EXOME AND PROTEOME PROFILING OF DEONI COWS WITH HIGH AND LOW REPRODUCTIVE PERFORMANCE

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. JOEL DEVADASAN M
M.V.Sc.

ANIMAL GENETICS AND BREEDING
SOUTHERN REGIONAL STATION
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(DEEMED UNIVERSITY)
BENGALURU-560030 (KARNATAKA), INDIA

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This is to certify that the thesis entitled, **"EXOME AND PROTEOME PROFILING OF DEONI COWS WITH HIGH AND LOW REPRODUCTIVE PERFORMANCE"** submitted by **Dr. JOEL DEVADASAN, M** in the partial fulfilment of the requirement for 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 him under my supervision, and guidance and no part of thesis has been submitted for any other degree or diploma.

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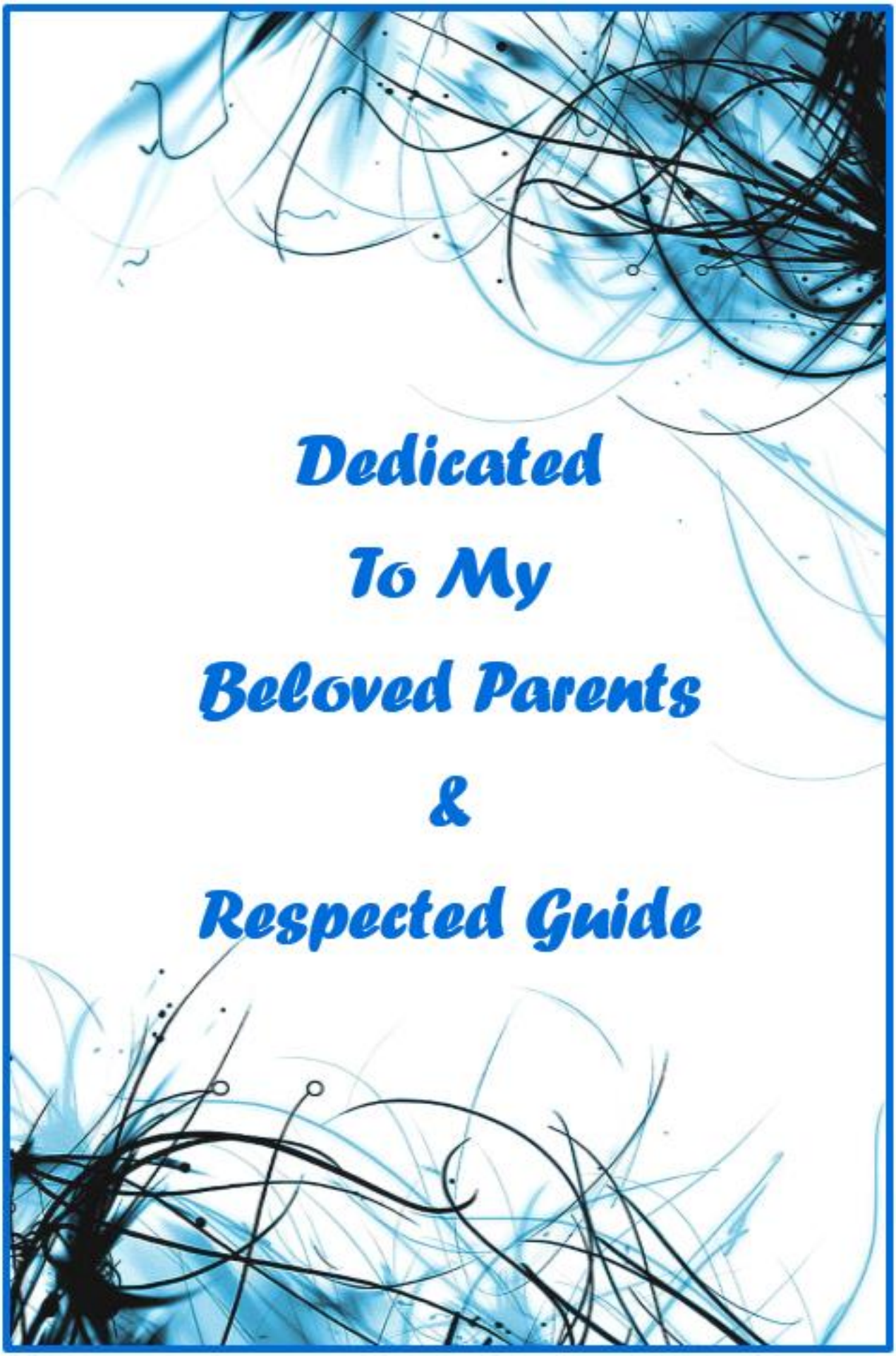
Dedicated
To My
Beloved Parents
&
Respected Guide

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M. Joel Devadasan

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CONTENTS

Chapter No.	TITLE		Page No.
1	**INTRODUCTION**		**1-2**
2	**REVIEW OF LITERATURE**		**3-24**
	2.1	Prologue	3
	2.2	Reproduction in dairy cattle	4
	2.3	Reproductive performance	4
	2.4	Challenges in reproduction traits	6
	2.5	Indicators of reproductive performance	6
	2.6	Economic loss due to poor reproductive performance	6
	2.7	Genetic markers	7
	2.8	Approaches to identifying genetic markers in complex traits	7
	2.9	Deoni cattle	9
	2.10	Whole exome sequencing	9
	2.11	Whole exome sequencing technology	10
	2.12	Applications of whole exome sequencing	11
	2.13	Exome sequencing studies in livestock	11
	2.14	Protein, proteome and proteomics	13
	2.15	Proteomics and its techniques	13
	2.16	Proteomics and animal reproduction	14
	2.17	Proteomic studies in animal reproduction	15
3	**MATERIALS AND METHODS**		**25-37**
	3.1	Location of study	25
	3.2	Experimental animals	25
	3.3	Sample preparation for whole exome sequencing	25
	3.3.1	Blood collection and sample preparation	25
	3.3.2	High salt DNA isolation	25
	3.3.3	DNA quality control	26

	3.3.4	Library preparation	28
	3.3.5	Illumina sequencing	28
	3.4	Whole exome sequencing data analysis	29
	3.5	Variant analysis	29
	3.6	Sample preparation for mass spectrometry proteomics analysis	31
	3.6.1	Plasma separation	31
	3.6.2	Protein extraction and digestion	31
	3.6.3	Strong cation exchange fractionation	31
	3.6.4	LC-MS/MS by data-independent acquisition (DIA) method	33
	3.6.5	DDA mode acquisition to generate spectral library	33
	3.6.6	Wide-window DIA of bovine plasma samples	33
	3.6.7	Gas phase fractionation (GPF) by narrow-window DIA	34
	3.7	Proteomics data analysis	34
	3.7.1	Building in-house plasma spectral library from DDA data	34
	3.7.2	Chromatogram library from GPF	35
	3.7.3	Spectral library search	35
	3.8	Statistical analysis	36
	3.9	Bioinformatics analysis	37
4	**RESULTS AND DISCUSSION**		**38-103**
	4.1	Selection of experimental animals	38
	4.2	Whole exome sequencing analysis	38
	4.2.1	Primary analysis	38
	4.2.2	Alignment and alignment postprocessing	39
	4.2.3	Variant calling, quality control of SNPs and annotation	41
	4.3	Gene Ontology of genes identified between high and low reproductive	44

		performance groups	
	4.4	Genes identified in high reproductive performance	44
	4.5	Genes identified in low reproductive performance	47
	4.6	Pathways identified in high and low reproductive performance groups	82
	4.6.1	Calcium signaling pathway	83
	4.6.2	Cholinergic synapse pathway	83
	4.6.3	GnRH signaling pathway	86
	4.7	Comparative plasma proteomics	88
	4.7.1	Comparative plasma proteomics of high and low reproductive performance in cyclical Deoni cows	88
	4.8	Gene Ontology analysis of altered plasma proteins between high and low reproductive performance cyclical groups	88
	4.9	Functional pathway analysis of altered plasma proteins between high and low reproductive performance cyclical groups	88
	4.10	Differentially regulated plasma proteins in relation to reproductive performance in cyclical groups	91
	4.11	Protein-protein interactions in cyclical Deoni cows	94
	4.12	Comparative plasma proteomics of high and low reproductive performance in pregnant Deoni cows	95
	4.13	Gene Ontology analysis of altered plasma proteins between high and low reproductive performance pregnant	95

		groups	
	4.14	Functional pathway analysis of altered plasma proteins between high and low reproductive performance pregnant groups	99
	4.15	Differentially regulated plasma proteins in relation to reproductive performance in pregnant groups	99
	4.16	Protein-protein interactions in pregnant Deoni cows	103
5	**SUMMARY AND CONCLUSIONS**		**104-107**
BIBLIOGRAPHY			**i-xxiv**

LIST OF TABLES

TABLE. No.	TITLE	Page No.
2.1	Genes associated with reproductive performance traits	8
2.2	Proteomic studies in farm animals	19
4.1	Reproductive performance of Deoni cows	39
4.2	Number of raw and processed reads in Deoni cows	40
4.3	Number of mapped and unique reads in Deoni cows	40
4.4	Number of SNPs and InDels identified at different filtration level	42
4.5	Chromosome-wise distribution of SNPs in Deoni cattle	42
4.6	List of genes identified in Deoni cows with high reproductive performance	49
4.7	List of Genes Identified in Deoni cows with low reproductive performance	64
4.8	List of pathways and genes identified using exome profiling in high and low reproductive performance groups	82
4.9	Differentially expressed proteins in the plasma proteome of high and low reproductive performance cyclical groups	90
4.10	Differentially expressed proteins in the plasma proteome of high and low reproductive performance pregnant groups	97

LIST OF FIGURES

Figure No	TITLE	Page No
3.1	Workflow of whole exome sequencing	27
3.2	Workflow of whole exome data analysis	30
3.3	Workflow of plasma proteome profiling and data analysis	32
4.1	Number of genes enriched in molecular functions in Deoni cows	45
4.2	Number of genes enriched in biological processes in Deoni cows	45
4.3	Calcium signaling pathway	84
4.4	Cholinergic synapse pathway	85
4.5	GnRH signaling pathway	87
4.6	Number of proteins enriched in molecular functions in cyclical Deoni cows	89
4.7	Number of proteins enriched in biological processes in cyclical Deoni cows	89
4.8	Volcano plot of differentially expressed proteins in cyclical Deoni cows	92
4.9	Protein-protein interaction in cyclical Deoni cows	94
4.10	Number of proteins enriched in molecular functions in pregnant Deoni cows	96
4.11	Number of proteins enriched in biological processes in pregnant Deoni cows	96
4.12	Volcano plot of differentially expressed proteins in pregnant Deoni cows	100
4.13	Protein-protein interaction in pregnant Deoni cows	103

LIST OF ABBREVIATIONS

AUC	:	Area under curve
ACN	:	Acetonitrile
APS	:	Ammonium per sulfate
BAM	:	Binary alignment format
BCA	:	Bicinchoninic acid
BCF	:	Binary calling format
bp	:	Base pair
CNV	:	Copy number variation
conc.	:	Concentration
db	:	Data base
DO	:	Days open
DNA	:	Deoxy ribonucleic acid
DDA	:	Data dependent acquisition
DIA	:	Data independent acquisition
DTT	:	Dithiothreitol
e.g.	:	As per example
EDTA	:	Ethylene diamine tetra acetic acid
et al.	:	Co-workers
GS	:	Genomic selection
GPF	:	Gas phase fractionation
HRP	:	High reproductive performance
IAA	:	Iodo acetamide
ICP	:	Inter-calving period
InDels	:	Insertion and deletion
iRT	:	Indexed retention time
LC	:	Liquid chromatography
LRP	:	Low reproductive performance
MAS	:	Marker assisted selection
mg/ml	:	Milligram per milliliter
ml	:	Milliliter
mM	:	Millimolar
MMT	:	Millionmetric tonnes

MQ	:	Milli-Q-water
MS	:	Mass spectrometry
ms	:	Minutes
MW	:	Molecular weight
m/z	:	Mass per charge
ng	:	Nanogram
nm	:	Nanometer
OD	:	Optical density
PAGE	:	Polyacrylamide gel electrophoresis
PCR	:	Polymerase chain reaction
QC	:	Quality control
QTL	:	Quantitative trait loci
RBC	:	Red blood corpuscles
RD	:	Read depth
RNA	:	Ribonucleic acid
RPM	:	Relative centrifugal force or G force
RT	:	Room temperature
SAM	:	Sequence alignment format
SCX	:	Strong cation exchange
SDS	:	Sodium do-decyl sulfate
SD	:	Standard deviation
Sec	:	Second
SNP	:	Single nucleotide polymorphism
TAE	:	Tris-acetate-EDTA
TE	:	Tris-EDTA
TEABC	:	Trimethyl ammonium bicarbonate
TFA	:	Trifluoro acetic acid
UTR	:	Untranslated region
VCF	:	Variant Calling Format
WBC	:	White Blood Corpuscles
WES	:	Whole exome sequencing
WGS	:	Whole genome sequencing
µg	:	Microgram
µg/L	:	Microgram per litre

µg/ml	:	Microgram per millilitre
µl	:	Microlitre
µM	:	Micro molar
%	:	Percentage
^{0}C	:	Degree centigrade / Celsius

ABSTRACT

Reproduction is an important factor for sustainable production in dairy cattle farming. Poor reproductive performance brings a detrimental effect on productivity and longevity in dairy cattle, thus improving the reproductive performance by all means is of paramount importance. Although all the reproduction processes were well defined and conserved, the genetic basis of controlling reproduction process is not fully understood, due to its complex nature. In the present study, we performed whole exome sequencing (WES) and comparative proteomics profiling in Deoni cows with differing reproductive performance to identify genes and protein related to reproductive performance. The Deoni cows of 6 to 9 years age were selected based on the days open and inter-calving period. In high reproductive performance (HRP) group cows mean days open and inter-calving period were 113±23.98 days and 398±24.85 days respectively. Likewise, in low reproductive performance (LRP) group cows mean days open and inter-calving period were 292±60.82 days and 576±58.90 days respectively. Using exome sequencing, we identified 312 and 301 SNPs in HRP and LRP cow groups respectively. A total of 221 genes from 312 SNPs in HRP cows group and 244 genes from 302 SNPs in LRP cows group were identified in the gene annotation. Gene Ontology revealed binding and catalytic activity in molecular functions while cellular, metabolic and biological processes in biological function were found to be enriched with genes identified in both groups. 12 different pathways enriched with genes involved in reproductive process were identified from the pathway analysis. In HRP cattle group ADAMTS19, TRPV4, HDGF, RANGAP1, ROR2, CNOT1, CELSR3, PTPRS, NFAM1, CTRC1, PLA2G4F, SHANK2, and SLIT3 while in LRP cattle group THBS4, SLC37A2, BRWD1, RABEP2, DGKI, ANKH, SCARF1, EPAS1, EIF4G3, and RABEP2 genes were found to be associated with reproduction related traits like calving ease, days open, implantation, calving interval and length of productivity. Using data independent acquisition (DIA) based LC-MS/MS, a total of 430 plasma proteins were identified in all biological replicate samples. In cyclical Deoni cows, a total of 20 proteins were differentially expressed ($P<0.05$) between high and low reproductive performance group; 15 upregulated while 5 downregulated. In pregnant Deoni cows, total of 35 proteins were differentially expressed ($P<0.05$) between high and low reproductive performance group; 23 upregulated while 12 downregulated. All these differentially expressed proteins were involved in oxidative stress control, autophagy, cell differentiation, cell migration, immune response, which are required for successful establishment of pregnancy. It is concluded that there is significant difference between Deoni cattle with differing reproductive performance at genome and proteome level. Further, our findings in this study contributes for understanding genetic basis of reproduction traits and aid in identifying probable candidate genes and proteins for enhancing the reproductive performance in dairy cattle.

सारांश

डेयरी पशुपालन में स्थायी उत्पादन के लिए प्रजनन एक महत्वपूर्ण कारक है। खराब प्रजनन प्रदर्शन डेयरी मवेशियों में उत्पादकता और दीर्घायु पर हानिकारक प्रभाव डालता है, इस प्रकार प्रजनन प्रदर्शन में हर तरह से सुधार सबसे महत्वपूर्ण है। यद्यपि सभी प्रजनन प्रक्रियाओं को अच्छी तरह से परिभाषित और संरक्षित किया गया था, प्रजनन प्रक्रिया को नियंत्रित करने के आनुवंशिक आधार को इसकी जटिल प्रकृति के कारण पूरी तरह से समझा नहीं गया है। वर्तमान अध्ययन में, हमने प्रजनन प्रदर्शन से संबंधित जीन और प्रोटीन की पहचान करने के लिए अलग–अलग प्रजनन प्रदर्शन के साथ देवनी गायों में संपूर्ण एक्सोम सीक्वेंसिंग (डब्ल्यूईएस) और तुलनात्मक प्रोटिओमिक्स प्रोफाइलिंग का प्रदर्शन किया। 6 से 9 वर्ष की आयु की देवनी गायों का चयन खुले दिनों और अंतर–ब्याने की अवधि के आधार पर किया गया था। उच्च प्रजनन प्रदर्शन (एचआरपी) समूह की गायों में औसत ओपन डे और इंटर ब्रीडिंग की अवधि क्रमशः 113 ± 23.98 दिन और 398 ± 24.85 दिन थी। इसी तरह, कम प्रजनन प्रदर्शन (एलआरपी) समूह में गायों का मतलब खुले दिन और अंतर–ब्याने की अवधि क्रमशः 292 ± 60.82 दिन और 576 ± 58.90 दिन थी। एक्सोम सीक्वेंसिंग का उपयोग करते हुए, हमने क्रमशः एचआरपी और एलआरपी गाय समूहों में 312 और 301 एसएनपी की पहचान की। एचआरपी गायों के समूह में 312 एसएनपी से कुल 221 जीन और एलआरपी गायों के समूह में 302 एसएनपी से 244 जीनों की जीन एनोटेशन में पहचान की गई थी। जीन ओन्टोलॉजी ने आणविक कार्यों में बाध्यकारी और उत्प्रेरक गतिविधि का खुलासा किया, जबकि जैविक कार्यों में सेलुलर, चयापचय और जैविक प्रक्रियाओं को दोनों समूहों में पहचाने गए जीन से समृद्ध पाया गया। मार्ग विश्लेषण से प्रजनन प्रक्रिया में शामिल जीनों से समृद्ध 12 विभिन्न मार्गों की पहचान की गई। उच्च प्रजनन प्रदर्शन (एचआरपी) गायो का समूह ADAMTS19, TRPV4, HDGF, RANGAP1, ROR2, CNOT1, CELSR3, PTPRS, NFAM1, CTRC1, PLA2G4F, SHANK2 और SLIT3 में जबकि कम प्रजनन प्रदर्शन (एलआरपी) गायो का समूह THBS4, SLC37A2, BRWD1, RABEP2, DGKI, ANKH, SCARF1, EPAS1, EIF4G3 और RABEP2 जीन प्रजनन संबंधी लक्षणों जैसे कि कालिवंग आयु , डे ओपन, इम्प्लांटेशन, कैल्विंग इंटरवल और उत्पादकता से जुड़े पाए गए। डेटा स्वतंत्र अधिग्रहण (डीआईए) आधारित एलसी–एमएस/एमएस का उपयोग करते हुए, सभी जैविक प्रतिकृति नमूनों में कुल 430 प्लाज्मा प्रोटीन की पहचान की गई थी। चक्रीय देवनी गायों में, उच्च और निम्न प्रजनन प्रदर्शन समूह के बीच कुल 20 प्रोटीन अलग–अलग व्यक्त किए गए (पी<0-05); 15 को अपग्रेड किया गया जबकि 5 को डाउनग्रेड किया गया। गर्भवती देवनी गायों में, उच्च और निम्न प्रजनन प्रदर्शन समूह के बीच कुल 35 प्रोटीन अलग–अलग व्यक्त किए गए (पी<0.05); 23 को अपग्रेड किया गया जबकि 12 को डाउनग्रेड किया गया। ये सभी विभेदित प्रोटीन ऑक्सीडेटिव तनाव नियंत्रण, स्वरभंग, कोशिका विभेदन, कोशिका प्रवास, प्रतिरक्षा प्रतिक्रिया में शामिल थे, जो गर्भावस्था की सफल स्थापना के लिए आवश्यक है। यह निष्कर्ष निकाला गया है कि जीनोम और प्रोटिओम स्तर पर अलग–अलग प्रजनन प्रदर्शन वाले देवनी गायो के बीच महत्वपूर्ण अंतर है। इसके अलावा, इस अध्ययन में हमारे निष्कर्ष प्रजनन लक्षणों के आनुवंशिक आधार को समझने में मदद करते हैं और डेयरी गायो में प्रजनन प्रदर्शन को बढ़ाने के लिए संभावित कैंडिडेट जीन और प्रोटीन की पहचान करने में सहायता करते हैं।

CHAPTER –1

Introduction

INTRODUCTION

India with 302.79 million bovines (about 17.5 % of the world) is the largest global milk producer with 209.9 MMT in 2020-21 and with the annual growth rate of 5.8% (Anonymous, 2020). Reproduction is very important for sustainable milk production in dairy cattle (Berglund, 2008; Sewalem *et al*., 2008). Since, low reproductive performance in dairy cattle has very detrimental effect on productivity, it results in economic losses due to an increase in the number of additional inseminations required, greater veterinary expenses, alter the lactations period, increase in inter-calving period, lesser number of calves, poor longevity and high involuntary culling rate with replacement costs (Boichard, 1990; Roxstrom and Strandberg, 2002). In addition to it, Indian dairy cattle have poor reproductive performance when compared to exotic cattle reproductive performance. Therefore, improving reproduction related traits is important for sustainable dairy cattle production.

Genetics, nutrition, and managemental practices all have significant effects on the improvement of reproductive traits. Genetics is considered as the fundamental requirement for improvement of reproductive performance in the livestock (Moore and Hasler, 2017). Overall genetic improvement of reproductive performance in cattle can be done by incorporating reproduction related traits into the selection programs (Berglund, 2008, Crowe *et al*., 2018; Ma *et al*., 2019). There are a number of selection techniques for improving reproductive performance in dairy cattle, including the conventional method, marker assisted selection (MAS), and genomic selection (GS). The most promising method is the genomic selection, which gives accurate results with a significant genetic gain when compared to other methods (Fathoni *et al*., 2022). However, reproduction related traits are complex in nature, low heritable, polygenic and influenced by environmental factors, due to this genomic selection became underpowered and found difficulty in improving reproduction traits in cattle (Berry *et al*., 2014; Ma *et al*., 2019).

Though the overall processes of reproduction which includes estrus, ovulation, fertilization, implantation of embryo, maintenance of gestation and calving are mostly found to be conserved and well defined, but the molecular and genetic basis behind each of these defined processes involved in the reproduction is much less known (Hufana-Duran and Duran, 2020; Shao *et al*., 2021). Hence, understanding the knowledge of molecular mechanism controlled by genes and its product in each specific reproduction process may improve the accuracy of genetic selection strategies and have larger impact on present and future genetic

gain for reproductive performance traits in cattle (Berglund, 2008; Berry *et al*., 2014; Shao *et al*., 2021).

In recent years, the development of high throughput platforms for structural (SNPs) and functional (RNA and protein) genome studies has made possibility for better understanding and identification of the genetic variation and its regulatory pathways affecting complex reproduction traits. Therefore, use of all omics resources and tools in the molecular genetics is crucial to identify genes and pathways having major effect on reproductive performance for the improvement of reproductive performance traits in dairy cattle (Veerkamp and Beerda, 2007).

Exome sequencing is an approach that enable us to identify and study the genetic variants (SNPs), Insertion/Deletion (InDels) and copy number variation (CNVs) in the protein-coding regions and 5'- and 3'- untranslated regions (UTR) in all over the genome (Warr *et al*., 2015; Menon *et al*., 2016). The exome sequencing studies of complex traits in livestock underpinned its success in the candidate gene studies (Shah *et al*., 2016; Whiston *et al*., 2017; Chen *et al*., 2018; Pereira *et al*., 2019) and also has stronghold in population genetic theory (Hirano *et al*., 2013; Sasaki *et al*., 2021). Similarly, proteomics is the study of set of proteins in a defined time and condition in an organism (Wang *et al*., 2019). Since protein act as a primary link between genotype and phenotype of the individual, it may provide valuable information on genetic basis of molecular mechanism controlling the reproduction traits. Comparative and quantitative proteomic studies have found to be promising in identification of significant proteins linked to the different stages of reproduction processes in livestock species (Soleilhavoup *et al*., 2016; Zhao *et al*., 2019; Bahuguna and Sharma, 2022).

Therefore, research in the fields of genomics and proteomics has promises for enhancing the effectiveness of reproduction traits in cattle (Cassandro, 2014). The development of new management techniques and tools to enhance the reproductive performance of cattle is now made possible by the combination of genomic and proteomics technologies to identify and interpret gene functions and protein expression related to reproductive traits in between the extreme phenotypic variation in cattle (Berry *et al*., 2014; Ma *et al*., 2019).

Therefore, the present study was planned with the following objectives:

1. **Whole exome capturing and comparative plasma proteomics profiling of Deoni cows with differing reproductive performance**
2. **Identification of potential exome regions and proteins associated with reproductive performance in cows**

CHAPTER –2

Review of Literature

Review of Literature

2.1 Prologue

The average milk production of dairy cows has significantly improved globally over the past 60 years, yet there is speculation that the pressure on genetic selection for milk production is also one of the factors responsible for the poor reproductive performance in dairy cows (Lucy, 2001). This decline in the reproductive performance of lactating dairy cows suggests that genetic selection for reproductive qualities has not been successful, in part (Desafios and Hansen, 2011). Fertility in dairy cows has decreased, although it is still high in dairy heifers. There is growing evidence to support the hypothesis that endometrial environment, corpus luteum (CL) function, and embryo quality are key determinants of reproductive status in dairy cows (Richard Pursley *et al.*, 1998). It is clear that milk production and the number of calves born are directly impacted by an animal's total reproductive performance, which also has an indirect impact on dairy farming profits. A number of factors, including low fertility rate, an increase in early embryonic death, the age at first calving, longer calving interval, and delayed maturity, lead to decline in overall reproductive performance. Reduced milk production and fewer calves being born are projected to have an impact on farm income as a result of these factors.

The pregnancy rate or number of calvings is thought to be a useful indicator of how well dairy animals in a herd are performing in terms of reproduction. The service per conception, the days open, and the calving interval are additional reproductive parameters that are taken into consideration when calculating the reproductive performance in dairy cows (Washburn *et al*., 2002). The general and reproductive health of the animal, genetic makeup, ovarian and endocrine functions, estrus expression, oocyte, sperm, and embryo quality, as well as the oviductal and uterine environments, are significant physiological factors that may have an impact on the reproductive performance of a post-partum dairy cow. Nutrition and environment are the other major contributing factors that may impede the dairy cow's ability to reproduce at its peak (Royal *et al*., 2000).

Exome and proteome profiling approaches have the capacity to explore genes and proteins related to the desired characters, it is therefore at the forefront of recent research,

among other methods and tools available to probe the physiological and genetic underpinnings of complex traits like the reproductive process. Numerous species, including cattle, have had extensive gene studies done on them to determine the genes and their involvement in the various stages of reproduction. Some of those discoveries are thought to be crucial for dairy cow reproductive performance improvement on developmental or functional level. The subset of proteins and the genes encoding these proteins, that have significant impact on dairy cows reproductive success can be analyzed to identify the protein in the plasma proteome at any particular stage. In this chapter, the role of whole exome sequencing (WES), proteomics, and applications in the identification of gene and protein markers for reproductive traits are reviewed. The review included information from both *Bos indicus* and *Bos taurus* cattle as well as from other livestock species due to scanty literature available on Deoni cattle, the focus breed.

2.2 Reproduction in Dairy Cattle

The ability of a female animal to produce healthy ova, fertilization, conceive, and give birth to the healthy young one, is known as reproductive efficiency (Berry *et al.*, 2014). When dairy cattle reproduce at the proper time in relation to ovulation, heat detection, and insemination, they should be able to conceive, maintain pregnancy, and give birth to a healthy calf is known as having good reproductive performance (Darwash *et al.*, 1997). Age at puberty, number of inseminations per conception, age at first calving, calving interval, etc. all affect a dairy cow's longevity and productivity. Cow reproductivity and the number of calves born during the course of the animal's productive life must be improved without compromising milk production. In a good environment, the reproductive performance of *Bos indicus* cows under standard selection and breeding management can be greatly boosted. Because they are crucial to production efficiency, reproductive characteristics in livestock are of utmost importance (Pryce *et al*., 2010). Genetic improvement by the traditional approach of selection has resulted in a slower annual genetic gain because of the reproduction traits' complex nature, long generation interval, and low heritability (Buddenberg *et al*., 1989).

2.3 Reproductive Performance

Indian dairy farming still has a problem with suboptimal reproductive performance in cattle, particularly in *Bos indicus* cattle. Reproductive inefficiency of dairy cattle directly affects the profitability of the entire herd by causing the system to make lower incomes. The complex trait of the reproduction process is impacted by a number of genetics and

environmental variables as there is a strong genetic support for the idea that genetics, the environment, and the interaction of G and E (GxE) (Berry *et al.*, 2014). Numerous theories, including those based on genetics, physiology, nutrition, and management, have been put out to explain this trait. These theories have been investigated at the animal, cellular, and molecular levels at key points in the productive lives of dairy cows (Walsh *et al.*, 2011).

The genetic trend for reproduction-related characteristics in dairy cows is steadily declining, primarily as a result of rigorous selection for increased milk production. Therefore, dairy cattle reproductive performance has declined as a result of it (Berglund, 2008; Cassandro, 2014; Ma *et al*., 2019). Poor reproductive performance usually results in involuntary culling of dairy cattle and decreased lifetime longevity (Sewalem *et al*., 2008; Berry *et al*., 2014). Studies have shown that in Holstein Frisian cattle population, where there is a steady decline in reproduction genetic trends in relationship to increased milk production (Ma *et al.,* 2019). Hence, utilizing all resources, techniques, and managemental approaches in order to reverse the genetic trends in the reproductive performance of dairy cows has now become paramount in the dairy cattle production system (Dillon *et al.*, 2006; Ma *et al.*, 2019).

