FINAL RESEARCH PROGRESS REPORT

For

PARB's CGS PROJECT NO.258

ENHANCEMENT OF COST EFFECTIVE MUTTON PRODUCTION THROUGH GENETICALLY ENHANCED PROLIFICACY MANAGEMENT

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(2015)

Table of contents

Sr. No	Title	Page No.
1	Table of contents	(i)
2	Basic information of the project	1
3	Executive summary	2
4	Introduction	4
5	Project Objective	5
6	Outputs planned for the project	6
7	Output 1	6
8	Output 2	6
9	Output 3	6
10	Output 4	7
11	Output 5	7
12	Detailed component wise methodology	8
13	References	29
14	Component wise salient achievements	30
15	Overall progress of the problem searched	33
16	Varieties, breeds, vaccines or products developed and patented	34
17	No. of national and international papers published	34
18	No. of Ph.D/M.Phil. produced	34
19	Current status of commercialization of the project	35
20	Impact of the project on strengthening of the institutional infrastructure, machinery, equipment and human resources	35
21	Constraints in the Implementation of the project and commercialization of the project	35
22	Suggestions for future research and development	36
23	Annexure I	37
24	Annexure II	41
25	Annexure III	45

26	**Annexure IV**	**48**
27	**Annexure V**	**50**
28	**Annexure VI**	**52**
29	**Annexure VII**	**54**
30	**Annexure VIII**	**57**
31	**Annexure IX**	**59**
32	**Annexure X**	**61**
33	**Annexure XI**	**63**
34	**Annexure XII**	**64**
35	**Annexure XIII**	**65**
36	**Annexure XIV**	**67**
37	**Annexure XV**	**68**

Basic Information of the project:

Name of the project	**ENHANCEMENT OF COST EFFECTIVE MUTTON PRODUCTION THROUGH GENETICALLY ENHANCED PROLIFICACY MANAGEMENT**
Project period (from-to)	01-07-2011 to 30-06-2015
Total project duration	48 Months
Total Project cost	Rs. 12.98 Millions
Total Expenditures	Rs. **???** Millions
Name of the Project Manager with designation	**Prof. Dr. Khalid Javed** **Chairman** Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore
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Overseas cooperating scientist and organization	NIL

Executive Summary

This project was aimed to improve sheep meat production and ultimately the rural incomes. Small ruminants play an important role in Pakistani economics by providing essential items of human diet (mutton and milk) and export goods (skins, leather, bones, wool etc). Human population is increasing double against the protein source exclusively from animal origin. Goats and sheep are kept for milk and meat production and contribute significantly to the income of the rural farmers.The target local sheep breed, the Lohi, is by far thedominant sheep breed in Punjab. The projectbuilt on the findings of PARB Project No. 258 that had established that low prolificacy can be improved in Lohi by using the Booroola fecundity gene (FecB).

The Booroola gene (FecB) is a dominant autosomal gene with an additive effect on ovulation rate.One copy of the Booroola gene increases ovulation rate (eggs shed per ewe ovulating) by about 1.5 and two copies by about 3.0. These extra ovulations typically increase litter size (lambs born per ewe lambing) by about 1.0 and 1.5, respectively. The effect of the Booroola gene has been shown to be due to a mutation in the bone morphogenetic protein 1B receptor (BMPR-1B) that is expressed in oocytes and granulosa cells and is located on chromosome 6. The utilization of (FecB) required DNA tests i.e. Restriction Fragment Length Polymorphism (RFLP) or DNA sequencing for Single Nucleotide Polymorphism (SNP) for the detection of mutation.

PARB Project No. 258 ran from 2011 to 2015 and involved collaborationbetween two institutions: University of Veterinary and Animal Sciences, Lahore (UVAS) and Livestock Production Research Institute, Bahadurnagar, Okara.The Animal Genetic Laboratory (AGL) and Small Ruminant Training and Research center (SRT&RC) of UVAS were utilized for genetic analysis and rearing/ breeding of Project animals respectively. The main thrust of the project was to test, under normal conditions, the performance of improved genotypes carrying the FecB mutation, and based on this, develop recommendations regarding the wider dissemination of the gene. Specifically the project objective was to investigate the regulation of expression of FecB in local Lohi sheep and to develop and maintain fecund nucleus Lohi sheep flock through screening of prolificacy gene(s) mutation in Lohi sheep.

The project animals were selected and purchased on the basis of phenotypic data of calving and birth status from two purebred nucleus flocks of Lohi sheep maintained at Livestock Production Research Institute, Bahadurnagar, Okara and Small Ruminant Training and Research center (SRT&RC), UVAS, Ravi Campus, Pattoki. The genetic analysis of project

animals was done at AGL and it included the RFLP marker analysis of bone morphogenetic protein receptor gene (BMPR1B) to find the A→G transition at position 746 (GenBank Acc. No. AF357007), substituting the glutamine present in the wild-type BMPR1B protein with an arginine. Animals were assigned genotypes i.e. carrying no transition (++), carrying one transition (B+) and individuals carrying transitions at both loci (BB).

After completing the genotyping the breeding plans were established to fix the genotype of coming generation. The breeding animals were maintained and bred at SRT&RC under semi stall feeding system. The screening/ breeding for FecB continued and two generations of Lohi sheep obtained. Breeding for FecB gene caused increase in twining rate from 21.71% to 35.71% within two generations, and if selection continues for FecB then rate of twining will double in four generations. Some future researches focusing nutritional and managemental requirements of twin carrying ewes must be conducted to boost the survivability of twins during developmental and growing period.

PROGRESS OF RESEARCH WORK

1. Introduction:

Small ruminants play an important role in Pakistan economics by providing essential items of human diet (mutton and milk) and export goods (skins, leather, bones, wool etc). Human population is increasing double against the protein source exclusively from animal origin. Goats and sheep are kept for milk and meat production and contribute significantly to the income of the rural farmers. These animals can be raised with very little supplemental concentrate/grains and with minimal shelter, and are generally an easy-care animal. Shortage of agricultural lands and migration of rural population towards cities is a serious matter of animal's protein shortage. Lack of commercial farming and shortage of animal feed resources are also important facts. To overcome this problem modern technology can be helpful through the establishment of high producing animals /flocks.

Small ruminants having ability of multiple births can be improved through marker assisted selection. It is established fact that an animal producing twins contributes more than 1.5 times towards meat than the animal producing single offspring per kidding or lambing. The search for DNA markers that significantly contribute to the variance of trait expression in livestock has been increasingly a focus in the field of livestock genetics. Defining a major gene as one where the difference between the homozygotes is 0.5 standard deviation from means, for prolificacy in sheep, that this includes genes where a single copy increases ovulation rate by more than about 0.2. The Booroola Merino was the first breed of sheep where ovulation rate and litter size were shown to be affected by a segregating major gene (Piper *et al.*, 1985). Records from prolific flocks in several countries have subsequently revealed further major genes that increase prolificacy in sheep. Current knowledge of major genes for prolificacy in sheep falls into three categories: (1) genes where the mutation has been identified and DNA testing is available; (2) genes where the mode of inheritance has been described but the mutation has not been identified; and (3) putative genes where there is evidence of apparent genetic segregation but there are insufficient records to ascertain the mode of inheritance.

The Booroola gene (FecB) is a dominant autosomal gene with an additive effect on ovulation rate (Piper *et al.*, 1985). One copy of the Booroola gene increases ovulation rate (eggs shed per

ewe ovulating) by about 1.5 and two copies by about 3.0. These extra ovulations typically increase litter size (lambs born per ewe lambing) by about 1.0 and 1.5, respectively. The effect of the Booroola gene has been shown to be due to a mutation in the bone morphogenetic protein 1B receptor (BMPR-1B) that is expressed in oocytes and granulosa cells and is located on chromosome 6 (Wilson *et al.*, 2001). Similarly some other genes i.e. BMP15, GDF9 were also found associated with litter size in different sheep breed (Morabandet *al.*, 2011). DNA tests are the key to the utilisation of these genes in the sheep/ goat industry and have also been a useful tool for determining the genetic basis of high prolificacy in distantly related breeds. A variety of inheritance patterns are associated with these genes and there is a wide range in the size of the effect on ovulation rate.

The increase in production associated with litter size (LS) is controlled by both genetic and environmental factors. Since the heritability of LS is low in sheep, attempts were made to discover the gene(s) controlling ovulation rate (OR) and thus LS. The OR analysis of the first recorded highly prolific breed, 'Booroola Merino' (BM) of Australia, provided strong evidence for the presence of a single gene governing OR (Davis *et al.* 1982). In 1993 the first DNA marker test for the Booroola gene was developed and two microsatellite markers, OarAE101 and OarHH55, were found to be linked to the FecB locus (Montogomery*et al.* 1993). This discovery helped to develop a DNA test to screen the mutation in other prolific breeds of sheep without prior knowledge of the pedigree (Wilson *et al.* 2001). The mechanism of action of the mutated gene has not yet been fully understood.

1. Project Objectives:

- To establish and maintain purebred fecund nucleus Lohi sheep flock through screening of prolificacy gene(s) mutation in Lohi sheep
- To increase mutton production by increasing lamb production through genetic management of Lohi sheep

2. Outputs planned for the project:

Output 1:

Selection of prolific Lohi ewes and Lohi rams on the basis of phenotypic and genotypic data from purebred herds of Lohi sheep maintained at LPRI Bahadurnagar and UVAS, SRT&RC Pattoki. After screening a flock of 100 mature females and 5 rams was developed, annexure I is attached, genetic screening resulted in the selection of two rams with desired genotypes i.e. B883 (BB) and (B+) 952.

Output 2:

Crop one from parent flock was obtained after planned breeding of selected rams with females in estrous. Genetic evaluation confirmed 39 lambs with heterozygous status of genotype, table below represents the numbers.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	47	34	30	9	37	35
952	50	18	14	2	17	4

Output 3:

To obtain second crop from parent flock the parent flock was selectively bred, new born were genotyped according to protocol followed for genotyping of parents. The detail of breeding and genotypes is as follows.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	47	40	40	5	45	42
952	49	26	26	5	31	11

Output4:

To obtain third crop from parent flock the parent flock was selectively bred, new born were genotyped according to protocol followed for genotyping of parents. The detail of breeding for crop 3 and genotypes of lambs is as follows.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	24	18	12	3	15	15
975	26	18	12	3	15	6

Output5:

Second generation (F2) was obtained after Inter-se crossing of F1 to analyze expression behaviorof FecB in Lohi sheep, genetic and phenotypic evaluation of F2 crop.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
2016	11	8	3	1	2	0
2030	12	8	3	0	3	1
2037	9	8	6	4	10	7
2015	5	4	2	0	2	1

Ram 2037 with heterozygous status of FecB produced twins at the rate of 66.66%. Comparative evaluation between parent generation and F1 for multiple births revealed 13.99% raise. Table below shows the figures.

