05$) association of SNP rs383429860 with visual observational traits, fore teat placement (FTPL), long term changes in teat-end condition (LTCTEC), rear teat placement (RTPL), skin condition (SC) and teat end shape (TES) (Table 4.63). The odds of finding genotype AA was found to be significantly higher to be associated with trait sub category 3, 3, 4 and 1 of FTPL, LTCTEC and RTPL, respectively, compared to AG and GG

genotype (Table 4.63). The genotype AG was found to be having higher odds of being associated with trait sub category 1of LTCTEC, SC and TES, respectively (Table 4.63).

Table 4.63 Association of SNP rs383429860 (RE: Sau96I) with udder and teat type traits (visual observations) in Karan Fries cattle

Trait		Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait subcategory	Exp (B)
1	Fore teat placement	16.488*	10	AA	3	>999.999
2	Front teat position	5.553	10	-	-	-
3	Long term changes in teat-end condition	11.581*	6	AA	3	2.625
				AG	1	1.25
4	Rear teat placement	22.431*	10	AA	4	6
5	Rear teat position	7.887	8	-	-	-
6	Skin condition	19.665*	4	AG	1	2.625
7	Teat end shape	11.498*	4	AG	1	1.4
8	Teat shape	6.528	4	-	-	-
9	Teat size	13.991	10	-	-	-
10	Udder shape	1.468	4	-	-	-
11	Udder suspension	3.966	6	-	-	-

* Significant at $P \leq 0.05$

In Sahiwal cattle, SNP rs383429860 genotypes were found to be significantly ($P \leq 0.05$) associated with udder width (UW), shortest distance from front teat end to floor (SDF) and shortest distance from rear teat end to floor (SDR) [Table 4.64 (a)]. The genotype AA was found to have highest mean value of UW (2283.496 ± 172.651 cm), this difference was statistically significant as compared to genotype AG and GG genotype [Table 4.64 (b)]. The genotype AG was having highest mean value of SDF (43.833 ± 1.028 cm) and SDR (44.542 ± 1.040 cm) respectively [Table 4.64 (b)]. This difference was found to be statistically non-significant as compared to genotype GG, but was significantly different from genotype AA ($P \leq 0.05$) [Table 4.64 (b)].

Table 4.64(a) Association of SNP rs383429860 (RE: Sau96I) with udder and teat type traits (measurements) in Sahiwal cattle

Dependent Variable			Sum of Squares	Mean Square	F	P-value
1	Rear udder height	Contrast	34.368	17.184	0.459	0.634
		Error	3147.486	37.470		
2	Rear udder width	Contrast	28.031	14.015	0.367	0.694
		Error	3206.156	38.169		
3	Udder width	Contrast	8531587.594	4265793.797	4.935	0.009
		Error	72613416.976	864445.440		
4	Fore udder attachment	Contrast	1189.331	594.665	1.619	0.204
		Error	30850.738	367.271		
5	Udder circumference	Contrast	214.775	107.387	0.895	0.412
		Error	10076.571	119.959		
6	Central ligament	Contrast	1.758	0.879	0.610	0.546
		Error	121.068	1.441		
7	Rear teat length	Contrast	83.893	41.946	0.958	0.388
		Error	3679.526	43.804		
8	Udder diameter	Contrast	15.006	7.503	0.248	0.781
		Error	2538.931	30.225		
9	Udder length	Contrast	97.320	48.660	1.294	0.280
		Error	3158.068	37.596		
10	Teat circumference	Contrast	23.551	11.775	0.447	0.641
		Error	2214.841	26.367		
11	Udder balance	Contrast	8.453	4.226	0.481	0.620
		Error	738.772	8.795		
12	Fore teat length	Contrast	63.283	31.642	0.870	0.423
		Error	3054.135	36.359		

Table 4.64(a) contd...

Dependent Variable			Sum of Squares	Mean Square	F	P-value
13	Distance between front and rear teat	Contrast	18.689	9.344	0.348	0.707
		Error	2255.311	26.849		
14	Distance between right and left teat	Contrast	80.561	40.280	1.363	0.262
		Error	2483.109	29.561		
15	Shortest distance from front teat end to floor	Contrast	242.852	121.426	3.284	0.042
		Error	3105.685	36.972		
16	Shortest distance from rear teat end to floor	Contrast	225.242	112.621	2.972	0.047
		Error	3182.606	37.888		
17	Teat diameter	Contrast	28.557	14.279	0.559	0.574
		Error	2146.067	25.548		

* Significant at $P \leq 0.05$

Table 4.64 (b) Pair-wise comparison of SNP rs383429860 (RE: Sau96I) genotypes with udder and teat type traits (measurements) in Sahiwal cattle

Trait		Genotype	Mean $\pm$ SE	P-value
1	Rear udder height	AA	23.377 $\pm$ 1.137	0.633
		AG	22.268 $\pm$ 1.035	
		GG	21.833 $\pm$ 1.276	
2	Rear udder width	AA	23.616 $\pm$ 1.147	0.693
		AG	22.626 $\pm$ 1.044	
		GG	22.216 $\pm$ 1.288	
3	Udder width	AA ^a	2283.496 $\pm$ 172.651	0.009*
		AG ^{a,b}	1919.200 $\pm$ 157.157	
		GG ^b	1467.953 $\pm$ 193.867	
4	Fore udder attachment	AA	110.621 $\pm$ 3.559	0.204
		AG	102.629 $\pm$ 3.239	
		GG	109.478 $\pm$ 3.996	
5	Udder circumference	AA	141.924 $\pm$ 2.034	0.412
		AG	143.869 $\pm$ 1.851	
		GG	139.963 $\pm$ 2.284	
6	Central ligament	AA	3.582 $\pm$ 0.223	0.545
		AG	3.701 $\pm$ 0.203	
		GG	3.346 $\pm$ 0.250	

Table 4.64 (b) contd...

Trait		Genotype	Mean $\pm$ SE	P-value
7	Rear teat length	AA	7.497 $\pm$ 1.229	0.387
		AG	5.606 $\pm$ 1.119	
		GG	5.196 $\pm$ 1.380	
8	Udder diameter	AA	49.277 $\pm$ 1.021	0.780
		AG	48.328 $\pm$ 0.929	
		GG	48.551 $\pm$ 1.146	
9	Udder length	AA	49.959 $\pm$ 1.139	0.279
		AG	52.112 $\pm$ 1.036	
		GG	49.950 $\pm$ 1.279	
10	Teat circumference	AA	9.360 $\pm$ 0.954	0.641
		AG	8.767 $\pm$ 0.868	
		GG	8.005 $\pm$ 1.071	
11	Udder balance	AA	-1.822 $\pm$ 0.551	0.620
		AG	-2.424 $\pm$ 0.501	
		GG	-2.551 $\pm$ 0.618	
12	Fore teat length	AA	8.062 $\pm$ 1.120	0.422
		AG	6.477 $\pm$ 1.019	
		GG	6.019 $\pm$ 1.257	
13	Distance between front and rear teat	AA	6.518 $\pm$ 0.962	0.707
		AG	6.227 $\pm$ 0.876	
		GG	5.346 $\pm$ 1.080	
14	Distance between right and left teat	AA	8.362 $\pm$ 1.010	0.261
		AG	7.094 $\pm$ 0.919	
		GG	5.864 $\pm$ 1.134	
15	Shortest distance from front teat end to floor	AA ^a	39.966 $\pm$ 1.129	0.042*
		AG ^b	43.833 $\pm$ 1.028	
		GG ^{a,b}	42.660 $\pm$ 1.268	
16	Shortest distance from rear teat end to floor	AA ^a	40.876 $\pm$ 1.143	0.046*
		AG ^b	44.542 $\pm$ 1.040	
		GG ^{a,b}	43.730 $\pm$ 1.283	
17	Teat diameter	AA	26.239 $\pm$ 0.939	0.573
		AG	25.444 $\pm$ 0.854	
		GG	26.851 $\pm$ 1.054	

* Significant at $P \leq 0.05$

^{a,b,c}Means with different letter superscript differ significantly

Among observational udder and teat type traits, the association study revealed significant ($P \leq 0.05$) association of SNP rs383429860 with visual observational trait long term changes in teat-end condition (LTCTEC), rear teat placement (RTPl) and teat size (TSi) (Table 4.65). The genotype AA was having higher odds of being significantly associated with trait sub category 1 and 4 of LTCTEC and TSi as compared to AG and GG genotypes (Table 4.65). The genotype AG was having higher odds to be significantly associated with trait sub category 4 of RTPl as compared to AA and GG genotypes (Table 4.65).

Table 4.65 Association of SNP rs383429860 (RE: Sau96I) with udder and teat type traits (visual observations) in Sahiwal cattle

	Trait	Chi-Square	df	Pair-wise estimation		
				Significant genotype	Trait sub category	Exp (B)
1	Fore teat placement	5.59	10	-	-	-
2	Front teat position	23.228	16	-	-	-
3	Long term changes in teat-end condition	11.047*	4	AA	1	1.467
4	Rear teat placement	11.703*	6	AG	4	>999.999
5	Rear teat position	17.461	12	-	-	-
6	Skin condition	0.227	2	-	-	-
7	Teat end shape	5.123	4	-	-	-
8	Teat shape	5.098	4	-	-	-
9	Teat size	49.58*	6	AA	4	7.333
10	Udder shape	1.129	4	-	-	-
11	Udder suspension	8.74	6	-	-	-

* Significant at $P \leq 0.05$

4.12.3.3 (b) Association of SNP rs383429860 with clinical mastitis and milk production traits in Karan Fries and Sahiwal cattle

In Karan Fries cattle, logistic regression analysis revealed genotypes of SNP rs383429860 had significant ($P \leq 0.05$) effect on incidence of clinical mastitis (Table 4.66). The odds ratio revealed genotype AA had 517.5% (confidence interval 1.713 - 15.631) and AG had 238.6% (confidence interval 0.897 - 6.345) more chance of occurring with healthy animals as compared to other genotype(s) (Table 4.66). In Sahiwal cattle, the SNP rs383429860 genotypes had significant effect ($P \leq 0.05$) on incidence of clinical mastitis

(Table 4.67). The odds ratio revealed genotype AA (Odds ratio =5.236) and AG (Odds ratio = 5.273) were having close to equal odds of being associated with healthy animals as compared to genotype GG (Table 4.67).

Table 4.66 Association of SNP rs383429860 (RE: Sau96I) genotypes with clinical mastitis in Karan Fries cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	06 (0.176)	58 (0.439)	0.010*	5.175 (1.713 – 15.631)
AG	10 (0.294)	43 (0.326)		2.386 (0.897 – 6.345)
GG	18 (0.529)	31 (0.235)		-
N	34	132		

* Significant at $P \leq 0.05$

Table 4.67 Association of SNP rs383429860 (RE: Sau96I) genotypes with clinical mastitis in Sahiwal cattle

Genotype	Mastitis	Healthy	P- value*	ODDS ratio estimation
AA	4 (0.222)	26 (0.377)	0.0048*	5.236 (1.479 – 18.534)
AG	5 (0.278)	31 (0.449)		5.273 (1.587 – 17.521)
GG	9 (0.500)	12 (0.174)		-
N	18	69		

* Significant at $P \leq 0.05$

For production traits, in Karan Fries cattle, the SNP rs383429860 genotypes had significant ($P \leq 0.05$) effect on 305-day milk yield and total milk yield [Table 4.68 (a)]. The genotype AG was found to be having higher mean values of 305-day milk yield (4575.299 ± 242.960 kg) and total milk yield (4531.896 ± 248.003 kg) [Table 4.68 (b)]. This higher mean value of genotype AG for both traits was found to be significantly ($P \leq 0.05$) different from other genotypes ([Table 4.68 (b)]).

Table 4.68(a) Association of SNP rs383429860 (RE: Sau96I) genotypes with milk production traits in Karan Fries cattle

Dependent Variable		Sum of Squares	Mean Square	F	P-value
Milk yield (305d)	Contrast	26406393.440	13203196.720	4.220*	0.016
	Error	509956157.293	3128565.382		
Monthly milk yield	Contrast	8512.658	4256.329	0.190	0.827
	Error	3649405.811	22388.993		
Test day milk yield	Contrast	7.683	3.841	0.159	0.853
	Error	3928.903	24.104		
Total milk yield	Contrast	26334537.999	13167269.000	4.039*	0.019
	Error	531344420.109	3259781.718		

*Significant at $P \leq 0.05$

Table 4.68 (b) Pair-wise comparison of SNP rs383429860 (RE: Sau96I) genotypes with milk production traits in Karan Fries cattle

Trait	Genotype	Mean $\pm$ SE	P-value
Milk yield (305 d)	AA ^a	4188.736 $\pm$ 217.721	0.016*
	AG ^b	4575.299 $\pm$ 242.960	
	GG ^a	3552.589 $\pm$ 258.002	
Monthly milk yield	AA	363.843 $\pm$ 18.418	0.827
	AG	372.811 $\pm$ 20.553	
	GG	381.345 $\pm$ 21.826	
Test day milk yield	AA	13.341 $\pm$ 0.604	0.853
	AG	12.884 $\pm$ 0.674	
	GG	12.923 $\pm$ 0.716	
Total milk yield	AA ^a	4117.083 $\pm$ 222.240	0.019*
	AG ^b	4531.896 $\pm$ 248.003	
	GG ^a	3507.170 $\pm$ 263.357	

* Significant at $P \leq 0.05$

^{a,b,c}Means with different letter superscript differ significantly

In Sahiwal cattle, SNP rs383429860 genotypes had significant ($P \leq 0.05$) effect on monthly milk yield, 305-day milk yield and total milk yield [Table 4.69 (a)]. For 305-day milk yield genotype AA (2282.547 ± 172.641 kg) was having maximum mean value, which was significantly different from genotype GG ($P < 0.05$) [Table 4.69 (b)]. A similar pattern was seen for total milk yield where genotype AA (2303.741 ± 213.013 kg) was having highest mean value which was significantly different from only genotype GG (1392.696 ± 239.189) ($P \leq 0.05$) [Table 4.69 (b)]. For monthly milk yield, the genotype GG was having significantly lowest mean value (108.065 ± 25.207) as compared to AA and AG genotypes (Table 4.69 b)

Table 4.69 (a) Association of SNP rs383429860 (RE: Sau96I) genotypes with milk production traits in Sahiwal cattle