In the recent years new research are being undertaken in the area of genomics transcriptomics, and proteomics; a multilple layer of genetics for better understanding of genotype and environment interactions in the complex reproduction process, as they are crucial for improving the reproduction-related traits to achieve good reproductive performance and productivity in dairy cattle farming (Veerkmap and Beerda, 2007). Another important field of present and future research is genomic selection, already available in many highly selected dairy cattle populations. The accuracy of selection for genetic improvement in cattle shown more in genomic selection method compared to traditional breeding methods or marker assisted selection using candidate genes or QTL region (Veerkmap and Beerda, 2007). Research in the areas of genomics and proteomics promises to make a genetic selection even more effective. The emergence of new management techniques and tools for enhancing reproductive performance is made possible by the integration of genomic and proteomic technologies with bioinformatics tools that help the interpretation of gene functioning and protein expression (Berry *et al*., 2014; Ma *et al*., 2019). For the benefit of the dairy industry and cow welfare, future initiatives to increase dairy cow fertility are required. These efforts should be based on an integrative approach of multi-omics events.

2.4 Challenges in Reproduction Traits

Several reports revealed that poor reproductive performance manifest longer calving intervals period, which could result in reduction in milk yield, increased replacement costs, and culling rates (Shao *et al*., 2021). Reproductive performance of dairy animals is decreasing for the past several years and calving rates for the first service are also declining significantly (Ma *et al*., 2019). The average fertility of dairy herd is declining, as more services are necessary for successful conception, which further resulted into lengthened calving intervals and increasing culling rate (Berry *et al.,* 2014). Though managemental practices influence majorly the reproductive performance of dairy cows, still genetic factors play an important role (Shao *et al*., 2021). However, recent improvements in reproductive efficiency of dairy cows can be ascribed to intensive efforts in development of new technologies to improve reproductive performance, particularly timed artificial insemination, early detection of pregnancy and increased emphasis on genetic selection for reproductive and longevity traits (Desafios and Hansen, 2011; Hufana-Duran and Duran, 2020).

2.5 Indicators of Reproductive Performance

Reproductive performance of an animal is showed by early heat detection, timed artificial insemination, successful implantation, embryonic development, early detection of pregnancy, foetal development, ease of calving and calf survival (Berglund, 2008). The number of days open, conception rate, and calving interval are some of the measures used to calculate reproductive performance. Days open and calving interval indicators are influenced by cow fertility as well as by other herd environmental factors, such as estrus detection and the length of the voluntary period (Ma *et al*., 2019).

2.6 Economic Loss Due to Poor Reproductive Performance

To determine the profitability of a dairy farm, the overall reproductive performance plays an important role. It is predicted that each dairy cow losses 2 to 3 lactation period due to poor reproductive performance, which have direct impact on the dairy farming economics. Pregnancy loss can have devasting effects on economic success in dairy farming (Thurmond *et al.,* 1990). Successful pregnancy is the first pre-requisite of dairy animal entering into the productive life and pregnancy rate determines directly to the profitability of dairy herd. Therefore, to run the dairy farm with maximum profitability, good reproductive performance is very crucial to increase the longevity and productivity of the dairy cows. In addition, Rezaee

Roodbari *et al*. (2015) reported that prolonged inter-calving period in dairy cows cause a mean loss in milk production and economic loss to dairy farmers.

2.7 Genetic Markers

The term "genetic markers" refers to a piece of DNA fragment that use particular alleles or locations within a genome as regulators or indicators for a particular feature of interest (Veerkmap and Beerda, 2007). These markers can be used to find specific chromosomal regions that include genes for quantitative traits and often contain variations in the genome's sequence (Ma *et al*., 2019). Genetic markers for reproductive qualities have been found, providing cattle producers with a way to perhaps identify superior animals, enhance selection efficiency, and further increase economic gains (Allan and Smith, 2008; Shao *et al*., 2021). Finding the genes responsible for genetic variation in the characteristic of interest requires the application of several genetic markers since the genetic regulation of quantitative traits is so complex. The lack of sufficient genetic markers is one of the reasons that hinder the researchers from fully understanding animal reproductive process (Ma *et al*., 2019).

2.8 Approaches to Identifying Genetic Markers in Complex Traits

The candidate gene strategy makes use of genes with established physiological functions to find genetic variations linked to an interest phenotype (Pimentel *et al*., 2011). This strategy gives the candidate gene coupled with a genuine causative mutation, and has proved very effective in discovering loci even with tiny effects on trait of interest (Andersson, 2001)

The most effective way to genotype many SNPs is via an SNP (Single Nucleotide Polymorphism) array chip, which is made up of a high-throughput test with many SNPs. In order to forecast a person's propensity for heritable genetic disease, high-density SNP genotyping has become a widely accessible biomedical diagnosis. Similar technology has been applied to cattle, where it has become widely available, improving herd health, animal productivity, and selection precision. These array chips use probe-labeled primers to locate the SNP of interest on the chromosome in relation to the trait of interest (Schmitt *et al*., 2010)

Whole genome sequencing (WGS) utilizes identified molecular markers dispersed all over the genome to make sure that every QTL is in linkage disequilibrium with at least one closest molecular marker (Andersson, 2001; Goddard and Hayes, 2007). Whole genome selection employs genomic data as a supplement to massive collections of performance data to estimate genetic merit values, enabling producers to make well-informed selection judgments.

WGS approach has the capacity to estimate the additive genetic merit also consider the epistatic and pleiotropic effects of genetic variant in all chromosomal region impacting the significant interest on traits. WGS also sums all loci across the genome affecting a trait indicating the genetic merit of an animal which can be predicted based on all the loci genotypes (Daetwyler *et al*., 2007). Genes associated with reproductive performance in cattle are enlisted in table 2.1.

Table 2.1 Genes Associated with Reproductive Performance Traits

Trait	Genes	References
Age at puberty	DGAT1	Collis *et al.*, 2012
	IRS1	Fortes *et al.*, 2013
	PIK3R1	
	CCHCR1	Melo *et al.*, 2019
	PKHD1	
	BPNT1	
	ADAMTS17	
	EPRS1	Zhou *et al.*, 2019
Age at first calving	STAT5A	Oikonomou *et al.*, 2011
	SELP	Chen *et al.*, 2017
	APP	Regatieri *et al.*, 2017
	ARHGEF3	
	SETD3	
	OAS1Z	Alex *et al.*, 2018
	LEP	Jecminkova *et al.*, 2018
Service period	IGFBP2	Pimentel *et al.*, 2011
	CAST	Hill *et al.*, 2016
	BCAS1	Ortega *et al.*, 2017
	BSP3	
	CD14	
	CD2	
	FYB	
	OCLN	
	GNRHR	Derecka *et al.*, 2010
	LTF	O'Halloran *et al.*, 2010
	GHR	Waters *et al.*, 2011
	GH1	Mullen *et al.*, 2011
	LEPR	Trakovicka' *et al.*, 2013
	RPS9	Moore *et al.*, 2016
	TLR4	Jecminkova *et al.*, 2018

2.9 Deoni Cattle

Deoni is a dual-purpose cattle breed found mainly in the Southern and Western regions of India, specifically in the districts of Bidar of northern Karnataka and Latur of south-eastern Maharashtra. Deoni cattle took its name from the Deoni taluk in Maharashtra's Latur district. Surti, Dongarpati, Wannera, Waghya, Balankya, and Shevera are some of the synonyms for this breed. The breed was formed through cross breeding with Gir cattle of Gujarat, Dangi cattle of Marathawada, and local desi cattle from Bidar and Osmanabad area (Singh *et al.*, 2002).

The Deoni cattle breed is further sub-divided into three strains based on morphological characteristics: Balankya, Wannera, and Waghya or Shevera (Das *et al.*, 2011). Balankya refers to animals with fully white coat color and no black spots on their bodies, whereas Wannera refers to animals with completely white coat color but black face. Waghya or Shevera stain, on the other hand, features white and black dots on the body. A detailed analysis of the production and reproductive performance of Deoni cattle was done by Das *et al.* (2011). The age at first calving was 38.73 ± 0.73 months; calving interval 447.22 ± 6.64 days; calving to first service 81.66 ± 1.63 days; first service to successful service 71.39 ± 4.01 days and service period 148.15 ± 3.71 days in Deoni cattle. Further they reported that the lactation performance was lactation yield 819.98 ± 16.50 kg and lactation length 195.2 ± 2.63 days (Das *et al.*, 2011; Kuralkar *et al*., 2014).

Due to continuous crossbreeding and less attention given to breed improvement of indigenous dual-purpose cattle breeds of India, the population of the Deoni cattle is declining and now lesser number of elite animals are available for their genetic improvement. Hence, the present investigation was taken up to identify molecular markers related to reproductive performance in Deoni cattle.

2.10 Whole Exome Sequencing

Whole exome sequencing (WES) involves capturing, sequencing and analysis of exome region present in the genome (Chen *et al*., 2018). WES usually on capturing the exon regions of the genome which are translated into functional proteins. This functional protein has direct impact on the trait of interest, if any mutation is found on the exon region (Bodi *et al*. 2013). Micro-RNA, long intergenic noncoding RNA as well as specific candidate loci can also be targeted in whole exome sequencing (Warr *et al.,* 2015).

The recent improvement in the high-throughput technologies allows exome sequencing approach to provide the data of all exon regions situated in between the genome. Hence, WES covers some of the limitations faced in the whole genome sequencing and hybridization genotyping array.

Since WES has become a reliable approach for identifying disease genes in humans, more and more livestock species are using it (Hirano *et al*., 2013; Chen *et al*., 2018). The use of molecular genetic approaches in species with complex traits is made possible by the applications of WES in livestock research. These techniques allow the identification of variations important for an economically important trait that can be put into marker assisted selection. The majority of the advantages of whole genome sequencing technology are now provided by WES, which is a well-established and powerful method for variation detection in the coding areas (Meynert *et al*., 2014).

2.11 Whole Exome Sequencing Technology

In whole exome sequencing there are two main approaches widely used to capture exome in the genome: solution-based and array-based exome capturing and sequencing approaches (Warr *et al.,* 2015). In solution-based approach, DNA samples are first fragmented and selective hybridization of target regions in the genome done using biotinylated oligonucleotide probes (baits). Magnetic streptavidin beads were used to bind only biotinylated hybridized probes and the polymerase chain reaction (PCR) step is done to amplify the hybridized sample for enrichment of target DNA regions. The sample is then sequenced using any high-throughput sequencing technology to acquire data for bioinformatic analysis.

Except that the probes are attached to a high-density microarray, array-based approaches are largely comparable to solution-based approaches. The array-based method was the first to be utilised in exome capture (Albert *et al*. 2007), but solution-based methods have largely supplanted it since they require less input DNA, can identify rare variants, are adaptable, and may thus be more effective than the array-based approach (Warr *et al*., 2015).

The major providers of exome capture kits are NimbleGen, Agilent, and Illumina, and each have different background and strengths. Capture Array-based methods performed better than the solution-based alternatives in low GC content regions; had high sensitivity and read mapping rates; and single-nucleotide polymorphism (SNP) detection from these reads was more specific to the target region. In addition, array-based capture has also been used successfully and accurately to identify rare and common variants and identify candidate genes in small population cohort studies (Ng *et al*. 2009).

2.12 Applications of Whole Exome Sequencing

Whole exome sequencing has been widely applied to humans in both research and clinical diagnosis of mono- or polygenic diseases. Likewise, it can be used to identify genomic variants with the potential to be associated with characteristics of economic importance in livestock animals. The amount of data generated from WES (Whole exome sequencing) is far more manageable than WGS (Whole genome sequencing), particularly for small research groups and groups studying organisms with large genomes (Schick *et al.,* 2015).

The exome data provides a handful of information such as depth of coverage, genotypic quality, allele frequency, and variant consequences. The filtering capabilities and a large number of exomes will simplify the process of prioritizing variants when using exome sequencing as a diagnostic tool. Analyzing the exome region shows how conserved an amino acid sequence is through evolution and between genes in a family can help to increase the confidence of having a deleterious effect (Wang *et al.,* 2011; Xu *et al*., 2014a).

Whole exome sequencing approach can be performed in groups of individuals with contrasting traits of interest in order to identify markers potentially able to alter the amino acid sequence of proteins and their structure and, consequently, phenotypes (Curi *et al.,* 2020). It has been used extensively to diagnose novel diseases and find novel causative mutations for known disease phenotypes. Markers identified in exome sequencing have the potential to use in improving complex economical traits in livestock species (Robert *et al.,* 2014). It is used to prioritize and identify strong candidate genes related to monogenic or polygenic traits of interest.

WES now especially useful for model organisms particularly whose genome is complex to improve the reference genome coding region for better annotation (Robert *et al.,* 2014). The amount of data produced by WES is far more manageable than WGS, particularly for small research groups and groups of species having larger genomes. WES can therefore be considered a much less costly and more efficient method of identifying all possible mutations in genes, compared to other methods such as genome-wide association studies or whole-genome sequencing (WGS) (Meynert *et al.,* 2014).

2.13 Exome Sequencing Studies in Livestock

In Japanese black cattle, the perinatal weak calf syndrome was reported frequently in the newborn calves. Hirano *et al*. 2013 investigated the genetic causes for perinatal weak calf

syndrome using the exome sequencing approach and identified the mutation in the IARS gene that was the major causative reason for the perinatal weak calf syndrome in Japanese black cattle population.

To identify the major genes responsible for the genetic basis for the difference between large and small size dog breeds, an exome capturing approach was performed and identified six major candidate genes for the size difference between large and small breed dogs (Rimbault *et al.,* 2013).

In Brown Swiss, Holstein Friesian, and Jersey breed cattle haplotype was found to have a negative effect on fertility traits. To identify the causative mutation responsible for defective bovine embryo development within these haplotype regions; an exome capturing approach combined with next-generation sequencing technology was performed and identified a putative mutation on the SMC2 gene which is responsible for the negative effect on defective bovine embryo development in Brown Swiss, Holstein Friesian, and Jersey breed cattle (Mc Clure *et al.,* 2014).

In dairy cattle, reproductive performance remains a critical issue underpinning sustainability in the dairy sector. Since bull is considered more than half of the herd, using the exome sequencing approach genes associated with bull fertility are identified and functionally characterized. In FOXJ3 gene identified a novel transcription factor and its role in the regulation of bull fertility (Whiston *et al.,* 2017).

Body measurement traits in cattle were found to have a positive correlation with reproductive traits like age at puberty and age at first calving traits. Hence understanding the association of the variants with body measurement traits is important. Therefore, exome sequencing was performed in cattle and identified that 7 genes out of 66 genes had a strong association with body measurement traits (Chen *et al.,* 2018).

In the English through breed horses, genes and SNPs associated with racing performance traits were identified using exome capturing and sequencing approach to select elite animals for racing purposes (Curi *et al.,* 2020). In Japanese black cattle, the study was carried out to find out the genetic background for risk of exposure to deleterious recessive traits due to intensive use of few elite sires and reported that the mutation in the IARS, CDC45, and CLDN16 genes were the major causative reason for incidence deleterious recessive traits in these cattle population (Sasaki *et al.,* 2021).

2.14 Protein, Proteome and Proteomics

Proteins regulate and execute many key biological processes in all living beings, and their multilayer actions in the physiological process not only dependent directly on its mRNA expression level but also on an individuals' translational control and regulation with response to environmental interaction. Therefore, proteins are considered as essential effectors of studying complex biological function, which carries most of the functional information translated from the gene of the genome. It also serves as a pivotal link between genotype and phenotype of the individual (Wang *et al.*, 2019).

In 1994, Marc Wilkins coined the term proteome to indicate the "PROTein complement of a genOME" and it describes "all proteins expressed by a genome" (Wilkins *et al.*, 1996). Since proteome considered as the most relevant mediator to characterize a biological system (Cox and Mann, 2007). Proteomics a branch of science emerged, which involves the study of gene-level protein expression. In simple, proteomics is the characterization of proteome, which includes expression, structure, functions, interactions and modifications of proteins at a given particular period of time (Domon and Aebersold, 2006; Wang *et al.*, 2019).

2.15 Proteomics and its Techniques

Proteomics is one of the most significant approaches to understand the gene function, because fluctuations in gene expression level can be determined by either analysis of transcriptome or proteome to discriminate between two biological states of the cell (Lander *et al.*, 2001; Aslam *et al.*, 2017). To understand the genetic basis as well as physiological function of gene product, two major approaches have followed; first is restricting the large-scale analysis of gene products to studies involving only proteins, and second is combining protein studies with analyses that have a genetic readout, such as mRNA analysis and genomics (Graves and Haystead, 2002).

Proteomic approaches can also be used for protein profiling, comparative expression analysis of two or more conditions, the localization and identification of post-translational modifications, and the determination of protein-protein interactions (Chandramouli and Qian, 2009). It also enables us to identify new drug target regions, which leads to the development of superior diagnostics and therapeutics techniques. It also correlates biological pathways and molecular mechanisms of complex biological traits (Ali-khan *et al.*, 2002).

The goal of proteomic study is to obtain a more global and integrated view of biology by studying protein and genome. As a result, broad analysis has been boosted by two methods

that have been invented in the last century (Patterson and Aebersold, 2003). In the mid-1970s, O'Farrell introduced two-dimensional polyacrylamide gel electrophoresis (2-D GE) for the separation of proteins (Laemmli, 1970). This technique combines isoelectric focusing (IEF) with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins are first separated based on their isoelectric point (pI) and in the second dimension according to their molecular weight (MW) (Scherp *et al.,* 2011). The later invention of immobilized pH gradients (IPG) increased the reproducibility and resolution of 2-D gels and made the technology available to a broader number of users (Arianmanesh *et al.,* 2016). Alongside, biological mass spectrometry (MS) has opened the door for the large-scale identification of peptides and proteins (Coskun, 2016). Biological MS has been made possible via the development of two ionization techniques, namely electrospray ionization (ESI) (Ahuja, 2003) and matrix-assisted laser desorption/ionization (MALDI) (Aslam *et al.,* 2017). Hence, in the past few decades, proteomics has been applied in various fields of life science to profile, study and analyze proteins in innumerable defined biological systems (Ramesha *et al.*, 2020).

2.16 Proteomics and Animal Reproduction

Farm animal proteomics studies in recent days have steadily increased and focused on how to improve and monitor animal health, production, and reproduction traits through relevant protein biomarkers (Danielsen *et al.*, 2010; Bendixen *et al.*, 2011; Ramesha *et al.*, 2020). Many ongoing studies also support that, proteomics has great potential in identifying functional genes and proteins related to the production and functional traits like reproduction, and longevity than genomics or transcriptomics studies. Thus, studying the proteins that are linked with reproductive traits might help us in understanding the physiological nature of the traits and improve reproductive performance in dairy cattle. However, most of reproduction-related traits are complex and transient in nature like the estrus cycle, conception, fertilization, implantation, embryo development, gestation, and parturition. Each stage of reproduction process is complex and has a multiple of biological functions and is controlled by many genes and proteins, so not easy to measure or interpret unambiguously. Therefore, understanding the patterns of inheritance governing this complex reproductive process is crucial (Marques *et al.*, 2009).

At a molecular level as well as physiological level, the reproductive performance of animals is a complex phenomenon that occurs through the interactions between different proteins. Although reproduction is understood at the molecular level, it is far from being understood in what way the reproductive phenotype and behavior relate to its molecular

foundations. Therefore, knowledge about female reproductive traits can be understood by studying proteome at different stages of the reproductive process like during estrus cycle, ovulation, fertilization, implantation, embryonic development, and gestation.

Many studies have been conducted using different proteomic approaches in the field of animal reproduction science. Now proteomics has become a helpful tool, allowing us to better understand the mechanism underlying the biology of reproduction in farm animals. The potential contributions of proteomics to the study of animal reproduction can be considered as two directional: first, in the diagnosis of reproductive diseases and the second, in the identification of individuals with high reproductive performance. Proteomics is also quite useful in the development and improvement of assisted reproductive biotechnologies by understanding the molecular as well as physiological mechanisms involved in assisted reproduction technologies (Ramesha *et al.,* 2020). It also has the capability to identify important pathways and networks related to reproduction-associated traits and to tackle existing problems in the areas of farm animal reproduction previously encountered in the conventional approaches of dairy cattle breeding (Shao *et al*., 2021).

Proteomic approaches are therefore well-suited for the study of complex traits like reproductive traits because most of the interactions and biological actions take place almost exclusively at the protein-protein interaction level without gene expression and genetic regulatory elements, which are finally expressed phenotypically in the animal (Karr, 2019). Since proteomics is considered as a powerful tool in deciphering the most dynamic and functionally interactive layer of the genome; a detailed study of the proteomics relevant to reproduction traits is required and it provides indispensable knowledge in the near future for the improvement of farm animal reproduction. In the light of the steadily increasing use of proteomics in farm animal reproduction, the global analysis of proteins, related to reproductive performance has become a crucial task to understand reproductive processes in more detail and to identify potential protein modulators and predictive markers (Itze-Mayrhofera and Brem, 2020).

2.17 Proteomic Studies in Animal Reproduction

Since reproductive traits are complex and low heritable in nature, dissection of reproductive traits genetic architecture will increase our understanding of the underlying reproductive physiological process and could probably allow the efficient genetic improvement of reproductive traits through the use of genes and proteins identified in the proteomic studies. For better understanding to utilize reproductive traits in cattle and breeding programs, classified

reproductive traits into ovulation, fertilization, and calving related traits based on the reproductive physiological point of view (Shao *et al*., 2021).

In dairy cattle Zhao *et al*. (2019) performed blood plasma proteomics to study proteins related to active and inactive ovaries in cattle and he reported PKM2, GPX3, ALDOB, RBP4 and AHSG proteins were differentially expressed between active and inactive ovaries groups of dairy cows. During estrus cycle in bovine, so many changes occur in the reproductive tract of an animal at cellular and molecular level. Therefore, Soleihavoup *et al.* (2016) studied protein-protein interaction in reproductive tract fluids like oviduct, cervix, and uterus of cattle during estrus cycle and reported heat shock proteins, mucins, complement cascade proteins, oviductin, osteopontin, and sperm adhesion proteins were more abundant at follicular phase and in luteal phase ceruloplasmin, lactoferrin, DMBT1, CD9 and galectin3 binding protein were abundant when compared to follicular phase of estrus cycle. Yu *et al*. (2019) reported oviduct-specific protein 1 and lactoferrin protein in bovine oviductal fluid were found involved in the pre-fertilization process in cattle. In bovine uterine fluid, ceruloplasmin, serotransferrin, and albumin proteins were identified as pregnancy-specific proteins after a few days of insemination (Lancheros-Buitrago *et al*., 2020). In low fertile dairy cows SERPINA1, TIMP2, ITIH1, HSPG2, C8A, COL1A2, F2, and IL1RAP proteins were reported differentially expressed in the follicular fluid when compared to high fertile dairy cows (Zachut *et al.*, 2016).

Identification of high and low fertile cows is vital for sustainable dairy cattle farming. Since follicular fluid provide suitable micro-environment and nourish the ovum from initial development till ovulation (Soleihavoup *et al*., 2016). Zachut *et al*. (2016) studied pre-ovulatory follicular fluid of high and low fertile dairy cows and reported SERPINA1, TIMP2, ITIH1, HSPG2, C8A, COL1A2, F2, and IL1RAP proteins were differentially expressed in the follicular fluid of low fertile cows. Likewise, Ferrazza *et al*. (2017) reported follistatin, inhibin, serglycin, spondin 1 and fibrinogen proteins in follicular fluid observed differentially altered in cows with divergent fertility rate.

In swine, oviductal fluid proteome profiling was studied to understand the impact of hormone regulation in the proteome composition of oviductal fluid and reported HSP90, OVGP1, ESR1, CD109, TSG101, ATP2B2, cytochrome, kinesin-like protein (KIF15), cartilage matrix protein (MATN) was altered during estrus period and cortactin, plasminogen activator, and urokinase (PLAU) were found altered after post ovulation (Laezer *et al*., 2020). Pyruvate kinase M1/2 (PKM), acyl-CoA synthetase long-chain family member 4 (ACSL4), olfactomedin 3 (OLFM3), Aquaporin 5 (AQP5), syndecan 2 (SDC2), heat shock protein

family, annexin protein family, myosin heavy chain 9 (MYH9), clathrin heavy chain (CLTC), alanyl aminopeptidase membrane (ANPEP), ezrin (EZR), and OVGP1 proteins were found in oviduct fluid, which are involved in the reproductive processes (Laezer *et al*., 2020).

In the endometrium of livestock, so many changes happen during the period of estrus cycle for successful implantation of embryo and establishment of pregnancy (Cammas *et al*., 2005). In equine, uterine fluid proteome profiling was performed by Maloney *et al.* (2019) to investigate the proteins changes that occur during estrus and diestrus period and its role in reproductive performance. They reported that immunoglobulin lambda-like polypeptide 1, haemoglobin subunit alpha, alpha-1B-glycoprotein, serotransferrin, apolipoprotein A-1, and haemoglobin subunit beta were identified abundant in estrus period, while during diestrus, immunoglobulin alpha-1 chain C region-related, complement factor I, CD 109 antigen and uterocalin, were found abundant (Maloney *et al*., 2019).

Early detection of pregnancy is an important component in successful reproduction management as well as crucial for sustainable milk production in dairy cattle (Hufana-Durand and Duran, 2020). Munoz *et al.* (2012) studied the uterine fluid proteome of cattle to differentiate pregnant and nonpregnant cows and he reported glycolysed tumor necrosis factor alpha (TNFα), interleukin 1 beta (Il-1B), insulin, lactotransferrin, non-phosphorylated peroxiredoxin, albumin, purine nucleoside phosphorylase, HSPA5 and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) proteins showed down regulation in pregnant cows. While, phosphorylated-peroxiredoxin, annexin A4 and nonglycosylated-TNFα proteins showed upregulation in pregnant cows when compared to nonpregnant cows.

In bovine blood serum proteomic study was done to establish early pregnancy specific diagnostic protein markers in cattle and identified lactotransferrin, golgin A4, MYRIP, PKD1, PWWP, and MUM1 proteins were differentially altered during early stage of pregnancy when compared to nonpregnant cows (Bahuguna and Sharma, 2022). Likewise in blood serum of pregnant and nonpregnant hefiers, SNX5, ACACA, CPSF6, DDB1, and SRFBP1 were reported as potential proteins to identify nonpregnant heifers from pregnant ones (Kusama *et al.*, 2022). Angiotensinogen, cadherin-5, fibronectin alpha chain, ADAM metallopeptidase with thrombospondin type 1 motif 13, and histidine-rich glycoprotein were reported to cause pregnancy failure in nonpregnant heifers when compared to the blood serum of pregnant heifers (Kusama *et al.*, 2022). Panda *et al*. (2020) observed the abundance of oligoadenylate synthetase-1 and interferon stimulated gene-15 ubiquitin like modifier proteins in the blood neutrophils of post artificial inseminated cows (day 18) and reported these proteins as pregnant

specific proteins in cattle. The salient findings of the proteomic studies in farm animal plasma, serum and other studies related to reproductive traits have been summarised in table 2.2.

The review of the work done in the area of whole exome sequencing and proteomic studies related to reproductive traits in bovines and other livestock clearly indicated that only few studies are available on reproductive traits in cattle particularly in indigenous cattle.