G0	Multiple birth %
Crop 1	25
Crop 2	15.15
Crop 3	25
Average	21.71667

G1	**35.71**
Improvement in multiple birth %	**13.99333**

Detailed component wise methodology adopted, data analyzed and results obtained

Phase-1 (Experiment I)

A. Selection of prolific ewes and rams

B. Selection and optimization of fecundity genes (BMPRIB, BMP15 & GDF9) markers

A)

Selection of flock:

The project animals were selected frompurebred herds maintained at LPRI Bahadurnagar and SRT&RC Center Pattoki on the basis of phenotypic and genotypic data. On the basis of phenotypic and pedigree records 100 prolific eves and 05 rams were purchased fromboth herds. The list of purchased animals is attached as Annexure I.

These animals were brought to and maintained at SRT&RC UVAS Pattoki for further breeding and selection on the basis of EBVs and GEBVs. Followings are some images of project animals at SRT&RC Pattoki.

B)

Lab activity: Selection and optimization of fecundity genes (BMPRIB, BMP15 & GDF9) markers

Collection of blood samples:

Five ml blood was collected from every animal cautiously from jugular vein in falcon tubes having EDTA as anticoagulant. Blood samples were brought to the Animal Genetics Lab, A-Block, UVAS Ravi Campus, Pattoki and stored at temperature -20°C before DNA extraction.

Extraction of DNA:

DNA extraction was done from whole blood samples by using modified method of Sambrook et al. (1989). The extracted DNA was stored at -20ºC. Agarose gel electrophoresis technique was used to confirm the extracted DNA.

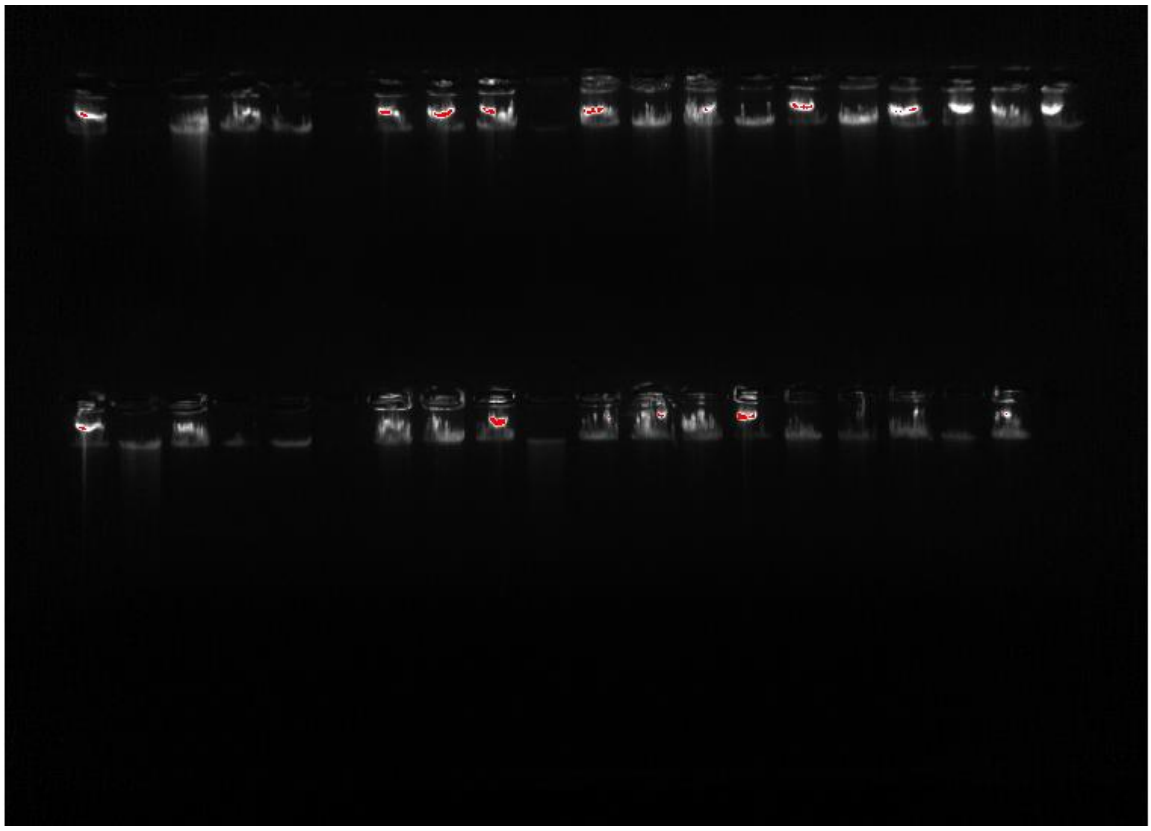
Following method was used for the extraction of DNA.

1. Initially, frozen blood samples were thawed for lysis of RBCs and washing was carried out with Tris-EDTA buffer (T.E. buffer or washing buffer). For making one liter washing buffer, ten ml of TrisHCl (1 M, pH 8) was poured in addition to four ml of EDTA (0.5 M, pH 8) in a flask and then sterilized water (ampules) was added to make final volume to one liter.
2. In first washing, T.E. buffer was added in sample tube up to mark 45 ml and the tube was placed on table undisturbed for ten minutes
3. Tube was centrifuged at 4500 rpm for about 15 minutes at 4°C.
4. Supernatant was discarded and the pellet was broken by tapping gently. Tube was shaken strongly.
5. Washing buffer was added up to mark 35 ml and placed on table undisturbed for ten minutes
6. Tube was centrifuged again at 4500 rpm for about 15 minutes at 4°C.
7. Washing process was repeated if necessary.
8. Supernatant was discarded and pellet was re-suspended in three ml of TNE (Buffer A1) for five ml of blood, Ten percent SDS (150 µl/5 ml of blood) 50 µl of Proteinase K (100 µg/ml for 5 ml of blood) was added later.
9. Sample was incubated at 55°C for overnight.

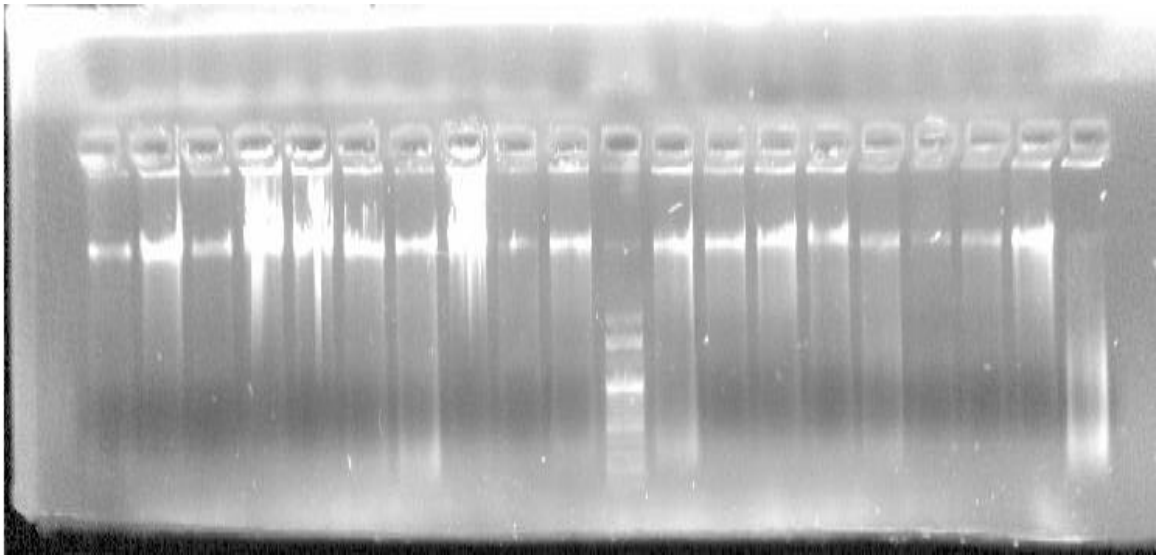
10. On following day, one ml of saturated NaCl (6 M) was added, tube was shaken vigorously and placed on table to let the foam disappear.
11. Tube was placed in ice for 25 minutes.
12. Tube was centrifuged at 4500 rpm for about 15 minutes at 4°C to discard protein debris. Take the supernatant in another fresh, labelled falcon tube.
13. Equal volume of isopropanol was added and tube was gently inverted.
14. DNA was precipitated in tube like piece of thread.
15. Tube was centrifuged again at 4500 rpm for 10 minutes at 4°C.
16. The collected DNA was washed with 70 percent ethanol (5 ml/5 ml blood).
17. Tube was placed on shaker overnight.
18. Tube was centrifuged once again at 4500 rpm for about fifteen minutes at 4°C.
19. Supernatant was discarded with caution.
20. Tube was placed on table for some hours to evaporate ethanol.
21. Four hundred µl sterilized water was added in dried tube.
22. Tube was placed in water bath at 70°C for one hour.
23. DNA was collected and stored in 1.5 ml screwed eppendroff tube at -20°C.

Quantification and confirmation of DNA

The quantification and confirmation of DNA was done through UV Illuminator and agarose gel electrophoresis. Figures representing DNA on agarose are as follows.



1 % Agarose gel representing DNA (40 ng/µL)



1 % Agarose gel representing DNA (40 ng/µL)

Primer designing & optimization:

The sheep fecundity genes (BMPRIB, BMP15and GDF9) were amplified using the in-vitro gene cloning methodology(polymerase chain reaction) with primers designed from published sheep sequences (sheep genomic BMPRIB, BMP15 & GDF9) by using Primer-3 freeware (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi).

A demo screenshot of designing a primer with Primer3Plus

Designed primers are as follows.

Gene	Primer Sequence
BMPR1B-F1	5'-CCAGAGGACAATAGCAAAGCAAA-3'
BMPR1B-R1	5'-CAAGATGTTTTCATGCCTCATCAACACGGTC-3'
BMP15-F1	5’-CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3'
BMP15-R1	5'-GATGCAATACTGCCTG CTTG-3'
GDF9-F1	5’-GACTGGTATGGGGAAATG-3’
GDF9-R1	5’-CCAATCTGCTCCTACACACCT-3’
GDF9-F2	5’-CTTTAGTCAGCTGAAGTGGGACAAC-3’
GDF9-R2	5’-ATGGATGATGTTCTGCACCATGGTGTGAACCTG-3’

Polymerase chain reaction (PCR) optimization

Master-mix were prepared for one additional sample to cover pipetting error. Optimization of PCR reaction was carried out for $MgCl_2$ and annealing temperatures.dNTPs and Taq DNA polymerase concentrations were also minimized (150 mM and 0.5 U respectively) to conserve the PCR components without compromising the amplification.