Dependent Variable		Sum of Squares	Mean Square	F	P-value
Milk yield (305 d)	Contrast	8555202.977	4277601.489	4.949*	0.009
	Error	72604907.302	864344.135		
Monthly milk yield	Contrast	292351.537	146175.769	10.003*	0.000
	Error	1227557.365	14613.778		
Test day milk yield	Contrast	56.996	28.498	1.678	0.193
	Error	1426.241	16.979		
Total milk yield	Contrast	10993407.974	5496703.987	4.177*	0.019
	Error	110532806.923	1315866.749		

* Significant at $P \leq 0.05$

Table 4.69 (b) Pair wise comparison of SNP rs383429860 (RE: Sau96I) genotypes with milk production traits in Sahiwal cattle

Trait	Genotype	Mean $\pm$ Std. Error	P-value
Milk yield(305 d)	AA ^a	2282.547 ± 172.641	0.009*
	AG ^{a,b}	1917.891 ± 157.148	
	GG ^b	1465.876 ± 193.856	
Monthly milk yield	AA ^a	247.483 ± 22.448	0.000*
	AG ^a	231.171 ± 20.434	
	GG ^b	108.065 ± 25.207	
Test day milk yield	AA	8.444 ± 0.765	0.193
	AG	8.586 ± 0.697	
	GG	6.692 ± 0.859	
Total milk yield	AA ^a	2303.741 ± 213.013	0.019*
	AG ^{a,b}	1771.986 ± 193.897	
	GG ^b	1392.696 ± 239.189	

* Significant at $P \leq 0.05$

^{a,b,c} Means with different letter superscript differ significantly

The association of udder and teat type traits with IL22 gene in the present study might be due to the influence of bovine IL22 gene with cell growth, differentiation and motility which in turn plays a role in mammary gland development (Tolleson, 2016). IL22 mediates the action of Stat3 (Signal transducer and activator of transcription 3) which have important roles in lineage commitment, survival and death of mammary gland epithelium (Hynes and Watson, 2010). Stat3 is essential for the initiation of apoptosis and remodeling (Chapman *et al.*, 1999). It also has role in involution of mammary gland (Humphreys *et al.*, 2002). Interleukins are modulators of cellular behaviour and particularly important in stimulating immune responses, such as inflammation. IL22 appears to play an important role in maintaining barrier function on exposed body surfaces. It promotes antimicrobial immunity, inflammation and tissue repair, which could aid in mastitis prevention and resistance (Tizard, 2012), thus creating a possible link to milk production and udder type traits. GWAS for udder composition traits in *Bos indicus* X *Bos taurus* cows, reported a significant SNP (ARS-USMARC-614) within the IL-22 gene at position 45.7 Mb of BTA 5 for udder support (Tolleson, 2016). All these studies justify the association of SNPs in this gene with milk production and clinical mastitis.

4.13. Effect of udder and teat type traits on clinical mastitis in Karan Fries and Sahiwal cattle

The results of logistic regression analysis for effect of udder and type traits on incidence of clinical mastitis in Karan Fries and Sahiwal cattle have been presented in [Table 4.70 (a) and (b)] to [Table 4.71 (a) and (b)]. In Karan Fries cattle, rear udder height (RUH), rear udder width (RUW), udder width (UW), fore udder attachment (FUA), rear teat length (RTL), udder diameter (UD), teat circumference (TC), fore teat length (FTL), teat diameter (TD) and distance between right and left teat (DLR) had statistically significant ($P \leq 0.05$) effect on incidence of clinical mastitis [Table 4.70 (a)]. Animals with clinical mastitis are having longer RUH (30.887 ± 0.880 cm), RUW (16.511 ± 0.422 cm), UW (77.584 ± 2.167 cm), larger FUA (153.308 ± 2.055 cm), RTL (6.348 ± 0.126 cm), UD (60.128 ± 0.980 cm), TC (9.367 ± 0.164 cm), FTL (7.575 ± 0.313 cm), DLR (10.731 ± 0.764 cm) and TD (2.673 ± 0.068 cm) as compared to healthy animals [Table 4.70(a)]. Among the observational udder and teat type traits, long term changes in teat-end condition (LTCTEC), teat shape (TS), rear teat placement (RTPI), front teat position (FTP), rear teat position (RTP), teat size (TSi) and teat-end shape (TES) had statistically significant ($P \leq 0.05$) effect on incidence of clinical mastitis [Table 4.70(b)]. Among the different classes of the traits, sub category 1 of LTCTEC, sub category 4 of RTPI, sub category 3 of TSi and sub category 2 of

FTP had higher odds (>999.999) of occurring in healthy animals [Table 4.70(b)]. The traits subcategory 1 of TES and sub category 4 of RTP had higher odds (13.623 and 13.395, respectively) of occurring in healthy animals [Table 4.70(b)].

Table 4.70(a) Effect of udder and teat type traits (measurement) on clinical mastitis in Karan Fries cattle

Trait		Mastitis	N	Mean $\pm$ SE	Wald χ^2	Pr > χ^2 *
1	Rear udder height	0	132	21.995 $\pm$ 0.346	28.842*	<0.001
		1	34	30.887 $\pm$ 0.880		
2	Rear udder width	0	132	11.865 $\pm$ 0.164	27.366*	<0.001
		1	34	16.511 $\pm$ 0.422		
3	Udder width	0	132	70.218 $\pm$ 0.658	10.126*	0.002
		1	34	77.584 $\pm$ 2.167		
4	Fore udder attachment	0	132	127.115 $\pm$ 1.079	25.491*	<0.001
		1	34	153.308 $\pm$ 2.055		
5	Udder circumference	0	132	143.328 $\pm$ 2.184	1.852	0.174
		1	34	139.717 $\pm$ 4.398		
6	Udder balance	0	132	1.045 $\pm$ 0.495	0.000	0.990
		1	34	0.983 $\pm$ 0.949		
7	Central ligament	0	132	3.162 $\pm$ 0.120	0.044	0.834
		1	34	3.095 $\pm$ 0.299		
8	Udder depth	0	132	52.387 $\pm$ 0.447	27.400*	<0.001
		1	34	60.128 $\pm$ 0.980		
9	Udder length	0	132	60.021 $\pm$ 0.590	0.145	0.704
		1	34	62.753 $\pm$ 1.829		
10	Fore teat length	0	132	4.861 $\pm$ 0.079	17.510*	<0.001
		1	34	7.575 $\pm$ 0.313		
11	Rear teat length	0	132	4.331 $\pm$ 0.074	21.178*	<0.001
		1	34	6.348 $\pm$ 0.126		

Table 4.70(a) contd...

Trait		Mastitis	N	Mean $\pm$ SE	Wald χ^2	Pr > χ^2 *
12	Teat circumference	0	132	7.188 $\pm$ 0.118	20.628*	<0.001
		1	34	9.367 $\pm$ 0.164		
13	Teat diameter	0	132	2.134 $\pm$ 0.021	23.660*	<0.001
		1	34	2.673 $\pm$ 0.068		
14	Distance between front and rear teat	0	132	6.888 $\pm$ 0.346	3.026	0.082
		1	34	6.059 $\pm$ 0.472		
15	Distance between right and left teat	0	132	8.655 $\pm$ 0.412	5.300*	0.021
		1	34	10.731 $\pm$ 0.764		
16	Shortest distance from front teat end to floor	0	132	46.515 $\pm$ 0.450	2.014	0.156
		1	34	49.058 $\pm$ 1.185		
17	Shortest distance from rear teat end to floor	0	132	46.707 $\pm$ 0.456	0.085	0.771
		1	34	47.204 $\pm$ 1.345		

*Significant at $P \leq 0.05$ **Table 4.70 (b) Effect of udder and teat type traits (visual observations) on clinical mastitis in Karan Fries cattle**

Trait		Chi-Square*	df	Pair wise estimation	
				Trait sub category	Exp (B)
1	Skin condition	1.344	2	-	-
2	Long term changes in teat-end condition	17.123*	3	1	>999.999
3	Teat shape	9.115*	2	-	-
4	Fore teat placement	30.199*	5	3	>999.999
5	Rear teat placement	21.601*	5	4	>999.999
6	Front teat position	13.886*	5	2	>999.999
7	Rear teat position	9.223*	4	4	13.395
8	Udder shape	4.731	-	-	-
9	Udder suspension	2.478	3	-	-
10	Teat size	22.077*	5	3	>999.999
11	Teat end shape	7.663*	2	1	13.623

*Significant at $P \leq 0.05$

In Sahiwal cows, rear udder height (RUH), rear udder width (RUW), udder width (UW), fore udder attachment (FUA), udder circumference (UC), central ligament (CL) and rear teat length (RTL) had statistically significant ($P \leq 0.05$) effect on incidence of clinical mastitis (Table 4.71 a). Animal with clinical mastitis were having higher mean values of RUH (27.355 ± 1.336 cm), FUA (125.087 ± 3.336 cm), RTL (8.934 ± 2.576 cm), UC (153.188 ± 1.436 cm) and CL (4.760 ± 0.166 cm) as compared to healthy animals (Table 4.71 a). Healthy animals are having longer RUW (25.311 ± 0.523 cm) and UW (2290.133 ± 101.907 cm) as compared to mastitic animals (Table 4.71 a). Among the visual observational udder and teat type traits, teat shape (TS), rear teat placement (RTPl) and udder shape (US) had statistically significant ($P < 0.05$) effect on incidence of clinical mastitis (Table 4.71 b). The odds of trait subcategory 1 of TS (1.786), 4 of RTPl (>999.999) and trait subcategory 1 of US (1.455) with healthy udder were higher as compared to mastitic udder (Table 4.71 b).

Table 4.71 (a) Effect of udder and teat type traits (measurements) on clinical mastitis in Sahiwal cattle

Trait		Mastitis	N	Mean $\pm$ SE	Wald χ^2	Pr $> \chi^2$ *
1	Rear udder height	0	69	20.786 ± 0.611	14.631*	0.000
		1	18	27.355 ± 1.336		
2	Rear udder width	0	69	25.311 ± 0.523	17.448*	<0.001
		1	18	22.847 ± 1.144		
3	Udder width	0	69	2290.133 ± 101.907	17.859*	<0.001
		1	18	895.121 ± 111.990		
4	Fore udder attachment	0	69	100.641 ± 2.011	19.401*	<0.001
		1	18	125.087 ± 3.336		
5	Udder circumference	0	69	138.235 ± 1.164	18.697*	<0.001
		1	18	153.188 ± 1.436		
6	Central ligament	0	69	3.139 ± 0.126	19.077*	<0.001
		1	18	4.760 ± 0.166		
7	Rear teat length	0	69	5.120 ± 0.199	6.942*	0.008*
		1	18	8.934 ± 2.576		
8	Udder diameter	0	69	48.846 ± 0.605	0.681	0.409
		1	18	48.305 ± 1.456		
9	Udder length	0	69	50.549 ± 0.770	2.928	0.087
		1	18	51.584 ± 1.291		

Table 4.71 (a) contd...

Trait		Mastitis	N	Mean $\pm$ SE	Wald χ^2	Pr > χ^2 *
10	Teat circumference	0	69	8.601 $\pm$ 0.709	0.000	0.984
		1	18	9.214 $\pm$ 0.634		
11	Udder balance	0	69	-2.147 $\pm$ 0.340	0.004	0.948
		1	18	-2.562 $\pm$ 0.741		
12	Fore teat length	0	69	6.751 $\pm$ 0.865	0.179	0.672
		1	18	7.256 $\pm$ 0.440		
13	Distance between front and rear teat	0	69	6.237 $\pm$ 0.701	0.264	0.607
		1	18	5.685 $\pm$ 0.753		
14	Distance between right and left teat	0	69	7.261 $\pm$ 0.760	0.031	0.861
		1	18	6.999 $\pm$ 0.679		
15	Shortest distance from front teat end to floor	0	69	42.380 $\pm$ 0.856	0.454	0.501
		1	18	41.826 $\pm$ 0.876		
16	Shortest distance from rear teat end to floor	0	69	43.510 $\pm$ 0.840	1.181	0.277
		1	18	41.979 $\pm$ 1.019		
17	Teat diameter	0	69	25.757 $\pm$ 0.588	0.003	0.953
		1	18	26.982 $\pm$ 1.220		

*Significant at $P \leq 0.05$ **Table 4.71 (b) Effect of udder and teat type traits (visual observations) on clinical mastitis in Sahiwal cattle**

Effect		χ^2	df	Pair-wise estimation	
				Trait subcategory	Exp (B)
1	Skin condition	0.393	1	-	-
2	Long term changes in teat-end condition	0.080	2	-	-
3	Teat shape	6.176*	2	1	1.786
4	Fore teat placement	4.604	5	-	-
5	Rear teat placement	7.632*	3	4	>999.999
6	Front teat position	13.141	8	-	-
7	Rear teat position	3.304	6	-	-
8	Udder shape	5.515*	2	1	1.455
9	Udder suspension	4.739	3	-	-
10	Teat size	6.559	3	-	-
11	Teat end shape	0.093	2	-	-

*Significant at $P \leq 0.05$

Results of the present study corroborate with the findings of Sorensen *et al.* (2000) who reported that cows having weaker fore udder attachment and lower udder depth had high risk of clinical mastitis. Singh *et al.* (2014) reported longer rear udder depth, deeper udder and less udder to floor distance was related to high risk of clinical mastitis. Nash *et al.* (2002) studied the same and reported that stronger fore udder attachment, shallower udders, deeper udder cleft was related to less number of TCE. Nemcova (2007) who reported that cows with deep udders, weak central ligaments, weak fore udder attachments and low rear udder height has the highest SCC during the entire lactation. The deeper udders were found to be at higher risk of developing mastitis due to their increased tendency to become soiled (Lopez-Benavides *et al.*, 2005) and more susceptible to lesions (Singh *et al.*, 2014), hence, being contaminated with environmental pathogens and developed mastitis. DeGroot *et al.* (2002) and Kadarmideen (2004) concluded that selection for higher udders with tighter attachments would be favourable for reducing SCC. Higher SCC was determined in cows with larger as well as smaller distance between teats. Chrystal *et al.* (2001) revealed less pointed and more inverted teat-ends have been associated with increased susceptibility to mastitis as they retain milk, which can act as a substrate for bacterial growth. Similar to present findings, Singh *et al.* (2014) also found that cows with long and thick teats had higher occurrence of subclinical mastitis. In contrary to this, few studies have showed higher value of the somatic cell score for shorter teats (Nemcova *et al.*, 2007) and a negative correlation of somatic cell number with teat diameter (Orban *et al.*, 2009). Bharti *et al.* (2015) reported pendulous udder, flat and inverted teat-end, very long and thick teat were more susceptible to intra-mammary infection in cows. Ahlawat (2007) studied relation of teat types and mastitis in Sahiwal cows and reported that bottle shaped teats are related to high SCC. Though our study revealed significant association of teat shape and teat end shape with clinical mastitis, Okano *et al.* (2016) reported that teat Shape and teat tip Shape has no relation with SCC in Holstein cows.