Table 2.2 Proteomic Studies in Farm animals

Condition	Sample	Findings	Reference
Protein changes in oviductal fluid due to gametes	Oviductal fluid	Fibrinogen, complement C3, retinol binding protein (RBP), and oviduct-specific glycoprotein (OGP)	Georgiou *et al*., 2005
Placenta of SCNT and AI cow	Placenta	Interleukin-18, annexin-XI, tissue inhibitor of metalloproteinase, vimentin, and tropomyosin beta chain.	Kim *et al*., 2005
Last month of pregnancy	Neutrophils (PBS)	Beta-actin, profilin I, calgranulin B, lactotransferrin, septin 6, albumin, annexin A1, histones, α-enolase, and cystatin C	Lippolis and Reinhardt, 2005
Pregnancy (End term) and post-partum	Blood serum	Kininogen, alpha-2-HS-glycoprotein, apolipoprotein A-IV, alpha-1-antichymotrypsin, and orosomucoid, haptoglobin.	Cairoli *et al*., 2006
Pregnant	Blood serum	Transferrin, albumins, IgG, gamma globulins.	Jin *et al*., 2006
Proteome profiling of porcine intrauterine protein during pregnancy	Uterine fluid	Albumin, Transferrin, Alpha-2-macroglobulin, matrix metalloproteinase-2, Tissue inhibitor of metalloproteinase-2, Superoxide dismutase	Kayser *et al*., 2006
Embryonic stem cells	Binuclear trophoblast giant cells	Pregnancy-associated glycoproteins and prolactin-related protein I	Klisch *et al*., 2006
Pregnant vs non-pregnant	Milk whey protein	Lactoferrin, NADH dehydrogenase subunit 2, albumin, serum albumin precursor and transferrin.	Han *et al*., 2008

First trimester (45days)	Amniotic and allantoic fluids	Cathepsin I, cystatin B and C, alpha1-antitrypisin, tissue inhibitor of metalloproteinase 2, ubiquitin B precursor protein, osteonectin and procollagen type I alpha 1 and type III alpha 1.	Riding *et al*., 2008
Pregnant and non-pregnant (Day 16 and 18)	Uterine luminal fluid	Carbonic anhydrase, ezrin, heat shock protein 70, isocitrate dehydrogenase, nucleoside diphosphate kinase, peroxiredoxin 1, purine nucleoside phosphorylase, thioredoxin, triosephosphate isomerase, cystatin E/M, legumain, retinol-binding protein (RBP) and tissue inhibitor of matrix metalloproteinase 2 (TIMP-2)	Ledgard *et al*., 2009
Pregnant vs non-pregnant	Milk	Serum albumin precursor, IgG1 heavy chain constant region, conglutinine precursor, epithelial keratin10 and kelch-like ECH-associated protein	Han *et al*., 2010
Placental Proteome	Placental tissue of healthy cow	Leucine aminopeptidase, JM-27 protein, cytochrome P450	Kim *et al*., 2010
Pregnant vs non-pregnant	Uterine luminal fluid (Ewes)	CC5 (Complement component 5), Adenosylhomocysteinase, GPI (Glucose 6 phosphate isomerase), paraoxonase 1, A1BG (alpha-1-B glycoprotein), IGHM, ACTN4 protein (Actinin 4), BCL-like 15, CA II (Carbonic anhydrase II), Transgelin, CC4A (Complement component 4A), PP9 (aldose reductase/ placental protein 9), A2M protein (Alpha 2 Microglobin), Ceruloplasmin (ferroxidase) isoform 1, APO-AI (apolipoprotein A-I)	Koch *et al*., 2010
Healthy vs milk fever cow	Blood Plasma	Angiotensin, endopin 2B, serum albumin, fibrinogen beta chain and IgG heavy-chain C-region.	Xia *et al*., 2012

Primiparous cow	Blood Serum	Orosomucoid, haptoglobin, amyloid A, transthyretin, apolipoprotein E and immunoglobulin gamma 1.	Yang *et al*., 2012
Pregnant vs non-Pregnant	Uterine fluid	Glycolysed tumor necrosis factor alpha (TNFα), interleukin 1 beta (Il-1B), insulin, lactotransferrin, non-phosphorylated peroxiredoxin, albumin, purine nucleoside phosphorylase, HSPA5, nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), phosphorylated-peroxiredoxin, annexin A4, nonglycosylated-TNFα, twinfilin, hepatoma-derived growth factor, and synaptotagmin-binding cytoplasmic RNA interacting protein.	Munoz *et al*., 2012
Preimplantation period	Uterine fluid	RPB4, TIMP2, IDH1, CST6, GDI2, ALDOA, CO3, GSN, HSP90A1, SERPINA3, VCN, purine nucleoside phosphorylase (PNP), HSPA8, isoforms of serotransferrin precursor (TF), isocitrate dehydrogenase [NADP] cytoplasmic (IDHA), purine nucleoside phosphorylase (PNP), cystatin-M precursor (CST6), retinol-binding protein 4, aldose reductase (ALDR), cathepsin D (CATD), heat-shock cognate 71 kDa protein (HSP7C), actin, cytoplasmic 1 (ACTB), prepro complement, component C3 (CO3), and cathepsin B precursor (CATB).	Forde *et al*., 2014
Comparative proteome study of pregnant and lactating cows	Plasma protein	Clusterin precursor, apolipoprotein A-IV precursor, apolipoprotein A-I precursor, apolipoprotein E precursor, conglutinin precursor, complement C4 precursor, mannose-binding protein C precursor, fibrinogen alpha chain precursor, fibrinogen gamma B-chain precursor, fibrinogen beta chain precursor, fibrinogen like protein-1 precursor, endopin-1 precursor, 72 kDa type IV collagenase precursor, actin cyto-plasmic 1, Kelch-like ECH associated protein 1, pigment epithelium-derived factor precursor, gluthathione peroxidase 3 precursor.	Kurpinska *et al*., 2014

Pregnant vs non-pregnant	Blood serum	Conglutinin precursor, modified bovine fibrinogen, IgG1, hemoglobin, complement component 3, and IgG2a	Lee *et al*., 2015
To understand protein-protein interaction in reproductive tract during estrus cycle	Oviduct, cervix and uterus fluid	Heat shock proteins, mucins, complement cascade proteins, oviductin, osteopontin, spermadhesin, ceruloplasmin, lactoferrin, DMBT1, CD9 and galectin3.	Soleilhavoup *et al*., 2016
Comparative proteome study pre-ovulatory follicular fluid to identify low fertile cows	Follicular fluid	SERPINA1, TIMP2, ITIH1, HSPG2, C8A, COL1A2, F2, and IL1RAP	Zachut *et al*., 2016
To understand microenvironment of follicular fluid proteome	Follicular Fluid	Follistatin, inhibin, serglycin, spondin-1 and fibrinogen	Ferrazza *et al*., 2017
Gestational specific response	Blood Plasma	Interleukin-16, Connective tissue growth factor, COL1A2, EGR1, FGB, HRG, RBP4 and APOC3	Pereira-fantini *et al.*, 2018
Proteome profile of histotrophic in early pregnancy	Histotroph	Uterocalin precursor (UP), Haptoglobin (HP), Chloride intracellular channel protein (CLIC1), F-actincapping protein subunit beta isoform X2 (CAPZB),14-3-3 protein epsilon-like protein (14-3-3), Alcohol dehydrogenase (NADPþ) like protein (AKR1A1), Actin, cytoplasmic 1, (ACTB), Isocitrate dehydrogenase [NADP] cytoplasmic (IDH1), Creatine kinase B-type (CKB), Enolase 1 (ENO1), Vitamin D-binding	Bastos *et al*., 2019

		protein (GC), Heat shock cognate 71 kDa protein (HSPA8) and Heat shock protein HSP 90-alpha (HSP90AA1)	
Oestrus vs dioestrus	Uterine flush	Immunoglobulin lambda-like polypeptide 1, haemoglobin subunit alpha, alpha-1B-glycoprotein, serotransferrin, apolipoprotein A-1, haemoglobin subunit beta, immunoglobulin alpha-1 chain C region-related, complement factor I, CD 109 antigen and uterocalin.	Maloney *et al*., 2019
Proteins associated to pre-fertilization process	Oviductal fluid	OVGF1 (oviduct specific protein), and Lactoferrin (LTF)	Yu *et al*., 2019
Inactive ovary vs Active ovary	Blood Plasma	PKM2, GPX3, ALDOB, RBP4 and AHSG	Zhao *et al*., 2019
Genetic merit for fertility alters the bovine uterine luminal fluid proteome	Uterine luminal fluid	SORD (Sorbitol dehydrogenase), GFPT1 (Glutamine-fructose—6-phosphate aminotransferase 1), IFNT2 (interferon tau-2), IFNT (interferon tau), FOLR1(folate receptor alpha), CHGA (Chromogranin A), LGMN (legumain), CTSH (cathepsins), TINAGL1, TIMP2 (tissue inhibitor of matrix metalloproteinase 2), LTF (Lactotransferrin), RBP (retinol-binding protein)	Gegenfurtner *et al*., 2020
Normal vs Over conditioned cow	Blood Plasma	Adiponectin, Angiotensinogen, Apolipoprotein C-III, ApoN protein, Complement C3, CD59 molecule, Conglutinin, Collectin-43, CPN2 protein, Prothrombin, Fibrinogrn alpha chain, Hepatitis A virus cellular receptor 1, Hemoglobin subunit delta, Inter-alpha-trypsin inhibitor heavy chain, Anthithrombin-III, Serotransferrin, Alpha-2-HS-glycoprotein, Apolipoprotein B, Apolipoprotein C-III, Collectin-43, Haptoglobin, Plasma kallikrein, Serpin A3-1.	Ghaffari *et al*., 2020

To study hormonal regulation by dynamic profile of EVs in porcine oviductal fluid	Oviductal fluid	HSP90,OVGP1,ESR1,CD109,TSG101,ATP2B2,Cytochromec,Kinesin-like protein (KIF15), cartilage matrix protein (MATN). Cortactin (CTTN), Plasminogen activator, urokinase (PLAU), Pyruvate kinase M1/2 (PKM), acyl-CoA synthetase long-chain family member 4 (ACSL4) and olfactomedin 3 (OLFM3), Aquaporin 5 (AQP5), syndecan 2 (SDC2). heat shock protein family (HSP90AA1, HSP90B1, HSPA1A, HSPA4, HSPB1, and HSPA8), annexin family (ANXA1, ANXA2, ANXA4, ANXA5, ANXA6, ANXA7, and ANXA11), myosin heavy chain 9 (MYH9), clathrin heavy chain (CLTC), alanyl aminopeptidase membrane (ANPEP), ezrin (EZR), and OVGP1, syntaxin and syntaxin-binding protein (STX2 and STXB1), ATPase plasma membrane Ca2+ transporting 2 (ATP2B2), talin 1 (TLN1). 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), scavenger receptor class B member 1 (SCARB1) and PDZ domain-containing 1 (PDZK1).	Laezer *et al*., 2020
Pregnant associated proteins	Uterine fluid	Ceruloplasmin, serotransferrin and albumin	Lancheros-Buitrago *et al*., 2020
Pregnant specific Proteins	Blood Neutrophils	Oligoadenylate synthetase-1 (OAS1) and Interferon stimulated gene-15 ubiquitinlike modifier (ISG15).	Panda *et al*., 2020
Early Pregnancy	Blood serum	Lactotransferrin, Golgin A4, MYRIP, PKD1, PWWP, and MUM1	Bahuguna and Sharma, 2022
Pregnant vs non-pregnant	Blood Serum	SNX5, ACACA, CPSF6, DDB1, SRFBP1, AGT, CDH5, FGA, ADAMTS13, HRG, THBS1, BRD9, PF4, C3, TG, CLEC12B, ASB17, and APOH.	Kusama *et al*., 2022

CHAPTER –3

Materials & Methods

MATERIALS AND METHODS

The present study was carried out to explore the possibility of identifying genetic variants and blood plasma proteins associated with reproductive performance traits in Deoni cows differing in their reproductive performance, using Whole Exome Sequencing (WES) and Data-Independent Acquisition (DIA) based LC-MS/MS respectively.

3.1 Location of Study

The study was conducted at Proteogenomic lab, Dairy Production Section, Southern Regional Station of ICAR- National Dairy Research Institute (NDRI), Adugodi, Bengaluru.

3.2 Experimental Animals

Deoni cows (n = 12) maintained in the Livestock Research Complex, Southern Regional Station of ICAR-National Dairy Research Institute, Bengaluru (12°57'01.0" N Latitude and 77°36'17.8" E Longitude) were utilized for this study. The age of Deoni cows ranged from 6 to 9 years. Utilizing the farm data, days open (DO) and inter-calving period (ICP) were calculated for all the cows maintained in the farm. The Deoni cows having DO and ICP more than mean - 1 SD were selected and grouped into the high reproductive performance group (n = 6). The Deoni cows having DO and ICP less than mean + 1 SD were selected and grouped into the low reproductive performance group (n = 6).

3.3 Sample Preparation for Whole Exome Sequencing

3.3.1 Blood Collection and Sample Preparation

Approximately 10 ml of blood was collected aseptically using a sterile vacutainer tube coated with 0.5% EDTA (Ethylene diamine tetra acetic acid) from the Jugular vein of 12 Deoni cows (Pregnant =6; Cyclical = 6); after we got authorization from Institutional Animal Ethics Committee (IAEC) (28.01.2021; CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2021/No.06). The blood samples collected from the selected animals were mixed properly along with EDTA to avoid clotting and labelled properly. The collected samples were then stored in the ice pack for further process.

3.3.2 High Salt DNA Isolation

Isolation of DNA was carried out using Modified High Salt Extraction method (Miller *et al*., 1988) (Fig 3.1). The following protocol was followed:

- Blood samples collected from jugular vein of selected cows using EDTA containing sterile vacutainer tubes were stored at 4ºC till processed.
- 10 ml of blood was transferred to 50 ml centrifuge tube and 2.5 volume of RBC lysis buffer was added, mixed and incubated in ice for 10-15 minutes.
- Subjected to Centrifugation at 5000 rpm for 10 minutes, supernatant was discarded.
- Previous step was repeated 2-3 times again till clear white pellet was formed.
- Pellet resuspended with a pasteur pipette, washed two times with 10 ml of Tris Buffer Saline (TBS). Centrifuged at 4000 rpm for 10 minutes. Supernatant was discarded.
- Pellet was suspended in 09 ml of TE-buffer (pH-8), vortex and 50µl proteinase-K then 0.5ml of 0.5M EDTA is added and mixed well.
- To the above 0.5 ml of SDS was added gently. Incubated at 50ºC in water bath for overnight.
- To the above 4.3ml of saturated NaCl solution was added and mixed by shaking vigorously then equal volume of Chloroform: isoamyl alcohol (24:1) is added and mixed well.
- Followed by centrifugation at 4000 rpm for 15 minutes.
- The upper aqueous phase was then transferred to fresh tube and chloroform: isoamyl alcohol (24:1) was again added and centrifuged at 4000 rpm for 10 minutes.
- The step was repeated twice, to reduce as much as protein contamination in aqueous layer containing DNA.
- Aqueous layer containing DNA was transferred into 100ml beaker, then double the volume of 95% ethanol was added to precipitate DNA.
- Precipitated DNA was spooled out using clear glass rod and washed with 70% ethanol to remove salts and dissolved it in 400µl of TE buffer.
- Isolated DNA was stored in the deep freezer at -20ºC until further processing for exome sequencing.

3.3.3 DNA Quality Control

The quantification and quality of the genomic DNA were assessed using Nanodrop2000 (Thermo Scientific, USA), Qubit (Thermo Scientific, USA) and agarose gel electrophoresis.

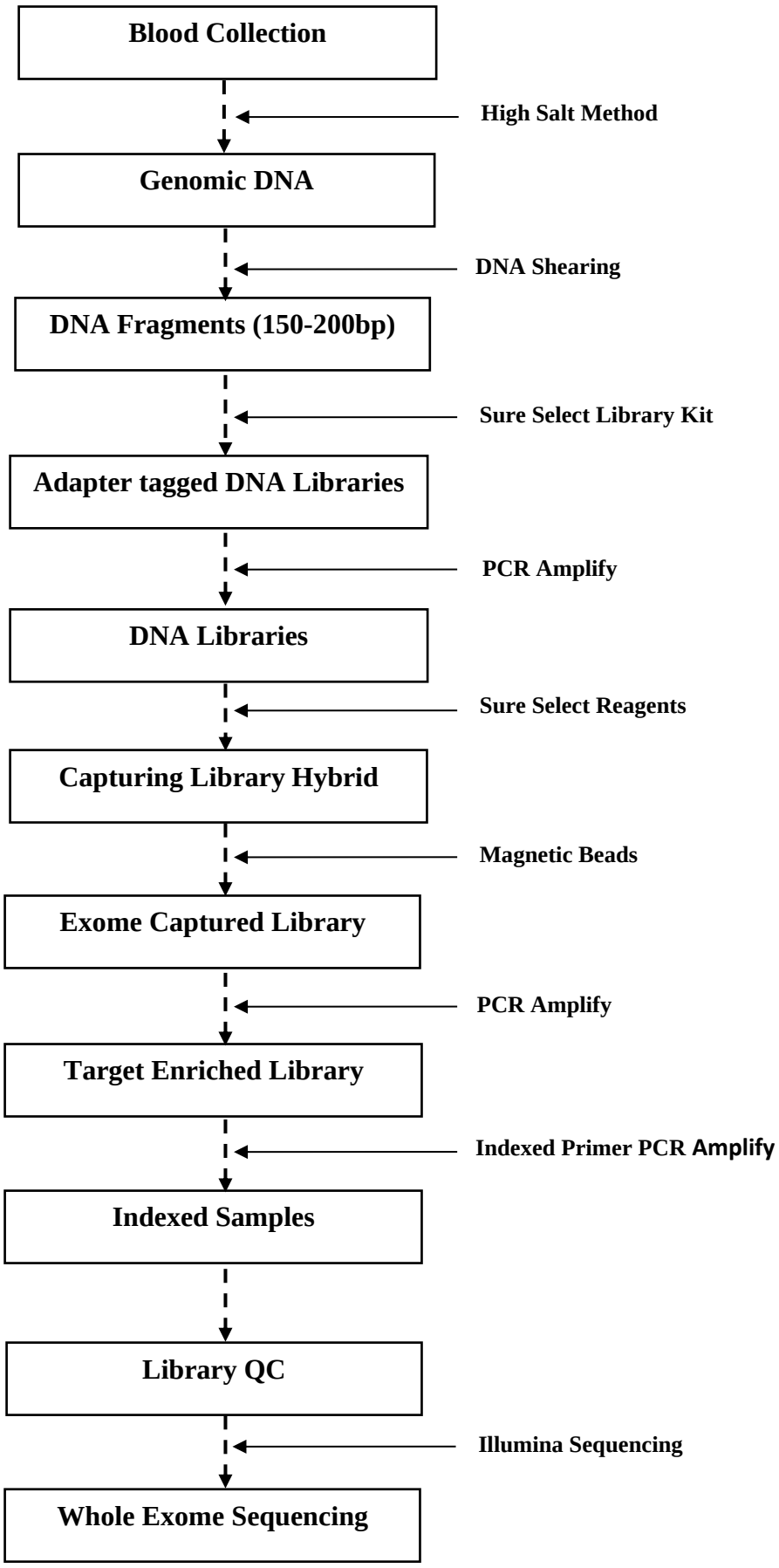

Fig 3.1 Workflow of Whole Exome Sequencing

3.3.4 Library Preparation

Library construction was carried out using Targeted exome sequencing library and it was prepared with Illumina-compatible Sure Select XT Library Prep Reagent Kit (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's instructions. 200 ng of Qubit quantified genomic DNA was sheared by adaptive focused acoustics using a Covaris S220 system (Covaris, Woburn, Massachusetts, USA). The fragment size distribution was verified on Agilent 2200 Tape Station. The fragments were end-repaired, adenylated and ligated to Illumina adaptors as per Sure Select XT library preparation kit protocol (Fig 3.1).

Adapter-ligated DNA was purified using Jet Seq Beads (Bioline, Cat # BIO-68031) and then amplified for 10 cycles of PCR using Illumina-compatible primers provided in the Sure Select XT kit. The amplified fragments were purified using Jet Seq Beads and the concentration was measured by Qubit fluorometer (Thermo Fisher Scientific, MA, USA).

Target enrichment was performed according to the manufacturer's instructions using Sure Select Bovine all exon capture baits. In-solution hybridization was then performed for 20 hours at 65°C. After hybridization, the captured target was pulled down by biotinylated probe-target hybrids using streptavidin-coated magnetic beads (Dyna beads My One Streptavidin T1 (Thermo Fisher scientific Inc).

The magnetic bead was washed according to the manufacturer's instructions and resuspended in 15 µl of nuclease free water. The captured DNA library was amplified for 10 cycles of PCR including appropriate indexing primer for each sample. The final PCR product (sequencing library) was purified with Jet Seq Beads followed by library quantification by Qubit fluorometer (Thermo Fisher Scientific, MA, USA) and its fragment size distribution was again analyzed on Agilent 2200 TapeStation.

3.3.5 Illumina Sequencing

The libraries were paired end sequenced on Illumina HiSeq X Ten (Illumina, San Diego, USA) for 150 cycles following manufacturer's instructions to generate 100X coverage per sample.

3.4 Whole Exome Sequencing Data Analysis

The Illumina-compatible sequencing library for the sample showed an average fragment size of 380 bp, as well as sufficient concentration for obtaining desired sequencing data. The sequenced data obtained using Illumina Hiseq (150 x 2 chemistry) was then demultiplexed using Bcl2fastq1 software v2.20 and Fast Q files were generated based on the unique dual barcode sequences. The sequencing quality was assessed using Fast QC2 v0.11.8 software. The raw reads were trimmed for adapter sequence, quality (Q30) and length (20bp) using Trim galore software v 0.6.4 to obtain good quality processed reads for downstream analysis (Fig 3.2). The adapter clipped; high quality reads were aligned against *Bos indicus* reference genome (Bos_indicus_1.0) using Bowtie2 v2.3.4.1 (Langmead and Salzberg, 2012) alignment tool. Alignment pre-processing was performed using SAMtools v1.9. It is a set of utilities that manipulate alignments in the SAM (Sequence Alignment Map), BAM, and CRAM formats, which converts between the formats, does sorting, merging and indexing, and can retrieve reads in any regions (Li *et al*., 2009) (Fig 3.2). SAM files were obtained, which were further processed to BAM files, using SAMtools v1.9 (Li *et al*., 2009), then PCR duplicates were reomoved using Picard. The reads were merged and piled up using SAMtools v1.9 for all the samples. The aligned files were used to predict the variants using VCFtools v0.1.15 (Danecek *et al*., 2011).

3.5 Variant Analysis

The variant files were called from the binary calling files separately for SNPs and InDels using VCFtools v0.1.15 (Danecek *et al*., 2011) (Fig 3.2). The genetic variants (SNPs and InDels) were filtered at minimum read depth (RD) of 10 and a mapping quality of 30 in order to minimize false positive rates. In addition to it, second quality checks were performed for filtering SNPs and InDels: Hardy Weinberg equilibrium (0.001), Minor allele frequency (0.01), Missing genotypes (0.7) and linkage disequilibrium (0.5) in order to obtain high quality SNPs and InDels.

Filtered genetic variants were annotated using SnpEff v4.4 (Cingolani *et al*. 2012). Candidate gene list for high reproductive performance and low reproductive performance were collected and analysed in the PANTHER and KEGG Database for Gene Ontology and pathway analysis (Mi and Thomas, 2009). Gene Ontology (GO) gave biological process (BP), cellular components (CC) and molecular functions as well as functional pathways for the genes identified from filtered genetic variant. This information was used to enrich the genes annotated

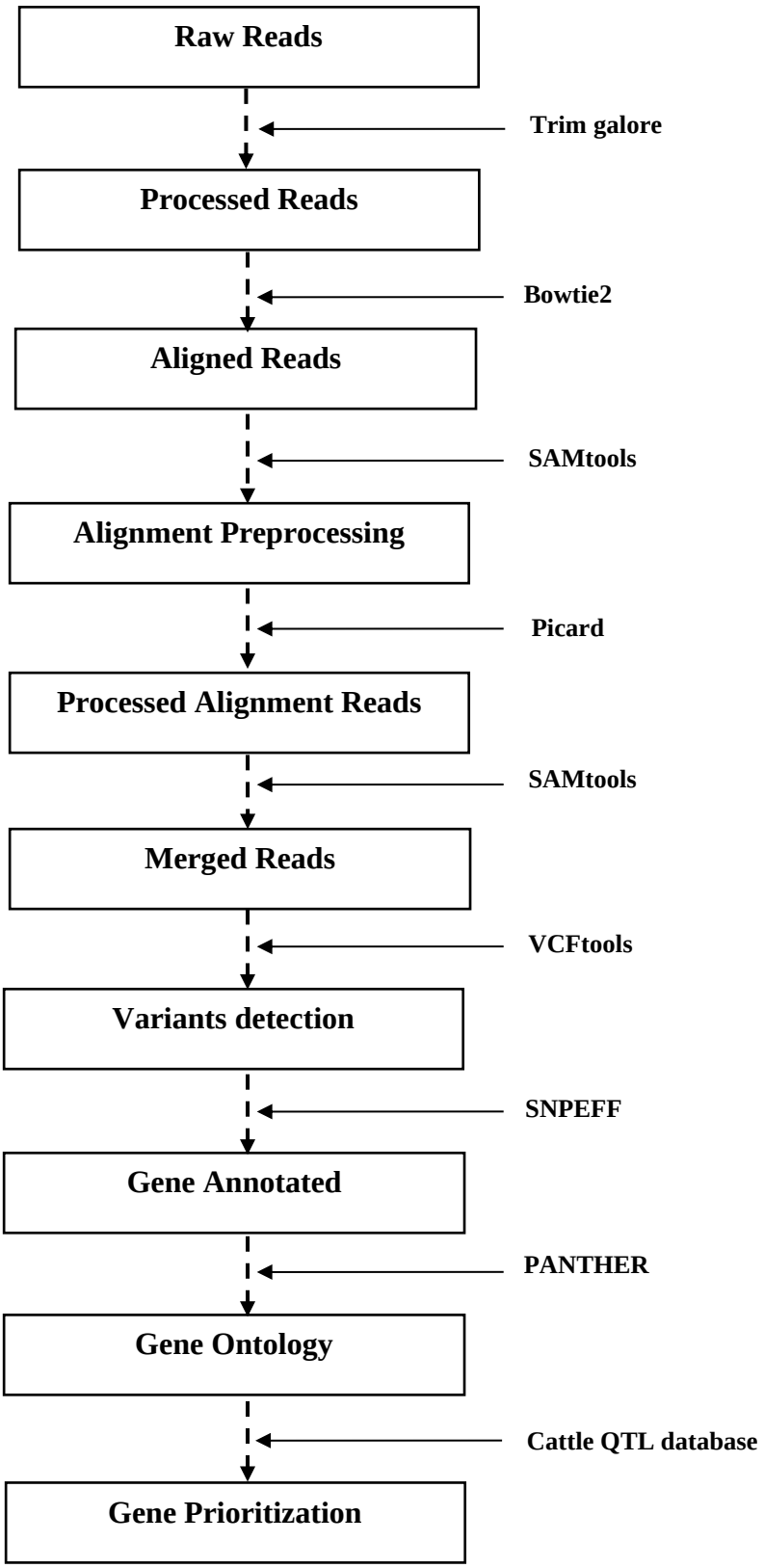

Fig 3.2 Workflow of Whole Exome Data Analysis

in this study. The genes identified in exome regions were cross referenced to the cattle QTLs for reproduction performance traits. The QTL information retrieved from the Cattle QTL database were used to prioritize genes related to reproductive performance traits (Hu *et al*., 2013).

3.6 Sample Preparation for Mass Spectrometry Proteomics Analysis

3.6.1 Plasma Separation

The blood sample was transferred carefully to 10 mL falcon tube for plasma separation. The plasma was separated at 5000 rpm for 10 mins in refrigerated centrifuge at 4°C (Eppendorf centrifuge 5810R). The plasma sample obtained were aliquoted in 500 µL cryovials and the samples were stored at -80 °C for further processing before the mass spectrometry analysis.

3.6.2 Protein Extraction and Digestion

Plasma samples (500µL from each biological replicate) were subjected to protein purification by acetone precipitation (at -20°C overnight). Subsequently acetone was removed and protein pellet was dried and resuspended in 50Mm TEABC. Protein quantity was estimated by biocinchoninic acid (BCA) assay (Pierce, Waltham, MA). Equal amounts of protein (200µg) were reduced using 100mM DTT (incubated at 60°C for 30 minutes) followed by alkylation using 100mM IAA (incubated at room temperature for 1 hour). Proteins were digested with modified sequencing-grade trypsin (Promega, Madison, WI) with 1:20 substrate/enzyme ratio at 37°C overnight. An aliquot of resulting peptides was run on 10% SDS-PAGE gel to confirm digestion. Peptides were vacuum dried and resuspended in 0.1% Formic acid and desalted by C-18 stage-tip method, as described previously (Subbannayya *et al*., 2015). Peptide estimation was carried-out using pierce quantitative peptide assay kit (Pierce, Waltham, MA).

3.6.3 Strong Cation Exchange (SCX) Fractionation

To build a bovine plasma spectral library, 25µg of peptides from each sample were pooled, dried and reconstituted in 2% trifluoroacetic acid (TFA). Peptide fractionation was carried out using an in-house prepared Stage Tip column-based protocol as discussed previously (Karthikkeyan *et al*., 2020) with minor changes. In short, the C18 material was stacked in to 200µl tip and activated by adding 100µl of 100% ACN followed by equilibration with 2% TFA. The reconstituted samples were loaded in to the activated stage tips to bind

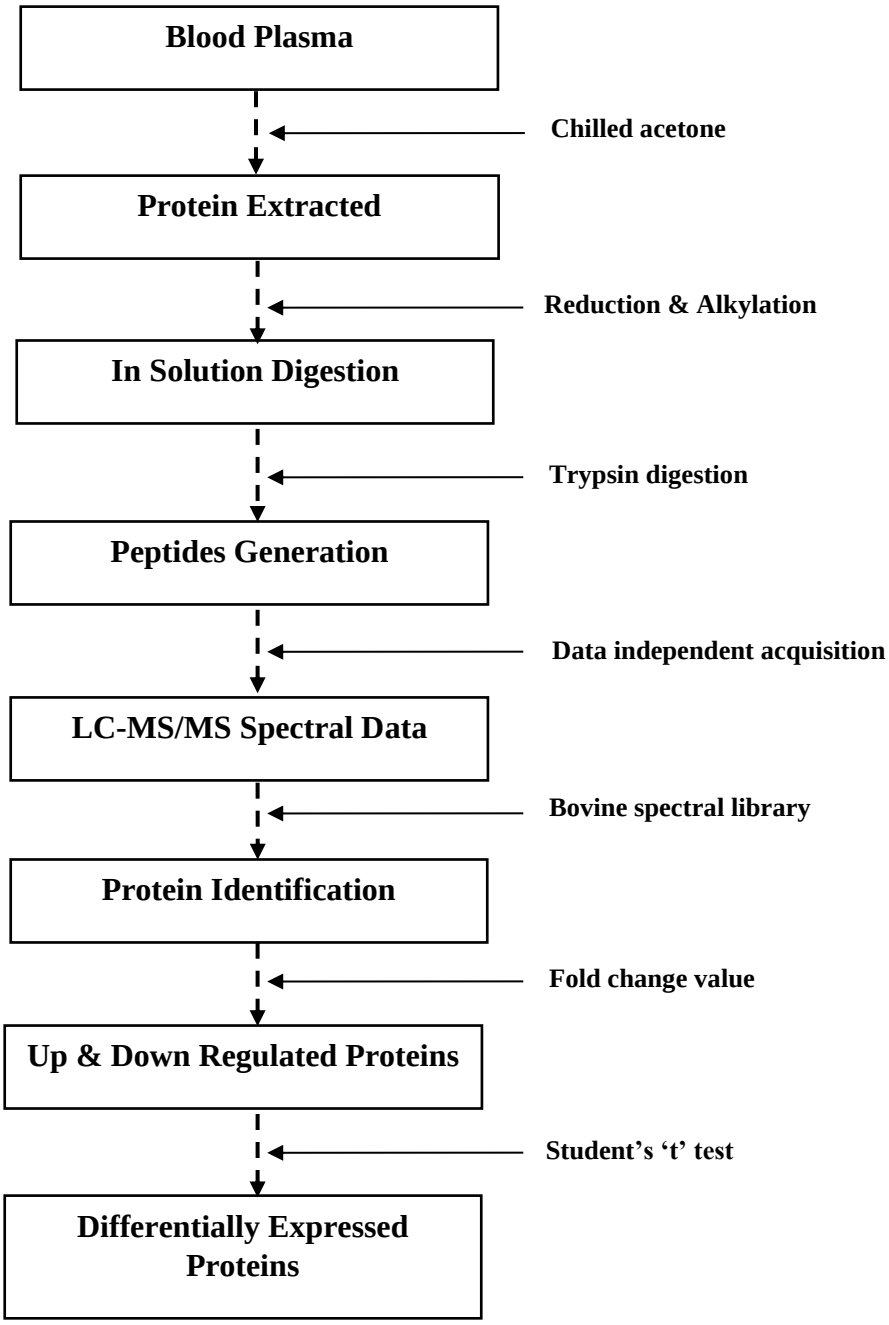

Fig 3.3 Workflow of Plasma Proteome Profiling and Data Analysis

peptides to C18 material. The flow-through was passed twice, followed by washing with 0.2% TFA. Peptides were eluted into 12 fractions using 2-80% gradient of ACN and 10mM TEABC. All the 12 fractions were analysed in DDA mode of LC-MS/MS to build an extensive spectral library.