All the reactions were carried out in 0.2 ml thin wall PCR tubes. The tubes were placed in a thermal cycler (BioRad-iCycler) and subjected to PCR.

The PCR of genes was performed in a reaction mixture of 25 µl, containing 2 µl of genomic DNA, 1 µl of each 10 pM oligonucleotide primers, 2 µl of 2mM $MgCl_2$, 2.5 µl of 25mM deoxynucleotide triphosphate mixture,0.2µl of 5U/µl*Taq* DNA polymerase and distilled water up to the total volume 25 µl.

Reaction recipe:

Reagents	**Volume**
DNA (50ng/ul)	2.0µl
PCR Buffer (2mM)	2.5µl
$MgCl_2$ (2Mm)	2µl
Primer F (10pM)	1µl
Primer R(10pM)	1µl
dNTPs (25mM)	2.5µl
Taq Polymerase (5U/µl)	0.2 µl
Distilled Water	13.8µl
Total	25µl

Reaction conditions:

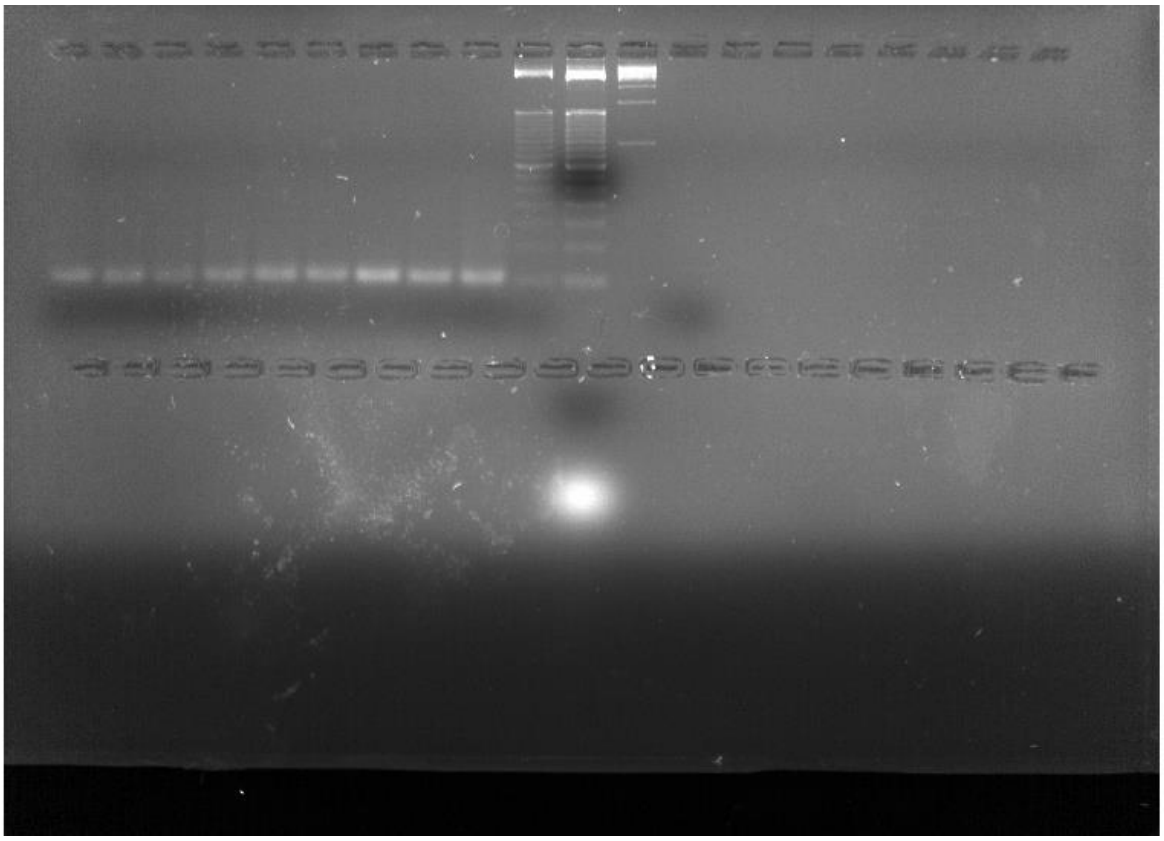
Initial denaturation of gDNA at 94 °C for 5 min and then 35 cycles of 94 °C for 45 sec, 57 °C for 45 sec, and 72 °C for 45 sec, with a final extension step for 10 min at 72 °C.

Primers were optimized at 60-61^{o}C.

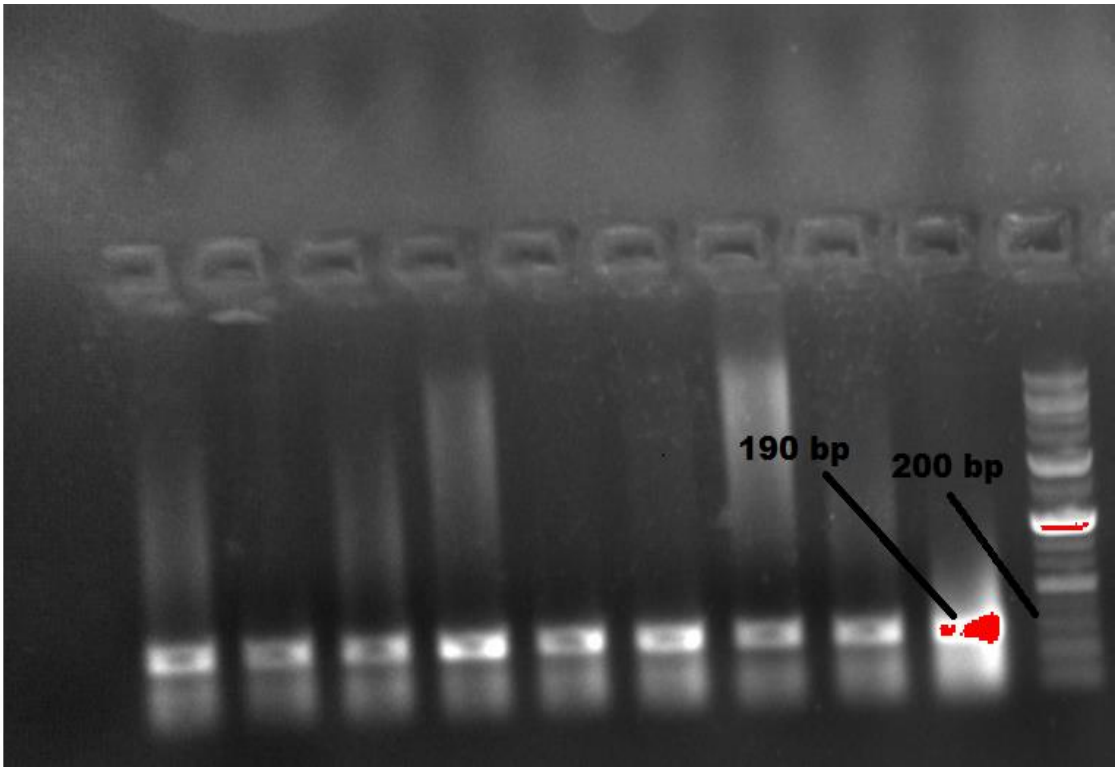
PCR protocol:

Step	**Temperature**	**Time**
Initial denaturation	94 ^{o}C	5 mint
1. Denaturation	94 ^{o}C	45 sec
2. Annealing	60 ^{o}C	45sec
3. Extension	72 ^{o}C	45sec
Repeat step1 to 3 for 35 cycles		
Final extension	72 ^{o}C	10min

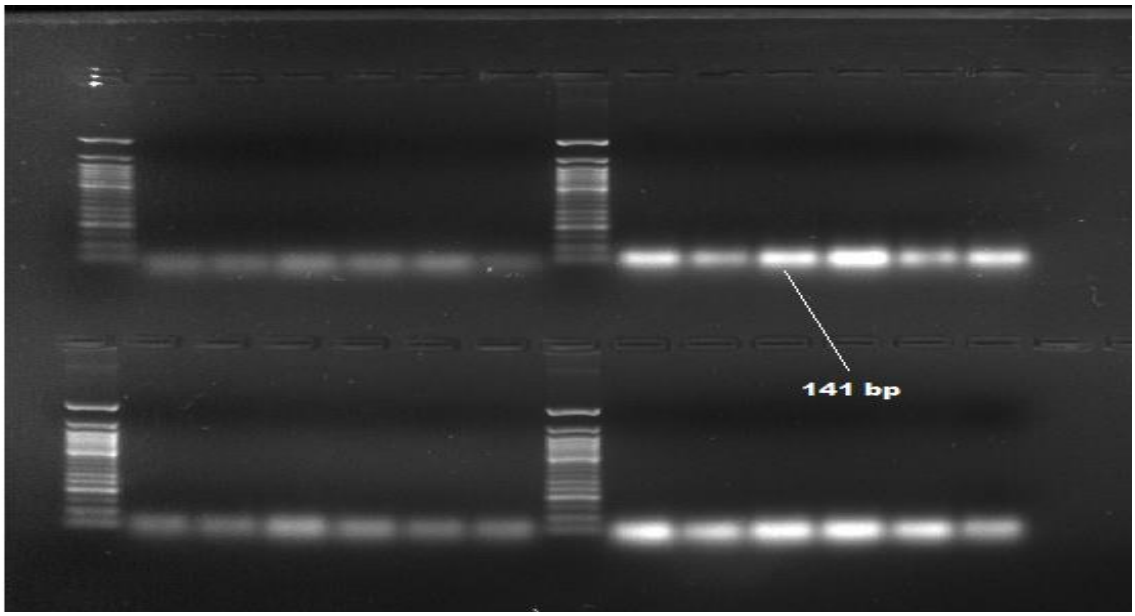
PCR amplifications were confirmed by running, 5 µl of PCR product mixed with 1µl of 6X gel loading dye from each tube on 3 per cent agarose gel (depending on the expected size of amplified product) at a constant voltage 80 V for 30 min in 0.5X TBE buffer. The amplified products were visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (Syngene, Gene Genius Bio Imaging) as shown in figures below.