4.14. Effect of udder and teat type traits on milk production in Karan Fries and Sahiwal cattle

Effect of continuous udder type traits on milk yield was estimated using GLM in SPSS. In Karan Fries cattle, RUH, RUW, UW, UC, FTL and RTL had statistically significant effect ($P \leq 0.05$) on 305-day milk yield [Table 4.72 (a)]. RUH, RUW, UW, UC, CL, FTL, TC and FUA had statistically significant ($P \leq 0.05$) effect on monthly milk yield [Table 4.72 (a)]. The traits RUH, FUA, UW, UC, FTL and TC had statistically significant ($P \leq 0.05$) effect on test day milk yield. Similarly, the measurement type traits RUH, RUW, UW, UC, FTL and RTL had statistically significant ($P \leq 0.05$) affect on total milk yield [Table 4.72 (a)].

**Table 4.72(a) Effect of udder and teat type traits (measurements) on milk production in
Karan Fries cattle**

Udder type trait	Production trait	Mean Square	F	Significance
Rear udder height	MY (305 d)	508.900	127.890*	0.000
	Monthly MY	86461.008	4.615*	0.033
	Test day MY	109.035	5.276*	0.023
	Total MY	4222666.142	21.973*	0.000
Rear udder width	MY (305 d)	497.780	133.230*	0.000
	Monthly MY	121350.762	10.094*	0.001
	Test day MY	8.244	0.399	0.529
	Total MY	4330699.055	19.656*	0.000
Fore udder attachment	MY (305 d)	146050.300	0.046	0.830
	Monthly MY	66613.450	7.964*	0.,001
	Test day MY	21.451	1.038	0.310
	Total MY	2254.113	0.001	0.979
Udder diameter	MY (305 d)	7391190.693	2.343	0.128
	Monthly MY	38888.931	2.076	0.152
	Test day MY	28.803	1.394	0.240
	Total MY	8193180.707	2.487	0.117
Udder balance	MY (305 d)	1237928.008	0.392	0.532
	Monthly MY	153.218	0.008	0.928
	Test day MY	11.000	0.532	0.467
	Total MY	550689.727	0.167	0.683
Udder length	MY (305 d)	149526.110	0.047	0.828
	Monthly MY	1880.104	0.100	0.752
	Test day MY	0.324	0.016	0.901
	Total MY	108477.952	0.033	0.856
Udder width	MY (305 d)	61044.177	2017.165*	0.001
	Monthly MY	59349.353	3.168*	0.050
	Test day MY	66.699	3.228*	0.050
	Total MY	62463072.730	819.584*	0.001
Udder circumference	MY (305 d)	11245705.016	3.564*	0.049
	Monthly MY	141116.475	7.532*	0.007
	Test day MY	138.585	6.706*	0.011
	Total MY	12028610.985	3.652*	0.047

Table 4.72(a) contd...

Udder type trait	Production trait	Mean Square	F	Significance
Central ligament	MY (305 d)	6434911.492	2.040	0.155
	Monthly MY	76263.236	4.070*	0.045
	Test day MY	38.235	1.850	0.176
	Total MY	6025039.906	1.829	0.178
Distance between right and left teat	MY (305 d)	322956.623	0.102	0.749
	Monthly MY	9381.372	0.501	0.480
	Test day MY	9.689	0.469	0.495
	Total MY	241038.406	0.073	0.787
Distance between front and rear teat	MY (305 d)	6217441.674	1.971	0.162
	Monthly MY	429.026	0.023	0.880
	Test day MY	0.001	0.000	0.994
	Total MY	7062861.079	2.144	0.145
Shortest distance from front teat end to floor	MY (305 d)	7366249.250	2.335	0.129
	Monthly MY	34112.763	1.821	0.179
	Test day MY	35.125	1.700	0.194
	Total MY	7188952.955	2.182	0.142
Shortest distance from rear teat end to floor	MY (305 d)	2674360.063	0.848	0.359
	Monthly MY	21798.725	1.163	0.282
	Test day MY	31.326	1.516	0.220
	Total MY	2638135.343	0.801	0.372
Fore teat length	MY (305 d)	18854765.709	5.976*	0.016
	Monthly MY	61464.492	3.281*	0.042
	Test day MY	80.676	3.904*	0.050
	Total MY	18926047.943	5.746*	0.018
Rear teat length	MY (305 d)	13161687.928	4.172*	0.043
	Monthly MY	10855.201	0.579	0.448
	Test day MY	8.857	0.429	0.514
	Total MY	13582203.960	4.123*	0.044
Teat circumference	MY (305 d)	989383.361	0.314	0.576
	Monthly MY	101810.280	5.434*	0.021
	Test day MY	81.994	3.968*	0.048
	Total MY	1137004.184	0.345	0.558
Teat diameter	MY (305 d)	4043.581	0.001	0.971
	Monthly MY	23150.880	1.236	0.268
	Test day MY	29.131	1.410	0.237
	Total MY	8703.859	0.003	0.959

*Significant at $P \leq 0.05$; MY = Milk yield

Among the visual observational udder and teat type traits, long term changes in teat-end condition (LTCTEC) had statistically significant ($P \leq 0.05$) effect on 305-day milk yield, monthly milk yield, test day milk yield and total milk yield [Table 4.72(b)]. Teat shape (TS), teat size (TSi) and udder suspension (USus) had significant ($P \leq 0.05$) effect on test day milk yield. In addition, udder suspension was also having significant effect on monthly milk yield ($P \leq 0.05$) [Table 4.72(b)].

Table 4.72 (b) Effect of udder and teat type traits (visual observations) on milk production in Karan Fries cattle

Observational trait	Production trait	Type III Sum of Squares	Mean Square	F	P-value
Fore teat placement	MY (305 d)	19042377.285	3808475.457	1.178	0.322
	Monthly MY	193285.357	38657.071	1.785	0.119
	Test day MY	198.028	39.606	1.695	0.139
	Total MY	23741485.564	4748297.113	1.423	0.219
Front teat position	MY (305 d)	9421508.709	1884301.742	0.572	0.721
	Monthly MY	18708.010	3741.602	0.165	0.975
	Test day MY	40.106	8.021	0.329	0.895
	Total MY	8928553.238	1785710.648	0.521	0.760
LTCTEC	MY (305 d)	26305672.812	8768557.604	2.785*	0.043
	Monthly MY	266276.227	88758.742	4.240*	0.006
	Test day MY	327.905	109.302	4.907*	0.003
	Total MY	28656403.534	9552134.511	2.925*	0.036
Rear teat placement	MY (305 d)	21991260.296	4398252.059	1.368	0.239
	Monthly MY	69030.894	13806.179	0.616	0.688
	Test day MY	84.078	16.816	0.698	0.625
	Total MY	28588442.367	5717688.473	1.729	0.131
Rear teat position	MY (305 d)	5820388.540	1455097.135	0.442	0.778
	Monthly MY	153493.284	38373.321	1.763	0.139
	Test day MY	190.239	47.560	2.044	0.091
	Total MY	7852953.905	1963238.476	0.575	0.681

Table 4.72 (b) contd...

Observational trait	Production trait	Type III Sum of Squares	Mean Square	F	P-value
Skin condition	MY (305 d)	12530011.918	6265005.959	1.949	0.146
	Monthly MY	88822.883	44411.441	2.028	0.135
	Test day MY	62.980	31.490	1.325	0.269
	Total MY	10319983.816	5159991.908	1.537	0.218
Teat end shape	MY (305 d)	7216139.824	3608069.912	1.111	0.332
	Monthly MY	5382.838	2691.419	0.120	0.887
	Test day MY	14.726	7.363	0.306	0.737
	Total MY	7498439.926	3749219.963	1.111	0.332
Teat shape	MY (305 d)	15067214.015	7533607.007	2.356	0.098
	Monthly MY	87004.488	43502.244	1.986	0.141
	Test day MY	194.082	97.041	4.227*	0.016
	Total MY	12591839.374	6295919.687	1.883	0.155
Teat size	MY (305 d)	20254998.645	4050999.729	1.256	0.286
	Monthly MY	240098.373	48019.675	2.248	0.052
	Test day MY	319.597	63.919	2.828*	0.018
	Total MY	21017113.051	4203422.610	1.253	0.287
Udder shape	MY (305 d)	4907624.264	2453812.132	0.753	0.473
	Monthly MY	3195.986	1597.993	0.071	0.931
	Test day MY	11.673	5.836	0.242	0.785
	Total MY	4518154.472	2259077.236	0.666	0.515
Udder suspension	MY (305 d)	10169907.702	3389969.234	1.044	0.375
	Monthly MY	337402.747	112467.582	5.487*	0.001
	Test day MY	219.651	73.217	3.191*	0.025
	Total MY	13573205.838	4524401.946	1.347	0.261

*Significant at $P \leq 0.05$; MY = Milk yield

For LTCTEC, the trait subcategory 2 was having highest means for 305-day milk yield (4463.546 kg), monthly milk yield (403.311 kg), test day milk yield (14.181 kg) and total milk yield (4395.395 kg), however, multiple comparison could not identify any single trait subcategory to be significantly different from others [Table 4.72(c)]. For USus, trait subcategory 4 was having highest monthly milk yield and subcategory 2 was having highest test day milk yield, for TS and TSi the subcategory 1 and 3 respectively were having highest means for test day milk yield, however, multiple comparison could not identify any single trait subcategory to be significantly different from others [Table 4.72(c)].

Table 4.72 (c) Pair wise comparison of significant udder and teat type traits (visual observations) with milk production in Karan Fries cattle

Type trait		Sub category	Production traits			
			MY (305 d)	Monthly milk yield	Test day milk yield	Total milk yield
1	LTCTEC	1	4118.366 ^{a,b}	379.9 ^{a,b}	13.343 ^b	4100.000 ^{a,b}
		2	4463.546 ^b	403.311 ^b	14.181 ^b	4395.395 ^b
		3	3722.429 ^{a,b}	306.661 ^a	11.051 ^{a,b}	3692.435 ^{a,b}
		4	3055.709 ^a	304.362 ^a	9.994 ^a	2858.136 ^a
2	USus	1	N.S.	296.34 ^a	11.532 ^a	N.S.
		2	N.S.	421.019 ^b	14.560 ^b	N.S.
		3	N.S.	342.634 ^{a,b}	12.248 ^{a,b}	N.S.
		4	N.S.	429.971 ^b	14.256 ^b	N.S.
3	TS	1	N.S.	N.S.	14.025 ^b	N.S.
		2	N.S.	N.S.	12.639 ^{a,b}	N.S.
		3	N.S.	N.S.	11.071 ^a	N.S.
4	TSi	1	N.S.	N.S.	12.157 ^{a,b}	N.S.
		2	N.S.	N.S.	12.265 ^{a,b}	N.S.
		3	N.S.	N.S.	15.115 ^b	N.S.
		4	N.S.	N.S.	13.298 ^{a,b}	N.S.
		5	N.S.	N.S.	12.788 ^{a,b}	N.S.
		6	N.S.	N.S.	10.967 ^a	N.S.

N.S. – Non significant at $P \leq 0.05$; MY – Milk yield
Means with different letter superscript differ significantly ($P \leq 0.05$);

In Sahiwal cattle, RUH, RUW and UW, had statistically significant ($P < 0.05$) effect on 305-day milk yield [Table 4.73(a)]. The traits RUH, RUW, UW and FUA had statistically

significant effect ($P \leq 0.05$) on monthly milk yield [Table 4.73(a)]. The traits UW, UC, DLR had statistically significant ($P \leq 0.05$) effect on test day milk yield [Table 4.73(a)]. Similarly, the measurement type trait RUH, RUW, UW, and TD had statistically significant ($P \leq 0.05$) effect on total milk yield [Table 4.73(a)].

Table 4.73 (a) Effect of udder and teat type traits (measurements) on milk production in Sahiwal cattle

Udder type trait	Production trait	Mean Square	F	P-value
Rear udder height	MY (305 d)	408.041	137.182*	0.000
	Monthly MY	131824.439	11.828*	0.001
	Test Day MY	0.115	0.011	0.918
	Total MY	4302666.142	18.973*	0.000
Rear udder width	MY (305 d)	397.540	133.652*	0.000
	Monthly MY	101350.762	9.094*	0.004
	Test Day MY	0.041	0.004	0.951
	Total MY	4230699.055	18.656*	0.000
Udder width	MY (305 d)	61393044.177	20640127.165*	0.000
	Monthly MY	136173.851	12.218*	0.001
	Test Day MY	121.570	11.282*	0.001
	Total MY	72473072.730	319.584*	0.000
Fore udder attachment	MY (305 d)	0.027	0.009	0.924
	Monthly MY	77613.450	6.964*	0.010
	Test Day MY	23.892	2.217	0.141
	Total MY	496831.397	2.191	0.143
Udder circumference	MY (305 d)	1.526	0.513	0.476
	Monthly MY	29954.378	2.688	0.106
	Test Day MY	95.092	8.824*	0.004
	Total MY	440.637	0.002	0.965

Table 4.73 (a) contd...

Udder type trait	Production trait	Mean Square	F	P-value
Central ligament	MY (305 d)	8.431	2.835	0.097
	Monthly MY	3789.050	0.340	0.562
	Test Day MY	9.974	0.926	0.339
	Total MY	16151.706	0.071	0.790
Rear teat length	MY (305 d)	6.943	2.334	0.131
	Monthly MY	973.473	0.087	0.768
	Test Day MY	1.041	0.097	0.757
	Total MY	65725.452	0.290	0.592
Udder diameter	MY (305 d)	0.270	0.091	0.764
	Monthly MY	10074.894	0.904	0.345
	Test Day MY	2.183	0.203	0.654
	Total MY	190104.282	0.838	0.363
Udder ligament	MY (305 d)	0.231	0.078	0.781
	Monthly MY	24670.593	2.214	0.141
	Test Day MY	25.298	2.348	0.130
	Total MY	66914.965	0.295	0.589
Teat circumference	MY (305 d)	2.100	0.706	0.404
	Monthly MY	23972.469	2.151	0.147
	Test Day MY	17.573	1.631	0.206
	Total MY	1806.344	0.008	0.929
Udder balance	MY (305 d)	0.232	0.078	0.781
	Monthly MY	14961.976	1.342	0.251
	Test Day MY	2.091	0.194	0.661
	Total MY	210761.335	0.929	0.338

Table 4.73 (a) contd...