3.6.4 LC-MS/MS by Data-Independent Acquisition (DIA) Method

An Orbitrap Fusion Tribrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) connected to the Easy- nLC-1200 nanoflow liquid chromatography system (Thermo Scientific) was used for data acquisition. Each sample (~1µg) was loaded on to a trap column (Acclaim PepMap, 2 cm X 0.75 µ ID, 2µm) and using an analytical column (Acclaim PepMap, 15 cm X 0.75µ ID, 2µm) peptides were made to separate and elute by passing Mobile Phase B (0.1% FA in 80% ACN) in gradient mode for 120 min at 300 nL/min. The percentage of Mobile Phase B at 0 min was 5% then gradually increased to 40% at 90min, this was further increased to 100% in another 15min and then kept constant for the next 15min. The EASY-nLC 1200 liquid chromatogram system was used for the entire chromatography separation process. Throughout the experiment, the temperature of the analytical column was kept at 45°C.

3.6.5 DDA Mode Acquisition to Generate Spectral Library

The 12 peptide fractions were subjected to MS survey scan at a mass scan range of 400−1600 m/z (120000 mass resolution at 200 m/z) in a data-dependent mode using an Orbitrap mass analyzer. The maximum injection time was set as 50ms. Only peptides with a charge state of 2−7 were considered for analysis, and the dynamic exclusion rate was set to 45 s. For MS/MS analysis, data was acquired at top speed mode with 3 s cycle and subjected to higher collision energy dissociation with 35% normalized collision energy. MS/MS scans were carried out at a range of 100−2000 m/z using the Orbitrap mass analyzer at a resolution of 30000 at 100 m/z. The maximum injection time was 200 ms.

3.6.6 Wide-Window DIA of Bovine Plasma Samples

The digested plasma samples were resuspended in mobile Phase A (0.1% formic acid). Equal volume (~2µg/µL) of the sample and iRT peptide mixture (~250fmol) was spiked and loaded onto a 96 well plate. Data was acquired in data-independent acquisition (DIA) mode. For ionization, EASY-Spray nano source (NSI) was used with spray voltage and source temperature of 2.1kV and 275°C, respectively. The precursor molecules in the 400-1100 m/z

range were filtered in quadrupole with 25Da isolation window (0.5m/z margin) and were fragmented at 30% HCD. There were 30 isolation windows in each cycle of DIA with maximum injection time of 60 ms and an automatic gain control (AGC) target value of 1e6. The fragment ions within m/z 145-2000 range were detected in Orbitrap at resolution 30K. Between each 20 MS/MS scans, one Full MS scan for the range of 350-1500 m/z were performed in Orbitrap at 120K resolution, with a maximum injection time of 50 ms and an automatic gain control (AGC) target value of 1e6. Throughout the acquisition, the RF level on the S-Lens was set to 55. The precursor and fragment scans were acquired in profile and centroid mode, respectively.

3.6.7 Gas-Phase Fractionation (GPF) by Narrow-Window DIA

A small amount of plasma digest from all the samples were pooled and used for gas-phase fractionation (GPF) using a narrow-window DIA method as mentioned by Searl *et. al.* (2020) and Pino *et al.* (2020). For the precursors with m/z ranges of 400-1100, the pooled sample was fractionated into 7 groups (400-502, 500-602, 600-702, 700-802, 800-902, 900-1002, 1000-1102). Each fraction was acquired separately with 4 m/z X 52 isolation windows (generated with Skyline version 21.1.0.146) and the overlap deconvolution method (MacLean *et al*., 2010). GPF was performed in between fixed wide window DIA runs. The remaining LC-MS/MS parameters were the same as those used in the wide window DIA method described earlier.

3.7 Proteomics Data Analysis

3.7.1 Building in-House Plasma Spectral Library from DDA Data

Plasma proteome data acquired through DDA mode were used to build an in-house spectral library using Proteome Discoverer 2.2 (Thermo Fisher Scientific, Bremen, Germany). Data were searched against RefSeq reference proteome of *Bos Taurus* (version 106) and known contaminants (116 entries) using SequestHT and MASCOT algorithms. Static modifications included carbamidomethylation of cysteine. Oxidation of methionine and protein N-terminal acetylation were set as a dynamic modification. Minimum peptide length was set to six amino acids and up to two missed cleavages were allowed. Proteins were identified at 1% False Discovery Rate (PSM, peptide and protein level) with a mass tolerance of 20 ppm at MS level and 0.02 Da at MS/MS level.

3.7.2 Chromatogram Library from GPF

ProteoWizard was used to convert all 7 GPF raw data files into mzML format. During the conversion of the files, filter options were set to demultiplex overlapping windows with a mass error of 10 ppm and peak picking at MS levels 1 and 2. The mzML files were searched against the *Bos taurus* predicted spectral library from Prosit (https://www.proteomicsdb.org/prosit/libraries/) using EncyclopeDIA version 1.12.31 (https://bitbucket.org/searleb/encyclopedia/wiki/Home). The *Bos taurus* proteome database (version 106) was used in the background. The search was carried out using parameters such as Target/Decoy Approach: Normal Target/Decoy, and trypsin as an enzyme. CID/HCD (B/Y) fragmentation precursor mass tolerance of 10 ppm; fragment mass tolerance of 10 ppm; library mass tolerance of 10 ppm; and percolator version: v3-01. The number of quantitative ions was set to 5 with a minimum of 3 ions, and the number of cores was set to 5 with the additional command line option "-filter Peaklists true". Finally, at 1% FDR, the search results for each fraction were merged into a single chromatogram library.

ProteoWizard was used to combine all 7 GPF mzML files into a single mzML file, which was then used to generate a calibrated iRT calculator in Skyline (MacLean *et al*., 2010). This single mzML file was searched against the chromatogram library using Skyline. Finally, the peptide precursors were reintegrated with a decoy-trained mProphet model in which the "Retention time difference" feature score ignored during model training. After reintegrating the peptide precursors, 14 pierce iRT peptides (iRT-C18) were chosen and calibrated against the entire chromatogram library of reintegrated peptide precursors.

3.7.3 Spectral Library Search

Using Skyline version 21.1.0.146, all DIA raw files from the *Bos Indicus* plasma digest acquisition were searched against the in-house plasma spectral library and chromatogram library built from DDA and GPF data (MacLean *et al*., 2010). The calibrated iRT model was incorporated into the Skyline document. By inverting the sequence, decoy peptides were created and added to the document. In the peptide settings tab, the digestion parameters are set as; Enzyme: Trypsin [KR|P] and Max missed cleavages: 2. *Bos taurus* RefSeq V106 was set as the Background proteome and Enforce peptide uniqueness by: None option was selected. In the filter tab, Min length: 7, Max length: 50, Auto-select all matched transition options were given for selection criteria. Under the Library tab, for Pick peptides matching, Library and Filter option was selected and peptides ranking was done based on Picked intensity. For the structural modifications: Carbamidomethyl at cysteine (C) was selected for the fixed

modification, and Acetyl at N-terminal and Oxidation at methionine (M) were selected as variable modifications, respectively.

For the transition setting, Precursor charges of 2, 3, 4, 5 and 6 were selected and also, parameters such as ion charges (1 and 2), Ion types (y, b, and p) were set. Product ion selection range was given as "ion 3" to "last ion". Under the filter tab, Auto-select all matching transition and use DIA precursor window for exclusion options were selected. In the Library tab, the ion match tolerance parameter was set as 0.02 m/z and the option "if a library spectrum is available, pick its most intense ions" (Product ion range was given as 3-6 ions) from filtered product ions were selected. Min m/z of 145 m/z, Max m/z of 2000 m/z were set under the Instrument tab. Also, Min time: 3 min, Max time: 100 min were set. For MS1 filtering, an Orbitrap mass analyzer with a resolving power of 1,20,000 at 200 m/z was employed with 3 isotope peaks. For MS/MS filtering the acquisition method was set as DIA with isolation schema for 400-1100 m/z range with 25m/z window width and 0.5 m/z margin. Centroided with mass accuracy of 20 ppm was set for the product mass analyser. Use high-selectivity extraction option was selected and for retention time filtering, use only scans within 5 minutes of predicted RT was set.

After importing all the files into the Skyline document, peptide precursors which were having less than 6 transitions (at least three precursor and product ions each) were discarded. The remaining peptide precursors were reintegrated using a decoy-trained mProphet model. Finally, peptide precursors which were having q-value of less than 0.01 were considered as true identifications.

3.8 Statistical Analysis

The area under the curve (AUC) of all peptide precursor product ion transitions (q-value < 0.01) with corresponding protein information was exported and used for protein relative abundance calculation using iq (Pham *et al*., 2020). In iq, the AUC of fragment ion transitions were provided as input and a protein table with abundance values was generated using "maxLFQ" method. The log2 transformed abundance values were converted back and subjected to quantile normalization using R. Finally, p-value and fold change were calculated by applying Student's T-test and average normalized abundance values, respectively. Proteins with p-value < 0.05 and fold change ≥ 1.5 were considered as upregulated where fold change ≤ 0.66 were considered as downregulated.

3.9 Bioinformatics Analysis

Enrichment analysis of subcellular localization, molecular functions, and biological processes of differentially expressed proteins was carried out by PANTHER classification system database (http://pantherdb.org/). Protein-protein interaction and clustering analysis was performed using STRING: functional protein association networks database. For statistical comparison, data were analysed using student's t-test. The proteins with p-value < 0.05 were considered statistically significant.

CHAPTER –4

Results and Discussion

RESULTS AND DISCUSSION

4.1 Selection of Experimental Animals

A total of 12 Deoni cows (6 with high reproductive performance and 6 with low reproductive performance) maintained at Livestock Research Center, Southern Regional Station, ICAR-National Dairy Research Institute, Bengaluru were selected in order to identify the genes in exome region and plasma proteins associated with reproductive performance (RP) traits in Deoni cattle (Table 4.1). The data of Deoni cows viz. date of birth, date of calving, number of calving, service period, history of calving, and health history were collected from Livestock Research Complex, Southern Regional Station of ICAR-National Dairy Research Institute, Bengaluru. The number of days open and inter-calving period ranged from 80 to 377 days and 366 to 650 days respectively. In high reproductive performance (HRP) group, mean days open and inter-calving period were 113±23.98 days and 398±24.85 days respectively. Likewise, in low reproductive performance group mean days open and inter-calving period were 292±60.82 days and 576±58.90 days respectively.

4.2 Whole Exome Sequencing Analysis

4.2.1 Primary Analysis

Whole exome sequencing was done through outsourcing in genotypic technology on Illumina Hi-seq platform (150x2chemistry) to generate 100X coverage per sample. A total of ~96 million paired end raw reads were generated from all the Deoni samples. The raw reads were trimmed for adapter sequence and removed reads having phred score of 30 and below using Trimgalore to obtain good quality processed reads. After the quality control (QC), 99.50 % of raw reads were retained. The total numbers of raw and processed reads for all samples are given in table 4.2.

Table 4.1 Reproductive Performance of Deoni Cows

Animal No.	Days open (days)	Inter-calving period (days)	Group
D1	134	420	High RP
D2	114	402	High RP
D3	132	421	High RP
D4	88	369	High RP
D5	80	366	High RP
D6	127	412	High RP
D7	377	599	Low RP
D8	205	650	Low RP
D9	276	489	Low RP
D10	325	570	Low RP
D11	251	618	Low RP
D12	318	531	Low RP

4.2.2 Alignment and Alignment Postprocessing

The quality control (QC) passed reads were mapped to the reference genome of *Bos indicus* (*Bos-indicus*-1.0) using Bowtie2. The overall alignment rate for all the Deoni samples were 92.90%. A total of ~85 million paired end unique reads were obtained from all the samples (Table 4.3). The duplicate reads were removed using Picard. Mapped reads were sorted chromosome-wise for all the samples in SAMtools. Using maximum likelihood algorithm, high reproductive performance (HRP) group and low reproductive performance (LRP) group Deoni cows were merged separately. Finally, variants were called out using VCF tools in high reproductive performance (HRP) group and low reproductive performance (LRP) groups.

Table 4.2: Number of Raw and Processed Reads in Deoni cows

Sample number	Number of raw reads generated	Number of QC processed reads	Processed reads in %
D1	87301884	86810822	99.43
D2	97374764	96907364	99.51
D3	95778926	95199198	99.39
D4	87239798	86710162	99.39
D5	98211418	97631750	99.40
D6	90624072	90131424	99.45
D7	96172584	94416556	98.17
D8	100961258	100363706	99.40
D9	96731400	96249118	99.50
D10	103161368	102598082	99.45
D11	90852286	90397398	99.49
D12	108990126	108358746	99.42
Average	**96116657**	**95481194**	**99.33**

Table 4.3: Number of Mapped and Unique Reads in Deoni Cows

Sample number	Total no of mapped reads	Total mapped reads in %	Uniquely mapped reads	Uniquely mapped reads in %
D1	80484254	92.71	77350952	89.10

D2	89586245	92.45	87489866	90.28
D3	88039712	92.48	85133298	89.43
D4	80317246	92.63	77153320	88.98
D5	90559631	92.76	87553268	89.68
D6	83388504	92.52	80427020	89.23
D7	87186038	92.34	77579036	82.17
D8	92714261	92.38	88257160	87.94
D9	90140660	93.65	87669032	91.09
D10	96039206	93.61	93390988	91.03
D11	84657656	93.65	81577140	90.24
D12	101409873	93.59	98706438	91.09
Average	**88710274**	**92.90**	**85190627**	**89.18**

4.2.3 Variant Calling, Quality Control of SNPs and Annotation

A total of 297,398,612 variants were identified in the high reproductive performance group and 162,565,301 variants were identified in the low reproductive performance group using the following parameters read depth (RD) 10 and mapping quality (MQ) 30 in VCFtools (Table 4.3). Additionally, a second quality filtration such as Hardy Weinberg equilibrium (HWE) 0.001, Minor allele frequency (MAF) 0.01, Missing genotypes 0.7 and Linkage disequilibrium (LD) 0.5 were applied to exclude false positive SNPs and InDels from all the samples. As a result of second filtration, a total of 312 SNPs obtained in the high reproductive performance group and 301 SNPs obtained in the low reproductive performance group (Table 4.4), are in agreement with earlier report of Whiston *et al*. (2017) on SNP identification in bulls with divergent in the fertility. Similarly, whole exome sequencing was performed in Quarter Horses with contrasting maximum speed index and only 24 SNPs were identified related to racing performance in Quarter Horses (Pereira *et al*., 2019).

Table 4.4: Number of SNPs and InDels Identified at Different Filtration Level

Reproductive performance group	High		Low	
Filtration level	**RD10* MQ30***	**HWE*, MAF*, Missing genotypes, LD***	**RD10* MQ30***	**HWE*, MAF*, Missing genotypes, LD***
SNPs	5,743,171	312	4,717,904	301
Insertions	291,480,110	0	157,744,275	0
Deletions	175,331	0	103,122	0
Total Variants	**297,398,612**	**312**	**162,565,301**	**301**

*RD-Read depth; *MQ-Mapping quality; *HWE-Hardy Weinberg equilibrium; *MAF-Minor allele frequency; *LD-Linkage disequilibrium

Chromosome-wise distribution of SNPs identified in high and low reproductive performance groups are given in table 4.5. SNPs identified and filtered were annotated against *Bos indicus* reference genome using SNPEFF program. A total of 221 genes were identified specific to the high reproductive performance group and 244 genes were identified specific to the low reproductive performance group.

Table 4.5: Chromosome-wise Distribution of SNPs in Deoni Cattle

Chromosome no.	High reproductive performance cow	Low reproductive performance cow
1	8	12
2	18	16
3	20	17
4	9	9
5	10	23
6	3	4

7	16	10
8	3	11
9	7	4
10	16	12
11	31	13
12	7	9
13	16	13
14	7	8
15	7	10
16	4	7
17	14	13
18	21	20
19	13	26
20	9	5
21	9	8
22	7	4
23	7	6
24	8	1
25	13	9
26	9	3
27	5	3
28	2	7
29	4	11
X	9	7
Total	**312**	**301**

4.3 Gene Ontology of Genes Identified between High and Low Reproductive Performance Groups

Using the Panther software, the genes identified with SNPs in the high and low reproductive performance groups were analyzed to find out the molecular function, biological process, and cellular component involvement. The majority of molecular functions activities were enriched in binding activity (38.5%), catalytic activity (29.3%), transcription regulation activity (7.5%), transporter activity (7.5%), molecular function regulator (5.2%) and molecular transducer activity (4.6%) (Fig 4.1). Likewise in biological processes: cellular process (29.9.%), metabolic process (16.2%), biological regulation (16.0%), response to stimulus (7.2%), localization (6.5%), multicellular organismal process (5.8%), signaling activities (5.2%), developmental process (4.9%) and biological adhesion (2.5%) genes were found to be enriched (Fig 4.2). In cellular components, genes with identified genetic variants were enriched in the cellular anatomical entity (76.30%) and protein-containing complex (23.70%).

4.4 Genes Identified in High Reproductive Performance

A total of 221 genes with 312 SNPs were identified from the high reproductive performance group in the gene annotation. The details of identified gene and their protein class are given in table 4.6.

ADAM metallopeptidase with thrombospondin Type 1 motif 19 (ADAMTS19) gene encodes a disintegrin metalloproteinase with thrombospondin motifs and it is a part of several protein modules. ADAMTS enzymes were localized in extracellular matrix and regulates various roles like matrix formation, resorption, angiogenesis, ovulation and coagulation (Demircan *et al*., 2014). Knauff *et al*. (2009) and Pyun *et al*. (2013) identified a polymorphism in the exon region of ADAMTS19 gene and found to have association with premature ovarian failure in humans. In this study a SNP was identified at locus c.23130951 in ADAMTS19 gene on chromosome 7 with a substitution of A>CTG.

Transient receptor potential vanilloid 4 (TRPV4) gene is a member of calcium permeable and non-selective cation ion channel. In the present study a variant was identified at locus c.66732516 of TRPV4 gene with a substitution of T>AGC. This gene is reported to modulate systemic osmotic pressure in the smooth muscle tissues. In pregnant animals, expression of TRPV4 gene was up-regulated when compared to non-pregnant animals. TRRV4

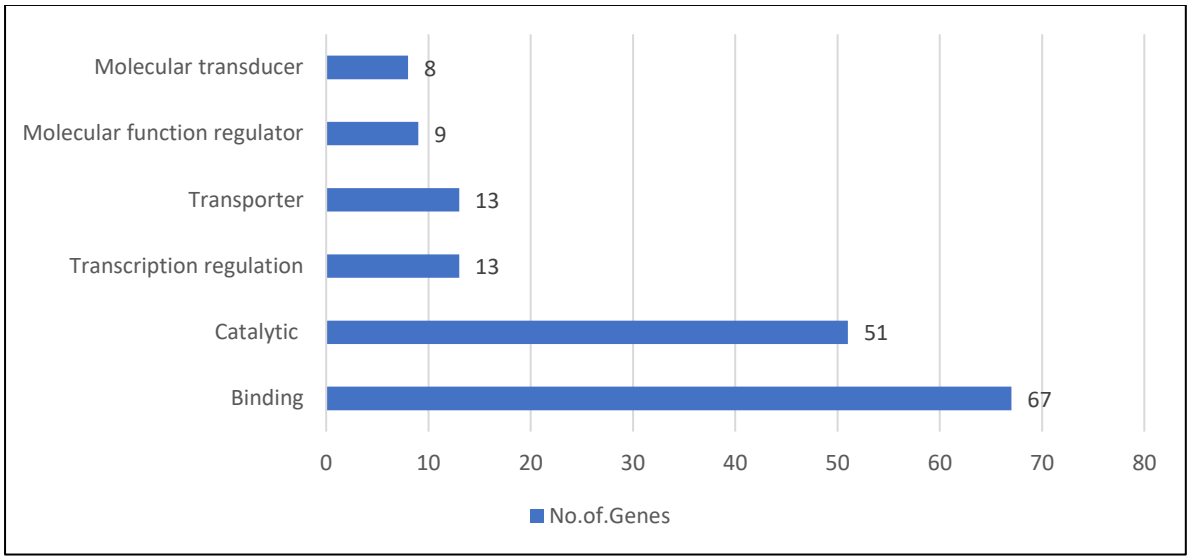

Fig 4.1 Number of Genes Enriched in Molecular Functions in Deoni Cows

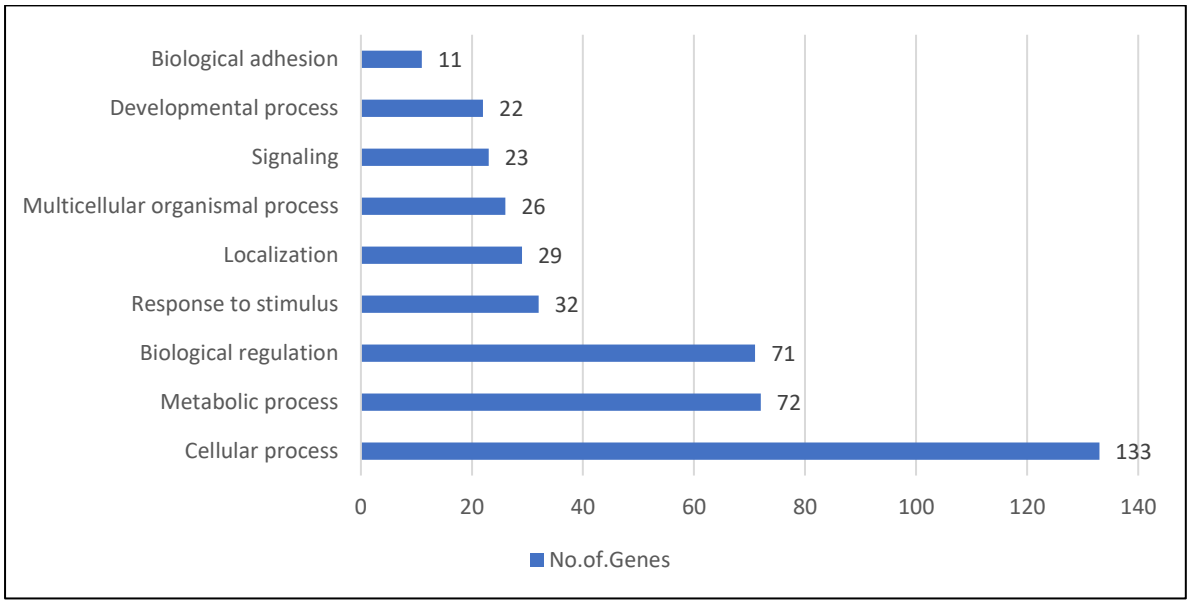

Fig 4.2 Number of Genes Enriched in Biological Processes in Deoni Cows

Gene was also found to alter the uterine contractility through calcium channel in response to endometrial osmotic pressure shift (Ying *et al*., 2015). Ducza *et al*. (2019) observed the co-expression of TRPV4 and AQP5 genes in the regulatory effect of the endometrium contraction at the time of parturition.

Hepatoma-derived growth factor (HDGF) gene have mitogenic and DNA-binding activity and involved in the cellular proliferation and differentiation. A genetic variant was identified in HDGF gene at locus c.15504496 with a substitution of A>TGC in this study. HDGF was reported as one of the potential embryokines in the bovine reproductive tract for the development of preimplant embryo (Tribulo *et al*., 2018). This gene was identified to be localized in the endometrium of cattle and it has significant effect at early developing stage of bovine blastocyst (Gomez *et al*., 2017).

Ran GTPase-activating protein 1 (RANGAP1) gene play a significant role in the nucleo-cytoplasmic transport pathway. A variant was identified at a locus c.114353282 of RANGAP1 gene with a substitution of C>TAG. The protein encoded by this gene interacts with the nuclear pore complex and controls nuclear transport in the cell. In oocyte, RANGAP1 gene regulates spindle functions, chromosome alignment and pronuclei nuclear envelope assembly at the time of oocyte fertilization (Weijie *et al*., 2018).

Receptor tyrosine kinase like orphan receptor 2 (ROR2) gene is a receptor protein belonging to the ROR subfamily present on the cell surface receptors. In the present study, a genetic variant was identified in ROR2 gene at locus c.90418480 with a substitution of T>CAG. ROR2 gene receptor was reported to control the cell polarity, asymmetric cell division, and cell movements during the early stage of embryonic development linked through non-canonical Wnt signaling pathway (Denicol *et al*., 2013). ROR2 receptor located in the endometrial tissue was found to be up regulated during the period of implantation in the cattle (Rabaglino and Kadarmideen, 2020).

ADAM with thrombospondin type-1 motif member 13 (ADAMTS13) gene produce an enzyme which particularly cleaves von Willebrand factor and also involved in matrix formation, ovulation and embryo implantation (Demicran *et al*., 2014; Dubail and Apte, 2015). Xiao *et al*. (2017) observed the significant role of ADAMTS13 gene in angiogenesis and placenta development, when this gene expression was compared between normal and abnormal placentae. Similarly, Feng *et al*. (2021) found association between down regulation of

ADAMST13 and recurrent pregnant loss in human. In our study, a missense variant was predicted at a locus c. 108359385 with substitution of A>GTC.

CCR4-NOT transcription complex subunit 1 (CNOT1) gene facilitates the stability of embryonic stem cells and it has been found to have association with fertility trait in cattle (Pimentel *et al*., 2011). Lysine methyl transferase (KMT2A) gene plays an important role during the time of early development and hematopoiesis and it has been found to have association with caving ease trait and also with length of productivity trait in Holstein-Friesian cattle population (Cole *et al*., 2011). Protein tyrosine phosphate receptor type S (PTPRS) gene was identified to have signal molecules that would signal a variety of cellular process activity in different tissues and it has been found to have association with fertility traits in cattle (Galliou *et al*., 2020).

4.5 Genes Identified in Low Reproductive Performance

A total of 244 genes with 302 SNPs were identified from the low reproductive performance group in the gene annotation. The details of identified gene and their protein class are given in table 4.7.

Thrombospondin 4 (THBS4) gene is the family member of glycoprotein. A genetic variant at locus c.10797357 with a substitution of A>GTC in THBS4 gene was identified in the present study. Thrombospondin glycoprotein has an adhesive property which mediate cell-to-cell and cell-to-matrix interactions. THBS4 gene reported to play a role in the cell attachment of embryo in the bovine endometrium due to its adhesive property. In repeat breeders' cow, THBS4 gene expression was observed to be down regulated in the endometrial tissue, when compared to non-repeat breeders (Hayashi *et al*., 2017).

Solute Carrier Family 37 Member 2 (SLC37A2) gene is an integral component of endoplasmic reticulum membrane. SLC37A2 reported to be involved in the glucose-6-phosphate transport and phosphate ion transmembrane transport. In Ayrshire, Brown Swiss, Holstein, Jersey, Montbéliarde, and Normande breeds of cattle, lethal mutation was reported in SLC37A2, causing negative influence on calving rate and prenatal lethality (Fritz *et al*., 2013). In our study, we identified a genetic variant in SLC37A2 at locus with a substitution of G>CAT.

Bromodomain and WD Repeat Domain Containing 1 (BRWD1) gene is a member of the WD repeat protein family. Genes in this family member were involved in a variety of

cellular processes such as cell cycle progression, signal transduction, apoptosis, and transcription regulation. BRWD1 gene is crucial for maintaining stability during epigenetic control of meiotic chromosome stability, any mutation in BRWD1 gene would cause impaired reproduction performance in mice (Pattabiraman *et al*., 2015). In Nordic Holstein-Friesian dairy cattle, BRWD1 gene was identified to have association with fertility traits (Cai *et al*., 2019). In the present study, genetic variant was identified at locus c.141614782 on BRWD1 gene with a substitution of A>TCG.

RAB GTPase Binding Effector Protein 2 (RABEP2) gene involved in the membrane trafficking, GTPase activator activity and growth factor activity and it has been found to have association with days open related reproductive traits in Holstein-Friesian cattle (Cochran *et al*., 2013; Ortega *et al*., 2017). Endothelial PAS Domain Protein 1 (EPAS1) gene regulates oxygen homeostasis by controlling transcription factors of gene and it has been found to have association with fertility trait in dairy cattle (Jiang *et al*., 2019).

Forkhead box H1 (FOXH1) gene was found to control the embryonic gene expression pattern in the early embryonic stage (Kofron *et al*., 2004). A missense genetic variant was predicted at locus c.240004 with substitution of T>CAG in FOXH1 gene in this study. FOXH1 transcription factor is one of the major transcription factors required at different stages of folliculogenesis process in oocyte and also in somatic theca and granulosa cell for normal ovarian function (Uhlenhaut and Treier, 2011). Tao *et al*. (2020) reported that mutation in FOXH1 gene cause arrest of oogenesis resulted in infertility in tilapia fish.

Branched Chain Keto Acid Dehydrogenase E1 Subunit Alpha (BCKDHA) gene is an inner mitochondrial enzyme complex that catalyze one of the major steps in the catabolism of the amino acids leucine, isoleucine, and valine and this gene was found to have association with conception rate in Holstein-Friesian dairy cattle (Jiang *et al*., 2019). Hoglund *et al*. (2015) found association between EIF4G3 gene and interval from first insemination to successful insemination in Nordic Red cattle.