3% Agarose gel representing PCR product (141 bp)



3% Agarose gel representing PCR product (190 bp)

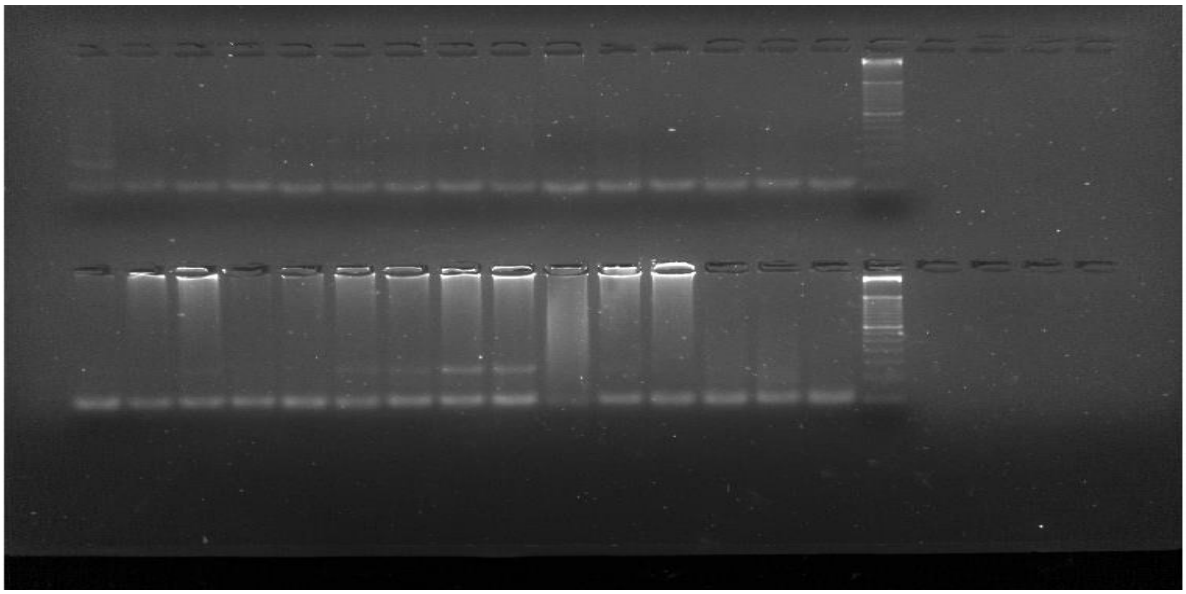


3% Agarose gel representing PCR product (141bp)

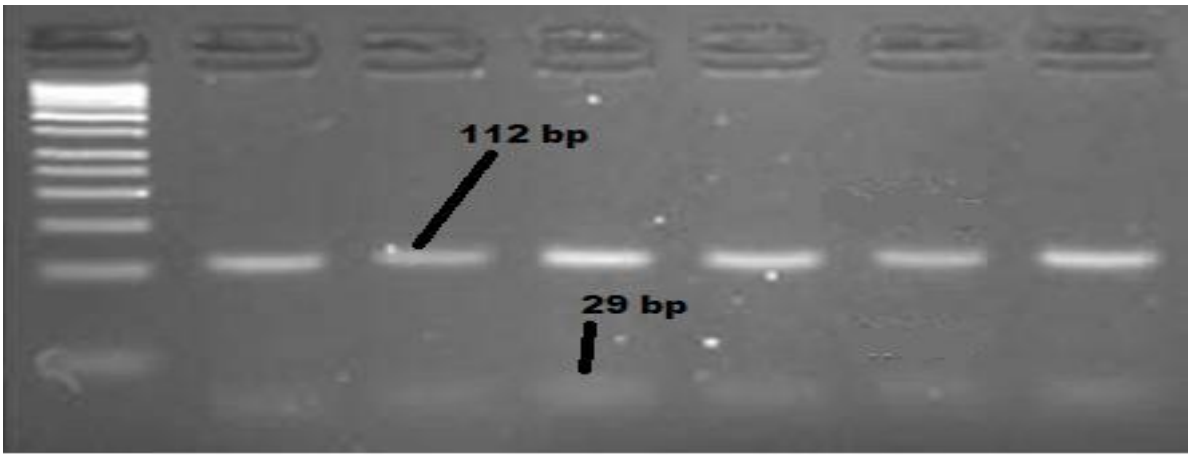
Genotyping:

RFLP

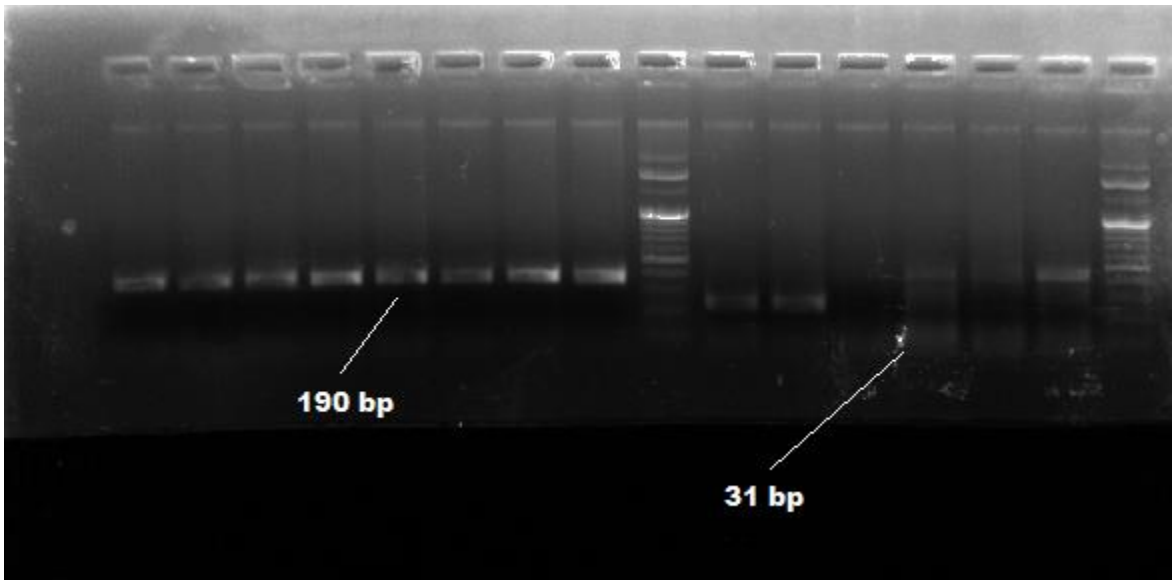
Amplicons were digested at 37 °C for an overnight period with 10 U of restriction enzymes. After digestion the restriction digests were electrophoresed for 1.5 hours at 80 Volts on a 4% agarose gel with ethidium bromide. PCR-RFLP fragment sizes in each sample were quantified and qualified by using a standard DNA molecular weight marker, by viewing the banding pattern under UV light on the transiluminatoras shown in figures below. The genotypic data is attached as annexure II.



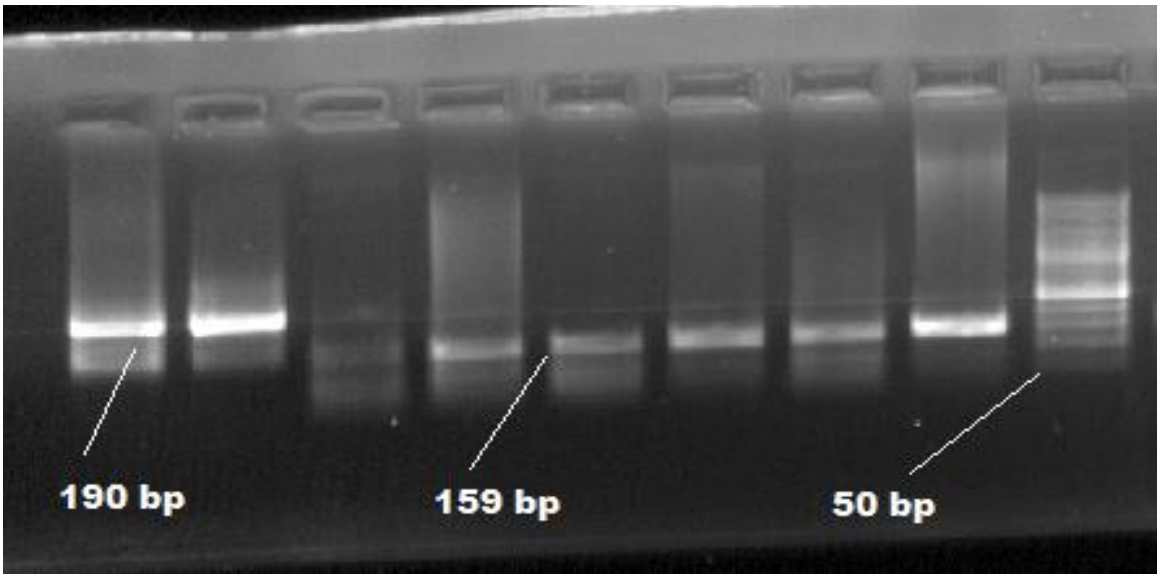
4% agarose gel representing restriction digests of PCR product (141 bp)



4% agarose gel representing restriction digests of PCR product (141 bp)



4% agarose gel representing restriction digests of PCR product (190 bp)