Udder type trait	Production trait	Mean Square	F	P-value
Fore teat length	MY (305 d)	7.303	2.455	0.122
	Monthly MY	3030.027	0.272	0.604
	Test Day MY	0.598	0.056	0.814
	Total MY	107150.971	0.473	0.494
Distance between right and left teat	MY (305 d)	0.788	0.265	0.608
	Monthly MY	4757.194	0.427	0.516
	Test Day MY	126.303	11.721*	0.001
	Total MY	139946.045	0.617	0.435
Distance between front and rear teat	MY (305 d)	3.153	1.060	0.307
	Monthly MY	3667.963	0.329	0.568
	Test Day MY	34.393	3.192	0.078
	Total MY	10119.615	0.045	0.833
Shortest distance from front teat end to floor	MY (305 d)	2.376	0.799	0.375
	Monthly MY	38255.132	3.432	0.068
	Test Day MY	12.461	1.156	0.286
	Total MY	49927.399	0.220	0.640
Shortest distance from rear teat end to floor	MY (305 d)	0.085	0.029	0.866
	Monthly MY	10454.838	0.938	0.336
	Test Day MY	0.305	0.028	0.867
	Total MY	3.197	0.000	0.997
Teat diameter	MY (305 d)	9.337	3.139	0.081
	Monthly MY	78.337	0.007	0.933
	Test Day MY	30.617	2.841	0.096
	Total MY	979280.908	4.318*	0.041

*Significant at $P \leq 0.05$; MY = Milk yield

Among the visual observational traits, teat end shape (TES) and teat shape (TS) had statistically significant ($P \leq 0.05$) effect on 305-day milk yield [Table 4.73(b)] and additionally, TES had significant effect on total milk yield [Table 4.73(b)].

Table 4.73 (b) Effect of udder and teat type traits (visual observations) on milk production in Sahiwal cattle

Observational trait	Production trait	Mean Square	F	P-value
Fore teat placement	MY (305 d)	961872.088	1.020	0.411
	Monthly MY	23936.811	1.385	0.239
	Test Day MY	19.690	1.152	0.340
	Total MY	1162524.720	0.814	0.543
Front teat position	MY (305 d)	795290.336	0.829	0.579
	Monthly MY	19621.720	1.123	0.357
	Test Day MY	15.376	0.882	0.536
	Total MY	1170302.411	0.814	0.593
LTC TEC	MY (305 d)	1124516.288	1.197	0.307
	Monthly MY	11074.502	0.621	0.540
	Test Day MY	2.099	0.119	0.888
	Total MY	1673332.340	1.189	0.309
Rear teat placement	MY (305 d)	652510.661	0.684	0.564
	Monthly MY	13732.637	0.771	0.514
	Test Day MY	20.121	1.174	0.325
	Total MY	777339.088	0.541	0.655
Rear teat position	MY (305 d)	379906.029	0.385	0.886
	Monthly MY	18491.844	1.050	0.400
	Test Day MY	16.158	0.932	0.477
	Total MY	490078.598	0.331	0.919
Skin condition	MY (305 d)	2217.211	0.002	0.962
	Monthly MY	380.520	0.021	0.884
	Test Day MY	21.811	1.269	0.263
	Total MY	164267.009	0.115	0.735

Table 4.73 (b) contd...

Observational trait	Production trait	Mean Square	F	P-value
Teat end shape	MY (305 d)	2943394.426	3.285*	0.042
	Monthly MY	33299.541	1.925	0.152
	Test Day MY	28.704	1.691	0.191
	Total MY	5339713.027	4.046*	0.021
Teat shape	MY (305 d)	2747868.719	3.051*	0.050
	Monthly MY	27240.153	1.561	0.216
	Test Day MY	29.978	1.769	0.177
	Total MY	1721036.020	1.224	0.299
Teat size	MY (305 d)	864760.796	0.914	0.438
	Monthly MY	21270.145	1.212	0.310
	Test Day MY	3.340	0.188	0.904
	Total MY	817854.586	0.570	0.636
Udder shape	MY (305 d)	236036.857	0.246	0.783
	Monthly MY	10880.116	0.610	0.546
	Test Day MY	29.482	1.739	0.182
	Total MY	454112.747	0.316	0.730
Udder Suspension	MY (305 d)	1936539.574	2.133	0.102
	Monthly MY	6205.895	0.343	0.794
	Test Day MY	22.898	1.344	0.266
	Total MY	2362154.107	1.713	0.171

*Significant at $P \leq 0.05$; MY = Milk yield

The multiple comparison grouping showed that for trait TS subcategory 2 was having maximum mean value of 305-day milk yield (2104.307 kg) which was statistically different from subcategory 3 [Table 4.73(c)]. For trait TES's subcategory 1 was having maximum mean value for 305-day milk yield (2737.305 kg) and total milk yield (2902.000 kg) which was significantly ($P \leq 0.05$) higher than other subcategories [Table 4.73(c)].

Table 4.73 (c) Pair wise estimation of significant udder and teat type traits (visual observations) with milk production in Sahiwal cattle

Type trait	Subcategory	Production traits			
		MY (305 d)	Monthly milk yield	Test day milk yield	Total milk yield
TES	1	2737.305 ^b	N.S.	N.S.	2902.000 ^b
	2	1834.165 ^a	N.S.	N.S.	1835.622 ^a
	3	1841.157 ^a	N.S.	N.S.	1618.852 ^a
TS	1	2104.307 ^{a,b}	N.S.	N.S.	N.S.
	2	1688.541 ^a	N.S.	N.S.	N.S.
	3	2480.008 ^b	N.S.	N.S.	N.S.

N.S. – Non significant at $P \leq 0.05$; MY – Milk yield

Means with different letter superscript differ significantly ($P \leq 0.05$);

These results corroborate with Bhuiyan *et al.* (2004) who observed that maximum test day milk yield was found in bowl shaped udder whereas udder length, width and depth were significantly and positively correlated with milk yield on test day. Rao (2006) and Singh (2005) reported that trough shaped udder, udder circumference and length was significantly and positively correlated with milk yield. Bermingham *et al.* (2007) explored taller, wider and deeper udders are likely to produce more milk. Khan and Khan (2016) reported that udder length had highest phenotypic correlation with test day yield at first stage of lactation followed by udder width, udder circumference and udder depth. Mingoas *et al.* (2017) showed udder size, udder depth and height has strong and positive correlation with milk yield. Fore udder attachment, udder depth, central ligament, rear udder height, teat length and front teat placement had significant effect on milk production (Foster *et al.*, 1989; Misztal *et al.*, 1992; Short and Lawlor, 1992). DeGroot *et al.* (2002) stated higher values of milk were found for high rear udder height and weak central ligament, *i.e.* connection with large udders.

4.15. Effect of clinical mastitis on milk production traits in Karan Fries and Sahiwal cattle

In Karan Fries cows mastitis had statistically significant effect ($P \leq 0.05$) on all the milk production traits i.e. monthly milk yield, test day milk yield, 305-day milk yield and total milk yield (Table 4.74). Animal having clinical mastitis were having higher mean 305 day milk yield (4631.09 ± 330.17 kg), monthly milk yield (381.34 ± 23.27 kg), test day milk yield (13.10 ± 0.77 kg) and total milk yield (4460.61 ± 331.14 kg) as compared to healthy animals (Table 4.74).

Table 4.74 Effect of clinical mastitis on milk production traits in Karan Fries cattle

Production trait	Mastitis	Mean $\pm$ SE	P- value*
Milk yield (305 d)	0	4003.50 ± 152.73	0.000
	1	4631.09 ± 330.17	
Monthly milk yield	0	369.16 ± 13.27	0.001
	1	381.34 ± 23.27	
Test day milk yield	0	13.06 ± 0.43	0.000
	1	13.10 ± 0.77	
Total milk yield	0	3977.98 ± 157.38	0.000
	1	4460.61 ± 331.14	

*Significant at $P \leq 0.05$

These results are in agreement with the studies in Holstein cows by Rajala-Schultz *et al.* (1999) who reported daily loss during the first 2 wk after the occurrence of mastitis varied from 1.0 to 2.5 kg and the total loss over the entire lactation varied from 110 to 552 kg. Hagnestam *et al.* (2007) reported that the magnitude of loss of yield varied from 0 to 902 kg (0 to 11 % of the 305-day milk yield) and a reduction of 1 to 8 kg in daily milk yield on the day of clinical mastitic onset. Boujenane *et al.* (2015) showed milk yields of cows with mastitis were 549.6 kg per 305 days of lactation which is lower than healthy animals.

In Sahiwal mastitis has statistically significant effect ($P \leq 0.05$) on all the milk production traits i.e. monthly milk yield, test day milk yield, 305-day milk yield and total milk yield (Table 4.75). Animal having clinical mastitis were having higher mean 305-day milk yield (2466.36 ± 245.51 kg) and total milk yield (2680.94 ± 348.25 kg) as compare to healthy animals (Table 4.75).

Table 4.75 Effect of clinical mastitis on milk production traits in Sahiwal cattle

Trait	Mastitis	Mean $\pm$SE	P-value*
Milk yield (305 d)	0	1777.39 $\pm$ 109.189	0.000
	1	2466.36 $\pm$ 245.51	
Monthly milk yield	0	210.02 $\pm$ 15.59	0.000
	1	181.19 $\pm$ 34.65	
Test day milk yield	0	8.03 $\pm$ 0.49	0.001
	1	8.04 $\pm$ 1.10	
Total milk yield	0	1631.92 $\pm$ 121.01	0.000
	1	2680.94 $\pm$ 348.25	

*Significant at $P \leq 0.05$

Similar observation was reported by Kumar *et al.* (2016) that losses of milk production were 10 to 15% in early stage. Effect of clinical mastitis on the milk yield in Indian dairy cows was also reported by Bangar *et al.* (2018) as a reduction of 1.64 kg overall mean daily milk yield after the occurrence of mastitis. The overall milk yield due to mastitis was reduced from 10.37 kg to 8.01 kg. The crossbred cows (2.37 kg/day) showed significantly higher reduction in milk yield due to mastitis than the indigenous (0.91 kg/day) lactating cows (Kumar *et al.*, 2010; Bangar *et al.*, 2018). The high losses in crossbred cows may relate to high milk production. Additionally, crossbred cows are more susceptible to intra-mammary infection as compared to indigenous cows (Slettbaek *et al.*, 1995; Radostits *et al.*, 2000; Sharma and Maiti, 2010).

CHAPTER –5

Summary and Conclusions

5. Summary and Conclusions

Udder conformation is an important aspect in dairy enterprise. The health, body conformation and type of udder affect milk production in dairy animals. Selection for dairy animals is classically directed towards production and reproduction traits, however, udder health and conformation are seldom given enough weightage. In developed nations daughter-dam comparison, daughter-herd mate comparison was initially started for animal improvement. However, udder type traits gained little focus as most of the low producing cows had poor udder and/or foot problems so were culled on the basis of production parameters.

Linear scoring of type traits started in 1980s with Ayreshire, Jersey and Guernsey cattle breeds. Later on type traits became important criteria for animal selection for better production and in some instances for better udder health. The present investigation was aimed to study type traits in Karan Fries and Sahiwal cattle breeds and to explore the genetic polymorphism affecting the udder type traits as well as the udder health (as indicated by the incidence of clinical mastitis). Subsequently, association of genetic polymorphism with udder type traits, incidences of clinical mastitis and milk production was studied. Additionally, effect of udder type traits on incidences of clinical mastitis and milk production was also explored.

Genomic DNA was isolated from blood samples by phenol chloroform method and the quality and quantity was checked. Previous genome wide association study have indicated that bovine VDR, PTPRR, HMGA2 and IL-22 gene polymorphism had combined effect on both, the udder type traits and incidence of mastitis. We curated the specific SNPs from the used bovine SNP chip using Ensembl genome browser and NCBI database. Out of these, eight SNPs having significant effect on udder type traits and/or mastitis and/or milk production were identified for the present investigation. Primers for the specific SNPs were designed using Primer3 software.

The PCR conditions were optimized to obtain respective amplicon of each gene. The amplicons were then sequenced and assembled using Mega 6.0 software. The sequences were then virtually digested by using Neb cutter v2.0 and specific restriction enzymes targeting the desired SNP were selected. After standardization of PCR and restriction digestion protocol, total 4 genes with 8 different SNP loci were targeted for polymorphism and association study.

These loci constituted two SNP loci from each VDR and PTPRR gene, one from HMGA2 gene and three from IL-22 gene. The SNPs at gene HMGA2 were found to be monomorphic in present study hence association analysis could only be carried out with VDR, PTPRR and IL-22 gene.

Karan Fries (N=166) and Sahiwal (N=87) cattle population maintained at Livestock Research Centre, ICAR-NDRI were genotyped at eight SNP loci and were subsequently phenotyped for udder type traits, incidence of mastitis and milk production traits. The udder type traits constituted total 8 udder measurement traits, 6 teat measurement traits and eleven visual observational udder and teat type traits, viz., rear udder height (RUH), rear udder width (RUW), udder length (UL), udder depth (UD), udder circumference (UC), udder balance (UB), udder width (UW), central ligament (CL), rear teat length (RTL), front teat length (FTL), teat diameter (TD), teat circumference (TC), distance between front and rear teat (DFR), distance between left and right teat (DLR), teat size (TSi), shortest distance from front teat end to floor (SDF) and shortest distance from rear teat end to floor (SDR), similarly seven observational udder and teat type traits were also recorded viz., fore udder attachment (FUA), fore teat placement (FTPl), rear teat placement (RTPl), front teat position (FTP), rear teat position (RTP), udder shape (US), udder suspension (USus), teat shape (TS), long term changes in teat end condition (LTCTEC), skin condition (SC) and teat end shape (TES). The incidence of clinical mastitis was recorded from the datasheet maintained at Animal health complex, ICAR-NDRI. Four milk production traits, milk yield in 305 days, total milk yield, test day milk yield and monthly milk yield were also recorded for each animal. The udder and teat type traits were also recorded as per ICAR (2001).

The phenotypic data was first standardized to estimate the effect of non genetic factors on udder type traits and production traits using ANOVA and the least squares mean values in Sahiwal and Karan Fries cattle through the technique described by Harvey (1990). The effect of non genetic factors on incidence of clinical mastitis was analysed using the maximum likelihood method of LOGISTIC procedure of SAS Version 9.3. The non genetic factors included the effect season (summer, rainy, autumn and winter), parity (1st, 2nd, 3rd, 4th and >5th), stage of lactation (1st, 2nd and 3rd) and level of production (High, medium and low).