Table 4.6 List of Genes Identified in Deoni Cows with High Reproductive Performance

Gene Symbol	Gene name	Protein class
ADAMTS19	ADAM metallopeptidase with thrombospondin type 1 motif 19; ADAMTS19; ortholog	Metalloprotease
TRPV4	Transient receptor potential cation channel subfamily V member 4; TRPV4; ortholog	Ion channel
ZNF274	Neurotrophin receptor-interacting factor homolog; ZNF274; ortholog	C2H2 zinc finger transcription factor
HDGF	Hepatoma-derived growth factor; HDGF; ortholog	Transcription cofactor
KLK5	Kallikrein C; KLK5; ortholog	Serine protease
NSMAF	Neutral sphingomyelinase activation associated factor; NSMAF; ortholog	Scaffold/adaptor protein
RANGAP1	Ran GTPase activating protein 1; RANGAP1; ortholog	GTPase-activating protein
P2RY14	P2Y purinoceptor 14; P2RY14; ortholog	G-protein coupled receptor
WWC2	WW and C2 domain containing 2; WWC2; ortholog	Protein wwc2
GALNT16	Polypeptide N-acetylgalactosaminyl transferase; GALNT16; ortholog	Glycosyltransferase
HAPLN2	Hyaluronan and proteoglycan link protein 2; HAPLN2; ortholog	Extracellular matrix glycoprotein
SLC12A9	Solute carrier family 12 member 9; SLC12A9; ortholog	Secondary carrier transporter
MYH15	Myosin heavy chain 15; MYH15; ortholog	Actin or actin-binding cytoskeletal protein

CCDC134	Coiled-coil domain containing 134; CCDC134; ortholog	Coiled-coil domain-containing protein 134
SRP14	Signal recognition particle 14 kDa protein; SRP14; ortholog	RNA metabolism protein
MATK	Tyrosine-protein kinase; MATK; ortholog	non-receptor tyrosine protein kinase.
TMEM39B	Transmembrane protein 39B; TMEM39B; ortholog	transmembrane protein 39b
KCNC4	Potassium voltage-gated channel subfamily C member 4; KCNC4; ortholog	voltage-gated ion channel.
RAB5C	Ras-related protein Rab-5C; RAB5C; ortholog	small gtpase.
ZNF541	Zinc finger protein 541; ZNF541; ortholog	chromatin/chromatin-binding, or -regulatory protein.
GREB1L	GREB1 like retinoic acid receptor coactivator; GREB1L; ortholog	greb1-like protein.
EIF5B	Eukaryotic translation initiation factor 5B; EIF5B; ortholog	translation initiation factor.
CCDC183	Coiled-coil domain containing 183; CCDC183; ortholog	coiled-coil domain-containing protein 183
TFIP11	Tuftelin-interacting protein 11; TFIP11; ortholog	rna splicing factor.
WDR31	WD repeat domain 31; WDR31; ortholog	wd repeat-containing protein 31
TAF2	Transcription initiation factor TFIID 150 kDa subunit; TAF2; ortholog	general transcription factor.
ROR2	Receptor tyrosine kinase like orphan receptor 2; ROR2; ortholog	transmembrane signal receptor.
NUP210	Nucleoporin 210; NUP210; ortholog	transporter.

RFX8	RFX family member 8, lacking RFX DNA binding domain; RFX8; ortholog	winged helix/forkhead transcription factor.
DMKN	Dermokine; DMKN; ortholog	dermokine
CHEK2	Checkpoint kinase 2; CHEK2; ortholog	non-receptor serine/threonine protein kinase.
RRBP1	Ribosome binding protein 1; RRBP1; ortholog	scaffold/adaptor protein.
OCA2	OCA2 melanosomal transmembrane protein; OCA2; ortholog	primary active transporter.
NFAM1	NFAT activating protein with ITAM motif 1; NFAM1; ortholog	nfat activation molecule 1.
MRPL10	39S ribosomal protein L10, mitochondrial; MRPL10; ortholog	ribosomal protein.
CPA3	Carboxypeptidase A3; CPA3; ortholog	metalloprotease.
PTPRS	Protein-tyrosine-phosphatase; PTPRS; ortholog	protein phosphatase.
GGT5	Gamma-glutamyltransferase 5; GGT5; ortholog	protease.
JKAMP	JAMP protein; JKAMP; ortholog	scaffold/adaptor protein.
WASF2	Wiskott-Aldrich syndrome protein family member 2; WASF2; ortholog	non-motor actin binding protein.
CELSR1	Cadherin EGF LAG seven-pass G-type receptor 1; CELSR1; ortholog	cadherin.
VPS29	Vacuolar protein sorting-associated protein 29; VPS29; ortholog	vesicle coat protein.
IL1F10	Interleukin-1; IL1F10; ortholog	interleukin superfamily.

OSBPL7	Oxysterol-binding protein; OSBPL7; ortholog	transfer/carrier protein.
STAU1	Staufen double-stranded RNA binding protein 1; STAU1; ortholog	double-stranded rna-binding protein staufen homolog 1
ANO2	Anoctamin; ANO2; ortholog	ion channel.
PLK3	Serine/threonine-protein kinase PLK; PLK3; ortholog	non-receptor serine/threonine protein kinase.
EIF2AK4	Non-specific serine/threonine protein kinase EIF2AK4; ortholog	non-receptor serine/threonine protein kinase.
CTNND2	Catenin delta 2; CTNND2; ortholog	intermediate filament binding protein.
MBP	Myelin basic protein; MBP; ortholog	myelin protein.
SLC22A15	Solute carrier family 22 member 15; SLC22A15; ortholog	secondary carrier transporter.
CEP72	Centrosomal protein 72; CEP72; ortholog	centrosomal protein of 72 kda
KAT2A	Histone acetyltransferase; KAT2A; ortholog	histone acetyltransferase kat2a
ZNF683	Zinc finger protein 683; ZNF683; ortholog	c2h2 zinc finger transcription factor.
SHANK2	SH3 and multiple ankyrin repeat domains 2; SHANK2; ortholog	scaffold/adaptor protein.
KMT2A	Histone-lysine N-methyltransferase; KMT2A; ortholog	histone modifying enzyme.
ZNF638	Zinc finger protein 638; ZNF638; ortholog	rna processing factor.
TFIP11	Tuftelin-interacting protein 11; TFIP11; ortholog	rna splicing factor.
TMEM79	Transmembrane protein 79; TMEM79; ortholog	transmembrane protein 79.

PLA2G4B	Phospholipase A2; PLA2G4B; ortholog	phospholipase.
DNAH7	Dynein axonemal heavy chain 7; DNAH7; ortholog	microtubule binding motor protein.
RPL11	60S ribosomal protein L11; RPL11; ortholog	ribosomal protein.
FSCN1	Fascin; FSCN1; ortholog	non-motor actin binding protein.
RASSF4	Ras association domain family member 4; RASSF4; ortholog	scaffold/adaptor protein.
SLC24A3	Solute carrier family 24 member 3; SLC24A3; ortholog	secondary carrier transporter.
HTRA1	Serine protease HTRA1; HTRA1; ortholog	serine protease.
LARP1	La ribonucleoprotein 1, translational regulator; LARP1; ortholog	rna metabolism protein.
HSPB9	Heat shock protein beta-9; HSPB9; ortholog	heat shock protein beta-9
YLPM1	YLP motif containing 1; YLPM1; ortholog	dna metabolism protein.
PGPEP1	Pyroglutamyl-peptidase I; PGPEP1; ortholog	cysteine protease.
TCF7	Transcription factor 7; TCF7; ortholog	dna-binding transcription factor.
RABL6	Rab-like protein 6; RABL6; ortholog	small gtpase.
IRF2BPL	Interferon regulatory factor 2 binding protein like; IRF2BPL; ortholog	zinc finger transcription factor.
VAC14	Protein VAC14 homolog; VAC14; ortholog	scaffold/adaptor protein.
IDO2	Indoleamine 2,3-dioxygenase 2; IDO2; ortholog	oxygenase.

DERL3	Derlin; DERL3; ortholog	primary active transporter.
ATCAY	ATCAY kinesin light chain interacting caytaxin; ATCAY; ortholog	phosphodiesterase.
FNDC4	Fibronectin type III domain-containing protein 4; FNDC4; ortholog	fibronectin type iii domain-containing protein 4 .
ASXL3	ASXL transcriptional regulator 3; ASXL3; ortholog	polycomb group protein asxl3-related
PATZ1	POZ/BTB and AT hook containing zinc finger 1; PATZ1; ortholog	c2h2 zinc finger transcription factor.
AP3S1	AP-3 complex subunit sigma-1; AP3S1; ortholog	vesicle coat protein.
ARL2	ADP-ribosylation factor-like protein 2; ARL2; ortholog	adp-ribosylation factor-like protein 2
SDK2	Sidekick cell adhesion molecule 2; SDK2; ortholog	structural protein.
HOXA3	Homeobox protein Hox-A3; HOXA3; ortholog	homeodomain transcription factor.
GLMP	Glycosylated lysosomal membrane protein; GLMP; ortholog	glycosylated lysosomal membrane protein.
COMT	Catechol O-methyltransferase; COMT; ortholog	methyltransferase.
KCNT1	Potassium sodium-activated channel subfamily T member 1; KCNT1; ortholog	ion channel.
DUSP7	Dual specificity protein phosphatase; DUSP7; ortholog	protein phosphatase.
VAC14	Protein VAC14 homolog; VAC14; ortholog	scaffold/adaptor protein.
KRT19	Keratin, type I cytoskeletal 19; KRT19; ortholog	intermediate filament.

SUN5	Sad1 and UNC84 domain containing 5; SUN5; ortholog	non-motor microtubule binding protein.
TBC1D4	TBC1 domain family member 4; TBC1D4; ortholog	gtpase-activating protein.
PLCD3	Phosphoinositide phospholipase C; PLCD3; ortholog	phospholipase.
PTPRU	Protein-tyrosine-phosphatase; PTPRU; ortholog	protein phosphatase.
DPY19L1	Dpy-19 like C-mannosyltransferase 1; DPY19L1; ortholog	c-mannosyltransferase dpy19l1-related
CHGA	Chromogranin-A; CHGA; ortholog	chromogranin-a
CNTN4	Contactin 4; CNTN4; ortholog	scaffold/adaptor protein.
MYT1	Myelin transcription factor 1; MYT1; ortholog	zinc finger transcription factor.
SIPA1	Signal-induced proliferation-associated 1; SIPA1; ortholog	gtpase-activating protein.
HTRA1	Serine protease HTRA1; HTRA1; ortholog	serine protease.
TARBP1	SpoU_methylase domain-containing protein; TARBP1; ortholog	rna methyltransferase.
FAM188A	Ubiquitin carboxyl-terminal hydrolase MINDY-3; MINDY3; ortholog	ubiquitin carboxyl-terminal hydrolase mindy-3.
CDH18	Cadherin-18; CDH18; ortholog	cadherin.
ARID1A	AT-rich interaction domain 1A; ARID1A; ortholog	at-rich interactive domain-containing protein 1a
ARFGEF3	ARFGEF family member 3; ARFGEF3; ortholog	guanyl-nucleotide exchange factor.
KLHL36	Kelch-like protein 36; KLHL36; ortholog	scaffold/adaptor protein.

OPLAH	5-oxoprolinase; OPLAH; ortholog	hydrolase.
IFNLR1	Interferon lambda receptor 1; IFNLR1; ortholog	transmembrane signal receptor.
TBCD	Tubulin-specific chaperone D; TBCD; ortholog	chaperone.
CHRNB2	Cholinergic receptor nicotinic beta 2 subunit; CHRNB2; ortholog	ligand-gated ion channel.
DPY19L2	Dpy-19 like 2; DPY19L2; ortholog	c-mannosyltransferase dpy19l2-related
IL36G	Interleukin-1; IL36G; ortholog	interleukin superfamily.
CNGB1	Cyclic nucleotide-gated cation channel beta-1; CNGB1; ortholog	ligand-gated ion channel.
CDK5RAP1	CDK5 regulatory subunit associated protein 1; CDK5RAP1; ortholog	mitochondrial trna methylthiotransferase cdk5rap1
TEX29	Testis-expressed protein 29; TEX29; ortholog	testis-expressed protein 29
TLL2	Metalloendopeptidase; TLL2; ortholog	metalloprotease.
PCSK6	Proprotein convertase subtilisin/kexin type 6; PCSK6; ortholog	serine protease.
CELSR3	Cadherin EGF LAG seven-pass G-type receptor 3; CELSR3; ortholog	cadherin.
DERL3	Derlin-3; DERL3; ortholog	primary active transporter.
GREB1	Growth regulating estrogen receptor binding 1; GREB1; ortholog	protein greb1
INPP4A	Phosphatidylinositol-3,4-bisphosphate 4-phosphatase; INPP4A; ortholog	inositol polyphosphate-4-phosphatase type i a .

EHD1	EH domain-containing protein 1; EHD1; ortholog	membrane traffic protein.
RUNX2	Runt-related transcription factor; RUNX2; ortholog	runt transcription factor.
RPL11	60S ribosomal protein L11; RPL11; ortholog	ribosomal protein.
CRTC1	CREB regulated transcription coactivator 1; CRTC1; ortholog	transcription cofactor.
FKBP6	Inactive peptidyl-prolyl cis-trans isomerase FKBP6; FKBP6; ortholog	chaperone.
MEGF8	Multiple EGF like domains 8; MEGF8; ortholog	multiple epidermal growth factor-like domains protein 8
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha; CACNA1C; ortholog	voltage-gated ion channel.
CEMIP	Hyaluronoglucosaminidase; CEMIP; ortholog	cell migration-inducing and hyaluronan-binding protein
CROCC2	Ciliary rootlet coiled-coil, rootletin family member 2; CROCC2; ortholog	chromatin/chromatin-binding, or -regulatory protein.
RXRG	Retinoic acid receptor RXR-gamma; RXRG; ortholog	c4 zinc finger nuclear receptor.
USP42	Ubiquitin carboxyl-terminal hydrolase 42; USP42; ortholog	cysteine protease.
VWA2	von Willebrand factor A domain containing 2; VWA2; ortholog	extracellular matrix structural protein.
TFR2	Transferrin receptor 2; TFR2; ortholog	metalloprotease.
EFNA3	Ephrin RBD domain-containing protein; EFNA3; ortholog	membrane-bound signaling molecule.

HCN1	Hyperpolarization activated cyclic nucleotide gated potassium channel 1; HCN1; ortholog	ion channel.
PRR3	MHC class I region proline-rich protein CAT56; PRR3; ortholog	proline-rich protein 3-related.
ARHGEF7	Rho guanine nucleotide exchange factor 7; ARHGEF7; ortholog	guanyl-nucleotide exchange factor.
OPLAH	5-oxoprolinase; OPLAH; ortholog	hydrolase.
PCM1	Pericentriolar material 1; PCM1; ortholog	pericentriolar material 1 protein
ATG12	Ubiquitin-like protein ATG12; ATG12; ortholog	ubiquitin-like protein atg12
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats protein; UACA; ortholog	scaffold/adaptor protein.
KIAA1217	KIAA1217; KIAA1217; ortholog	sickle tail protein homolog
CRYBB3	Beta-crystallin B3; CRYBB3; ortholog	structural protein.
DECR2	2,4-dienoyl-CoA reductase 2; DECR2; ortholog	reductase.
GTF3C2	General transcription factor IIIC subunit 2; GTF3C2; ortholog	general transcription factor 3c polypeptide 2.
KLHL29	Kelch like family member 29; KLHL29; ortholog	scaffold/adaptor protein.
ANTXR1	ANTXR cell adhesion molecule 1; ANTXR1; ortholog	cell adhesion molecule.
SCAF1	SR-related CTD associated factor 1; SCAF1; ortholog	rna splicing factor.
ZC2HC1B	Zinc finger C2HC domain-containing protein 1B; ZC2HC1B; ortholog	c2h2 zinc finger transcription factor.

PIBF1	Progesterone immunomodulatory binding factor 1; PIBF1; ortholog	progesterone-induced-blocking factor 1
LOXHD1	Uncharacterized protein; LOXHD1; ortholog	lipoxygenase homology domain-containing protein 1
DIS3	DIS3 homolog, exosome endoribonuclease and 3'-5' exoribonuclease; DIS3; ortholog	exoribonuclease.
UACA	Uveal autoantigen with coiled-coil domains and ankyrin repeats protein; UACA; ortholog	scaffold/adaptor protein.
P3H3	Procollagen-proline 3-dioxygenase; P3H3; ortholog	extracellular matrix glycoprotein.
GTF3C3	General transcription factor IIIC subunit 3; GTF3C3; ortholog	general transcription factor.
ALG1	Uncharacterized protein; ALG1; ortholog	glycosyltransferase.
ATG12	Ubiquitin-like protein ATG12; ATG12; ortholog	ubiquitin-like protein atg12.
PTK7	Protein tyrosine kinase 7 (inactive); PTK7; ortholog	inactive tyrosine-protein kinase 7
BBS9	Uncharacterized protein; BBS9; ortholog	protein pthb1
GNL1	G protein nucleolar 1 (putative); GNL1; ortholog	guanine nucleotide-binding protein-like 1
TRIM29	Tripartite motif containing 29; TRIM29; ortholog	ubiquitin-protein ligase.
VWF	von Willebrand factor; VWF; ortholog	extracellular matrix protein.
UVRAG	UV radiation resistance associated; UVRAG; ortholog	uv radiation resistance-associated gene protein.
BIRC6	Baculoviral IAP repeat containing 6; BIRC6; ortholog	ubiquitin-protein ligase.

MCF2	MCF.2 cell line derived transforming sequence; MCF2; ortholog	guanyl-nucleotide exchange factor
OTOP3	Otopetrin 3; OTOP3; ortholog	transporter
MEI1	Meiotic double-stranded break formation protein 1; MEI1; ortholog	meiosis inhibitor protein 1
SNX29	Sorting nexin-29; SNX29; ortholog	scaffold/adaptor protein.
SYNJ2	Phosphoinositide 5-phosphatase; SYNJ2; ortholog	phosphatase.
FHOD3	Formin homology 2 domains containing 3; FHOD3; ortholog	fh1/fh2 domain-containing protein 3
TRIM41	Tripartite motif containing 41; TRIM41; ortholog	ubiquitin-protein ligase.
USP42	Ubiquitin carboxyl-terminal hydrolase 42; USP42; ortholog	cysteine protease.
VWF	von Willebrand factor (Fragment); VWF; ortholog	extracellular matrix protein.
PPP1R13B	Protein phosphatase 1 regulatory subunit 13B; PPP1R13B; ortholog	protein-binding activity modulator.
EFHB	EF-hand domain family member B; EFHB; ortholog	calmodulin-related
ANO10	Anoctamin; ANO10; ortholog	ion channel
PADI2	Protein-arginine deiminase; PADI2; ortholog	chromatin/chromatin-binding, or -regulatory protein
CPNE6	Copine-6; CPNE6; ortholog	calcium-binding protein
IQSEC2	IQ motif and Sec7 domain ArfGEF 2; IQSEC2; ortholog	guanyl-nucleotide exchange factor
MEOX1	MEOX1 protein; MEOX1; ortholog	homeodomain transcription factor

RIPK4	RIPK4 protein; RIPK4; ortholog	non-receptor serine/threonine protein kinase
CNOT1	CCR4-NOT transcription complex subunit 1; CNOT1; ortholog	mrna polyadenylation factor.
CFAP74	Uncharacterized protein; CFAP74; ortholog	cilia- and flagella-associated protein 74
TET3	Methylcytosine dioxygenase TET; TET3; ortholog	methylcytosine dioxygenase tet3
FNTB	Protein farnesyltransferase subunit beta; FNTB; ortholog	acyltransferase
TFF2	Trefoil factor 2; TFF2; ortholog	intercellular signal molecule
GCN1	GCN1 activator of EIF2AK4; GCN1; ortholog	scaffold/adaptor protein
GPR179	G protein-coupled receptor 179; GPR179; ortholog	g-protein coupled receptor
COPZ1	Coatomer subunit zeta-1; COPZ1; ortholog	vesicle coat protein
USP10	Ubiquitin carboxyl-terminal hydrolase 10; USP10; ortholog	cysteine protease.
PKNOX2	PBX/knotted 1 homeobox 2; PKNOX2; ortholog	homeodomain transcription factor.
ANXA5	Annexin A5; ANXA5; ortholog	calcium-binding protein.
B3GAT1	Galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase; B3GAT1; ortholog	glycosyltransferase.
KIRREL2	Kirre like nephrin family adhesion molecule 2; KIRREL2; ortholog	immunoglobulin superfamily cell adhesion molecule.
UPB1	Beta-ureidopropionase 1; UPB1; ortholog	hydrolase.

ADAMTS13	ADAM metallopeptidase with thrombospondin type 1 motif 13; ADAMTS13; ortholog	metalloprotease
SLIT3	Slit guidance ligand 3; SLIT3; ortholog	slit homolog 3 protein
CARMIL3	Capping protein regulator and myosin 1 linker 3; CARMIL3; ortholog	protein-binding activity modulator
ANAPC1	Anaphase-promoting complex subunit 1; ANAPC1; ortholog	ubiquitin-protein ligase
MACF1	Microtubule actin crosslinking factor 1; MACF1; ortholog	intermediate filament binding protein
CHGA	Chromogranin-A; CHGA; ortholog	chromogranin-a
SLC9A1	Sodium/hydrogen exchanger 1; SLC9A1; ortholog	secondary carrier transporter
ZC3H4	Zinc finger CCCH-type containing 4; ZC3H4; ortholog	zinc finger transcription factor
PLA2G4F	Phospholipase A2; PLA2G4F; ortholog	phospholipase
EMC1	ER membrane protein complex subunit 1; EMC1; ortholog	er membrane protein complex subunit 1
DUSP11	RNA/RNP complex-1-interacting phosphatase; DUSP11; ortholog	mrna capping factor
GLB1L3	Beta-galactosidase; GLB1L3; ortholog	galactosidase
ANXA5	Annexin; ANXA5; ortholog	calcium-binding protein
SPATA2	Spermatogenesis associated 2; SPATA2; ortholog	spermatogenesis-associated protein 2
MBP	Myelin basic protein; MBP; ortholog	myelin protein

SNX29	Sorting nexin-29; SNX29; ortholog	scaffold/adaptor protein
FAT1	FAT atypical cadherin 1; FAT1; ortholog	cadherin.
MINK1	Misshapen like kinase 1; MINK1; ortholog	non-receptor serine/threonine protein kinase.
IFT172	Intraflagellar transport 172; IFT172; ortholog	intraflagellar transport protein 172 homologs
PHACTR2	Phosphatase and actin regulator; PHACTR2; ortholog	phosphatase modulator.
SLC9A1	Sodium/hydrogen exchanger; SLC9A1; ortholog	secondary carrier transporter
RNF114	E3 ubiquitin-protein ligase RNF114; RNF114; ortholog	E3 ubiquitin-protein ligase rnf114
TCF25	Transcription factor 25; TCF25; ortholog	transcription factor 25
CNGB1	Cyclic nucleotide-gated cation channel beta-1; CNGB1; ortholog	ligand-gated ion channel.
SPATA46	Spermatogenesis-associated protein 46; SPATA46; ortholog	spermatogenesis-associated protein 46
DUSP11	RNA/RNP complex-1-interacting phosphatase; DUSP11; ortholog	mrna capping factor
SLC16A11	Solute carrier family 16 member 11; SLC16A11; ortholog	transporter
RARRES2	Retinoic acid receptor responder protein 2; RARRES2; ortholog	retinoic acid receptor responder protein 2
MYADM	Myeloid-associated differentiation marker; MYADM; ortholog	myeloid-associated differentiation marker

Table 4.7 List of Genes Identified in Deoni cows with Low Reproductive Performance

Gene Symbol	Gene name	Protein class
TLE1	TLE family member 1, transcriptional corepressor; TLE1; ortholog	transcription cofactor
RABL3	Rab-like protein 3; RABL3; ortholog	small gtpase
THBS4	Thrombospondin-4; THBS4; ortholog	cell adhesion molecule
THBS4	Thrombospondin-4; THBS4; ortholog	cell adhesion molecule
RNF214	Ring finger protein 214; RNF214; ortholog	ring finger protein 214
CCDC189	Coiled-coil domain containing 189; CCDC189; ortholog	coiled-coil domain-containing protein 189
GRIN2B	Glutamate receptor; GRIN2B; ortholog	transmembrane signal receptor
BCKDHA	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial; BCKDHA; ortholog	dehydrogenase
TBC1D2B	TBC1 domain family member 2B; TBC1D2B; ortholog	gtpase-activating protein
LPCAT3	Lysophospholipid acyltransferase 5; LPCAT3; ortholog	acetyltransferase
SF3A3	Splicing factor 3a subunit 3; SF3A3; ortholog	rna splicing factor
SCARF1	Scavenger receptor class F member 1; SCARF1; ortholog	membrane traffic protein

DRG2	Developmentally-regulated GTP-binding protein 2; DRG2; ortholog	developmentally-regulated gtp-binding protein 2
BRAT1	BRCA1 associated ATM activator 1; BRAT1; ortholog	brca1-associated atm activator 1
ZNF652	Zinc finger protein 652; ZNF652; ortholog	c2h2 zinc finger transcription factor
MRI1	Methylthioribose-1-phosphate isomerase; MRI1; ortholog	isomerase
SYCP2	Synaptonemal complex protein 2; SYCP2; ortholog	chromatin/chromatin-binding, or -regulatory protein
RAB36	RAB36, member RAS oncogene family; RAB36; ortholog	small gtpase
BRIP1	BRCA1 interacting protein C-terminal helicase 1; BRIP1; ortholog	dna helicase
ARL2BP	ADP-ribosylation factor-like protein 2-binding protein; ARL2BP; ortholog	adp-ribosylation factor-like protein 2-binding protein
NLRP5	NACHT, LRR and PYD domains-containing protein 5; NLRP5; ortholog	defense/immunity protein
UBP1	Ubiquitin carboxyl-terminal hydrolase 1; USP1; ortholog	cysteine protease
FTSJ3	pre-rRNA processing protein FTSJ3; FTSJ3; ortholog	rna methyltransferase
RABEP2	Rab GTPase-binding effector protein 2; RABEP2; ortholog	rab gtpase-binding effector protein 2
LTN1	E3 ubiquitin-protein ligase listerin; LTN1; ortholog	ubiquitin-protein ligase
TOM1L1	Target of myb1 like 1 membrane trafficking protein; TOM1L1; ortholog	tom1-like protein 1

UHRF1BP1L	UHRF1 binding protein 1 like; UHRF1BP1L; ortholog	uhrf1-binding protein 1-like
OBSL1	Obscurin like cytoskeletal adaptor 1; OBSL1; ortholog	obscurin-like protein 1
TNNI3	Troponin I, cardiac muscle; TNNI3; ortholog	non-motor actin binding protein
TRIM47	Tripartite motif containing 47; TRIM47; ortholog	ubiquitin-protein ligase
PHKG2	Phosphorylase b kinase gamma catalytic chain, liver/testis isoform; PHKG2; ortholog	non-receptor serine/threonine protein kinase
VPS37C	VPS37C subunit of ESCRT-I; VPS37C; ortholog	membrane trafficking regulatory protein
STAC2	SH3 and cysteine rich domain 2; STAC2; ortholog	sh3 and cysteine-rich domain-containing protein 2
CDC42BPG	Non-specific serine/threonine protein kinase; CDC42BPG; ortholog	non-receptor serine/threonine protein kinase
ITPR3	Inositol 1,4,5-trisphosphate receptor type 3; ITPR3; ortholog	ligand-gated ion channel
MAP3K6	Mitogen-activated protein kinase kinase kinase; MAP3K6; ortholog	non-receptor serine/threonine protein kinase
ODF3L1	Outer dense fiber protein 3-like protein 1; ODF3L1; ortholog	structural protein
PSAP	Pulmonary surfactant-associated protein A; SFTPA1; ortholog	surfactant
PSAP	Prosaposin; PSAP; ortholog	scaffold/adaptor protein
TMEM245	Transmembrane protein 245; TMEM245; ortholog	transmembrane protein 245

PRSS8	Serine protease 8; PRSS8; ortholog	serine protease
KCNC1	Potassium voltage-gated channel subfamily C member 1; KCNC1; ortholog	voltage-gated ion channel
EIF4B	Eukaryotic translation initiation factor 4B; EIF4B; ortholog	translation initiation factor
ZXDC	ZXD family zinc finger C; ZXDC; ortholog	c2h2 zinc finger transcription factor
ZMAT4	Zinc finger matrin-type protein 4; ZMAT4; ortholog	zinc finger matrin-type protein 4
ANAPC5	Anaphase-promoting complex subunit 5; ANAPC5; ortholog	anaphase-promoting complex subunit 5
AAK1	AP2-associated protein kinase 1; AAK1; ortholog	ap2-associated protein kinase 1
ADCYAP1R1	Pituitary adenylate cyclase-activating polypeptide type I receptor; ADCYAP1R1; ortholog	transmembrane signal receptor
RAB37	RAB37, member RAS oncogene family; RAB37; ortholog	small gtpase
SV2A	Synaptic vesicle glycoprotein 2A; SV2A; ortholog	synaptic vesicle glycoprotein 2a
DGKI	Diacylglycerol kinase; DGKI; ortholog	kinase
FAT3	FAT atypical cadherin 3; FAT3; ortholog	cadherin
VSX1	Visual system homeobox 1; VSX1; ortholog	homeodomain transcription factor
ODF3L1	Outer dense fiber protein 3-like protein 1; ODF3L1; ortholog	structural protein
SLC9A3R1	Na (+)/H (+) exchange regulatory cofactor NHE-RF1; SLC9A3R1; ortholog	scaffold/adaptor protein