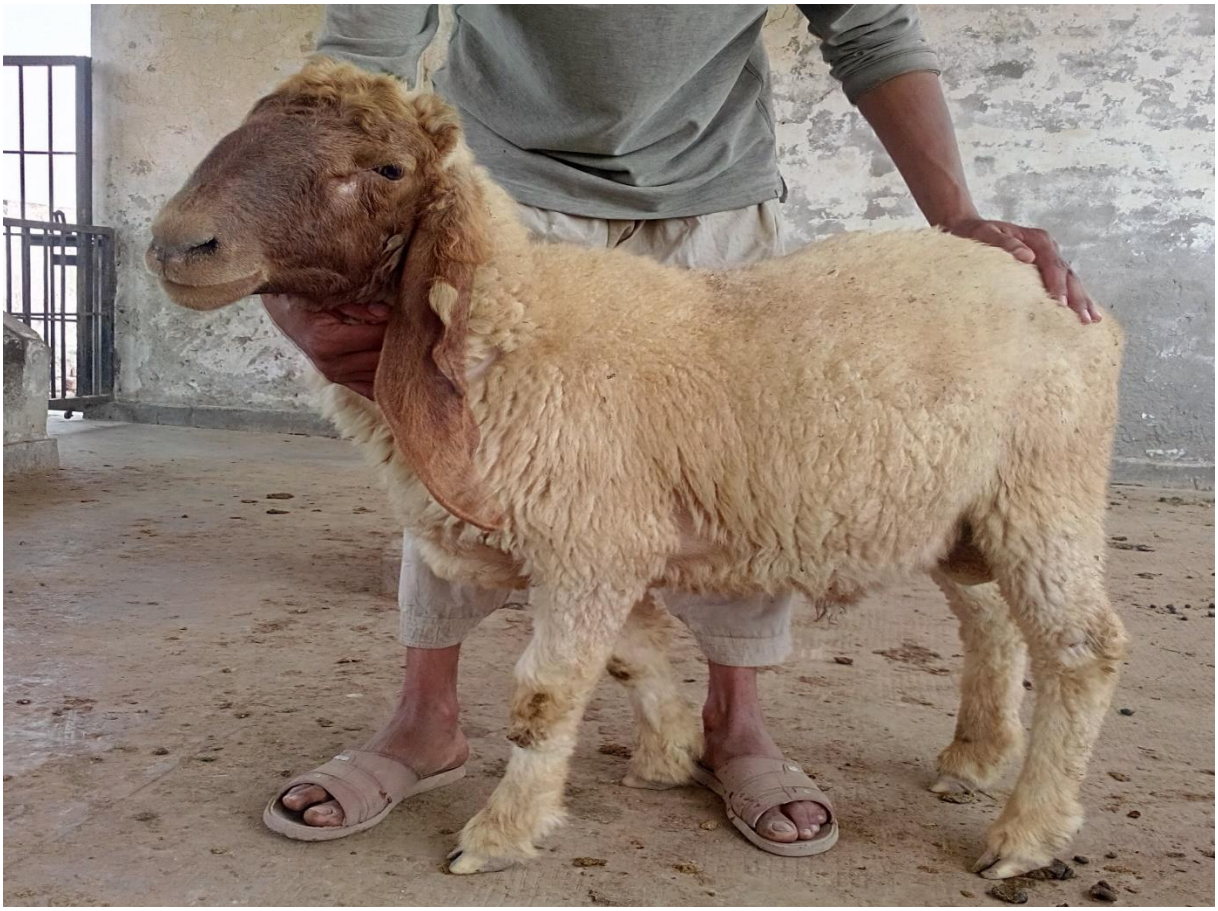
4% agarose gel representing restriction digests of PCR product (190 bp)

PHASE – II (Experiment II)

Breeding of selected stock and genotypic and phenotypic evaluation of F1 progeny

Breeding of the screened stock with selective rams was done, for this a total of 97 ewe were divided into two groups and each group was allotted a different ram i.e. B883 and 952. Detailed breeding groups are attached as annexure III.

RAM: B883 (BB)



RAM: 952 (B+)

The screening of males resulted in the screening out of two rams (ID No. B883 and 952), with BB and B+ allele, respectively. 52 ewes were successfully bred, out of which 44 were concieved, list is attached as annexure IV. All new born lambs (annexure V) were reared according to farm practices of SRT&RC, UVAS, Pattoki.

Genotypic and phenotypic study of F1 (Generation-I) crop 1 according to established markers (Experiment 1) of fecundity genes (BMPRIB and BMP15) was carried out. The genotypes of new born animals are attached as annexure VI. Summary is as follows.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	47	34	30	9	37	35
952	50	18	14	2	17	4

Breeding of selected stock for crop 2 (F1)

Breeding of parent stock was carried out in next breeding season for second crop, the detail of exposed, served and pregnant females is attached as annexure VII.

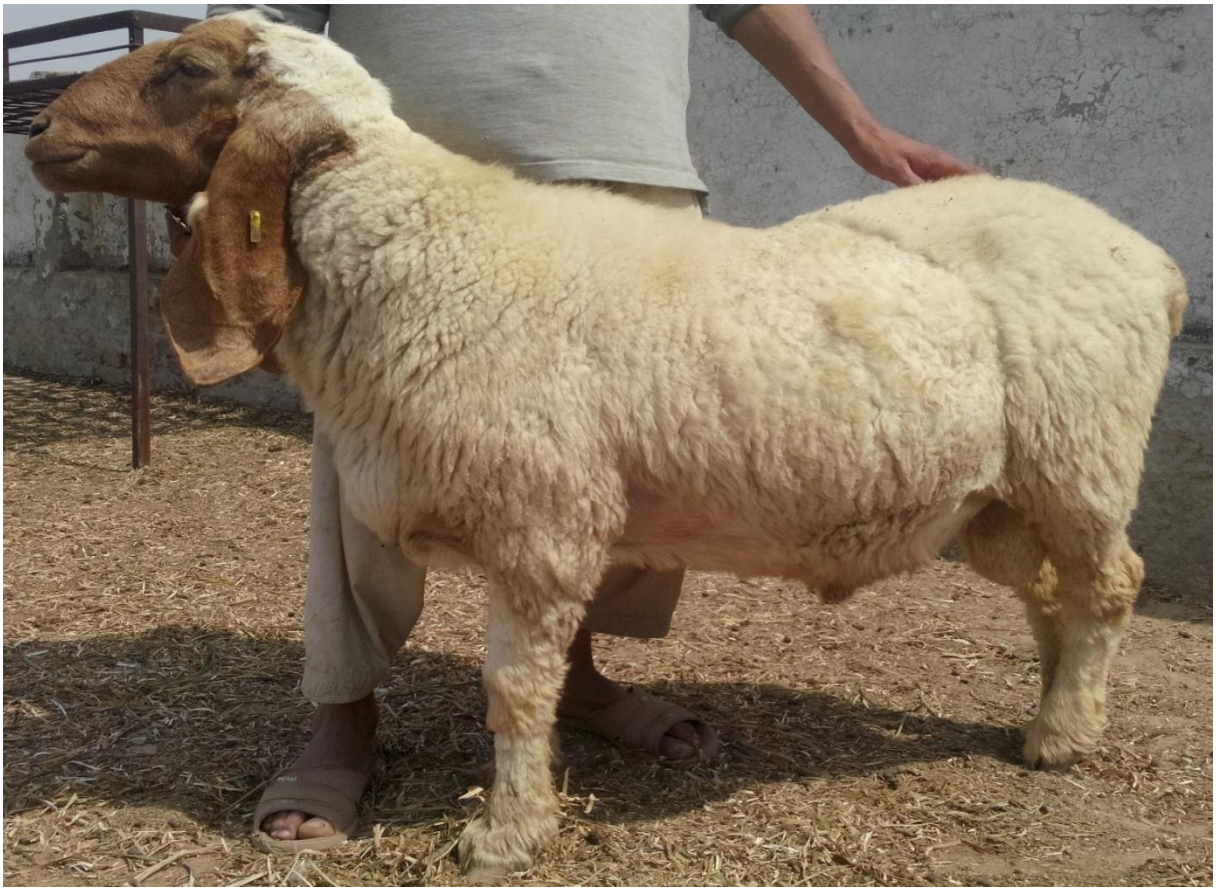
Lambing and Genotyping of crop 2 (F1)

Ewes served during breeding season lambed in lambing season, detail of new borns is attached as annexure VIII. Genotyping of crop 2 lambs was carried according to the protocol adopted for parent generation. Observed genotypes are attached as annexure IX. Summary is given below.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	47	40	40	5	45	42
952	49	26	26	5	31	11

Breeding of selected stock for crop 3 (F1)

A total of 50 females from parent flock were divided into two groups for breeding purposes in breeding season i.e. Sep – Oct, 2014 for third crop. Ram B883 and 975 were used, the detail of exposed and served females is attached as annexure X.



RAM: 975 (B+)

Lambing and Genotyping of crop 3 (F1)

Ewes served during breeding season lambed in lambing season, detail of new crop is attached as annexure XI. Genotyping of crop 3 lambs was carried according to the protocol adopted for parent generation. Observed genotypes are attached as annexure XII.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
B883	24	18	12	3	15	15
975	26	18	12	3	15	6

Experiment III

Inter-se crossing of F1 and genetic and phenotypic evaluation of F2 crop

A total of 35 females from F1generation of project were also subjected to breeding. Females were divided into four groups; each group was allotted a separate ram. Annexure XIII is attached.

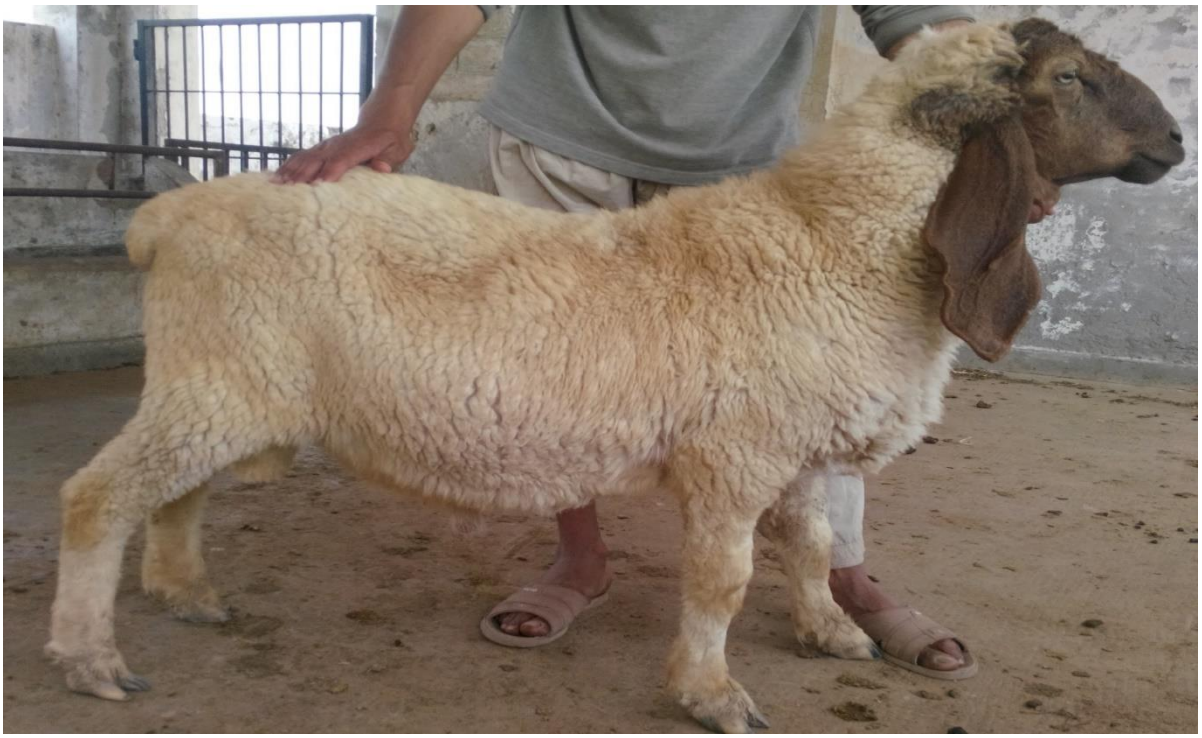
Lambing of F1 and genetic evaluation of F2

Successfully bred females lambed after completion of gestation period produced F2 crop 1, the new born were maintained at SRT&RC and genotyped using facilities of AGL following the defined protocol. The details of F2 and of genotypes of F2 are attached as annexure XIV and annexure XV. Brief detail of number of lambs per ram is given below.