The association between different genotypes identified after restriction digestion of specific SNPs and the phenotypic traits was done using appropriate model in SPSS 20 and SAS university edition. The incidence of mastitis was recorded in binary format (1 for mastitis and 0 for healthy) hence logistic regression was used to establish the effect of

genotypes on incidence of mastitis. The visual observational udder conformation traits were observed in discrete numbers categorizing each trait in number of subcategories hence, the effect of genotypes on these traits was analyzed by multinomial regression in SPSS.

Effects of non-genetic factor on production traits, type traits and incidence of mastitis were analysed and it was found that parity had significant effect on 305-day milk yield (305MY) in Karan Fries cows; while it had significant effect on monthly milk yield (MMY), test day milk yield (TDMY) and 305-day milk yield (305MY) in Sahiwal cows. Stage of lactation had also significant effect on monthly milk yield (MMY) and test day milk yield (TDMY) in Karan Fries cows.

For udder and teat type traits, in Karan Fries cattle, fore udder attachment (FUA), rear udder height (RUH), udder depth (UD), udder length (UL), udder width (UW), teat circumference (TC), fore teat length (FTL), shortest distance from front teat ends to floor (SDF), shortest distance from rear teat ends to floor (SDR), teat diameter (TD) were significantly affected by parity. Whereas, stage of lactation has significant effect on udder circumference (UC), distance between fore and rear teats (DFR) and distance between left and right teats (DLR). Similarly in Sahiwal cattle, fore udder attachment (FUA), rear udder width (RUW), udder depth (UD), udder width (UW) and teat diameter (TD) were significantly affected by parity. Teat type trait i.e teat circumference (TC) was significantly affected by season and stage of lactation. In addition, stage of lactation had significant effect on distance between fore and rear teats (DFR), distance between left and right teats (DLR), shortest distance from rear teat ends to floor (SDR) and udder circumference (UC).

For incidence of mastitis, in Karan Fries cattle, the percentage of incidence of mastitis was maximum in parity 4 (41.1%), similarly rainy season and high level of milk production showed maximum percentage of incidence of clinical mastitis viz., 30.5% and 31.0%, respectively. In Sahiwal cattle, the percentage of incidence of mastitis was maximum in parity 4 (52.8%), similarly, rainy season and high level of milk production showed maximum percentage of incidence of clinical mastitis viz., 30.4% and 31.3%, respectively.

Association analysis of SNPs (rs454303072 and rs470608846) in VDR gene was performed with udder type traits, milk production and incidence of clinical mastitis in KF and Sahiwal cattle. After genotyping of the animals through PCR-RFLP, rs470608846 locus was found to be monomorphic and hence, did not revealed any nucleotide variation both in KF and Sahiwal cattle. Hence further analysis was done only for rs454303072 locus (two

genotypes AA and GG) of which AA genotype was found to be superior with higher RUH, GG (rs454303072) genotype was found to have superior RUW, UC, DFR and DLR. Similarly for observational traits, AA genotype had significant effect ($P \leq 0.05$) on FTP, RTP, TS, TSi, US and USus in KF cattle. In Sahiwal cattle also, AA (rs454303072) genotype had highest RUH, RUW, UW and UC. AA genotype of VDR gene (rs454303072) also had more chances of occurring with healthy animals as compared to genotype GG (rs454303072) in both KF and Sahiwal cattle. Among the production traits, GG (rs454303072) genotype was found to be superior with higher 305-day milk yield, monthly milk yield, test day milk yield and total milk yield in Karan Fries cows. Whereas, the genotype AA (rs454303072) was found to be superior with higher 305-day milk yield, monthly milk yield, test day milk yield and total milk yield in Sahiwal cows.

SNPs in PTPRR gene and its association with udder type traits, production traits and incidence of mastitis revealed three genotypes (AA, AC and CC) at SNP locus rs382671389 and three genotypes CC, TC and TT at SNP locus rs435289107 in KF and Sahiwal cattle. The AA genotype (rs382671389) was found to be significantly ($P < 0.05$) associated with RUH, RUW, FUA, front teat position, rear teat position and teat size in KF cattle. Genotype AA (rs382671389) in Sahiwal cattle had significant effect on FUA, RUH, RUW, skin condition and teat shape. Genotype AC was having higher odds of being associated with LTC TEC and RTP. AC (rs382671389) genotype had more chances of occurring with healthy animals in both KF and Sahiwal cattle. Among the production traits, CC (rs382671389) genotype had higher 305-day milk yield, monthly milk yield, test day milk yield and total milk yield in Karan Fries cows. Whereas, the genotype AA (rs382671389) was superior with higher monthly milk yield in Sahiwal cows.

Genotype TT in KF cattle (SNP rs435289107) had significant effect ($P \leq 0.05$) on rear udder height (RUH) and shortest distance from rear teat end to floor (SDR). Genotype CC (rs435289107) had significant effect ($P \leq 0.05$) on udder width (UW), udder circumference (UC), udder length (UL), distance between right and left teat (DLR), front teat position (FTP), long term changes in teat-end condition (LTC TEC) and teat shape (TS). Among the observational udder type traits, genotype CC had significant effect on front teat position (FTP), long term changes in teat-end condition (LTC TEC) and teat shape (TS). The genotype TC (rs435289107) of Sahiwal cattle had significant effect ($P \leq 0.05$) on udder width (UW), teat end shape (TES). Genotype CC (rs435289107) had significant effect ($P \leq 0.05$) on teat shape (TS). CC (rs435289107) genotype had more chances of occurring with healthy animals

and also had higher monthly milk yield and test day milk yield in KF. TC (rs435289107) genotype had higher 305-day milk yield and monthly milk yield in Sahiwal cows.

SNPs in IL-22 gene (rs135884509, rs136785910 and rs383429860) and their association study with udder type traits, milk production traits and incidence of mastitis in KF and Sahiwal cattle exhibited polymorphism in all the loci in both breeds. The AA genotype (rs135884509) had significant effect ($P \leq 0.05$) on RUH, RUW, UC, FTP, RTP, UW, CL, rRTL, DFR, FTP, RTP, TS_i and US_{us} in KF cows. Genotype CC (rs135884509) had significant effect ($P \leq 0.05$) on teat shape (TS). AA (rs135884509) genotype had more chances of occurring with healthy animals in KF cows. The genotype AA (rs135884509) had higher 305-day milk yield and total milk yield. Genotype CC (rs135884509) had higher monthly milk yield and test day milk yield the in KF cows. Genotype TC (rs135884509) had higher 305-day milk yield and monthly milk yield in KF cows. In Sahiwal cows, the genotype TC (rs135884509) had more value of UW, FUA, UC and TC and was significantly associated with RTP, US and US_{us}. Genotype TC (rs135884509) had more chance of occurring with healthy animals and had higher values of 305-day milk yield, test day milk yield and total milk yield.

Genotype TT (rs136785910) had significant effect ($P \leq 0.05$) on RUW, UC, TC, DLR and TD in Karan Fries cows. The genotype TC (rs136785910) had significant effect ($P \leq 0.05$) on UB, FTP, LTC_{TEC}, RTP_I and TS_i in KF cows. TC (rs136785910) showed more chance of occurring with healthy KF animals. The genotype TT (rs136785910) was found to have higher values of 305-day milk yield, monthly milk yield, test day milk yield and total milk yield in KF cows. In Sahiwal cows, the genotype TC (rs136785910) had significant effect ($P \leq 0.05$) on UW, FUA, UC, TC RTP, US and US_{us}. In Sahiwal cattle, genotype TC (rs136785910) had more chance of occurring with healthy animals. The genotype TC (rs136785910) was found to be having higher values of 305-day milk yield, test day milk yield and total milk yield.

Genotype AG (rs383429860) was significantly associated with RUH and CL in KF cows. The GG (rs383429860) genotype was found to have highest UC. The AA (rs383429860) genotype was found to be superior with higher UB, SDR, FTP_I, LTC_{TEC} and RTP_I. The genotype AG (rs383429860) was found to have associated with LTC_{TEC}, SC and TES in KF cows. The genotype AG (rs383429860) had higher mean values for 305-day milk yield and total milk yield. The genotype AA and AG (rs383429860) were less susceptible to clinical mastitis in KF cows. In Sahiwal cows, the genotype AA (rs383429860) was

significantly associated with UW, LTC TEC and TSi. The genotype AG (rs383429860) was significantly associated with RTPL. The genotype AA and AG (rs383429860) had higher mean values for 305-day milk yield in Sahiwal cows.

5.1 Conclusions:

- ✓ In Karan Fries cattle, genotype AA at SNP locus rs454303072, genotype AA at SNP locus rs383429860 and rs135884509 as well were found to be less susceptible to mastitis and associated with desirable sub category of major udder type traits.
- ✓ Genotype TT at SNP locus rs136785910 was found to be significantly associated with desirable udder type traits and higher milk production in Karan Fries cattle.
- ✓ Genotype CC at SNP locus rs435289107 in Karan Fries cattle was found to be associated with healthy udder and teat type traits.
- ✓ The genotype AA at SNP locus rs454303072, rs135884509 and rs382671389 was found to be less susceptible to mastitis and associated with major udder type traits in Sahiwal cattle.
- ✓ The genotype CC (rs435289107) in Sahiwal cattle was found to have desirable udder type traits, higher 305-day milk yield and monthly milk yield and less susceptibility to clinical mastitis.
- ✓ The genotype AA and AG (rs383429860) were found to be less susceptible to mastitis and have positive effects on udder type traits in Sahiwal cattle.
- ✓ Genotype CT at SNP locus rs136785910 in Sahiwal cattle had a positive effect on type traits however it was not having significant effect on mastitis.

5.2 Recommendations:

From the present study genotype AA (rs135884509 and rs454303072) was found to be associated with desirable udder type traits and less susceptibility to clinical mastitis in Karan Fries cattle. In Sahiwal cattle, the genotype AA at SNP locus rs454303072 of VDR gene and in Karan Fries cattle genotype AA (rs135884509 and rs383429860) of IL-22 gene may be used as an aid to selection for higher milk production with desired udder conformation and lesser susceptibility to mastitis after validation in larger population. In Karan Fries cattle the genotype AA at SNP locus rs135884509 of IL-22 gene was associated with healthy udder and major type traits. The same SNP showed the highest LD with other loci in Sahiwal cattle. Therefore, other regions of this gene need to be studied for their possible association with type traits and mastitis preferably in larger herds of cattle.

Bibliography

Bibliography

- Abdou, H., Marichatou, H., Beckers, J.F., Dufrasne, I. and Hornick, J.L. (2012). Physiology of reproduction and chemical composition of colostrum of domestic mammals. *Ann. Med. Vet.* 156: 87-98.
- Abdullah, M., Khalid, J., Muhammad, S.K., Nisar, A., Jalees, A.B. and Umair, Y. (2013). Relationship of Udder and Teat Morphology with Milk Production in Nili-Ravi Buffaloes of Pakistan. *Buffalo Bull.* 32 (2): 1335-1338.
- Abi Habib, W., Brioude, F., Edouard, T., Bennett, J.T., Lienhardt-Roussie, A. and Tixier, F., et al. (2018). Genetic disruption of the oncogenic HMGA2–PLAG1–IGF2 pathway causes fetal growth restriction. *Genet. Med.* 20: 250–258.
- Aguiar, T.S., Torrecilha, R.B.P., Milanesi, M., Utsunomiya, A.T.H., Trigo, B.B., Tijjani, A., Musa, H.H., Lopes, F.L., Ajmone-Marsan, P., Carvalheiro, R., de Rezende Neves, H.H., do Carmo, A.S., Hanotte, O., Sonstegard, T.S., Garcia, J.F. and Utsunomiya, Y.T. (2018). Association of copy number variation at intron 3 of HMGA2 with navel length in *Bos indicus*. *Front. Genet.* 9: 627
- 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.
- Ahmad, S.S., Khan, M.A., Ali, A. and Muhammad, M.S. (1993). Effect of some environmental factors on lactation curve, 305-day milk yield and persistency in Nili-Ravi buffaloes. *The J Ani Pl Sci.* 3: 110-12.
- Ahmed, S. and Barbary, E. (2000). Dairy and meat cattle. College of Agric., Univ. of Alexandria, Egypt, Dar El-Maarif Printing Press. pp: 73.
- Akutsu, N., Lin, R., Bastien, Y., Bestawros, A., Enepekides, D.J., Black, M.J. and White, J.H. (2001). Regulation of gene expression by 1 alpha, 25-dihydroxyvitamin D3 and its analog EB1089 under growth-inhibitory conditions in squamous carcinoma cells. *Mol. Endocrinol.* 15: 1127–1139.

- Amin, A.A., Gere, T. and Kishk, W.H. (2002). Genetic and environmental relationship among udder conformation traits and mastitis incidence in Holstein Friesian into two different environments. *Archiv Tierzucht Dummerstorf*. 45: 129–138.
- Anand, A. and Chada, K. (2000). In vivo modulation of Hmgic reduces obesity. *Nature Genet*. 24: 377–80.
- Anderson, R.R. (1985). Mammary gland. In: Lactation (Ed.: Larson, B. L.). Ames, The Iowa State University Press p. 276.
- Angelova, T. (2006). Study for opportunities to optimize the selection of Brown cattle in Bulgaria. Ph.D. Thesis, Institute of Agriculture, Stara Zagora, Bulgaria, 133.
- Anonymous (2012a). ICAR-National Dairy Research Institute. News letter.17: 1.
- Anonymous (2012b). National Mastitis Council. A global organization of mastitis control and milk quality. Columbus Avenue South New Prague, Minnesota 56071, USA.
- Aoki, N., Kawamura, M., Yamaguchi-Aoki, Y., Ohira, S. and Matsuda, T. (1999). Down regulation of protein tyrosine phosphatase gene expression in lactating mouse mammary gland. *J. Biochem*.125: 669–675.
- Ashwell, M.S., Heyen, D.W., Weller, J.I., Ron, M., Sonstegard, T.S., Van Tassell, C.P. and Lewin, H.A. (2005). Detection of quantitative trait loci influencing conformation traits and calving ease in Holstein Friesian cattle. *J. Dairy Sci*. 88: 4111–4119.
- Atkins, G. (2007). Using conformational anatomy to identify functionality in dairy cows. 27th European Holstein and Red Holstein Conference, Denmark.
- Atkins, G. and Shannon, J. (2002). Minimizing lameness through genetic selection. *Advances in Dairy Techno*. 14: 93.
- Bagnato, A. and Oltenacu, P.A. (1994). Phenotypic evaluation. *J Dairy Sci*. 77: 874-82.
- Bangar, Y.C., Verma, M.R. and Sharma, V.B. (2018). Effect of Clinical Mastitis on the Milk Yield in Dairy Cows in India. *Int. J. Livest. Res*. 8(8):171-177.
- Bardakcioglu, H.E., Sekkin, S. and Oral Toplu, H.D. (2011). Relationship between some teat and body measurements of holstein cows and sub-clinical mastitis and milk yield. *J. Anim. Vet. Adv*10:1735–1737.