PAQR9	Progestin and adipoQ receptor family member 9; PAQR9; ortholog	g-protein coupled receptor
SH2D4A	SH2 domain containing 4A; SH2D4A; ortholog	defense/immunity protein
CCDC60	Coiled-coil domain containing 60; CCDC60; ortholog	coiled-coil domain-containing protein 60
PTPRCAP	Protein tyrosine phosphatase receptor type C-associated protein; PTPRCAP; ortholog	protein tyrosine phosphatase receptor type c-associated protein
ADAMTS7	ADAM metallopeptidase with thrombospondin type 1 motif 7; ADAMTS7; ortholog	metalloprotease
TTC39A	Tetratricopeptide repeat domain 39A; TTC39A; ortholog	tetratricopeptide repeat protein 39a
ARFGAP3	ADP-ribosylation factor GTPase-activating protein 3; ARFGAP3; ortholog	gtpase-activating protein
TMEM98	Transmembrane protein 98; TMEM98; ortholog	transmembrane protein 98
THAP8	THAP domain containing 8; THAP8; ortholog	thap domain-containing protein 8
BRD3	Bromodomain containing 3; BRD3; ortholog	chromatin/chromatin-binding, or -regulatory protein
PLAGL2	PLAG1 like zinc finger 2; PLAGL2; ortholog	c2h2 zinc finger transcription factor
ZNF575	Uncharacterized protein; ZNF575; ortholog	c2h2 zinc finger transcription factor
SEZ6L	Seizure related 6 homologs like; SEZ6L; ortholog	seizure 6-like protein
CXCL14	C-X-C motif chemokine ligand 14; CXCL14; ortholog	chemokine

KCNH6	Potassium voltage-gated channel subfamily H member 6; KCNH6; ortholog	ion channel
COL15A1	Collagen type XV alpha 1 chain; COL15A1; ortholog	extracellular matrix structural protein
DYSF	Dysferlin; DYSF; ortholog	membrane trafficking regulatory protein
PAPLN	Papilin, proteoglycan like sulfated glycoprotein; PAPLN; ortholog	metalloprotease
SCN5A	Sodium channel protein; SCN5A; ortholog	voltage-gated ion channel
PLXND1	Plexin D1; PLXND1; ortholog	transmembrane signal receptor
SLC37A2	Glucose-6-phosphate exchanger SLC37A2; SLC37A2; ortholog	transporter
KCNT2	Potassium sodium-activated channel subfamily T member 2; KCNT2; ortholog	ion channel
LAD1	Ladinin 1; LAD1; ortholog	structural protein
SUPT6H	Transcription elongation factor spt6; SUPT6H; ortholog	general transcription factor
NLRP5	NACHT, LRR and PYD domains-containing protein 5; NLRP5; ortholog	defense/immunity protein
SLC9A2	Sodium/hydrogen exchanger; SLC9A2; ortholog	secondary carrier transporter
AMOTL1	Angiomotin-like protein 1; AMOTL1; ortholog	angiomotin-like protein 1
NALCN	Sodium leak channel, non-selective; NALCN; ortholog	ion channel
KCTD15	BTB/POZ domain-containing protein KCTD15; KCTD15; ortholog	scaffold/adaptor protein

ZFAT	Zinc finger and AT-hook domain containing; ZFAT; ortholog	c2h2 zinc finger transcription factor
ITGB7	Integrin beta; ITGB7; ortholog	integrin
PPP1R16A	Protein phosphatase 1 regulatory subunit 16A; PPP1R16A; ortholog	phosphatase modulator
PRR11	Proline rich 11; PRR11; ortholog	proline-rich protein 11
TNS2	Tensin 2; TNS2; ortholog	non-motor actin binding protein
DPP6	Dipeptidyl aminopeptidase-like protein 6; DPP6; ortholog	serine protease
ANKH	ANKH inorganic pyrophosphate transport regulator; ANKH; ortholog	progressive ankylosis protein homolog
POLR2C	DNA-directed RNA polymerase II subunit RPB3; POLR2C; ortholog	dna-directed rna polymerase
ALKBH8	Alkylated DNA repair protein alkB homolog 8; ALKBH8; ortholog	alkylated dna repair protein alkb homolog 8
ZSWIM8	Zinc finger SWIM domain-containing protein 8; ZSWIM8; ortholog	zinc finger swim domain-containing protein 8
LLGL1	Lethal (2) giant larvae protein homolog 1; LLGL1; ortholog	membrane trafficking regulatory protein
ATP4A	Sodium/potassium-transporting ATPase subunit alpha; ATP4A; ortholog	primary active transporter
MCM3AP	Minichromosome maintenance complex component 3 associated protein; MCM3AP; ortholog	scaffold/adaptor protein
MTF1	Metal regulatory transcription factor 1; MTF1; ortholog	c2h2 zinc finger transcription factor

ZFYVE19	Zinc finger FYVE-type containing 19; ZFYVE19; ortholog	abscission/nocut checkpoint regulator
CIT	Citron Rho-interacting kinase; CIT; ortholog	non-receptor serine/threonine protein kinase
P3H1	Procollagen-proline 3-dioxygenase; P3H1; ortholog	extracellular matrix glycoprotein
SLC41A3	Solute carrier family 41 member 3; SLC41A3; ortholog	secondary carrier transporter
HNRNPA3	Heterogeneous nuclear ribonucleoprotein A3; HNRNPA3; ortholog	rna processing factor
PADI1	Protein-arginine deiminase; PADI1; ortholog	chromatin/chromatin-binding, or -regulatory protein
RASGRF1	Ras protein specific guanine nucleotide releasing factor 1; RASGRF1; ortholog	guanyl-nucleotide exchange factor
SAP130	Sin3A associated protein 130; SAP130; ortholog	histone modifying enzyme
PRKAG1	5'-AMP-activated protein kinase subunit gamma-1; PRKAG1; ortholog	kinase modulator
CTNS	Cystinosin; CTNS; ortholog	amino acid transporter
IQCD	Dynein regulatory complex protein 10; IQCD; ortholog	dynein regulatory complex protein 10
ACTN2	Alpha-actinin-2; ACTN2; ortholog	actin or actin-binding cytoskeletal protein
BEST1	Bestrophin; BEST1; ortholog	ion channel
NDST2	[Heparan sulfate]-glucosamine N-sulfotransferase; NDST2; ortholog	transferase

TMEM245	Transmembrane protein 245; TMEM245; ortholog	transmembrane protein 245
KRT80	Keratin, type II cytoskeletal 80; KRT80; ortholog	keratin, type ii cytoskeletal 80
SATB1	DNA-binding protein SATB; SATB1; ortholog	lambda repressor-like transcription factor
ZFHX2	Zinc finger homeobox 2; ZFHX2; ortholog	c2h2 zinc finger transcription factor
RHOB	Rho-related GTP-binding protein RhoB; RHOB; ortholog	small gtpase
RAB17	RAB17, member RAS oncogene family; RAB17; ortholog	small gtpase
TNNT1	Troponin T, slow skeletal muscle; TNNT1; ortholog	actin binding motor protein
ADAMTS2	A disintegrin and metalloproteinase with thrombospondin motifs 2; ADAMTS2; ortholog	metalloprotease
CCR4	C-C motif chemokine receptor 4; CCR4; ortholog	cell adhesion molecule
MROH1	Maestro heat-like repeat-containing protein family member 1; MROH1; ortholog	maestro heat-like repeat-containing protein family member 1
ZFYVE26	Zinc finger FYVE domain-containing protein 26; ZFYVE26; ortholog	zinc finger fyve domain-containing protein 26
CLEC19A	C-type lectin domain containing 19A; CLEC19A; ortholog	c-type lectin domain family 19-member a
IL2RB	High affinity IL-2 receptor subunit beta; IL2RB; ortholog	transmembrane signal receptor
CFI	Complement factor I; CFI; ortholog	serine protease
PLA2G4E	Phospholipase A2; PLA2G4E; ortholog	phospholipase

MLXIP	BHLH domain-containing protein; MLXIP; ortholog	basic helix-loop-helix transcription factor
HPD	4-hydroxyphenylpyruvate dioxygenase; HPD; ortholog	oxygenase
CTBS	Di-N-acetylchitobiase (Fragment); CTBS; ortholog	di-n-acetylchitobiase
SEMA5A	Semaphorin 5A; SEMA5A; ortholog	membrane-bound signaling molecule
PTPN9	Protein tyrosine phosphatase non-receptor type 9; PTPN9; ortholog	protein phosphatase
CBX7	Chromobox 7; CBX7; ortholog	chromobox protein homolog 7
RHCG	Ammonium transporter Rh type C; RHCG; ortholog	primary active transporter
EXOSC5	Exosome component 5; EXOSC5; ortholog	exoribonuclease
RHCG	Ammonium transporter Rh type C; RHCG; ortholog	primary active transporter
RIMS1	Regulating synaptic membrane exocytosis 1; RIMS1; ortholog	membrane traffic protein
KAT8	Histone acetyltransferase; KAT8; ortholog	histone modifying enzyme
EMG1	EMG1 N1-specific pseudouridine methyltransferase; EMG1; ortholog	methyltransferase
UBP1	Upstream binding protein 1; UBP1; ortholog	dna-binding transcription factor
ANKRD17	Ankyrin repeat domain 17; ANKRD17; ortholog	defense/immunity protein
SGSM1	Small G protein signaling modulator 1; SGSM1; ortholog	gtpase-activating protein
FAM175A	BRCA1-A complex subunit Abraxas 1; ABRAXAS1; ortholog	brca1-a complex subunit abraxas 1

MED24	Mediator complex subunit 24; MED24; ortholog	general transcription factor
SLC17A4	Solute carrier family 17 member 4; SLC17A4; ortholog	secondary carrier transporter
PROCA1	Protein PROCA1; PROCA1; ortholog	protein proca1
MMP28	Matrix metallopeptidase 28; MMP28; ortholog	metalloprotease
RILP	Rab interacting lysosomal protein; RILP; ortholog	non-motor microtubule binding protein
OSCAR	Osteoclast associated Ig-like receptor; OSCAR; ortholog	immunoglobulin receptor superfamily
CASC3	Protein CASC3; CASC3; ortholog	protein casc3
CLPTM1	Cleft lip and palate transmembrane protein 1 homolog; CLPTM1; ortholog	cleft lip and palate transmembrane protein 1
PAX5	Paired box 5; PAX5; ortholog	homeodomain transcription factor
MYO5A	Myosin VA; MYO5A; ortholog	actin binding motor protein
ELL	Elongation factor for RNA polymerase II; ELL; ortholog	general transcription factor
MRI1	Methylthioribose-1-phosphate isomerase; MRI1; ortholog	isomerase
PSD4	Pleckstrin and Sec7 domain containing 4; PSD4; ortholog	guanyl-nucleotide exchange factor
BRWD1	Bromodomain and WD repeat domain containing 1; BRWD1; ortholog	bromodomain and wd repeat-containing protein 1
GPR160	G protein-coupled receptor 160; GPR160; ortholog	g-protein coupled receptor

CCDC57	Coiled-coil domain containing 57; CCDC57; ortholog	coiled-coil domain-containing protein 57
PANK1	Pantothenate kinase 1; PANK1; ortholog	kinase
MCMBP	Mini-chromosome maintenance complex-binding protein; MCMBP; ortholog	mini-chromosome maintenance complex-binding protein
APOM	Apolipoprotein M; APOM; ortholog	apolipoprotein
EPAS1	Endothelial PAS domain protein 1; EPAS1; ortholog	basic helix-loop-helix transcription factor
FAM234A	Protein FAM234A; FAM234A; ortholog	protein fam234a
PRPF8	PRPF8 protein; PRPF8; ortholog	rna splicing factor
CTBS	Di-N-acetylchitobiase; CTBS; ortholog	di-n-acetylchitobiase
DNAJC17	DnaJ homolog subfamily C member 17; DNAJC17; ortholog	chaperone
PKP4	Plakophilin 4; PKP4; ortholog	intermediate filament binding protein
PLA2G6	Phospholipase A2 group VI; PLA2G6; ortholog	phospholipase
CD34	CD34 molecule; CD34; ortholog	cell adhesion molecule
NBEA	Neurobeachin; NBEA; ortholog	scaffold/adaptor protein
BOP1	Ribosome biogenesis protein BOP1; BOP1; ortholog	ribosomal protein
BAG6	BCL2-associated athanogene 6; BAG6; ortholog	chaperone
TAX1BP3	Tax1-binding protein 3; TAX1BP3; ortholog	tax1-binding protein 3

PTPRF	Receptor-type tyrosine-protein phosphatase F; PTPRF; ortholog	protein phosphatase
USP2	Ubiquitin carboxyl-terminal hydrolase 2; USP2; ortholog	cysteine protease
SLC39A11	Zinc transporter ZIP11; SLC39A11; ortholog	secondary carrier transporter
PACS1	Phosphofurin acidic cluster sorting protein 1; PACS1; ortholog	phosphofurin acidic cluster sorting protein 1
FRMD1	FERM domain containing 1; FRMD1; ortholog	ferm domain-containing protein 1
ZFYVE26	Zinc finger FYVE domain-containing protein 26; ZFYVE26; ortholog	zinc finger fyve domain-containing protein 26
CORO1B	Coronin; CORO1B; ortholog	non-motor actin binding protein
TMEM117	TMEM117 protein; TMEM117; ortholog	transmembrane protein 117
HBZ	Hemoglobin subunit zeta; HBZ; ortholog	globin
KIFC2	Kinesin family member C2; KIFC2; ortholog	microtubule binding motor protein
PPM1G	Protein phosphatase 1G; PPM1G; ortholog	protein phosphatase
COL16A1	Collagen type XVI alpha 1 chain; COL16A1; ortholog	extracellular matrix structural protein
RRM2B	Ribonucleotide reductase regulatory TP53 inducible subunit M2B; RRM2B; ortholog	reductase
AMOTL1	Angiomotin-like protein 1; AMOTL1; ortholog	angiomotin-like protein 1
RNF40	E3 ubiquitin protein ligase; RNF40; ortholog	e3 ubiquitin-protein ligase bre1b

TADA1	Transcriptional adapter 1; TADA1; ortholog	transcriptional adapter 1
LEMD3	LEM domain containing 3; LEMD3; ortholog	inner nuclear membrane protein man1
MROH1	Maestro heat-like repeat-containing protein family member 1; MROH1; ortholog	maestro heat-like repeat-containing protein family member 1
SPTA1	Spectrin alpha, erythrocytic 1; SPTA1; ortholog	actin or actin-binding cytoskeletal protein
LPCAT1	Lysophosphatidylcholine acyltransferase 1; LPCAT1; ortholog	acyltransferase
SLC22A11	Solute carrier family 22 member 11; SLC22A11; ortholog	secondary carrier transporter
FAM160A2	FHF complex subunit HOOK interacting protein 1B; FHIP1B; ortholog	fhf complex subunit hook interacting protein 1b
SETD1B	[Histone H3]-lysine (4) N-trimethyltransferase; SETD1B; ortholog	histone modifying enzyme
BCKDHA	2-oxoisovalerate dehydrogenase subunit alpha; BCKDHA; ortholog	dehydrogenase
HDAC7	Histone deacetylase; HDAC7; ortholog	histone modifying enzyme
POU6F2	Uncharacterized protein; POU6F2; ortholog	pou domain, class 6, transcription factor 2
PHLDB1	Pleckstrin homology like domain family B member 1; PHLDB1; ortholog	pleckstrin homology-like domain family b member 1
SNX17	Sorting nexin-17; SNX17; ortholog	scaffold/adaptor protein
TMEM184B	Transmembrane protein 184B; TMEM184B; ortholog	transporter
VSX1	Visual system homeobox 1; VSX1; ortholog	homeodomain transcription factor

NDUFA3	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 3; NDUFA3; ortholog	dehydrogenase
TMEM98	Transmembrane protein 98; TMEM98; ortholog	transmembrane protein 98
SCUBE1	Signal peptide, CUB domain and EGF like domain containing 1; SCUBE1; ortholog	extracellular matrix glycoprotein
CCDC180	Uncharacterized protein; CCDC180; ortholog	coiled-coil domain-containing protein 180
MLLT3	MLLT3 super elongation complex subunit; MLLT3; ortholog	protein af-9
SCX	Scleraxis bHLH transcription factor; SCX; ortholog	basic helix-loop-helix transcription factor
KIF1C	Kinesin family member 1C; KIF1C; ortholog	microtubule binding motor protein
AK9	Nucleoside-diphosphate kinase; AK9; ortholog	nucleotide kinase
FOXH1	Forkhead box H1; FOXH1; ortholog	forkhead box protein h1
ITPR3	Inositol 1,4,5-trisphosphate receptor type 3; ITPR3; ortholog	ligand-gated ion channel
OC90	Uncharacterized protein; OC90; ortholog	phospholipase
FHOD1	Formin homology 2 domains containing 1; FHOD1; ortholog	fh1/fh2 domain-containing protein 1
TFPT	TCF3 fusion partner homolog; TFPT; ortholog	tcf3 fusion partner
SFI1	SFI1 centrin binding protein; SFI1; ortholog	protein sfi1 homolog
SLC7A9	Solute carrier family 7 (Cationic amino acid transporter, y+ system), member 9; SLC7A9; ortholog	transporter

AAK1	AP2-associated protein kinase 1; AAK1; ortholog	ap2 associated kinase 1
NDST2	Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 2; NDST2; ortholog	transferase
RSPRY1	RSPRY1 protein; RSPRY1; ortholog	ring finger and spry domain-containing protein 1
DYSF	Dysferlin; DYSF; ortholog	membrane trafficking regulatory protein
SEC16B	Protein transport protein Sec16B; SEC16B; ortholog	protein transport protein sec16b
SULF1	Sulfatase 1; SULF1; ortholog	hydrolase
DNAJB2	DnaJ heat shock protein family (Hsp40) member B2; DNAJB2; ortholog	chaperone
MYOM3	Myomesin 3; MYOM3; ortholog	structural protein
KLHDC8A	Kelch domain containing 8A; KLHDC8A; ortholog	kelch domain-containing protein 8a
COTL1	Coactosin-like protein; COTL1; ortholog	non-motor actin binding protein
CHD5	DNA helicase; CHD5; ortholog	chromatin/chromatin-binding, or -regulatory protein
ATP2A1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1; ATP2A1; ortholog	primary active transporter
DPP10	Dipeptidyl peptidase like 10; DPP10; ortholog	serine protease

ZSWIM8	Zinc finger SWIM domain-containing protein 8; ZSWIM8; ortholog	zinc finger swim domain-containing protein 8
SEC16B	Protein transport protein sec16; SEC16B; ortholog	protein transport protein sec16b
MLXIPL	MLX interacting protein like; MLXIPL; ortholog	basic helix-loop-helix transcription factor
PSAP	Prosaposin; PSAP; ortholog	scaffold/adaptor protein
PLSCR3	Phospholipid scramblase; PLSCR3; ortholog	transporter
CFAP161	Cilia and flagella associated protein 161; CFAP161; ortholog	structural protein
TNK1	Tyrosine kinase non receptor 1; TNK1; ortholog	non-receptor tyrosine protein kinase
FER1L5	Fer-1 like family member 5; FER1L5; ortholog	membrane trafficking regulatory protein
ZNF513	Zinc finger protein 513; ZNF513; ortholog	c2h2 zinc finger transcription factor
RGS11	Regulator of G protein signaling 11; RGS11; ortholog	regulator of g-protein signaling 11
LLGL1	Lethal (2) giant larvae protein homolog 1; LLGL1; ortholog	membrane trafficking regulatory protein
CCDC130	Coiled-coil domain-containing protein 130; CCDC130; ortholog	rna splicing factor
TNC	Uncharacterized protein; TNC; ortholog	extracellular matrix protein
RASGEF1A	RasGEF domain family member 1A; RASGEF1A; ortholog	guanyl-nucleotide exchange factor
TMEM135	Transmembrane protein 135; TMEM135; ortholog	transmembrane protein 135

COQ9	Ubiquinone biosynthesis protein COQ9, mitochondrial; COQ9; ortholog	ubiquinone biosynthesis protein coq9, mitochondrial
DOCK6	Dedicator of cytokinesis 6; DOCK6; ortholog	guanyl-nucleotide exchange factor
DIP2A	Disco interacting protein 2 homolog A; DIP2A; ortholog	disco-interacting protein 2 homolog a
CMIP	C-Maf inducing protein; CMIP; ortholog	c-maf-inducing protein
ARHGDIG	Rho GDP-dissociation inhibitor 3; ARHGDIG; ortholog	g-protein modulator
TMEM208	Transmembrane protein 208; TMEM208; ortholog	transmembrane protein 208
CCDC185	Coiled-coil domain containing 185; CCDC185; ortholog	coiled-coil domain-containing protein 185
LPCAT3	Lysophospholipid acyltransferase 5; LPCAT3; ortholog	acetyltransferase
DDX42	RNA helicase; DDX42; ortholog	rna helicase
BBS1	BBS1 domain-containing protein; BBS1; ortholog	bardet-biedl syndrome 1 protein
DDN	DDN protein DDN; ortholog	dendrin
GAPDHS	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific; GAPDHS; ortholog	dehydrogenase
SLC37A2	Glucose-6-phosphate exchanger SLC37A2; SLC37A2; ortholog	transporter
SP1	Sp1 transcription factor; SP1; ortholog	c2h2 zinc finger transcription factor

4.6 Pathways Identified in high and low reproductive performance groups

The list of genes identified in the exome profiling of high and low reproductive performance groups were subjected to pathway analysis using gProfiler. Functional pathway analysis resulted in identification various pathways enriched with genes related to reproductive performance. The important pathways identified are given in table 4.8.

Table 4.8 List of Pathways and Genes Identified Using Exome Profiling in High and Low Reproductive Performance Groups.

Pathway	Gene count	Genes
GnRH signaling	5	CACNA1C, PLA2G4B, PLA2G4F, PLA2G4E, ITPR3
Progesterone mediated oocyte maturation	2	ANAPC1, ANAPC5
Oocyte maturation	3	ANAPC1, ANAPC5, ITPR3
Calcium signaling	5	CACNA1C, PLCD3, ITPR3, ATP2A1, PHKG2
Estrogen signaling	7	KRT19, ARID1A, PADI2, CNOT1, SP1, ITPR3
Glutamatergic synapse	10	PTPRS, CACNA1C, PLA2G4B, PLA2G4F, SHANK2, SV2A, DGKI, GRIN2B, PLA2G4E, ITPR3
Regulation of action cytoskeleton	12	WASF2, CELSR1, FHOD3, FSCN1, SLC9A1, ARHGEF7, SPAT1, COTL1, FHOD1, ACTN2, CORO1B, ITGB7
Cholinergic synapse	3	CHRNB2, CACNA1C, ITPR3
cAMP signaling	7	CACNA1C, SLC9A1, CNGB1, ADCYAP1R1, GRIN2B, TNNI3, ATP2A1
Focal adhesion	9	VWF, TADA1, ITGB7, TNS2, ACTN2, TNC, THBS4, TNK1, RASGRF1

PI3K-Akt signaling	7	ENFNA3, VWF, ITGB7, EIF4B, IL2RB, TNC, THBS4
Ovarian Steroidogenesis	3	PLA2G4B, PLA2G4F, PLA2G4E

4.6.1 Calcium Signaling Pathway

In this study, five candidate genes viz CACNA1C, PLCD3, ITPR3, ATP2A1, PHKG2 were observed to be enriched in the calcium signaling pathway (Fig 4.3). Increased level of Ca2+ ions were observed to be correlated with excitation of GnRH neurons, which resulted in the secretion of GnRH (Liu *et al*., 2008). High Ca^{2+} ions levels in GnRH neurons are achieved through two mechanisms including, the voltage-dependent Ca^{2+} influx through extracellular resources and the secretion of Ca^{2+} from the intracellular (Tahir *et al*., 2021). Calcium voltage-gated channel subunit alpha1 C (CACNA1C) gene encodes an alpha-1 subunit of a voltage-dependent calcium channel, mediating the influx of calcium ions into the cell upon membrane polarization (Bootman *et al*., 2001). Likewise, phospholipase C delta 3 (PLCD3) gene was found to be a member of the phospholipase C family, catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce the second messenger's diacylglycerol and inositol 1,4,5-trisphosphate (IP3). These second messengers increase Ca^{2+} concentrations in the cytosol in response to extracellular stimuli induced by protein kinase C through the cellular response which result in excitation of GnRH neurons (Zhang *et al*., 2009). Similarly, ATP2AL, ITPR3 and PHKGA also reported to increase the Ca^{2+} ions levels. In short, CACNA1C, ATP2AL, ITPR3, PHKGA and PLCD3 genes were found to be involved in regulating GnRH secretion mechanism by activating GnRH neurons through calcium signaling pathway, which is required for normal GnRH secretion for better reproductive performance.

4.6.2 Cholinergic Synapse Pathway

Cholinergic neurons regulate GnRH neurons through their neurotransmitter nicotine and acetylcholine by activating signaling cascade comprises of calcium signaling, cAMP signaling and MAPK signaling in GnRH neurons to secrete GnRH (Iremonger *et al*., 2010; Arai *et al*., 2017). CHRNB2, CACNA1C and ITPR3 genes were identified to be enriched in the cholinergic synapse pathway in the present study (Fig 4.4). Earlier researchers have reported that CACNA1C and ITPR3 genes regulate GnRH neurons through calcium signaling pathway which result in GnRH secretion (Bootman *et al*., 2001; Liu *et al*., 2008).

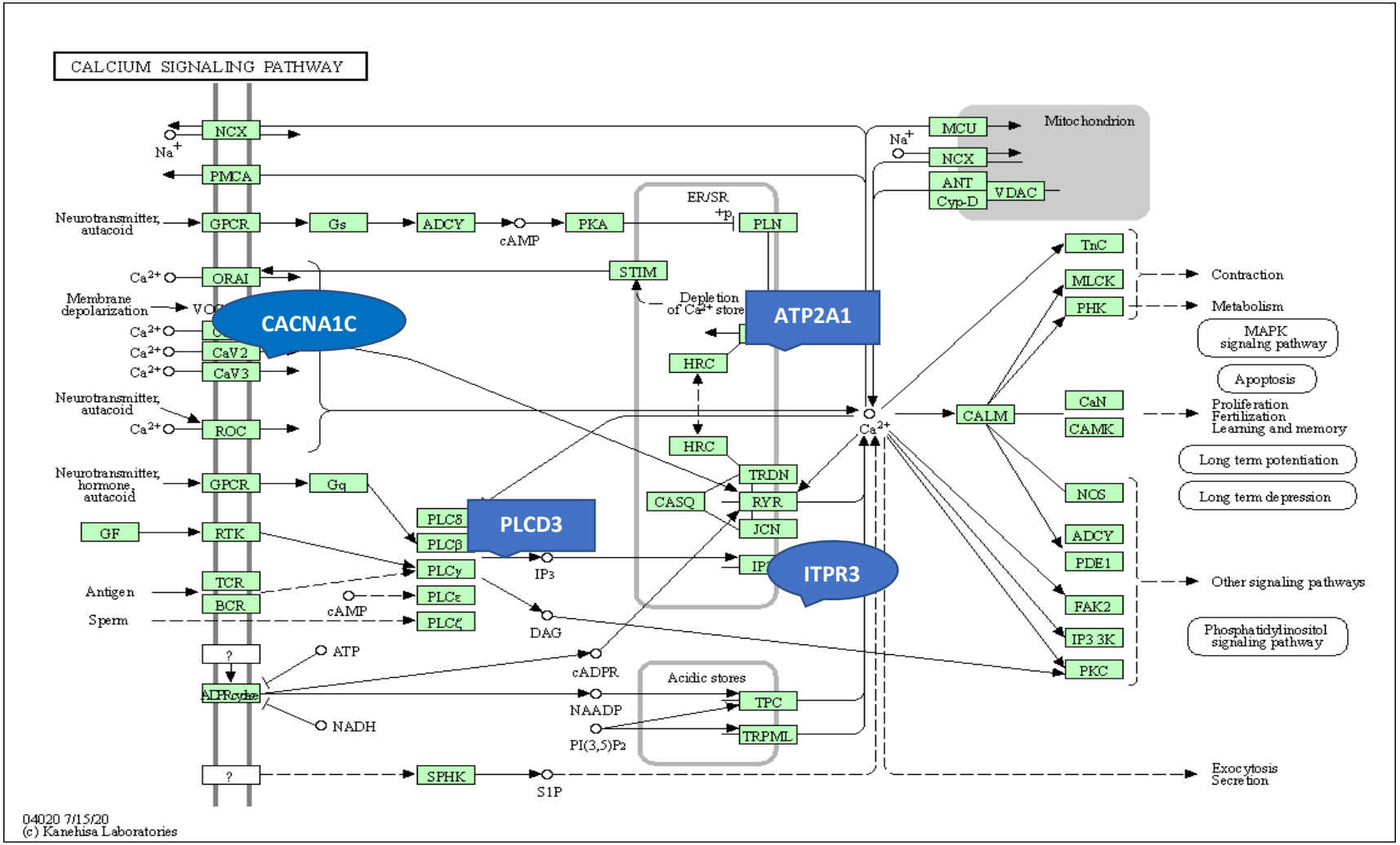

Fig 4.3 Calcium Signaling Pathway

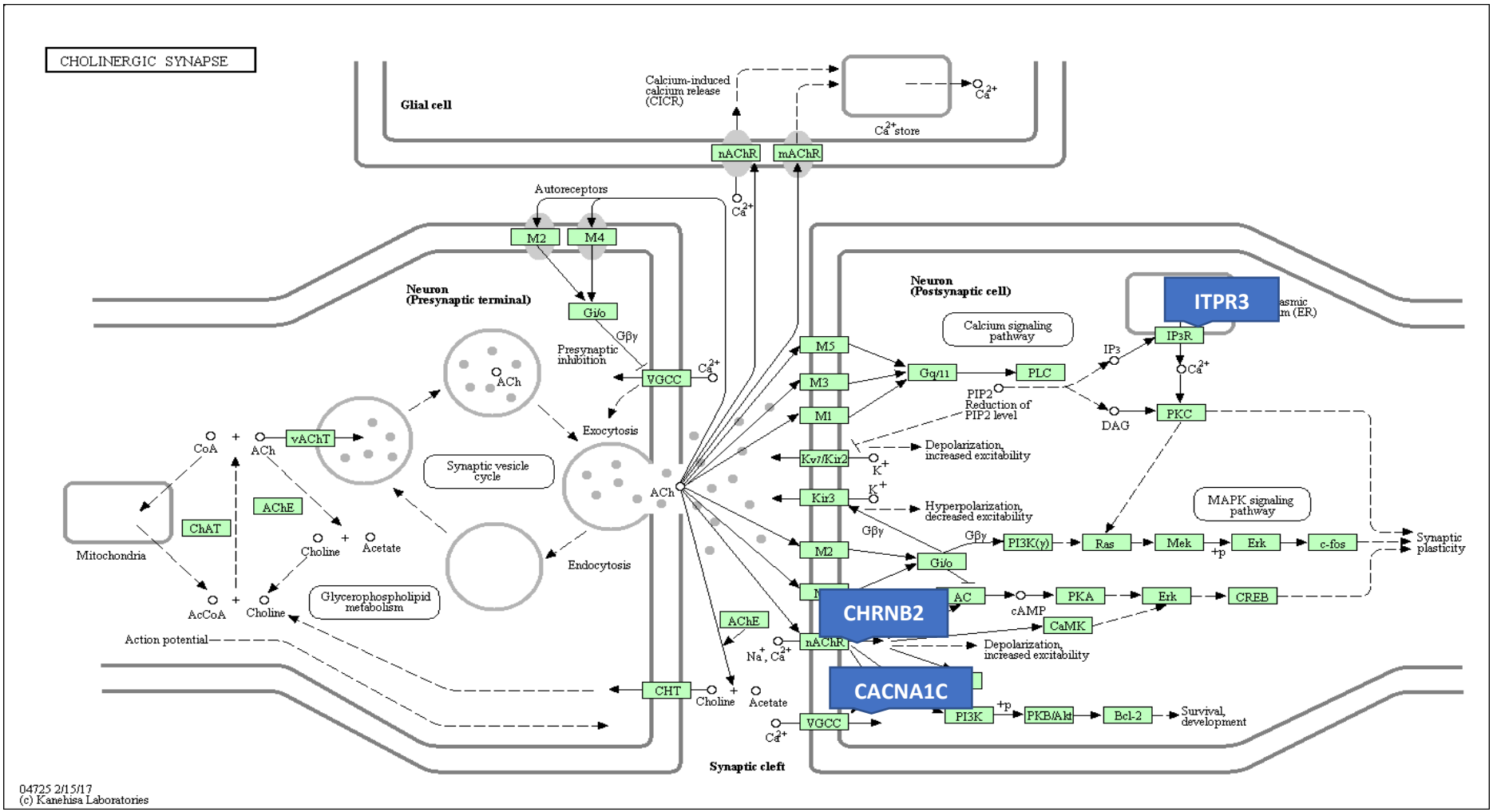

Fig 4.4 Cholinergic Synapse Pathway

While CHRNB2 was a nicotinic acetylcholine receptor which regulates GnRH neurons through cholinergic neuron by the neurotransmitter to secrete GnRH hormone (Krsmanovic *et al*., 2009; Arai *et al*., 2017). CHRNB2, CACNA1C and ITPR3 genes linked to cholinergic synapse were integrated in the mechanism that contribute to GnRH secretion, and therefore could be related to reproductive performance.