Ram	No. of Ewes exposed	No. of Ewes bred	Lambed	% Multiple births	No. of lambs	No. of heterozygotes
2016	11	8	3	1	2	0
2030	12	8	3	0	3	1
2037	9	8	6	4	10	7
2015	5	4	2	0	2	1

The ram 2037 showed highest rate of multiple births (66.66%).

RAM: 2015 (++)



RAM: 2016 (B+)

RAM: 2030 (B+)

RAM: 2037 (B+)

References:

- Davis. G. H. 2009. The Booroola gene: origin, distribution, use and management of the FecB mutation. Proceeding Australian Centre for International Agricultural Research (ACIAR) 133.
- Montgomery G.W., Crawford A.M., Penty J.M., Dodds K.G., Ede A.J., Henry H.M., Pierson C.A., Lord E.A.,Galloway S.M., Schmack A.E., Sise J.A., Swarbrick P.A., Hanrahan V., Buchanan F.C. and Hill D.F. 1993. The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. Nature Genetics 4, 410–414.
- Moradband F, Rahimi G, Gholizadeh M. 2011. Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. Asian-Aust J Anim Sci. 24. 1179-1183.DOI: 10.5713/ajas.2011.10453
- Piper L.R., Bindon B.M. and Davis G.H. 1985. Single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed.
- Sambrook J, Fritsch EF, Miniatis T. 1989. Molecular cloning: A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press. p. 999.
- Wilson T., Wu Xi-Yang, Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Component wise salient achievements

- Screened Lohi Sheep for the presence of fecundity genes based on phenotypic, genotypic and pedigree records
- Selected 100 prolific ewes and 05 rams from LPRI BahadarnagarDistt. Okara and SRT&RC, Ravi Campus
- DNA was extracted and genotyping of selected animals was completed
- Selection, optimization and amplification of fecundity gene BMPRIB primers of all selected Lohi sheep
- RFLP analysis, SNP detection and calculation of frequency distribution pattern
- Selected animals maintained and bred at SRT&RC
- Screening/ genotyping of 1^{st} generation (F1) for the heterozygous status of fecundity genes and selection for further breeding
- Screening/ genotyping of 2^{nd} generation (F2) for the heterozygous status of fecundity genes and selection for further breeding is going on

A twin carrying ewe

A twin carrying ewe

A twin carrying ewe

A twin carrying ewe

3. Overall progress of the problem searched

As this project was aimed to increase mutton production through enhancing twinning rate in Lohi sheep, at field conditions it is 18 to 20 % and was 21.71 % of generation zero of project animals but after this project we are be able to get a flock (Generation one) having rate upto 35.71 %, ultimately with increased number of lambs/lambing season. A flock of 100 ewes at 80 % lambing with 35 % twining rate willproduce 108 lambs/lambing season that have monetary value on an average 18000*100= 18,000,00/-Rs at the age of one year and weighing 45 kg/animal (assuming 7 % mortality before market age and @ 400 Rs/kg market rate).

Table: Overall progress in multiple birth rate within one generation of selection

G0	**Multiple birth %**
Crop 1	25
Crop 2	15.15
Crop 3	25
Average	21.71667
G1	**35.71**
Improvement in multiple birth %	**13.99333**

Testing of rams of F1 resulted in the screening of a breeding ram i.e. 2037 with 66.66% twinning rate. The increased performance of G1 over G0 can be attributed to introduction of FecB through planned breeding of G0 for F1 while the confirmation of prolific ram will be done by introducing heterozygous rams to field ewes for more prolific generation, the lambing performance of resultant animals will confirm the prolific status of rams i.e. (2037).

30 % increase in twinning rate yields 24 more lambs having economic value 18000*24 = 4,32,000/- Rs per lambing season and if selection and mating continues for 2 to 3 generations then twining rate may be increased upto 50 % producing 40 more lambs as compared to flock having 20 % twinning rate. 40 lambs weighing on an average 45 kg at the age of one year would have market value about 7, 20,000/- Rs. (assuming 8 % mortality before market age and @ 400 Rs/kg market rate).

A thorough analysis of behavior of twining genes in local Lohi sheep became possible. Analysis concludes that if selection remain continuously practiced for Fec gene(s) mutations generation after generation the rate of twin births will increase after each generation.

4. Varieties, breeds, vaccines or products developed and patented

N/A

5. No. of national and international papers published

6. No. of Ph.D/M.Phil. produced

The following students get benefited from resources developed by this project, they worked with project animals and used genetic lab facility extended with the help of PARB.

Sr. No.	Name of student	Degree	Year	Thesis title
1	Ali HaiderSaleem	M. Phil (Animal Breeding and Genetics)	2014	Association Study Of Leptin Gene With Growth Trait In Lohi Sheep
2	Sadaqat Ali Chatha	M.Phil (Livestock Management)	2014	Effect of rumen protected methionine and sulphur supplementation on productive and quality traits in Lohi sheep

7. Current status of commercialization of the project. How many stakeholders adopted this technology along with monitory benefits

Meat is major animal protein source and its demand is increasing rapidly with the increase in human population load in Pakistan and also around the globe. An effective approach of increasing the animal protein availability is by improving meat production. Keeping in view the increasing demand of animal protein this project was aimed to increase meat production through genetic management. Genetic management caused the increase in number of lambs and after each generation the surplus animals were public auctioned for monitory benefits.

As detection of desired genotype of lamb for its ability to produce multiple ovum require special facility and technical expertise so, the AGL of UVAS, Ravi campus Pattoki will provide service to stakeholders from private sector for genetic screening of rams.

8. Impact of the project on strengthening of the institutional infrastructure, machinery, equipment and human resources

This project have great impact in strengthening of departmental infrastructure by making possible the purchase of costly equipments i.e. Thermal cycler, Refrigerated centrifuge machine, water distillation plant etc. for Animal Genetic Laboratory. Project also helped in the extension of chemical facility of AGL, in the development of technique/ expertise and in the provision of training opportunity to the personnel working with project animals.

Infrastructure of SRT&RC was also improved with the funding of PARB as it helped in repairing of animal sheds, helped in the extension of farm resources i.e. feed managers, molasses sprinkler.

Moreover the project animals are also an asset to the institute and the continuous selection for twining will make it more prolific herd.

9. Constraints in the:

(a) Implementation of the project

NIL

(b) Commercialization of the project

NIL

10. Suggestions for future research and development

Based upon the experience and findings of this project some recommendation for future researchers may be advised i.e.

- Wider introduction of fecundity genes in the Lohi sheep
- Other heavy breeds i.e. Kajli may also be brought under the selection for increase in prolificacy
- Introduction of FecB should be preferred through introducing heterozygous males in herds having little litter size
- Selection for FecB must be continued only if there is no evidence of adverse effects at homozygous condition
- Researches may be planned on standardization of nutritional requirements of twin carrying ewe, and also some management based to increase the rate of survivability of twin born
- Nutritional management should focus on the practical questions "when is supplementation indicated?", "which animals to supplement?", "when and for how long?", "with what?" and at "what level"? For forage supplements, identifying the optimum stage of growth for feeding as a supplement is critical. The practical potential of fodder conservation practices should also be explored.

Dated: _____ _____

(**Signature of Project Manager)**

Dated:_____ **_____**

(Signature of Head of Organization)

Annexure 1.

The list of purchased animals

Sr. No.	Tag No.	Date of birth	Sex	Weight (Kg)	Rate/ Kg	Amount
1	B875	29.06.10	M	47	275	12925
2	B881	01.02.11	M	51	275	14025
3	B883	01.02.11	M	48	275	13200
4	B98	09.03.11	M	37	275	10175
5	B102	10.03.11	M	40	275	11000
6	B565	25.02.09	F	37	275	10175
7	B618	14.03.09	F	39	275	10725
8	B474	09.09.08	F	52	275	14300
9	B542	12.02.09	F	47	275	12925
10	B422	17.03.08	F	45	275	12375
11	B473	08.09.08	F	49	275	13475
12	B476	12.02.05	F	52	275	14300
13	B604	08.03.09	F	45	275	12375
14	B507	06.02.09	F	41	275	11275
15	B672	26.03.09	F	45	275	12375
16	B716	21.02.10	F	33	275	9075
17	B831	15.03.10	F	36	275	9900
18	B873	27.06.10	F	34	275	9350
19	B895	05.02.11	F	37	275	10175
20	B896	05.02.11	F	34	275	9350
21	B898	06.02.11	F	37	275	10175
22	B3	08.02.11	F	34	275	9350
23	B20	19.02.11	F	36	275	9900
24	B26	20.02.11	F	32	275	8800
25	B37	24.02.11	F	37	275	10175
26	B46	25.02.11	F	37	275	10175
27	B54	26.02.11	F	39	275	10725
28	B58	26.02.11	F	39	275	10725
29	B59	26.02.11	F	37	275	10175
30	B62	27.02.11	F	42	275	11550
31	B63	27.02.11	F	35	275	9625
32	B66	27.02.11	F	39	275	10725
33	B72	28.02.11	F	32	275	8800
34	B73	28.02.11	F	40	275	11000