- Basic Animal Husbandry & Fisheries Statistics. (2017). Available at: http://dahd.nic.in/sites/default/files/Basic%20Animal%20Husbandry%20and%20Fisheries%20Statistics%202017%20%28English%20version%29_5.pdf [Accessed 12 Nov 2018]
- 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, 12-Mar-2007: 77.
- Berry, D.P. (2007). Evaluation and optimal utilization of the international linear type classification schemes. Project No. 5258. Dairy Production Research centre, Moorepark, Germany, Co.Cork.
- Bharti, P., Bhakat, C., Pankaj Prabhat, K., Bhat Showkat, 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.
- Biffa, D., Debela, E. and Beyene, F. (2005). Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia. *Int J Appl Res Vet M.* 3: 189-198.
- Boettcher, P.J., Dekkers, J.C.M. and Kolstad, B.W. (1998). Development of an udder health index for sire selection based on somatic cell score, udder conformation, and milking speed. *J. Dairy Sci.* 81, 1157–1168.
- Bolormaa, S., Pryce, J.E., Reverter, A., Zhang, Y., Barendse, W., Kemper, K., Tier, B., Savin, K., Hayes, B.J. and Goddard, M.E. (2014). A multi-trait, meta-analysis for detecting pleiotropic polymorphisms for stature, fatness and reproduction in beef cattle. *PLoS Genet.* 10(3), p.e1004198.
- Bostein, D., White, R.L., Sholnick, M. and David, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am J Hum Genet.* 32:314–331.
- Boujenane, I., Aïmani, J.El. and Khalid (2015). Effects of clinical mastitis on reproductive and milk performance of Holstein cows in Morocco. *Trop Anim Health Prod.* 47:207-211.

- Bradford, H.L., Moser, D.W., Minick Bormann, J. and Weaber, R.L. (2015). Estimation of genetic parameters for udder traits in Hereford cattle. *J. Anim. Sci.* 93:2663– 2668.
- Breed Survey. (2013). Available at: [http:// dahd.nic.in/ sites/ default/ filess/ Breeding% 20Survey% 20Book% 20-%20Corrected.pdf](http://dahd.nic.in/sites/default/files/Breeding%20Survey%20Book%20-%20Corrected.pdf) [Accessed 15 Nov 2017]
- Britt, J.S. and Farnsworth, R. (2011). Hoard's Dairyman. Available at: [http:// milkquality.wisc.edu/ wp-content/ uploads/ 2011/09/ teat-end-condition-scoring-chart.pdf](http://milkquality.wisc.edu/wp-content/uploads/2011/09/teat-end-condition-scoring-chart.pdf). [Accessed on 25 Sep 2017].
- Campanile, G., Di Palo, R., De Rosa, C., Peretti, V., Amante, L., Ciotola, F. and Coletta A. (2003). Preliminary results on Mediterranean Italian Buffalo morfometry. *Ital. J. l Anim. Sci.* 2(1): 337-339.
- Capuco, V.A., Wood, D.L., Baldwin, Mcleod, R.K. and Paape, M.J. (2001). Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of Sub-Tropical. *J Dairy Sci.* 84: 2177–2187.
- Chachare, R.A. and Walkunde, T.R. (2011). Udder and teat measurements influenced by milking, udder shape and texture in gaolao cows. *Res. J. Animal Hus. & Dairy Sci.* 2(1&2) : 40-42.
- Chaki, E.K., Ghosh, N. and Majumdar, S.C. (1999). Relationship of udder and teat types to part lactation yield in primiparous crossbred cows. *Indian Vet. J.* 76: 58-60.
- Chapman, R.S., Lourenco, P.C., Tonner, E., Flint, D.J., Selbert, S., Takeda, K., Akira, S., Clarke, A.R. and Watson, C.J. (1999). Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. *Gene. Dev.* 13:2604–2616.
- Chishty, M.A., Arshad, M., Avais, M., Hameed, S. and Ijaz, M. (2007). Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of Tehsil Gojra, Pakistan. *Buffalo Bull.* 26: 50-55.
- Chrystal, M.A., Seykora, A.J. and Hansen, L.B. (1999). Heritabilities of teat end shape and teat diameter and their relationships with somatic cell score. *J. Dairy Sci.* 82(9): 2017–2022.
- Chrystal, M.A., Seykora, A.J., Hansen, L.B., Freeman, A.E., Kelley, D.H. and Healey, M.H. (2001). Heritability of teat-end shape and the relationship of teat-end shape with somatic cell score for an experimental herd of cows. *J. Dairy Sci.* 84: 2549–2554.

- Chyr, S.C. (1973). Differences between pedigree groups of Holstein cows in rate of milk flow and udder measurements and their relation to udder health and milk production. M.S. Thesis, Iowa State Univ.
- Chyr, S.C., Berger, P.J. and Freeman, A.E. (1974). Relation among milk flow rates, udder measurements, and udder health in pedigree selected high and low producing cows. *J. Dairy Sci.* 57: 616.
- Cleynen, I. and Ven, V.D.W.J.M. (2008). The HMGA proteins: A myriad of functions (Review). *Int. J. Oncol.* 32: 289-305.
- Cole, J.B., Wiggans, G.R., Ma, L., Sonstegard, T.S., Lawlor, T.J.Jr., Crooker, B.A., Van Tassell, C.P., Yang, J., Wang, S., Matukumalli, L.K. and Da, Y. (2011). Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U.S. Holstein cows. *BMC Genomics.* 12: 408–424.
- Corbett, R. (2009). Minimizing the effects of immune suppression through management and nutrition. In: Proc. NMC Annual Meeting. 113-119.
- Dahiya, S.P. (2005). Appraisal of linear type traits for reproductive efficiency in Sahiwal cows. *Indian J. Anim. Sci.* 75: 945-948.
- Dahiya, S.P. and Rathi, S.S. (2002). Linear scoring of type conformation for milk production in Tharparkar cattle. *Indian J. Dairy Sci.* 72 (10): 911-913.
- 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.
- Das, K.S., Tomer, O.S., Sinha, M.A. and Bhattacharya, T.K. (2002). Correlation of prepartum udder and teat measurement with milk yield in primiparous crossbred dairy cows. *Indian J. Anim. Prod. Mgmt.* 18 (1-2): 17-19.
- DeGroot, B.J., Keown, J. F., Van Vleck, L.D. and Marotz, E.L. (2002). Genetic parameters and responses of linear type, yield traits, and somatic cell scores to divergent selection for predicted transmitting ability for type in Holsteins. *J. Dairy Sci.* 85, 1578–1585.
- 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. *Opin. Anim. Vet. Sci.* 2(1): 49-54.

- Dhanda, M.R. and Sethi, M.S. (1962). Investigation of Mastitis in India. ICAR Res. Series No. 35. New Delhi, India.
- Donald, H.P. (1960). Genetic aspects of maximum rate of flow during milking. *J. Dairy Res.* 27: 361–372.
- Dubey, A. (2010). Studies on linear type traits in Sahiwal. Thesis. Indira Gandhi Krishi Vishwavidyalaya, Raipur, India.
- Dubey, A., Mishra, S., Khune, V., Gupta, P.K., Sahu, B.K. and Nandanwar, A.K. (2012). Improving linear type traits to improve production sustainability and longevity in purebred Sahiwal cattle. *J. Agr. Sci. Tech.* 5: 636–639.
- Dux, M., Muranowicz, M., Siadkowska, E., Robakowska-Hyzorek, D., Flisikowski, K., Bagnicka, E., Zwierzchowski, L. (2018). Association of SNP and STR polymorphisms of insulin-like growth factor 2 receptor (IGF2R) gene with milk traits in Holstein-Friesian cows. *J Dairy Res.* 85(2), pp.138-141.
- Emre, B. and Alacam, E. (2015): The Occurrence of teat hyperkeratosis in cows and its effect on milk somatic cell counts Türkiye Klinikleri. *J Vet Sci.* 6(1): 1–6.
- Ensemble genome browser. URL - <https://asia.ensembl.org/index.html>
- Epaphras, A., Karimuribo, E.D. and Msellem, S.N. (2004). Effect of season and parity on lactation of crossbred Ayrshire cows reared under coastal tropical climate in Tanzania. *Livestock Res Rural Dev.* 16(6).
- Fadlemoula, A., Dughyam, A.M., Mohame, d.G.E., AlDeib, M.K. and AlZubaidy, A.J. (2009). Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. *BJVM.* 12: 199-206.
- Falconer, D.S. and Mackay, T.D. (1996). Introduction to Quantitative Genetics. 4th ed., Addison Wesley Longman Limited, Essex, U.K.
- Fink, T., Tiplady, K., Lopdell, T., Johnson, T., Snell, R.G., Spelman, R.J., Davis, S.R. and Littlejohn, M.D. (2017). Functional confirmation of PLAG1 as the candidate causative gene underlying major pleiotropic effects on body weight and milk characteristics. *Sci. Rep.* 7, p.44793.
- Flury, C., Boschung, C., Denzler, M., Bapst, B., Schnyder, U., Gredler, B. and Signer-Hasler, H. (2014). Genome-wide association study for 13 udder traits from linear type

- classification in cattle. Proceedings, 10th World Congress of Genetics Applied to Livestock Production.
- Fortes, M., Reverter, A., Nagaraj, S., Zhang, Y., Jonsson, N., Barris, W., Lehnert, S., Boe Hansen, G. and Hawken, R. (2011). A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle. *J. Animal Sci.* 89: 1669-1683.
- Foster, W.W., Freeman, A.E., Berger, P.J. and Kuck, A. (1989). Association of type traits scored linearly with production and herd life of holsteins. *J. Dairy Sci.* 72: 2651–2664.
- Freeman, A.E. (1976). Management traits in dairy cattle, dystocia, and udder characteristics related to production, and a review of other traits. *Livest. Prod. Sci.* 3: 13–26.
- Frisch, J.E. (1982). The use of teat-size measurements or calf weaning weight as an aid to selection against teat defects in cattle. *Anim. Prod.* 32: 127-133.
- Fuerst-Waltl, B., Sölkner, J., Essl, A., Hoeschele, I. and Fuerst, C. (1998). Non linearity in the genetic relationship between milk yield and type traits in Holstein cattle. *Livest. Prod. Sci.* 57: 41–47.
- 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.
- Gao, Y., Dong, L., Wei, M., Aimin, L., Xianyong, L., Zhang, C., Lei, 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: e53–e59.
- Garg, D.N. (2001). Emergence of bovine mycoplasmal mastitis in India. Round table conference on mastitis (IAAVR) . No.2:1-9.
- Ghosh, B. and Prasad, J. (1998). Milk yield and composition as influenced by udder measurements, in Jersey × Red Sindhi crosses. *Indian J. Anim. Prod. Mgmt.* 14(1): 23-25.
- Grohn, Y.T., Wilson, D.J., Gonzalez, R.N., Hertl, J.A., Schulte, H., Bennett, G. and Schukken, Y.H. (2004). Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 87: 3358 -3374.

- Guarin, J.F. and Ruegg, P. (2016). Pre and postmilking anatomical characteristics of teats and their associations with risk of clinical mastitis in dairy cows. *J. Dairy Sci.* 99: 1–7.
- Gubbawar, S.G., Shelke, R.R., Chavan, S.D. and Pohare, S.R. (2012). Phenotypic characteristics of Gaolao Strain of Nagpuri Buffalo Breed. *Asian J. Animal Sci.* 7(1): 6-14.
- Gupta, R., Singh, R.P. and Tomar, S.S. (1991). Udder and teat measurement and their association with milk production in Karan-Fries cows. *Indian J. Anim. Res.* 25(1): 23-28.
- 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 Likelihood Computer Program. PC-2 version. The Ohio State University, Columbus, USA.
- Haussler, M.R., Whitfield, G.K., Haussler, C.A., Hsieh, J.C., Thompson, P.D., Selznick, S.H., Dominguez, C.E. and Jurutka, P.W. (1998). The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone Miner. Res.* 13: 325–349.
- Hewitt, D. (2001). International Committee for Animal Recording; A Report on ICAR Conformation Working Group. Section 5-1.
- Heyen, D.W., Weller, J.I., Ron, M., Band, M., Beever, J.E., Feldmesser, E., Da, Y., Wiggans, G.R., VanRaden, P.M. and Lewin, H.A. (1999). A genome scan for QTL influencing milk production and health traits in dairy cattle. *Physiol. Genomics.* 1: 165–175.
- Hickman, C.G. (1964). Teat shape and size in relation to production characteristics and mastitis in dairy cattle. *J. Dairy Sci.* 47: 777–782.
- Holstein Association of USA. (2016). Available at [http:// www.holsteinusa.com/ genetic_ evaluations /ss_linear.html](http://www.holsteinusa.com/genetic_evaluations/ss_linear.html).
- Hortet, P. and Seegers, H. (1998). Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. *Prev Vet Med.* 37: 1–20.
- Humphreys, R.C., Bieri, B., Zhao, L., Raz, R., Levy, D. and Hennighausen, L. (2002). Deletion of Stat3 blocks mammary gland involution and extends functional

- competence of the secretory epithelium in the absence of lactogenic stimuli. *ENDOAO*. 143: 3641–3650.
- Hussain, R., Khan, A., Tariq, J. M. and Rizvi, F. (2012). Possible Factors Associated with Mastitis in Indigenous Cattle in Punjab, Pakistan. *Pakistan Vet. J.* 32(4): 605-608.
- Hynes, N.E. and Watson, C.J. (2010). Mammary gland growth factors: Roles in normal development and in cancer. Cold Spring Harb. Perspect. *Biol.* 2: a003186.
- ICAR (2001). Conformation Recording of Dairy Cattle. Available at: www.icar.org/Documents/.../Guidelines/...../Section/205.0-5.10.pdf. [Accessed 2 Dec. 2016].
- ICAR (2012). ICAR guidelines for recording, evaluation and genetic improvement of health traits. Available at: <http://www.icar.org/Documents/Rules%20and%20regulations/Amendments%202012/Recording,%20Evaluation%20and%20Genetic%20Improvement%20of%20health%20traits.pdf> [Retrieved 10 December 2017]
- Japheth, K.P., Mehla, R.K., Imtiwati 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.
- Javed, K., Afzal, M., Ahmad, M. and Hussain, R. Genetics studies on Cholistani cows: I. Productive traits. (2001). *The J Ani Pl Sci.* 11(2): 48-52.
- 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. *JBINO*. 3(3): 117-123.
- Juozaityene, V., Juozaitis, A. and Micikeviciene, R. (2006). Relationship between somatic cell count and milk production or morphological traits of udder in Black-and-White cows. *Turk J Vet Anim Sci.* 30: 47–51.
- Juozaityene, V., Juozaitis, A., Zymantiene, J., Oberauskas, V., Aniulienė, 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.
- Kadarmideen, H.N. (2004). Genetic correlations among body condition score, somatic cell score, milk production, fertility and conformation traits in dairy cows. *Anim. Sci.* 79: 191–201.