4.6.3 GnRH Signaling Pathway

A gonadotropin-releasing hormone is a neuropeptide hormone produced in the hypothalamus and reported as a key regulator of female reproductive function required for normal reproductive performance in animals (Tahir *et al*., 2021). In the present study CACNA1C, PLA2G4B, PLA2G4F, PLA2G4E, and ITPR3 genes were identified to be linked with GnRH signaling pathway (Fig 4.5). These genes regulate GnRH secretion by activating GnRH neurons through calcium signaling pathway (Bootman *et al*., 2001; Liu *et al*., 2008). Bliss *et al*. (2010) reported increased level of Ca2+ ions with positive correlation of GnRH secretion. Therefore, CACNA1C, PLA2G4B, PLA2G4F, PLA2G4E, and ITPR3 genes might improve reproductive performance in cattle, because these genes are linked to GnRH signaling pathway.

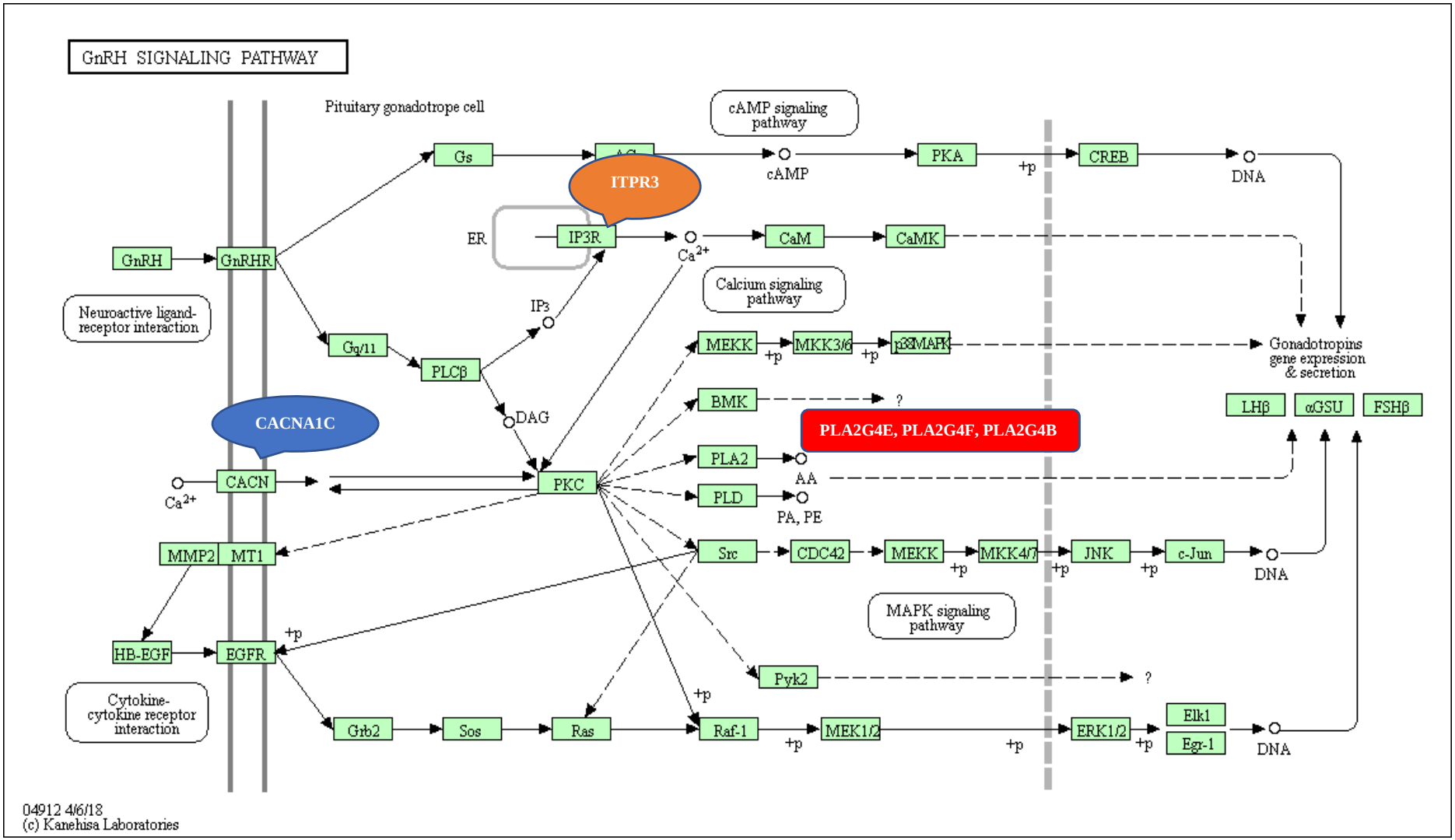

Fig 4.5 GnRH Signaling Pathway

4.7 Comparative Plasma Proteomics

4.7.1 Comparative Plasma Proteomics of High and Low reproductive performance in Cyclical Deoni Cows

Data-independent acquisition (DIA) based LC-MS/MS plasma proteomics was carried out in normal cyclical Deoni cows with differing reproductive performance to identify the differentially expressed proteins related to reproductive performance. A total of 430 proteins were identified across all the biological replicate samples. The fold change value of identified proteins were calculated in the cyclical high reproductive performance (HRP) and low reproductive performance (LRP) groups. Proteins with fold change of ≥1.5 and p- value ≤0.05 were considered to be upregulated, while those with fold change of ≤0.66 and p- value ≤0.05 were considered to be downregulated statistically. Using this cut-off value, we identified 20 (15 upregulated and 5 downregulated) differentially expressed proteins with respect to LRP and 20 (5 upregulated and 15 downregulated) differentially expressed proteins with respect to HRP groups (Table 4.7).

4.8 Gene Ontology Analysis of Altered Plasma Proteins between High and Low Reproductive Performance Cyclical Groups

The differentially expressed proteins of the plasma proteome in the high- and low-reproductive-performance groups were further analysed using the Panther software to reveal the molecular function, biological process, and cellular component role of identified proteins in the present study. In molecular function: binding (57.10%), catalytic (28.60%), and structural molecule (14.30%) activities were found to be enriched (Fig 4.6). In biological process, identified proteins were found to be mostly involved in cellular process (35.30%), metabolic process (15.80%), localization (10.50%), biological regulation (5.30%), response to stimulus (5.30%), multicellular organismal process (5.30%), immune system process (5.30%), developmental process (5.30%) and biological adhesion (5.30%) (Fig 4.7). In cellular components: cellular anatomical entity (75.00%) and protein-containing complex (25.00%) were enriched in this study.

4.9 Functional Pathway Analysis of Altered Plasma Proteins between High and Low Reproductive Performance Cyclical Groups

Pathway analysis of differentially expressed plasma protein in relation to reproductive performance revealed 3 proteins involved in 3 different pathways: B2M protein involvement

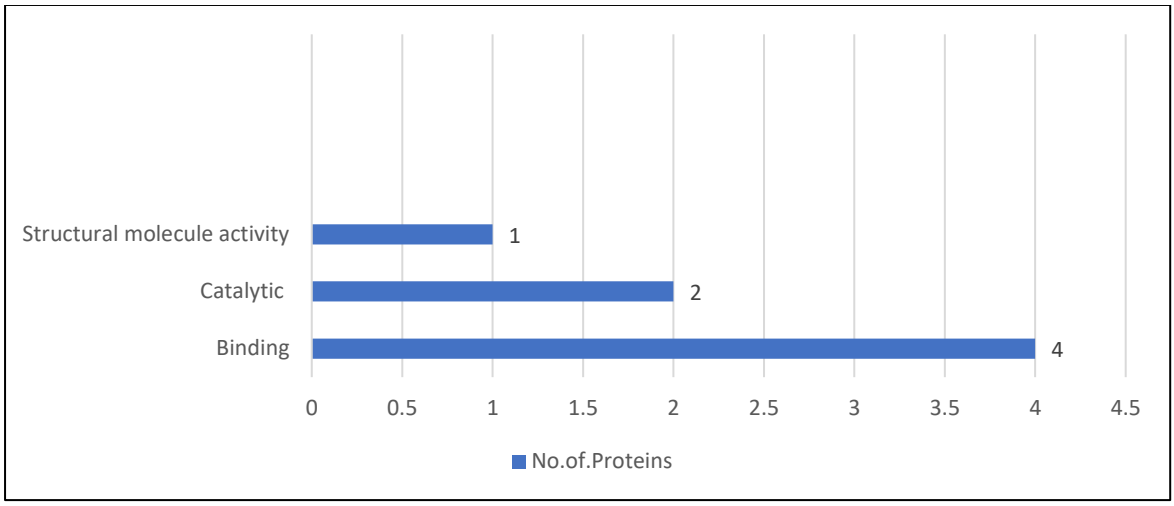

Fig 4.6 Number of Proteins Enriched in Molecular Functions in Cyclical Deoni Cows

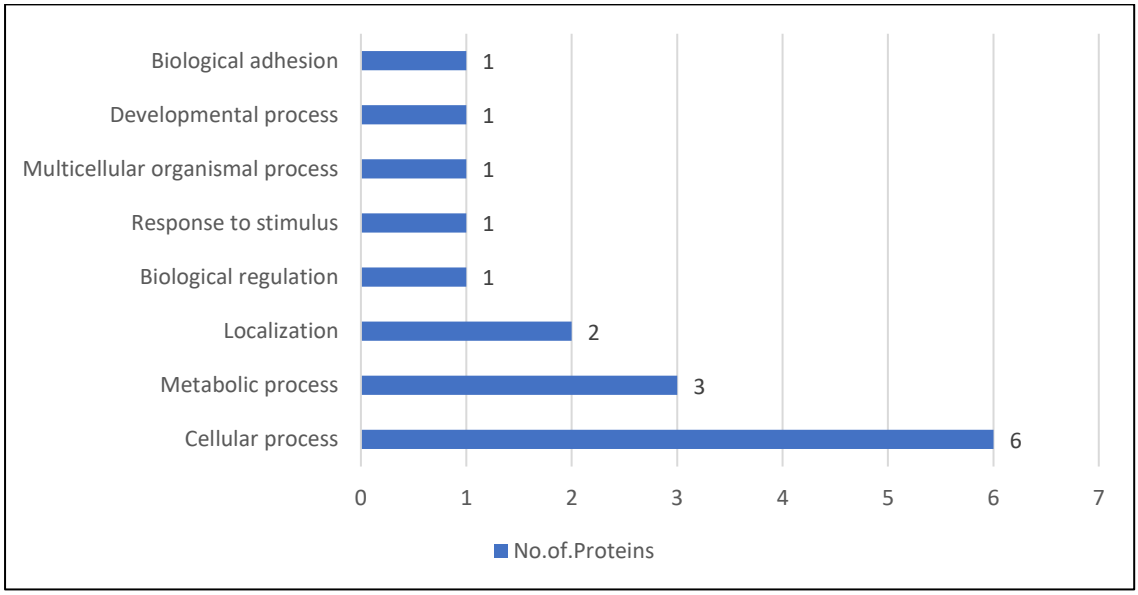

Fig 4.7 Number of Proteins Enriched in Biological Processes in Cyclical Deoni Cows

Table 4.9 Differentially Expressed Proteins in the Plasma Proteome of High and Low Reproductive Performance Cyclical Groups

Protein Accession	Gene Symbol	Protein Name	HRP Fold change value	LRP Fold change value	*p*-value
NP_001029434.1	AFP	Alpha fetoprotein	0.314560196	3.179041759	0.035477983
XP_002700507.3	SHROOM2	Shroom family member 2	0.146397155	6.830733853	0.045801953
NP_001094733.1	FAM72A	Family with sequence similarly 72 member A	0.415673272	2.405735629	0.017837486
NP_001159984.1	PROC	Protein C	0.565194401	1.769302735	0.020486414
NP_001177353.1	WC1.3	WC1.3 molecule	189.8319657	0.005267817	0.042470951
NP_001179630.2	BARD1	BRCA1 associated ring domain 1	0.507467246	1.970570528	0.033875185
NP_001192362.2	SETD1A	SET domain containing 1A, histone lysine methyltransferase	15.56745486	0.064236576	0.012267205
NP_001229511.1	CSTF2T	Cleavage stimulation factor subunit 2 tau	0.206982474	4.831326916	0.01477757
NP_776318.1	B2M	Beta-2-microglobulin	0.439137316	2.277192039	0.044006115
NP_776502.1	GPX3	Glutathione peroxidase 3	1.670850932	0.598497437	0.007944394
NP_777128.1	TNXB	Tenascin XB	0.34582091	2.891670139	0.014031902
XP_002693921.4	LOC100336868	Complement factor H	0.46802068	2.13665772	0.048208937
XP_002696910.2	LOC615277	Acyl-coenzyme A thioesterase THEM4	0.375185928	2.665345169	0.006719851
XP_002696910.2	CFHR5	Complement factor H related 5	0.375185928	3.92222628	0.006719851
XP_015327629.1	ADIPOQ	Adiponectin	2.389176907	0.418554188	0.00158708
XP_024833616.1	LOC112441499	Immunoglobulin lambda-1 light chain-like	0.548276784	1.823896303	0.026871682
XP_024852719.1	TBC1D32	TBC1 domain family member 32	0.089506464	11.1723774	0.026426736
XP_024856930.1	APCDD1L	APC downregulated 1 like	4.532284623	0.220639276	0.017700431
NP_001068592.1	LAMP1	Lysosomal associated membrane protein 1	0.17780414	5.624166022	0.007867071
NP_001076979.1	KRT6A	Keratin 6A	0.149343015	6.695994447	0.014803416

in T cell activation. Protein C in blood coagulation pathway and adiponectin protein in gonadotropin-releasing hormone receptor pathway.

4.10 Differentially Regulated Plasma Proteins in Relation to Reproductive Performance in Cyclical Groups

A total of 20 proteins were found to be differentially expressed in between high and low reproductive performance groups. Among the differentially expressed proteins, 5 were upregulated and 15 proteins were found to be downregulated in high reproductive performance group as compared to low reproductive performance group (Table 4.7). Similarly, 15 proteins were upregulated and 5 proteins were downregulated in low reproductive performance group as compared to high reproductive performance group as shown in volcano plot (Fig 4.8).

Alpha fetoprotein (AFP) is produced in the yolk sac and the liver of the foetus in the pregnant cow. AFP is physiologically and structurally similar to the function of serum albumin. AFP plays an important role in anti-oxidant effect, growth regulation in different cells, immune modulator and transport lipids and estrogen hormone to target tissue. Rizzo *et al*. (2019) reported that AFP along with Anti-Mullerian hormone plays a significant role in sexual differentiation due to its binding ability for estrogen hormone. Vincze *et al*. (2018) correlated AFP level with foetal age, conception failure, placentitis and embryonic loss in equine. AFP was also considered as acute phase reactant and it elevated during acute inflammatory response in pregnancy (Mizejewski, 2015). This protein was found to be overexpressed in the low reproductive performance group in our study as compared to high reproductive performance group.

Tenascin x (TNXB) protein is an extracellular glycoprotein regulate cell differentiation and having anti-adhesive effect. TNXB was suggested as a one of the important extracellular proteins for oocyte maturation (Hatzirodos *et al*., 2014). In our study, TNXB protein was found to be downregulated in the high reproductive performance group in agreement with earlier findings of Tahir *et al*. (2019) where they also reported tenascin x protein downregulation in the post-pubertal heifers as compared to pre-pubertal heifers.

BRCA1- associated ring domain-1 (BARD1) protein plays a major role in the control of the cell cycle in response to any DNA damage in the cell. BRCA1 protein was found to be an important marker in determining oocyte maturation and its quality (Gasca *et al*., 2007; Huang and wells, 2010). In the present study BARD1 protein was found to be upregulated in

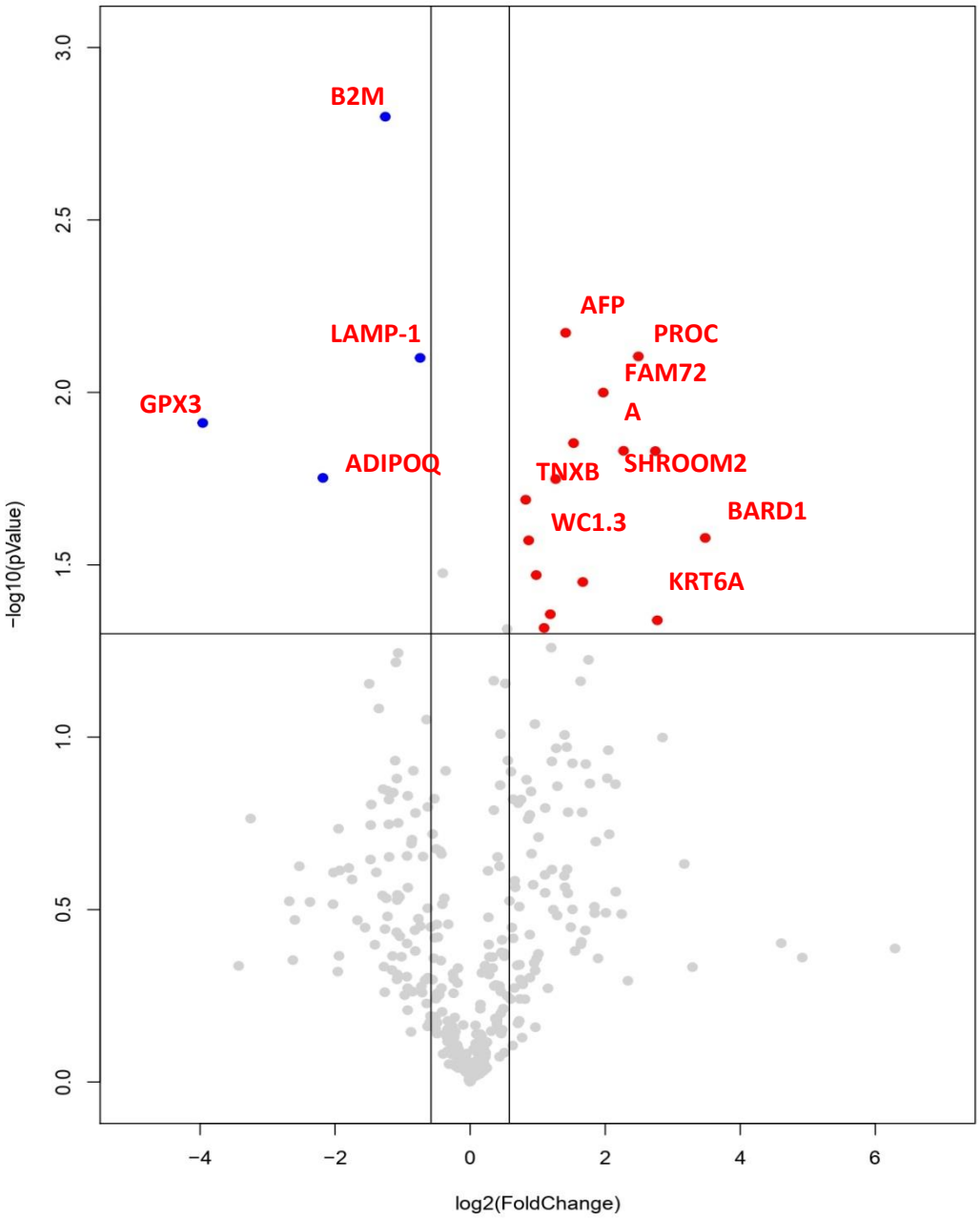

Fig 4.8 Volcano Plot of Differentially Expressed Proteins in Cyclical Deoni Cows

low reproductive performance group which is in agreement with result observed in the study by Gasca *et al*. (2008).

Keratin 6A (KRT6A) protein is involved in repair of the damaged epithelial cell and regulate migration of epithelial cells to the site of tissue damage. KRT6A protein expression was found to be enriched in the oesophagus, cervix and vagina tissues (Yip *et al*., 2013; Li *et al*., 2018). In this study the expression of KRT6A was found to be upregulated in the low reproductive performance group.

Beta-2 Microglobulin (B2M) protein found in association with the major histocompatibility complex (MHC) class I on the cell surface of all nucleated cells. This protein was found to regulate early pregnancy and progesterone secretion in ovine endometrium (Gray *et al*., 2006). In mice B2M expression was associated with reduced reproductive performance (Cooper *et al*., 2007). In our study B2M protein was found to be upregulated in the low reproductive performance group.

Lysosome associated membrane glycoprotein-1 (LAMP1) participate in autophagy, cell proliferation, cell apoptosis and cell attachment in the endometrium to prepare the uterine environment for implantation of embryo (Rehman *et al*., 2003). Significant association between LAMP1 expression with successful implantation of embryo was reported in the ovine uterus (Yang *et al*., 2021). In the present study this protein was found upregulated in the low reproductive performance group.

Glutathione peroxidase-3 (GPX3) protein protect the cells from oxidative stress damage. GPX3 protein was reported to play an important role in reducing oxidative stress damage during the transport of fertilized ovum in the oviduct and embryo during implantation stage in the uterus (Xu *et al*., 2014b). In our study GPX3 protein was upregulated in the high reproductive performance group.

Adiponectin (ADIPOQ) protein expression was observed in the adipose tissue and circulated in the plasma for controlling metabolic and hormonal processes. ADIPOQ protein mediates its action in the periphery by AdipoR1 and AdipoR2 receptors (Michalakis and Segars, 2010). Adiponectin receptors were located in hypothalamic-pituitary-gonadal axis, oviduct, uterus, embryo, placental tissues and found to have significant association with production and reproductive performance in the livestock (Dobrzyn *et al*., 2018; Mohammed

et al., 2022). In our study ADIPOQ protein was upregulated in the high reproductive performance group.

Earlier studies discussed above support that the identified proteins in our study are related to the reproductive performance in dairy cattle.

4.11 Protein-protein Interactions in Cyclical Deoni Cows

The differentially expressed plasma proteins in cyclical Deoni cows were analyzed using STRING software for protein interactions. The proteins identified in our study were found to be not much interacted suggesting that these proteins have independent functions. Most of the identified proteins were interacting with B2M and AFP proteins (Fig 4.9).

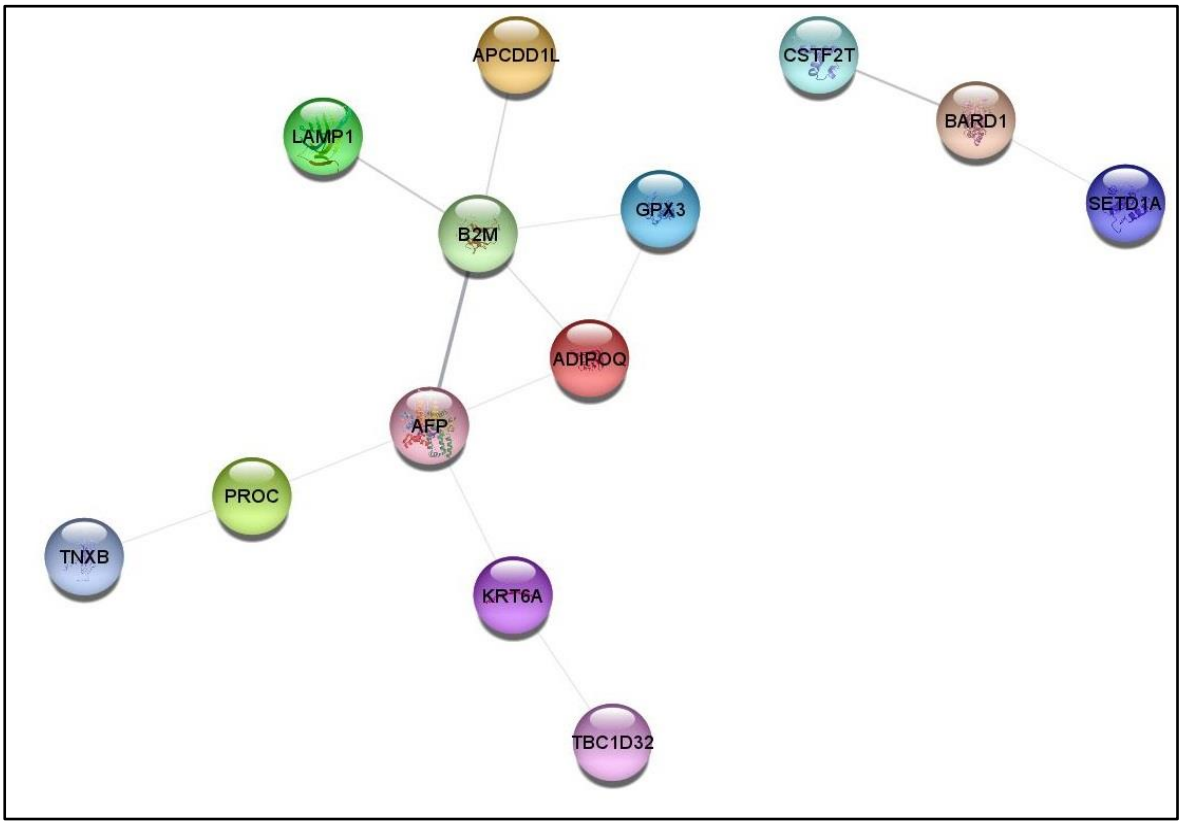

Fig 4.9 Protein-protein Interaction in Cyclical Deoni Cows

4.12 Comparative Plasma Proteomics of High and Low Reproductive Performance in Pregnant Deoni Cows

Data-independent acquisition (DIA) based LC-MS/MS plasma proteomics was carried out in pregnant Deoni cows with differing reproductive performance to identify the differentially expressed proteins related to reproductive performance. A total of 430 proteins were identified across all the biological replicate samples. The fold change value of identified protein was calculated in the pregnant high reproductive performance (HRP) and low reproductive performance (LRP) groups. Proteins with fold change of ≥1.5 and p- value ≤0.05 were considered to be upregulated, while those with fold change of ≤0.66 and p- value ≤0.05 were considered to be downregulated. Using this cut-off value, we identified 35 (23 upregulated and 12 downregulated) differentially expressed proteins in comparison to LRP and 35 (12 upregulated and 23 downregulated) differentially expressed proteins in comparison to HRP groups (Table 4.8).

4.13 Gene Ontology Analysis of Altered Plasma Proteins between High and Low Reproductive Performance Pregnant Groups

The differentially expressed proteins identified from the plasma proteomics in relation to the high- and low-reproductive-performance groups were further analysed using the Panther software to find out the molecular function, biological process, and cellular component role of proteins. In molecular function: binding activity (38.90%), catalytic activity (25.00%), molecular function regulator (16.70%), transporter activity (5.60%), transcription regulator activity (2.80%), cytoskeletal motor activity (2.80%), ATP-dependent activity (2.80%), and molecular adaptor activity (2.80%) were enriched (Fig 4.10). In biological process: cellular process (23.30%), biological regulation (17.80%), metabolic process (16.4%), response to stimulus (22.20%), localization (6.80%), signaling (4.10%), locomotion (1.40%), growth (2.70%), multicellular organismal process (5.50%), immune system process (2.70%), developmental process (4.10%) and biological adhesion (4.10%) identified proteins were enriched (Fig 4.11). In Cellular components: cellular anatomical entity (63.90%) and protein-containing complex (13.90%) were enriched.