35	B76	28.02.11	F	31	275	8525
36	B79	01.03.11	F	35	275	9625
37	B81	02.03.11	F	36	275	9900
38	B82	02.03.11	F	36	275	9900
39	B85	03.03.11	F	36	275	9900
40	B88	04.03.11	F	33	275	9075
41	B91	05.03.11	F	39	275	10725
42	B95	06.03.11	F	27	275	7425
43	B101	09.03.11	F	38	275	10450
44	B103	10.03.11	F	35	275	9625
45	B108	11.03.11	F	33	275	9075
46	B114	03.03.11	F	36	275	9900
47	B121	15.03.11	F	34	275	9350
48	B127	15.03.11	F	38	275	10450
49	B129	16.03.11	F	37	275	10175
50	B135	17.03.11	F	37	275	10175
51	B138	18.03.11	F	37	275	10175
52	B146	20.03.11	F	38	275	10450
53	B152	21.03.11	F	39	275	10725
54	B159	24.06.11	F	36	275	9900
55	B177	30.06.11	F	33	275	9075
56	5	Oct.07	F	43	275	11825
57	8	03.03.07	F	39	275	10725
58	10	Oct.07	F	43	275	11825
59	17	Oct.07	F	40	275	11000
60	24	14.03.10	F	37	275	10175
61	25	14.03.07	F	54	275	14850
62	26	23.03.07	F	54	275	14850
63	34	22.03.07	F	50	275	13750
64	62	23.03.07	F	41	275	11275
65	67	12.03.07	F	44	275	12100
66	99	29.12.04	F	42	275	11550
67	115	12.11.06	F	45	275	12375
68	131	28.10.08	F	38	275	10450
69	148	12.01.09	F	38	275	10450
70	152	20.01.09	F	40	275	11000
71	153	22.01.09	F	44	275	12100
72	157	27.01.09	F	37	275	10175
73	159	07.03.12	F	44	275	12100
74	160	07.02.09	F	37	275	10175

75	186	05.10.09	F	42	275	11550
76	192	19.10.09	F	44	275	12100
77	215	11.03.10	F	43	275	11825
78	233	21.07.10	F	38	275	10450
79	236	23.07.10	F	44	275	12100
80	244	31.12.10	F	44	275	12100
81	248	16.02.11	F	35	275	9625
82	249	17.02.11	F	42	275	11550
83	250	17.02.11	F	35	275	9625
84	251	18.02.11	F	39	275	10725
85	252	19.02.11	F	35	275	9625
86	253	19.02.11	F	41	275	11275
87	254	19.02.11	F	36	275	9900
88	255	20.02.11	F	44	275	12100
89	257	22.02.11	F	34	275	9350
90	258	22.02.11	F	39	275	10725
91	260	23.02.11	F	37	275	10175
92	261	25.02.11	F	30	275	8250
93	262	26.02.11	F	34	275	9350
94	263	27.02.11	F	39	275	10725
95	264	28.02.11	F	49	275	13475
96	265	28.02.11	F	35	275	9625
97	266	28.02.11	F	48	275	13200
98	267	01.03.11	F	35	275	9625
99	273	07.03.11	F	44	275	12100
100	280	29.02.12	F	35	275	9625
101	281	29.02.12	F	31	275	8525
102	286	31.03.11	F	46	275	12650
103	289	15.08.11	F	34	275	9350
104	290	15.08.11	F	32	275	8800
105	301	25.12.11	F	25	275	6875
106	308	26.02.12	F	20	275	5500
107	311	28.02.12	F	17	275	4675
108	316	05.03.12	F	24	275	6600
109	317	05.03.12	F	20	275	5500
110	321	08.03.12	F	26	275	7150
111	325	10.03.12	F	21	275	5775
112	326	11.03.12	F	13	275	3575
113	327	09.03.12	F	16	275	4400
114	330	18.03.12	F	18	275	4950

115	331	18.03.12	F	18	275	4950
116	966	28.09.11	M	27	275	7425
117	967	28.09.11	M	35	275	9625
118	973	25.12.11	M	24	275	6600
119	975	26.02.12	M	17	275	4675
120	979	28.02.12	M	27	275	7425
121	980	29.02.12	M	25	275	6875
122	981	29.02.12	M	23	275	6325
123	987	05.03.12	M	9	275	2475
124	988	05.03.12	M	21	275	5775
125	989	9.03.12	M	25	275	6875
126	992	10.03.12	M	20	275	5500
127	993	10.03.12	M	20	275	5500
128	994	10.03.12	M	16	275	4400
129	995	10.03.12	M	18	275	4950
130	997	10.03.12	M	32	275	8800
131	998	11.03.12	M	24	275	6600
Total						**1282600/--**

Annexure II.

Genotypes of parent generation for BMPR1B and BMP15

Sr. No.	Tag No.	Date of birth	Sex	BMPR1B			BMP15		
				BB	B+	++	BB	B+	++
1	B875	29.06.10	M			++			++
2	B881	01.02.11	M			++			++
3	B883	01.02.11	M	BB					++
4	B98	09.03.11	M			++			++
5	B102	10.03.11	M			++			++
6	B565	25.02.09	F			++			++
7	B618	14.03.09	F			++			++
8	B474	09.09.08	F			++			++
9	B542	12.02.09	F	BB					++
10	B422	17.03.08	F			++			++
11	B473	08.09.08	F			++			++
12	B476	12.02.05	F			++			++
13	B604	08.03.09	F			++			++
14	B507	06.02.09	F			++	BB		
15	B672	26.03.09	F			++			++
16	B716	21.02.10	F			++			++
17	B831	15.03.10	F			++			++
18	B873	27.06.10	F			++			++
19	B895	05.02.11	F			++			++
20	B896	05.02.11	F			++			++
21	B898	06.02.11	F			++			++
22	B3	08.02.11	F			++			++
23	B20	19.02.11	F			++			++
24	B26	20.02.11	F			++			++
25	B37	24.02.11	F			++			++
26	B46	25.02.11	F	BB					++
27	B54	26.02.11	F			++			++
28	B58	26.02.11	F			++			++
29	B59	26.02.11	F			++			++
30	B62	27.02.11	F			++	BB		
31	B63	27.02.11	F			++			++
32	B66	27.02.11	F			++			++

33	B72	28.02.11	F			++			++
34	B73	28.02.11	F			++			++
35	B76	28.02.11	F			++			++
36	B79	01.03.11	F			++			++
37	B81	02.03.11	F			++			++
38	B82	02.03.11	F			++			++
39	B85	03.03.11	F			++			++
40	B88	04.03.11	F			++			++
41	B91	05.03.11	F			++			++
42	B95	06.03.11	F			++			++
43	B101	09.03.11	F			++			++
44	B103	10.03.11	F			++			++
45	B108	11.03.11	F			++			++
46	B114	03.03.11	F			++			++
47	B121	15.03.11	F			++			++
48	B127	15.03.11	F			++			++
49	B129	16.03.11	F			++			++
50	B135	17.03.11	F			++			++
51	B138	18.03.11	F			++			++
52	B146	20.03.11	F			++			++
53	B152	21.03.11	F			++			++
54	B159	24.06.11	F			++			++
55	B177	30.06.11	F			++			++
56	5	Oct.07	F	BB					++
57	8	03.03.07	F			++			++
58	10	Oct.07	F			++	BB		
59	17	Oct.07	F			++			++
60	24	14.03.10	F			++			++
61	25	14.03.07	F			++			++
62	26	23.03.07	F			++			++
63	34	22.03.07	F			++			++
64	62	23.03.07	F			++			++
65	67	12.03.07	F			++			++
66	99	29.12.04	F			++			++
67	115	12.11.06	F			++			++
68	131	28.10.08	F			++			++
69	148	12.01.09	F			++			++
70	152	20.01.09	F			++			++
71	153	22.01.09	F			++			++

72	157	27.01.09	F			++			++
73	159	07.03.12	F	BB					++
74	160	07.02.09	F			++			++
75	186	05.10.09	F			++			++
76	192	19.10.09	F			++			++
77	215	11.03.10	F			++			++
78	233	21.07.10	F			++			++
79	236	23.07.10	F			++			++
80	244	31.12.10	F			++			++
81	248	16.02.11	F			++			++
82	249	17.02.11	F			++			++
83	250	17.02.11	F			++			++
84	251	18.02.11	F			++			++
85	252	19.02.11	F			++			++
86	253	19.02.11	F			++			++
87	254	19.02.11	F			++			++
88	255	20.02.11	F			++			++
89	257	22.02.11	F			++			++
90	258	22.02.11	F			++			++
91	260	23.02.11	F	BB					++
92	261	25.02.11	F			++			++
93	262	26.02.11	F			++			++
94	263	27.02.11	F			++			++
95	264	28.02.11	F			++			++
96	265	28.02.11	F			++			++
97	266	28.02.11	F			++			++
98	267	01.03.11	F			++			++
99	273	07.03.11	F			++			++
100	280	29.02.12	F			++			++
101	281	29.02.12	F			++			++
102	286	31.03.11	F			++			++
103	289	15.08.11	F			++			++
104	290	15.08.11	F			++			++
105	301	25.12.11	F			++			++
106	308	26.02.12	F			++			++
107	311	28.02.12	F			++			++
108	316	05.03.12	F			++			++
109	317	05.03.12	F			++			++
110	321	08.03.12	F			++			++

111	325	10.03.12	F			++			++
112	326	11.03.12	F			++			++
113	327	09.03.12	F			++			++
114	330	18.03.12	F			++			++
115	331	18.03.12	F			++			++
116	966	28.09.11	M			++			++
117	967	28.09.11	M			++			++
118	973	25.12.11	M			++			++
119	975	26.02.12	M		B+				++
120	979	28.02.12	M			++			++
121	980	29.02.12	M			++			++
122	981	29.02.12	M			++			++
123	987	05.03.12	M			++			++
124	988	05.03.12	M			++			++
125	989	9.03.12	M			++			++
126	992	10.03.12	M			++			++
127	993	10.03.12	M			++			++
128	994	10.03.12	M			++			++
129	995	10.03.12	M			++			++
130	997	10.03.12	M			++			++
131	998	11.03.12	M			++			++

Annexure III
Breeding of parent flock

a.) Group 1

RAM B883

Sr. No.	Tag #	Category	DOB	Ram
1	5	EWE	Oct.07	B883
2	8	EWE	03.03.07	B883
3	10	EWE	Oct.07	B883
4	17	EWE	Oct.07	B883
5	24	EWE	14.03.10	B883
6	25	EWE	14.03.07	B883
7	26	EWE	23.03.07	B883
8	34	EWE	22.03.07	B883
9	62	EWE	23.03.07	B883
10	67	EWE	12.03.07	B883
11	99	EWE	29.12.04	B883
12	115	EWE	12.11.06	B883
13	131	EWE	28.10.08	B883
14	148	EWE	12.01.09	B883
15	152	EWE	20.01.09	B883
16	153	EWE	22.01.09	B883
17	157	EWE	27.01.09	B883
18	159	EWE	01.02.09	B883
19	160	EWE	07.02.09	B883
20	186	EWE	05.10.09	B883
21	192	EWE	19.10.09	B883
22	215	EWE	11.03.10	B883
23	233	EWE	21.07.10	B883
24	236	EWE	23.07.10	B883
25	244	EWE	31.12.10	B883
26	248	FYS	16.02.11	B883
27	249	FYS	17.02.11	B883
28	250	EWE	17.02.11	B883
29	251	FYS	18.02.11	B883
30	252	FYS	19.02.11	B883
31	253	FYS	19.02.11	B883
32	254	FYS	19.02.11	B883
33	255	FYS	20.02.11	B883