- 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. *Int. J. Livest. Res.* 7(7): 190-199.
- Kamboj, M.L., Singh, A. and Shiv Prasad. (2007). Effect of udder and teat shapes and their measurements on somatic cell counts in milk of Karan Fries cows. *Res. J. Animal Hus. & Dairy Sci.* 60(6): 435-440.
- Kasap, A., Skorput, D., Kompan, D., Gorjanc, G., Mioč, B. and Potočnik, K. (2014). The effect of time elapsed from milking to scoring on udder linear scores and estimation of genetic parameters. *Vet. arhiv.* 84 (1): 9-18.
- Kavitha, K., Lakshmi, K., Rajesh, K., Suresh Satheesh, K. and Syama Sundar, N. (2009). Buffalo mastitis- risk factors. *Buffalo Bull.* 28: 134-137.
- 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.
- Khate, K. and Yadav, B.R. (2010). Incidence of mastitis in Sahiwal cattle and Murrah buffaloes of a closed organized herd. *Indian J Anim Sci.* 80 (5): 467–69.
- Khatib, H.W., Huang, D., Mikheil, V., Schutzkus and Monson, R.L. (2009). Effects of signal transducer and activator of transcription (STAT) genes STAT1 and STAT3 genotypic combinations on fertilization and embryonic survival rates in Holstein cattle. *J. Dairy Sci.* 92(12): 6186–6191.
- Khatri, S.B., Trivedi, M.M., Patel, Y.G. and Rajpura, R.M. (2017). Udder and teat measurements and their relation with milk production. *Int J Adv Biotechnol Res.* 7(3): 582-584.
- 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 Sci.* 72: 296–302.
- Klemke, K., Muller, M.H., Wosniok, W., Markowski, D.N., Nimzyk, R. and Maria Helmke, B.M., et al. (2014). Correlated expression of HMGA2 and PLAG1 in thyroid tumors, uterine leiomyomas and experimental models. *PLoS One.* 9: 88126.
- Kocak, O. (2006). Influence of mastitis on milk yield in Holstein cows. *Acta Vet. Brno.* 75: 507-513.

- Kramer, C.Y. (1957). Extension of multiple range tests to group correlated adjusted means. *Biometrics*. 13(1): 13-18.
- Krastanov, J. (1995). Study for opportunities for increasing the efficiency of selection in the main dairy cattle breeds in Bulgaria, Ph.D. Thesis, Institute of Agriculture, Stara Zagora, Bulgaria. 154 Pp.
- Kshatriya, P.S., Trivedi, M.M. and Dhama, A.J. (2009). Association of udder biometry and skin thickness with milk yield in Kankrej and Crossbred cows. *Indian J. Field Vet.* 5(1): 11-13.
- Kuczaj, M. (2003). Analysis of changes in udder size of high-yielding cows in subsequent lactations with regard to mastitis. *Elect. J. Polish Agricult. Univ.* 6(1): <http://www.ejpau.media.pl/volume6/issue1/animal/art-02.html>
- Kuhn, C., Bennewitz, J., Reinsch, N., Xu, N., Thomsen, H., Looft, C., Brockmann, G.A., Schwerin, M., Weimann, C., Hiendleder, S., Erhardt, G., Medjugorac, I., Forster, M., Brenig, B., Reinhardt, F., Reents, R., Russ, I., Averdunk, G., Blumel, J. and Kalm, E. (2003). Quantitative trait loci mapping of functional traits in the German Holstein cattle population. *J. Dairy Sci.* 86: 360–368.
- Kumar, G.S.N., Apannavar, M.M., Surnagi, M.D. and Kotresh, A.M. (2010). Study on incidence and economics of clinical mastitis. *Karnataka Journal of Agricultural Science*. 23(2): 407-408.
- Kumar, M., Singh, S., Kumar, D., Sharma, M., Pandey, R.K. and Rai, D.C. (2016). Impact of mastitis on milk production and composition in lactating cows at the dairy farm. *Res. Environ. Life Sci.* 9(9): 1117-1118.
- Lande, M.H. (2018). Relationship of size and shape of udder and teats with milk yield in red kandhari cattle of nanded district (Doctoral dissertation, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani).
- Larroque, H. and Ducrocq, V. (2001). Relationship between Type and Longevity in the Holstein Breed. *Genet. Sel. Evol.* 33: 39-59.
- Lavania, P., Khadda, B.S. and Pathodiya, O.P. (2011). Studies on Udder Measurement Traits in Surti Buffaloes. *Journal of Progressive Agriculture*. 2(1): 70- 72.
- Lee, Y.S. and Dutta, A. (2007). The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev.* 21: 1025-103.

- Lopez-Benavides, M.G., Williamson, J.H., Cursons, R.T., Lacy-Hulbert, S.J. and Woolford, M.W. (2005). *Streptococcus uberis* population dynamics in the New Zealand pastoral dairy farm. In: Hogeveen H, editor. Proceedings of the 4th IDF International Mastitis Conference. Maastricht: Wageningen Academic Publishers. pp. 649–655.
- Lund, M.S., Guldbrandtsen, B., Buitenhuis, A.J., Thomsen, B. and Bendixen, C. (2008). Detection of quantitative trait loci in Danish Holstein cattle affecting clinical mastitis, somatic cell score, udder conformation traits, and assessment of associated effects on milk yield. *J. Dairy Sci.* 91: 4028–4036.
- MacNeil, M.D. and Mott, T.B. (2006). Genetic analysis of gain from birth to weaning, milk production, and udder conformation in Line 1 Hereford cattle. *J. Anim. Sci.* 84: 1639–1645.
- Marinov, I., Penev, T. and Gergovska, Z. (2015). Factors Affecting Linear Type Traits in Black-and-White Cows. *Int.J.Curr.Microbiol.App.Sci.* 4(10): 374-383.
- Mayer, D.G., Davison, T.M., McGowan, M.R., Young, B.A., Matschoss, A.L., Hall, A.B., Goodwin, P.J., Jonsson, N.N. and Gaughan, J.B. (1999). Extent and economic effect of heat loads on dairy cattle production in Australia. *Aust. Vet. J.* 77(12): 804-808.
- Mazza, S., Sartori, C., Berry, D. and Mantovani, R. (2013). Factors affecting linear type traits of Valdostana cattle. *Agric. conspec. sci.* 78(3): 207 211.
- Mein, G.A., Neijenhuis, F., Morgan, W.F., Reinemann, D.J., Hillerton, J.E., Baines, J.R., Ohnstad, I., Rasmussen, M.D., Timms, L., Britt, J.S., Farnsworth, R., Cook, N. and Hemling, T. 2001. Evaluation of bovine teat condition in commercial dairy herds: 1. Non-infectious factors. ‘Teat Club International’. AABP-NMC International Symposium on Mastitis and Milk Quality in Vancouver, BC, Canada.
- Mein, G.A., Reinemann, D.J., Schuring, N. and Ohnstad, I. (2004). Milking machines and mastitis risk: A storm in a teat-cup. Proceedings of the 43rd Annual Meeting of the National Mastitis Council, USA.
- Mingoas, K.J., Awah Ndukum, J., Dakyang, H. and Zoli, P.A. (2017). Effects of body conformation and udder morphology on milk yield of zebu cows in North region of Cameroon. *Vet. World.* 10(8): 901-905.

- Misztal, I., Lawlor, T.J., Short, T.H. and Van Raden, 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. *Int J Sci Environ Technol.* 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: 493–501.
- Nakov, D., Hristov, S. and Andonov, S., et al. (2014). Udder-related risk factors for clinical mastitis in dairy cows. *Veterinarski archive.* 84: 2, 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.
- Nelson, C.D., Reinhardt, T.A., Lippolis, J.D., Sacco, R.E. and Nonnecke, B.J. (2012). Vitamin D signaling in the bovine immune system: A model for understanding human vitamin D requirements. *Nutrients.* 4: 181–196.
- Nelson, C.D., Reinhardt, T.A., Thacker T.C., Beitz, D.C. and Lippolis, J.D. (2010b). Modulation of the bovine innate immune response by production of 1 α ,25-dihydroxyvitamin D(3) in bovine monocytes. *J. Dairy. Sci.* 93:1 041–1049.
- 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., Mohammad, W.A. and Wright, J.R. (1983). Effect of Herd and Sire on Uniform Type Trait Appraisal Scores for Ayrshires, Guernseys, Jerseys and Milking Shorthorns. *J. Dairy Sci.* 66: 2173.
- Norman, H.D., Powell, R.L., Wright, J.R. and Cassell, B.G. (1988). Phenotypic and genetic relationship between linear functional type traits and milk yield for five breeds. *J. Dairy Sci.* 71: 1880-1896.
- Ogorevc, J., Kunej, T. A., Razpet 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., Filho, L.C.N., Bronkhorst, D.E., Borges, M.H.F., Junior, F.A.B., Diniz, M.D.S., de Santana, E.H.W. and da Silva, C.B. (2016). Relationship between Shape of Teat and Teat Tip and Somatic Cell Count (SCC) in Dairy Cows. *Acta Sci Vet.* 43: 1276.
- Orban, M., Gulyas, L., Nemeth, S. and Gergacz, Z. (2009). Morphometric evaluation of udders in jersey cows. *Sci. Pap. Anim. Sci. Biotechnol.* 42(2): 327–332.
- Pander, B.L. and Chopra, S.C. (1989). Effect of Udder and Teat Conformation on Milk Flow Rate and Milk Production in Crossbred Dairy Cattle. *Dairy Sci. Abstr.* 51(11): 4602.
- Patel, Y.G., Trivedi, M.M., Rajapura, 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. Environ. Technol.* 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(1), p.14.
- Pawar, H.N., Ravi Kumar, G.V.P.P.S. and Narang, R. (2012). Effect of Year, Season and Parity on Milk Production Traits in Murrah Buffaloes. *J Buffalo Sci.* 1: 122-125.
- Payne, W.J.A. and Wilson, T.R. (1999). An introduction to animal husbandry in the tropics. 5th ed. Blackwell Publishing Ltd, Iowa State University Press, U.S. pp 826.
- Pegolo, S., Dadousis, C., Mach, N., Ramayo-Caldas, Y., Mele, M., Conte, G., Schiavon, S., Bittante, G. and Cecchinato, A. (2017). SNP co-association and network analyses identify E2F3, KDM5A and BACH2 as key regulators of the bovine milk fatty acid profile. *Sci Rep.* 7(1), p.17317.

- Perez-Cabal, M.A. and Alenda, R. (2002). Genetic relationship between life time profit and type traits in Spanish Holstein cows. *J. Dairy Sci.* 85(12): 3480-3491.
- Petkov, P. and Stoyanova, R. (2006). Study of some linear type traits in relation with cow age and period of lactation. *Anim. Sci.* XLIII (1): 22-25.
- Pfannkuche, K., Summer, H., Li, O., Hescheler, J. and Droge, P. (2009). The High Mobility Group Protein HMGA2: A Co-regulator of chromatin structure and pluripotency in stem cells. *Stem Cell Rev Rep.* 5: 224-230.
- 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.
- Prasad, R.M.V., Sudhakar, K., Raghava Rao, E., Ramesh Gupta, B. and Mahender, M. (2010a). Studies on the udder and teat morphology and their relationship with milk yield in Murrah buffaloes. *Livestock Res. Rural Dev.* 22(1): 101-108.
- Prasad, R.M.V., Raghava Rao, E., Sudhakar, K., Ramesh Gupta, B. and Mahender, M. (2010b). Studies on udder and teat measurements as affected by parity and their relationship with milk yield in Murrah buffaloes. *Buffalo Bull.* 29(3): 194-198.
- Radostits, O.M., Gay, C.C., Blood, D.C. and Hinchkliff, K.W. (2000). A Text Book of Veterinary Medicine. 9th Ed. W.B. Saunders, New York.
- Rajala-Schultz, P.J., Grohn, Y.T., McCulloch, C.E. and Guard, C.L. (1999). Effects of clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 82(6): 1213-1220.
- 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.
- Rasby, R. (2011). A Guide to Udder and Teat Scoring Beef Cows, Institute of Agriculture and Natural Resources. Available at: https://beef.unl.edu/learning/udder_score.shtml [Accessed 30 Nov. 2016].
- Ray, D.E., Halbach, T.J. and Armstrong, D.V. (1992). Season and lactation number effects on milk production and reproduction of dairy cattle in Arizona. *J. Dairy Sci.* Volume 75, Issue 11. 2976-2983.
- Riley, D.G., Chase, Jr.C.C., Olson, T.A., Coleman, S.W. and Hammond, A.C. (2004). Genetic and nongenetic influences on vigor at birth and preweaning mortality of purebred and high percentage Brahman calves. *J Anim Sci.* 82(6): 1581-1588.