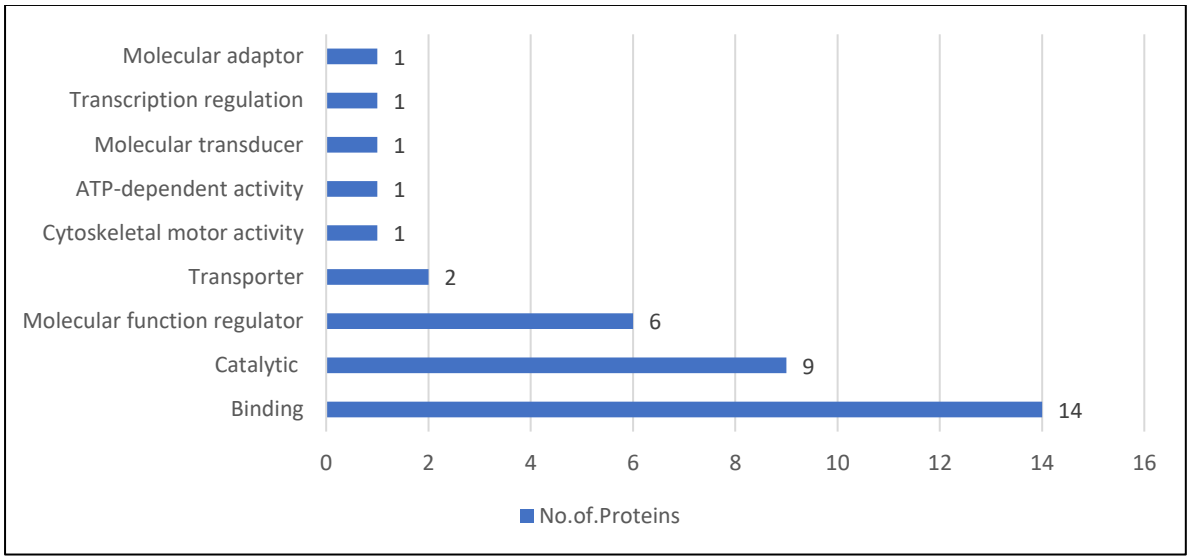

Fig 4.10 Number of Proteins Enriched in Molecular Functions in Pregnant Deoni Cows

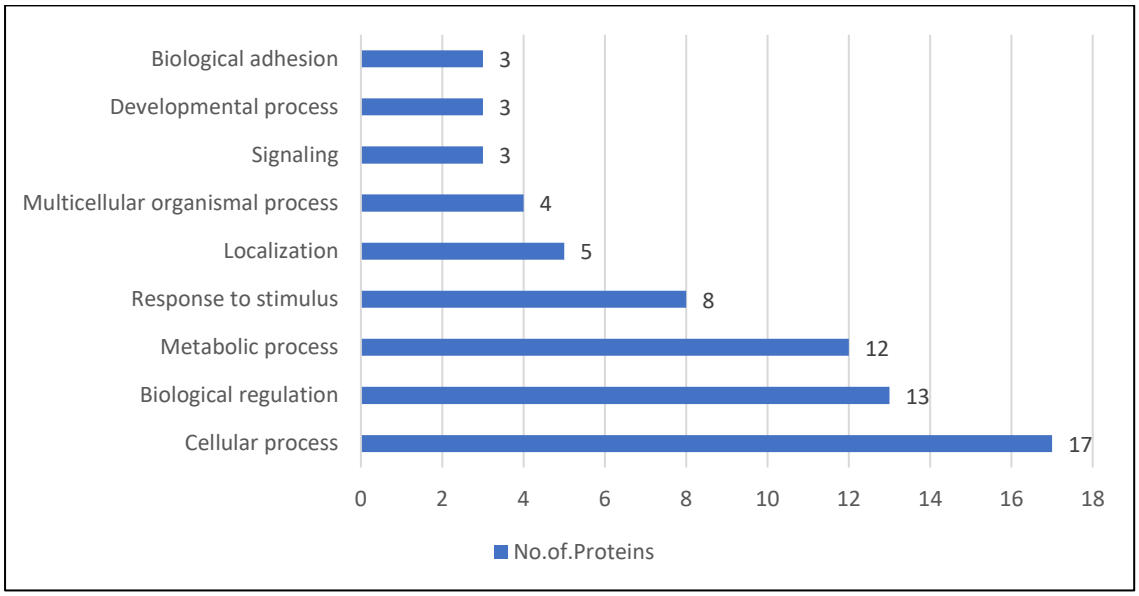

Fig 4.11 Number of Proteins Enriched in Biological Processes in Pregnant Deoni Cows

Table 4.10 Differentially Expressed Proteins in the Plasma Proteome of High and Low Reproductive Performance Pregnant Groups

Protein Accession	Gene Symbol	Protein Name	HRP Fold change value	LRP Fold change value	*p*-value
XP_010815611.1	SERPINA6	Serpin family A member 6	0.101068464	336615.7024	0.046849048
NP_001003907.1	APON	Apolipoprotein N	0.466334098	2.144385334	0.032072565
NP_001029208.1	PPARA	Peroxisome proliferator activated receptor alpha	0.596218461	1.677237566	0.04554098
NP_001029429.2	APOM	Apolipoprotein M	3.495483331	0.28608347	0.004581549
NP_001030209.2	CRYZL1	Crystallin zeta like 1	0.101068464	9.894283121	0.042844504
NP_001033119.1	SGTA	Small glutamine rich tetratricopeptide co-chaperone alpha	61.29621204	0.016314222	0.037757499
NP_001033185.1	CFI	Complement factor 1	0.574926493	1.739352791	0.013290095
NP_001033207.1	SLC17A2	Solute carrier family-17member 2	3.222340316	0.310333454	0.002202051
NP_001039562.1	FGL2	Fibrinogen like 2	3.581448576	0.279216629	0.028883731
NP_001068779.1	SFTPB	Surfactant protein B	0.124633396	8.023531641	0.021494462
NP_001095589.1	CD5L	CD5 molecule like	0.601319311	1.512128836	0.001256926
NP_001095850.1	APOC2	Apolipoprotein C2	0.314879502	3.175818031	0.034758719
NP_001098847.1	NANP	N-acetlyneuraminic acid phosphatase	3.168195167	0.315637121	0.007724222
NP_001159984.1	PROC	Protein C	0.377777196	2.647062898	0.032835444
NP_001177353.1	WC1.3	WC1.3 molecule	3.553471392	0.281414957	0.024721385
NP_001179860.1	CELSR2	Cadherin EGF LAG seven-pass G-type receptor 2	0.222915633	4.486002112	0.01086633

NP_001192331.1	TGFBI	Transforming growth factor beta induced	0.340611029	2.935900233	0.041536673
NP_001192645.1	ZNFX1	Zinc finger NFX1-type containing 1	2.923058339	0.342107438	0.008557462
NP_001268848.1	AOX4	Aldehyde oxidase 4	5.127825499	0.195014436	0.033332419
NP_001306813.1	LOC100297192	Ig heavy chain Mem5-like	0.207378205	4.822107504	0.026384222
NP_776327.1	CLU	Clusterin	0.500567104	1.997734155	0.027381457
NP_776409.1	AHSG	Alpha 2-HS glycoprotein	0.457386151	2.186336421	0.007480364
NP_776532.1	MBL2	Mannose binding lectin 2	4.580732889	0.218305678	0.026003819
NP_777048.1	TMSB10	Thymosin beta 10	3.631440683	0.275372803	0.035365784
NP_777095.1	SERPINF2	Serpin family F member 2	0.590618479	1.693140386	0.020039202
NP_786968.2	KNG1	Kininogen 1	0.464712467	2.151868245	0.010528805
XP_002691765.2	PPP1R26	Protein phosphatase 1 regulatory subunit 26	0.316778446	3.156780436	0.015844169
XP_005205346.3	RAPGEF5	Rap guanine nucleotide exchange factor 5	0.466042242	2.14572824	0.038456294
XP_010817657.1	VWA3A	Von Willebrand factor A domain containing 3A	0.568985134	1.757515163	0.040771066
XP_015327629.1	ADIPOQ	Adiponectin	0.207649298	4.815812102	0.028142558
XP_024839229.1	FSIP2	Fibrous sheath interacting protein 2	2.481634896	0.402960162	0.037837716
XP_024852035.1	LOC112447830	spermatogenesis-associated protein 31A3-like	11.52660107	0.086755844	0.004006129
XP_024856677.1	KIF16B	Kinesin family member 16B	0.26568155	3.76390457	0.010382469
NP_001071328.1	DGKA	Diacylglycerol kinase alpha	1.00344E-08	99656976.92	0.005522647
NP_001306832.1	VPREB1	V-set pre-B cell surrogate light chain 1	0.145995181	6.849541164	0.003141497

4.14 Functional Pathway Analysis of Altered Plasma Proteins between High and Low Reproductive Performance Pregnant Groups

Pathway analysis of differentially expressed plasma protein in relation to reproductive performance between high and low reproductive performances revealed 7 different pathways and proteins involved in it. Pathways were CCKR signaling map (CLU), Wnt signaling pathway (CELSR2), p53 pathway (DGKA), plasminogen activating cascade (SERPINF2), gonadotropin-releasing hormone receptor (ADIPOQ, PPARA), cadherin signaling pathway (CELSR2), and blood coagulation pathway (SERPINF2, KNG1, PROC).

4.15 Differentially Regulated Plasma Proteins in Relation to Reproductive Performance in Pregnant Groups

A total of 35 proteins were found to be differentially expressed between high and low reproductive performance groups. Among these differentially expressed proteins 12 were upregulated and 23 proteins were downregulated in high reproductive performance group as compared to low reproductive performance group (Table 4.8). Similarly, 23 proteins were upregulated and 12 proteins were downregulated in low reproductive performance group as compared to high reproductive performance group was shown in volcano plot (Fig 4.12)

Serpin family A member 6 (SERPINA6) protein is a corticosteroid-binding globin produced in the liver and found circulated in the plasma. It binds steroid hormones such as corticosterone, aldosterone and progesterone and transport it to target site (Gardill *et al*., 2012). Elevated level of glucocorticoids was found to have a negative influence on reproductive performance at ovary and uterus (Whirledge and Cidlowsk, 2010). In the present study, the expression of SERPINA6 was found to be upregulated in the low reproductive performance group.

Peroxisome proliferator activated receptor Alpha (PPARA) protein act as ligand activated transcription factor and it belongs to nuclear receptor family. These protein receptors were found to be situated in the hypothalamic-pituitary-gonadal axis and contribute a significant role in the reproduction system (Vitti *et al*., 2016). PPARA protein plays a key role in controlling the implantation process, differentiation of embryonic cells, and vascularization of placenta at the time of pregnancy period (Huang *et al*., 2007; Kang *et al*., 2011; Meher *et al*., 2015). PPARA was observed to be downregulated in the high reproductive

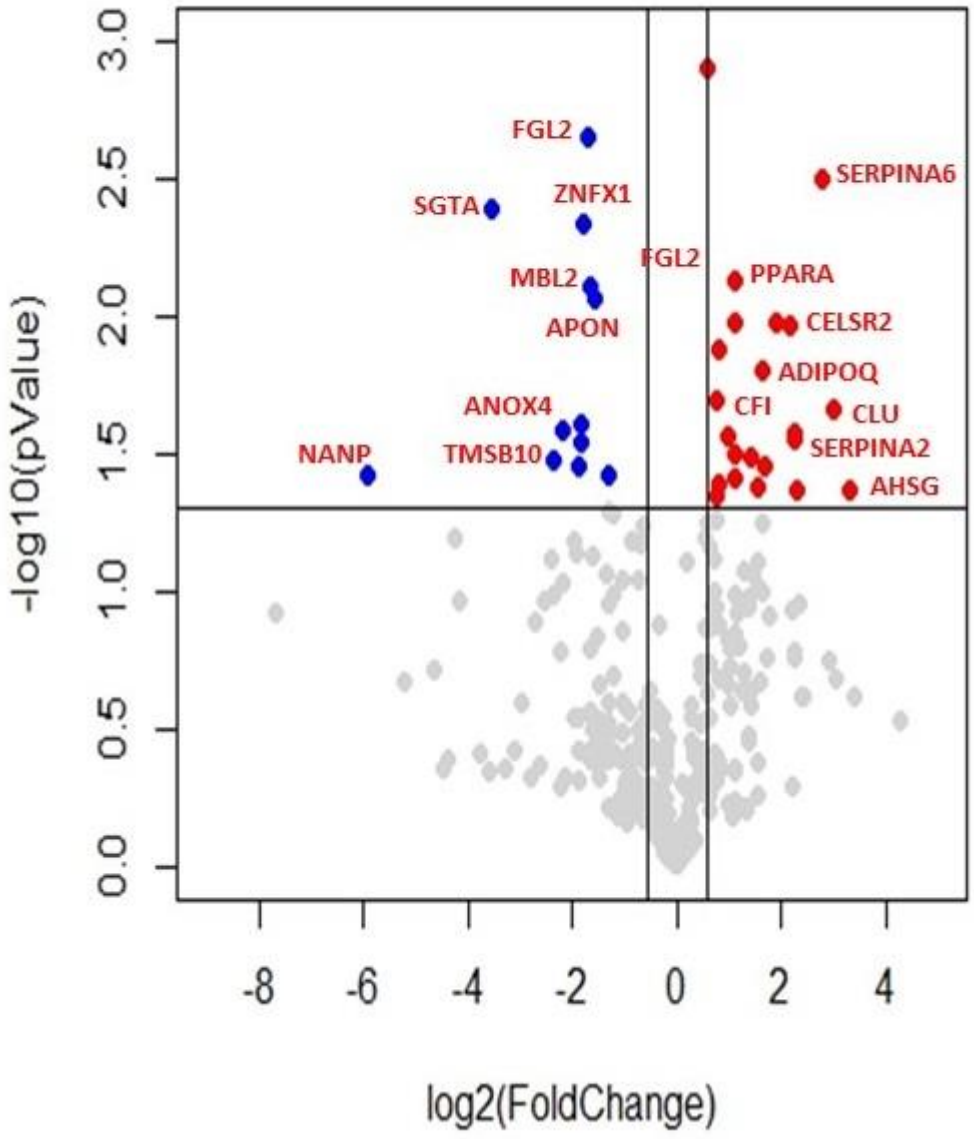

Fig 4.12 Volcano Plot of Differentially Expressed Proteins in Pregnant Deoni Cows

performance group in the present study which is in agreement with the earlier report by Gimble *et al*. (1998) in which PPARA was downregulated during gestation period.

Clusterin (CLU) protein play an important role in lipid transport, cell adhesion, immune tolerance mechanism for male antigen (sperm) in female reproductive tract and regulation of oxidative stress damage (Zalata *et al*. 2012). In the present study, CLU protein was found to be overexpressed in the low reproductive performance group. Janiszewska and Kratz, 2020 reported clusterin protein as sensitive marker for oxidative stress condition in the female reproductive tract during sperm capacitation, which cause fertilization failure.

Serpin family F member 2 (SERPINF2) protein is a key inhibitor of plasmin, which degrades fibrin and numerous other proteins. SERPINF2 plays a significant role in regulating blood clotting pathway (Rau *et al*., 2007). SERPINF2 protein was found to be upregulated in the low reproductive performance group in this study. SERPINF2 protein was found to be localized in the granulosa cells of bovine follicles and contribute its role in coagulation, protein c and fibrinolytic pathways which involved in the follicular growth mechanism (Hayashi *et al*., 2011). In the post-partum cow, association between over expression of SERPINF2 with reduced fertility was found in response to higher concentration of blood urea level (Cheng *et al*., 2015). Shao *et al*. (2019) correlated overexpression of SERPINF2 with imbalanced sex hormone in preeclampsia condition in human.

Kininogen 1 (KNG1) is an extracellular matrix protein participate in cell adhesion, proliferation and extracellular remodelling in different tissues (Barua *et al*., 2018). In the present study, KNG1 protein was found to be differentially expressed between high and low reproductive performance groups. Kaczynski *et al*. (2018) suggested that KNG1 protein promotes changes between maternal and placental tissues which is necessary to promote fertilization, endometrial remodelling, embryo implantation, placental invasion to support fetal development during pregnancy period.

Alpha-2-HS-glycoprotein (AHSG) is a negatively charged glycoprotein synthesised mainly in the liver and found circulating in the blood. Elevated level of AHSG protein was found to be highly related to negative energy balance, since reproduction and energy balance are highly correlated this might impair reproductive efficiency in dairy cows (Wathes *et al*., 2011). Similarly, upregulation of AHSG was found to be closely related to inactive ovaries when comparison with active ovaries in plasma proteins of dairy cows (Zhao *et al*., 2019). This protein was found to be upregulated in the low reproductive performance group in the present

study. So, this protein might be one of those proteins which are responsible for low reproductive performance in Deoni cows.

Fibrinogen like 2 proteins (FGL2) have a physiological role at mucosal membrane of different tissue. Yang and Hooper, (2013) reported the expression of FGL2 protein in the heart, lungs, intestine, spleen, ovary, uterus, liver and kidney. In this study, FGL2 protein was found to be upregulated in the high reproductive performance group when compared to the low reproductive performance group. FGL2 protein also act as immune suppressor to suppress the T-cell activation and significantly implant the embryo without any immune rejection (Mu *et al*., 2007). Furthermore, FGL2 protein act as prothrombinase and remodel the endometrial surface for successful implantation of embryo (Clark *et al*., 2004; Yang and Hooper, 2013). FGL2 protein level was found to be upregulated in the pregnant women when compared to non-pregnant women and its low level at the time of pregnancy reported to cause embryonic losses in mice's (Pan *et al*., 2003; Mu *et al*., 2007).

Zinc finger NFX-type containing 1 (ZNFX1) protein modulate maternal immune response in response to IFNT (Interferon Tau) which is required for uterine receptivity and successful implantation of embryo (Spencer *et al*., 2008). ZNFX1 protein is observed upregulated in the high reproductive performance group which is in agreement with the result observed in the study done by Forde *et al*. (2012) where ZNFX1 protein was upregulated in the uterus during early pregnancy.

Thymosin Beta 10 (TMSB10) protein contributes its major role in the cell migration, cell motility, cell differentiation and also regulate small proteins of actin cytoskeleton. TMSB10 protein was found to be upregulated in the high reproductive performance Deoni cow group in this study. Cammas *et al*. (2005) observed positive correlation between increased level of TMSB10 protein with elongation and implantation of ovine embryo in the endometrial tissue. TMSB10 was also reported as an important protein for bovine ovarian follicle maturation (Salhab *et al*., 2010).

Mannose Binding Lectin-2 (MBL2) protein belongs to the collectin family and a part of complement system found in the plasma proteins. MBL2 protein was reported to be positively correlated with lesser incident of occurrence of mastitis and reproductive tract related infection and diseases, which resulted in the improvement of the reproductive efficiency in an animal (Seta *et al*., 2007; Bulla *et al*., 2010; Moretti *et al*., 2021). MBL2 protein was observed to be upregulated in the high reproductive performance group which is in agreement

with the result observed in the study done by Sato *et al*. (2016) in which this protein was found to be associated with high reproductive performance group of Large White Yorkshire pig population.

All the above reports are in agreement with the identified differentially expressed proteins in the present study in Deoni cows with differing reproductive performances.

4.16 Protein-protein Interactions in Pregnant Deoni Cows

The differentially expressed plasma proteins in cyclical Deoni cows were analyzed using STRING software for protein interactions. The proteins identified in our study were found to be not much interacted suggesting that these proteins have independent functions. Most of the proteins were found to be interacted with AHSG (Fig 4.13).

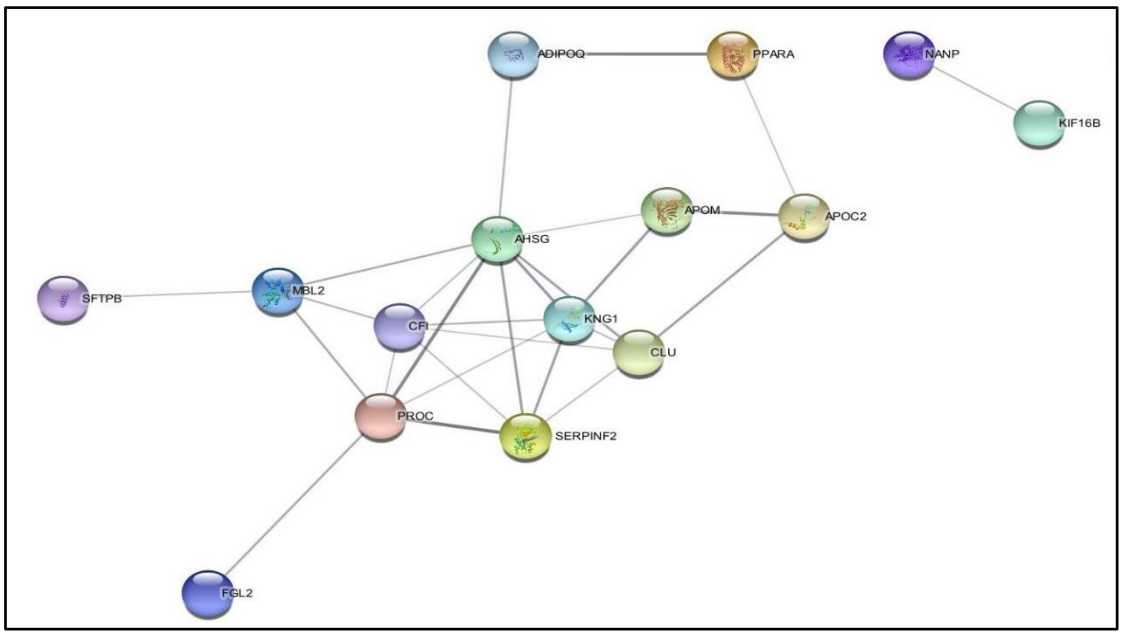

Fig 4.13 Protein-protein Interaction in Pregnant Deoni Cows

CHAPTER –5

Summary and Conclusions

SUMMARY AND CONCLUSIONS

Reproductive performance is one of the major determinants of efficient dairy cattle production. Continuous selection for milk production traits resulted in decline in the genetic trend of reproductive performance in the dairy cattle, which directly affect the productivity and longevity of the dairy cattle. The selection for better reproductive performance is the only way to reverse the declining genetic trends for reproductive traits in dairy cattle. However, the selection for improvement of reproductive traits is often based on phenotypic traits like age at puberty, age at first calving, conception rate, days open and inter-calving periods, which are having low heritability, polygenic in nature and controlled by several environmental factors resulting in hindering the phenotypic based selection for improvement of reproductive performance traits in cattle. Considering the above facts, incorporation of genetic information about genes and proteins related to reproductive characters in to the selection program would improve both the accuracy and selection of dairy cattle with good reproductive performance. For that purpose, understanding the genetic basis of reproductive traits and identification of genes and proteins associated with reproduction traits are crucial. Therefore, the present study was designed to profile the exome and proteome of the genomic DNA and blood plasma respectively in Deoni cows with differing reproductive performance and to identify the exons with genes, differentially expressed proteins in cows related to reproductive performance traits.

The Deoni cows with age ranged from 6 to 9 years were selected based on the days open and inter-calving period. In high reproductive performance (HRP) group mean days open and inter-calving period calculated were 113±23.98 days and 398±24.85 days respectively. Likewise, in low reproductive performance group, mean days open and inter-calving period calculated were 292±60.82 days and 576±58.90 days respectively. The blood samples were collected from selected Deoni cows and genomic DNA was isolated using high salt method. The quantity and quality of the genomic DNA were assessed using Nanodrop2000 and agarose gel electrophoresis. Library construction was carried out using targeted exome sequencing library and it was prepared with Illumina-compatible Sure Select XT Library Prep Reagent Kit. Finally, whole exome sequencing was performed on Illumina Hi-seq platform to generate 100X coverage per sample. On the other hand, plasma was separated from the blood samples and proteins were extracted, reduced, alkylated, digested, desalted and fractionated by following standard procedures. The fractionated protein samples were run in an orbitrap fusion tribrid mass spectrometry connected with easy- nLC-1200 nanoflow liquid chromatography system

to acquire data independent acquisition mass spectrometry data from all the biological replicate samples. In-house bovine plasma spectral library was built from the plasma proteome data acquired from data dependent acquisition as well as from gas-phase fractionation approach to identify the plasma protein. The mass spectrometry data were finally searched against in-house bovine plasma spectral library. The salient findings observed from whole exome sequencing analysis and plasma proteomics were:

- On an average, ~96 million paired end raw reads were generated for all the Deoni samples.
- 99.50 % of raw reads were retained for alignment process, after it was processed for adaptor sequences and phred score value below 30.
- Processed reads were aligned to *Bos indicus* reference genome and got overall alignment rate of 92.90% for all the samples.
- HRP and LRP group samples were merged separately using maximum likelihood method and called out for variants.
- At read depth of 10 and mapping quality 30, 297,398,612 variants in HRP group and 162,565,301 variants in LRP group were identified
- Additionally, Hardy Weinberg equilibrium (0.001), minor allele frequency (0.01), missing genotypes (0.7) and linkage disequilibrium were imposed to reduce the false positive rate and got 312 SNPs in HRP group and 301 SNPs in LRP group
- Identified SNPs were annotated and got 221 genes in HRP group and 244 genes in LRP group
- Gene Ontology revealed that binding and catalytic activity were the major molecular function while cellular, metabolic and biological regulation as the primary biological process
- Pathway enrichment of identified genes revealed 12 different pathways related to reproductive functions.
- There were no significant differences in the molecular function, biological process and cellular component between HRP and LRP group.
- ADAMTS13, TRPV4, HDGF, RANGAP1, ROR2, CNOT1, CELSR3, PTPRS, NFAM1, ADAMTS19, CTRC1, PLA2G4F, SHANK2, and SLIT3 were some of the genes found to be associated with reproductive traits like calving ease, insemination per conception, length of productivity and days open.

- In LRP group, THBS4, SLC37A2, FOXH1, BRWD1, RABEP2, DGKI, ANKH, SCARF1, EPAS1, EIF4G3, and RABEP2 were some of the genes involved in the calving ease, productivity, days open and calving interval traits.
- In plasma proteomics, a total of 430 plasma proteins were identified in all biological replicate samples. In that, 20 proteins were differentially expressed ($p<0.05$) between high and low reproductive performance cyclical group.
- Gene Ontology showed that binding, catalytic and structural molecule activities were mostly enriched in the molecular function while in the biological process cellular process, metabolic process and in localization differentially expressed proteins were enriched.
- Important proteins include AFP, TNXB, BARD, KRT6A, B2M, LAMP1, GPX3, and ADIPOQ were found to be involved in any of oxidative stress damage control, cell proliferation, cell differentiation, apoptosis, energy balance and autophagy which is crucial for transport of fertilized embryo from oviduct to uterus and prepare the uterine environment receptive for successful implantation.
- A total of 35 proteins were differentially expressed ($p<0.05$) between high and low reproductive performance pregnant groups.
- Gene Ontology revealed that binding, catalytic and molecular function regulator activities were enriched most in the molecular function while in the biological process cellular process, biological regulation and in metabolic processes, altered proteins were enriched.
- Important proteins include SERPINA6, PPARA, CLU, SERPINF2, KNG1, AHSG, FGL2, ZNFX1, TMSB10, and MBL2 were found to play a role in any of immune response, cell adhesion, energy balance, extracellular matrix, steroid binding protein, cell differentiation, cell migration and vascularization which is required for normal reproductive performance and establishment of pregnancy in an animal.

Conclusions

Based on the findings of the present study, the following conclusions are derived.

- Exome and proteome profiling of Deoni cows with differing reproductive performance revealed that there is significant difference between the high and low reproductive performance groups at genome and proteome level.
- ADAMTS, TRPV4, HDGF, RANGAP1, ROR2, CNOT1, CELSR3, PTPRS, NFAM1, CTRC1, PLA2G4F, SHANK2, SLIT3, THBS4, SLC37A2, BRWD1, RABEP2, DGKI, ANKH, SCARF1, EPAS, EIF4G3, and RABEP2 genes identified in the exome profiling and their association with reproductive performance traits suggest that these genes might serve as a candidate genetic marker for improving reproductive performance in dairy cattle.
- AFP, TNXB, BARD, KRT6A, B2M, LAMP1, GPX3, and ADIPOQ proteins in cyclical cows and SERPINA6, PPARA, CLU, SERPINF2, KNG1, AHSG, FGL2, ZNFX1, TMSB10, and MBL2 proteins in pregnant cows were reported to be involved in cell differentiation, cell migration, cell adhesion, immune response, oxidative stress control, autophagy, apoptosis, negative energy balance which is required for proper establishment of pregnancy in dairy cows.
- The findings in the present study contribute for better understanding of the genetic and physiological basis of reproductive performance and may aid to identify probable candidate genes and protein for better improvement of reproduction performance in dairy cattle.

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CURRICULUM VITAE

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Career Objective

Looking forward to employ my skills to contribute to the profession of veterinary and animal science.

Technical Skill sets on research

Wet Laboratory

DNA isolation

Plasma separation

Gel electrophoresis

Genomics

ddRAD Sequencing of DNA

Whole Exome profiling of DNA

Bioinformatics analysis of genomics data.

Proteomics

Proteomic profile analysis of bovine plasma.

Bioinformatics analysis of mass spectrometry data.

Bioinformatics

PRINSEQ, Stacks, Samtools, BCF tools, VCF tools, SNPEFF, DAVID, Cystoscope (Cluego).

Academic Qualifications

Degree	University/Board	Year	Percentage/ Grade
PhD	National Dairy Research Institute, Karnal	2022	8.4/10.0
PGDAW	Indira Gandhi Open University	2022	7.6/10
M.V.Sc.	National Dairy Research Institute, Karnal	2019	8.5/10.0
B.V.Sc & AH	Tamil Nadu Veterinary and Animal Sciences University, Chennai	2016	7.7/10.0
HSC (XII class)	Tamil Nadu State Board	2011	91.00%

Research Project

Masters' Programme: Whole Genome SNPs Identification and Annotation in Tharparkar Cattle.

Doctor of Philosophy Programme: Exome and Proteome Profiling of Deoni Cows with High and Low Reproductive Performance.

Awards and Fellowships:

1. Received Institutional Scholarship funded by ICAR (Indian Council for Agriculture & Research) during Post graduation.
2. Received Dean Merit Award From ICAR-National Dairy Research Institute during Post graduation.
3. Received Institutional Scholarship funded by ICAR during Doctor of Philosophy programme.

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(Joel Devadasan, M)