- Rogers, G.W. and Hargrove, G.L. (1993). Absence of quadratic relationships between genetic evaluations for somatic cell scores and udder linear traits. *J. Dairy Sci.* 76: 3601–3606.
- Rogers, G.W., Hargrove, G., Lawlor, T.J. and Ebersole, J.L. (1991). Correlations among linear type traits and somatic cell counts. *J. Dairy Sci.* 74: 1087-1091.
- 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: 2198–2204.
- Saatchi, M., Schnabel, R.D., Taylor, J.F. and Garrick, D.J. (2014). Large-effect pleiotropic or closely linked QTL segregate within and across ten US cattle breeds. *BMC Genet.* 15:442.
- Sahu Bhooshan, K., Mishra, S. and Dubey, A. (2016). Appraisal of linear type traits in graded Murrah buffaloes. *Buffalo Bull.* 35(2): 291- 298.
- Saini, A.L. and Gill, R.S. (1988). Proceedings of II world buffalo congress on milk production in relation to variation in size and shape of udder and teats in Murrah buffaloes. India. 12-16: pp. 70-75.
- Saiyad, I.H. and Patel, J.M. (1989). Biometrics of udder in relation to milk production in Jersey × Kankrej crossbreds. *Dairy. Sci. Abstr.* 57(11): 7104.
- Sambrook, J. and Russell, D. (2001). Molecular cloning: A laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York.
- SAS Institute Inc. (2011) SAS® 9.3 System Options: Reference. 2nd ed. SAS Institute Inc., Cary, NC.
- Schrooten, C., Bovenhuis, H., Coppieters, W. and Van Arendonk, J.A.M. (2000). Whole genome scan to detect quantitative trait loci for conformation and functional traits in dairy cattle. *J. Dairy Sci.* 83: 795–806.
- Schrooten, C., Bink, M.C. and Bovenhuis, H. (2004). Whole genome scan to detect chromosomal regions affecting multiple traits in dairy cattle. *J. Dairy Sci.* 87: 3550–3560.
- Schutz, M.M., VanRaden, P.M., Boettcher, P.J. and Hansen, L.B. (1993). Relationship of somatic cell score and linear type trait evaluations of Holstein sires. *J. Dairy Sci.* 76: 658–663.

- Sekerden, O. and Erden, H. 1993. The relationship between some udder conformation traits and milk yield in Jersey cows. *Anim. Breed. Abstr.* 61(7): 437.
- Sewalem, A., Kistemaker, G.I., Miglior, F. and Van Doormal, B.J. (2004). Analysis for the relationship between survival in Canadian Holsteins using a weibull proportional hazards model. *J. Dairy Sci.* 87: 3938-3946.
- Seykora, A.J. and McDaniel, B.T. (1986). Genetics statistics and relationships of teat and udder traits, somatic-cell counts, and milk-production. *J. Dairy Sci.* 69, 2395–2407.
- Sezenler, A., Ceyhan, A., Yuksel, M.A., Taner, O.A. and Yildirim, M. (2016). Effect of parity and type of lambing on performance and udder traits of Bandirma ewes. *Indian J Anim Sci.* 86 (5): 572–577.
- Shahmohammadi, A., Rezapour, A.K. and Zakeri, A. (2014). Effect of vitamin D supplementation in treatment of naturally occurring mastitis in cattle. *Eur. J. Exp. Biol.* 4(2): 132–135.
- Sharma, N. and Maiti, S.K. (2010). Incidence, etiology and antibiogram of subclinical mastitis in cows in durg, Chhattisgarh. *The Indian Journal of Veterinary Research.* 19: 45-54.
- Short, T.H. and Lawlor, T.J. (1992). Genetic-parameters of conformation traits, milk-yield, and herd life in Holsteins. *J. Dairy Sci.* 75, 1987–1998.
- Sharma, N., Singh, N.K. and Bhadwal, M.S. (2011). Relationship of somatic cell count and mastitis: An overview. *Asian-Australas J Anim Sci.* 24: 429–438.
- Short, T.H., Lawlor, Jr.T.J. and Lee, K.L. (1991). Genetic parameters for three experimental linear type traits. *J. Dairy Sci.* 74:2020–2025.
- Sid Ahmed, I.A. and El Barbary, A.S.A. (2000). Dairy and meat cattle. College of Agric. Un. of Alexandria, Egypt, Dar El-Maarif Printing Press. Pp: 73-99.
- Singh, A. (2005). Factor affecting incidence of mastitis in crossbred cows. M.V.Sc.Thesis, ICAR-National Dairy Research Institute, Karnal, India.
- Singh, A. and Kumar, A. (2007). Prediction of 305-day milk yield based on peak yield and Pre-peak period in karan fries cows. *Indian J. Anim. Res.* 41 (4) : 299-301.
- 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, M., Raju, S., Pundir, J.K., Chander, R.K., Tomar, P.S. and Ludhri, R.S. (2001). Effect of parity, stage of lactation and season on incidence of mastitis in cattle and buffaloes. *Inter. J. Anim. Sci.* 16 (2): 227-233.
- Singh, R.R., Dutt, T., Singh, M. and Kumar, M. (2010). Association of udder and teat dimensions with production traits in Vrindavani cattle. *Indian J. Dairy Sci.* 63(6): 455-458.
- Singh, R.S., Bansal, B.K. and Gupta, D.K. (2014). Udder health in relation to udder and teat morphometry in Holstein Friesian X Sahiwal crossbred dairy cows. *Trop. Anim. Health Prod.* 46: 93–98.
- Singh, S.K., Arya, H.P.S. and Pandey, H.S. (1999). Type of udder and teat shape in cross bred cows and their association with milk ability. *Indian J Anim Sci.* 33: 70 –72.
- Singh, S.K., Pandey, H.S., Suman, C.L. and Saxena, M.M. (1997). Milkability and milk flow rate in relation to udder and teat shapes of crossbred cows. *Indian J. Anim. Prod. Mgmt.* 13(1):13-18.
- 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.
- Slettбакк, T., Jorstad, A., Farver, T.B. and Holmes, J.C. (1995). Impact of milking characteristics and morphology of udder and teats on clinical mastitis in first and second lactation in Norwegian cattle. *Prev. Vet. Med.* 24: 235–244.
- Snedecor, G.W. and Cochran, W.G. (1994). Statistical methods (7th ed). Iowa State Uni,
- Solkner, J. and Petschina, R. (1999). Relationship between type trait and longevity in Austrian Simmental cattle. Proc. International Workshop on EU Concerted Action. Genetic Improvement of Functional Traits in Cattle; Longevity. Jouy-en-Josas, France. INETRBULL. 21: 91-95.
- 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.
- Sonwane, R.B. (2015). Morphometric characterization of Marathwadi Buffalo its breeding Tract. Thesis submitted to M.V.Sc. (LPM) Degree to M.A.F.S.U., Nagpur (M.S.).

- Snedecor, G.W. and Cochran, W.G. 1(967). Statistical Methods (7th ed). Iowa State Uni, Press, Iowa.
- Sørensen, M.K., Jensen, J. and Christensen, L.G. (2000). Udder conformation and mastitis resistance in Danish first lactation cows: heritabilities, genetic and environmental correlations. *Acta Agric. Scand. A Anim. Sci.* 50(2): 72-82.
- Sori, H., Zerihum, A. and Abdicho, S. (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. *Int. J. Appl. Res. Vet. Med.* 3: 332-338.
- Stamschror, J. (2000). Judging Dairy Cattle - USDA. University of Minnesota pp. 1-8.
- Steenefeld, W., Hogeveen, H., Barkema, H.W., van den Broek, J. and Huirne, R.B.M. (2008). The Influence of cow factors on the incidence of clinical mastitis in dairy cows. *J. Dairy Sci.* 91: 1391-1402.
- Strillacci, M.G., Frigo, E., Schiavini, F., Samoré, 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. *IJVA.* 25(2): 101-115.
- Tayade, U.D., Zinjarde, R.M., Rokde, S.N. and Ingole, A.S. (2010). Phenotypic characteristics of Gaolao Strain of Nagpuri buffaloes in Wardha district of Maharashtra. *Indian J. Animal sci.* 80(1): 67-68.
- The NCBI handbook. (2002). Chapter 18. The Reference Sequence (RefSeq) Project. Bethesda (MD): National Library of Medicine, National Center for Biotechnology Information. <http://www.ncbi.nlm.gov/books/NBK21091/>[Accessed 2 September 2015].
- Thompson, J.R., Freeman, A.E., Wilson, D.J., Chapin, C.A., Berger, P.A. and Kuck, A. (1981). Evaluation of a Linear Type Program in Holsteins. *J. Dairy Sci.*, 64: 1610-1617.
- Tizard, I. R. (2012). Veterinary Immunology. 9th ed. Saunders Elsevier. St. Louis, MO.
- Tolleson, M.W. (2016). Genome wide association for udder composition traits in Bos Indicus– Bos Taurus cows. M. Sc. Thesis. Texas A&M University.

- Trajcev, M. and Nakov, D. (2010). Distribution of abnormal secretion and subclinical mastitis among the udder quarters in dairy cows. *Yearbook of the faculty of agricultural science and food*. 55: 129–138.
- Trimberger, G.W., Etgen, W.M. and Gelton, D.M. (1985). *Dairy Cattle Judging Techniques*, 4th Edn., pp. 135-140.
- Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Res.* 40(15), pp.e115-e115.
- 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: 695–701.
- 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.
- Van Vleck, L.D. and Norman, H.D. (1972). Association of type traits with reasons for disposal. *J. Dairy Sci.* 55: 1698–1705.
- VanRadeq, P.M., Jensen, E.L., Lawlor, T.L. and Funk, D.A. (1990). Prediction of transmitting abilities for Holstein type traits. *J. Dairy Sci.* 73: 191.
- Voelker, D.E. (1981). Dairy herd improvement associations. *J. Dairy sci.* 64: 1269-1277.
- Vollema, A.R., Van der Beek, S., Harbers, A.G.F. and DeJong, 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 Holdeo crossbred cows. *Karnataka. J. Agric. Sci.* 13(3): 802-804.
- Wall, E., White, I.M.S., Coffey, M.P. and Brotherstone, S. (2005). The Relationship between fertility, rump angle and selected type information in Holstein Friesian cows. *J. Dairy Sci.* 88: 1521-1528.
- Wallenberg, G.J., Vanderpoel, H.M. and Vanior, J.T. (2002). Viral infection and bovine mastitis. *Vet. Microbiol.* 88: 27–45.
- Watson, C.J. (2006). Involution: Apoptosis and tissue remodeling that convert the mammary gland from milk factory to a quiescent organ. *Breast Cancer Res.* 8:203.

- West, J.W. (2003). Effects of heat-stress on production in dairy cattle. *J. Dairy Sci.* 86(6): 2131-2144.
- WHFF (2005). International type evaluation of dairy cattle. Available at: http://whff.info/documentation/documents/typetraits/type_en_2005-2.pdf [Accessed 29 Nov. 2016].
- Wiggans, G.R. (1991). National genetic improvement program for dairy cattle in the United States. *J. Anim. Sci.* 69: 3853-3860.
- Wilson, D.J., Gonzalez, R.N., Hertl, J.A., Schulte, H., Bennett, G.J., Schukken, Y.H. and Grohn, Y.T. (2004). Effect of clinical mastitis on the lactation curve: A mixed model estimation using daily milk weights. *J. Dairy Sci.* 8: 2073–2084.
- Wondifraw, Z., Thombre, B.M. and Bainwad, D.V. (2013). Effect of non-genetic factors on milk production of Holstein Friesian × Deoni crossbred cows. *Afr. J. Dairy Farming Milk Prod.* 1(4): 079-084.
- Wu, X., Fang, M., Liu, L., Wang, S., Liu, J., Ding, X., Zhang, S., Zhang, Q., Zhang, Y., Qiao, L. and Lund, M.S. (2013). Genome wide association studies for body conformation traits in the Chinese Holstein cattle population. *BMC genomics*, 14(1), p.897.
- Wu, X., Lund, M.S., Sahana, 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), p.50.
- Yeh, F.C., Yang, R. and Boyle, T. (1999). POPGENE. Version 1.31. Quick User Guide Univ. Alberta and Centre Int. Forestry Res.
- Yosbanis, E., Jose, C., Pastor, P. and Luis, N. (2013). Relationship between udder morphology, production and composition of buffalo milk. *Rev. electrón. vet.* 23(3): 220-225.
- Young, C.W., Legates, J.E. and Lecce, J.G. (1960). Genetic and phenotypic relationships between clinical mastitis, laboratory criteria and udder height. *J. Dairy Sci.* 43: 54–62.
- Zhang, R.F., Chen, H., Lei, C.Z., Fang, X.T., Zhang, Y.D., Hu, S.R. and Su, L.H. (2007). Association between PCR-RFLP polymorphisms of five gene loci and milk traits in Chinese Holstein. *Asian-Aust. J. Anim. Sci.* 20(2): 166 – 171.

- Zwald, N.R., Weigel, K.A., Chang, Y.M., Welper, R.D. and Clay, J.S. (2006). Genetic analysis of clinical mastitis data from on farm management software using threshold models. *J. Dairy Sci.* 89: 330–336.
- Zwertvaegher, I., Van Weyenberg, S., Piepers, S., Baert, J. and De Vlieghe, S. (2012). Variance components of teat dimensions in dairy cows and associated factors. *J. Dairy Sci.* 95: 4978-4988.

Web resources -

NEB cutter V2.0. URL - <http://www.labtools.us/nebcutter-v2-0/>

Primer3. URL- <https://primer3plus.com/cgi-bin/dev/primer3plus.cgi>

NCBI BLAST. URL - <https://blast.ncbi.nlm.nih.gov/Blast.cgi>

Ensemble genome browser. URL - <https://asia.ensembl.org/index.html>

Database of SNP (dbSNP). URL - <https://www.ncbi.nlm.nih.gov/projects/SNP/>

Appendix

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
Formaldehyde	:	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	:	Schimidzu 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		
<i>BsrI</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>BamHI</i> restriction enzyme (20 U/μl)	:	NEB Inc.
<i>Hinp1I</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>TaqI</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>HaeIII</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>HinfI</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>HpaII</i> restriction enzyme (10 U/μl)	:	NEB Inc.
<i>Sau96I</i> restriction enzyme (10 U/μl)	:	NEB Inc.
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

Appendix- II

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 water and 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 °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 °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 °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 °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 everytime)

NH ₄ Cl (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 4⁰C

RBC Lysis buffer (1X prepared freshly everytime)

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 ⁰C was liquefied by keeping in a water bath maintained at 65 ⁰C 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 ⁰C).

Proteinase-K

Proteinase-k (20 mg) was dissolved in 1ml of double distilled water. Aliquots of 400µl per vial made and stored at -20 ⁰C.

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 ₂ HPO ₄	:	0.7 gm
KH ₂ PO 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 0.6 %**

Agarose	:	0.60 gm
TBE (10 X)	:	10 ml
Double distilled water:		90 ml

Agarose 1.8 %

Agarose	:	1.80 gm
TBE (10X)	:	10 ml
Double distilled water:		90 ml

Agarose 2.0 %

Agarose	:	2 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 °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.