34	257	FYS	22.02.11	B883
35	258	FYS	22.02.11	B883
36	260	FYS	23.02.11	B883
37	261	FYS	25.02.11	B883
38	262	FYS	26.02.11	B883
39	263	FYS	27.02.11	B883
40	264	FYS	28.02.11	B883
41	265	FYS	28.02.11	B883
42	266	EWE	28.02.11	B883
43	267	FYS	01.03.11	B883
44	286	FYS	31.03.11	B883
45	289	FYS	15.08.11	B883
46	290	FYS	15.08.11	B883
47	301	FYS	25.12.11	B883

b.) Group 2

RAM 952

Sr. No.	Tag #	Category	DOB	Ram
1	B101	FYS	09.03.11	952
2	B103	FYS	10.03.11	952
3	B108	FYS	11.03.11	952
4	B114	FYS	03.03.11	952
5	B121	FYS	15.03.11	952
6	B127	FYS	15.03.11	952
7	B129	FYS	16.03.11	952
8	B135	FYS	17.03.11	952
9	B138	FYS	18.03.11	952
10	B146	FYS	20.03.11	952
11	B152	FYS	21.03.11	952
12	B159	FYS	24.06.11	952
13	B177	FYS	30.06.11	952
14	B20	FYS	19.02.11	952
15	B26	FYS	20.02.11	952
16	B3	FYS	08.02.11	952
17	B37	FYS	24.02.11	952
18	B422	EWE	17.03.08	952
19	B46	FYS	25.02.11	952
20	B473	EWE	08.09.08	952
21	B474	EWE	09.09.08	952
22	B476	EWE	12.02.05	952

23	B507	EWE	06.02.09	952
24	B54	FYS	26.02.11	952
25	B542	EWE	12.02.09	952
26	B565	EWE	25.02.09	952
27	B58	FYS	26.02.11	952
28	B59	FYS	26.02.11	952
29	B604	EWE	08.03.09	952
30	B618	EWE	14.03.09	952
31	B62	FYS	27.02.11	952
32	B63	FYS	27.02.11	952
33	B66	FYS	27.02.11	952
34	B672	EWE	26.03.09	952
35	B716	FYS	21.02.10	952
36	B72	FYS	28.02.11	952
37	B73	FYS	28.02.11	952
38	B76	FYS	28.02.11	952
39	B79	FYS	01.03.11	952
40	B81	FYS	02.03.11	952
41	B82	FYS	02.03.11	952
42	B831	FYS	15.03.10	952
43	B85	FYS	03.03.11	952
44	B873	EWE	27.06.10	952
45	B88	FYS	04.03.11	952
46	B895	FYS	05.02.11	952
47	B896	FYS	05.02.11	952
48	B898	FYS	06.02.11	952
49	B91	FYS	05.03.11	952
50	B95	FYS	06.03.11	952

Annexure IV
Service detail of breeding animals

Sr. No.	Animal ID	1st Service	2nd Service	Sire ID	Breeding status
1	5	7/11/2012		B883	✓
2	8	14/10/2012		B883	✓
3	10	13/10/2012		B883	✓
4	17	19/10/2012		B883	✓
5	24	9/10/2012		B883	✓
6	25	10/10/2012		B883	✓
7	26	6/10/2012		B883	✓
8	34	8/10/2012		B883	✓
9	67	8/10/2012		B883	✓
10	99	3/10/2012		B883	✓
11	115	12/11/2012		B883	✓
12	131	1/11/2012		B883	✓
13	148	13/10/2012		B883	✓
14	152	16/10/12		B883	✓
15	153	11/10/2012		B883	✓
16	157	1/10/2012		B883	✓
17	160	2/11/2012		B883	✓
18	236	12/10/2012		B883	✓
19	248	19/09/2012		B883	✓
20	250	4/10/2012		B883	✓
21	251	1/9/2012		B883	✓
22	253	4/10/2012		B883	✓
23	254	10/11/2012		B883	✓
24	255	18/10/2012		B883	✓
25	257	26/09/2012		B883	✓
26	260	18/10/2012		B883	✓
27	261	28/09/2012		B883	✓
28	263	18/09/2012		B883	✓
29	264	3//4/2012		B883	✓
30	267	20/09/12		B883	✓
31	273	1/11/2012		B883	✓
32	286	17/10/2012		B883	✓
33	289	4/10/2012		B883	✓
34	280	1/11/2012		B883	✓

35	B3	7/11/2012		952	✓
36	B20	12/10/2012		952	✓
37	B37	12/10/2012		952	✓
38	B46	7/11/2012	17/6/2012	952	✓
39	B62	13/10/2012		952	✓
40	B63	13/10/2012		952	✓
41	B73	2/11/2012		952	✓
42	B81	18/10/2012		952	✓
43	B95	21/10/2012		952	✓
44	B108	23/10/2012		952	✓
45	B121	5/10/2012		952	✓
46	B138	8/11/2012		952	✓
47	B152	23/10/2012		952	✓
48	B476	8/10/2012	9/10/2012	952	✓
49	B507	3/10/2012	4/10/2012	952	✓
50	B542	16/10/2012		952	✓
51	B565	31/10/2012		952	✓
52	B672	1/10/2012		952	✓

Annexure V

Lambing of generation one for crop 1 (F1)

Sr. No.	Lamb ID	Sex	Type of birth	Dam ID	Sire ID	Remarks
1	1138	F	Twin	5	B883	
2	2034	M	Twin	5	B883	
3	2021	M	Twin	8	B883	
4	1131	F	Twin	8	B883	
5	2020	M	Twin	10	B883	
6	1130	F	Twin	10	B883	
7	2031	M	Single	17	B883	
8	2023	M	Single	24	B883	
9	1143	F	Single	25	B883	
10	1141	F	Twin	26	B883	
11	1142	F	Twin	26	B883	
12	1126	F	Twin	34	B883	
13	1127	F	Twin	34	B883	
14	2039	M	Single	67	B883	
15	2028	M	Single	99	B883	
16	2033	M	Single	131	B883	
17	1123	F	Twin	148	B883	
18	1124	F	Twin	148	B883	
19	2024	M	Twin	152	B883	
20	2025	M	Twin	152	B883	
21	1128	F	Twin	153	B883	
22	2019	M	Twin	153	B883	
23	2018	M	Single	157	B883	
24	2035	M	Single	160	B883	
25	1122	F	Single	236	B883	
26	2014	M	Single	248	B883	
27	1117	M	Single	251	B883	
28	1135	F	Single	253	B883	
29	2038	M	Single	254	B883	
30	2022	M	Single	255	B883	
31	1118	F	Single	257	B883	
32	1125	F	Single	260	B883	

33	-	-	Single	261	B883	Still birth
34	2015	M	Single	263	B883	
35	2037	M	Single	264	B883	
36	2016	M	Twin	267	B883	
37	-	-	Twin	267	B883	Mummified
38	2036	M	Single	273	B883	
39	1136	F	Single	280	B883	
40	1140	F	Single	B3	952	
41	2027	M	Single	B37	952	
42	1137	F	Single	B46	952	
43	1129	F	Single	B63	952	
44	2032	M	Single	B73	952	
45	1132	F	Single	B81	952	
46	2030	M	Single	B108	952	
47	1121	F	Single	B121	952	
48	1144	F	Single	B138	952	
49	2029	M	Single	B152	952	
50	2017	M	Twin	B507	952	
51	1119	F	Twin	B507	952	
52	1133	F	Triplet	B542	952	
53	2026	M	Triplet	B542	952	
54	1134	F	Triplet	B542	952	
55	1139	F	Single	B565	952	
56	1120	F	Single	B672	952	

Annexure VI.

Genotypes of crop 1 (F1) of parent generation for BMPR1B and BMP15

Sr. No.	Tag No.	Sex	BMPR1B			BMP15		
			BB	B+	++	BB	B+	++
1	1138	F	BB					++
2	2034	M		B+				++
3	2021	M		B+				++
4	1131	F		B+				++
5	2020	M		B+			B+	
6	1130	F		B+			B+	
7	2031	M		B+				++
8	2023	M		B+				++
9	1143	F		B+				++
10	1141	F		B+				++
11	1142	F		B+				++
12	1126	F		B+				++
13	1127	F		B+				++
14	2039	M		B+				++
15	2028	M		B+				++
16	2033	M		B+				++
17	1123	F		B+				++
18	1124	F		B+				++
19	2024	M		B+				++
20	2025	M		B+				++
21	1128	F		B+				++
22	2019	M		B+				++
23	2018	M		B+				++
24	2035	M		B+				++
25	1122	F		B+				++
26	2014	M		B+				++
27	1117	M		B+				++
28	1135	F		B+				++
29	2038	M		B+				++
30	2022	M		B+				++
31	1118	F		B+				++
32	1125	F	BB					++

33	2015	M		B+				++
34	2037	M		B+				++
35	2016	M		B+				++
36	2036	M		B+				++
37	1136	F		B+				++
38	1140	F			++			++
39	2027	M			++			++
40	1137	F		B+				++
41	1129	F			++			++
42	2032	M			++			++
43	1132	F			++			++
44	2030	M			++			++
45	1121	F		B+				++
46	1144	F		B+				++
47	2029	M			++			++
48	2017	M			++		B+	
49	1119	F			++		B+	
50	1133	F	BB					++
51	2026	M		B+				++
52	1134	F	BB					++
53	1139	F			++			++
54	1120	F			++			++

Annexure VII

Breeding status of parental generation for crop 2 (F1)

Sr. No.	Female exposed	Service status	Pregnant	Male
1	B3			
2	B20	✓	✓	952
3	B26	✓	✓	952
4	B37			
5	B46	✓	✓	952
6	B54			
7	B58	✓	✓	952
8	B59	✓	✓	952
9	B62			
10	B63	✓	✓	952
11	B66			
12	B72			
13	B73	✓	✓	952
14	B76	✓	✓	952
15	B79	✓	✓	952
16	B81	✓	✓	952
17	B82			
18	B85			
19	B95			
20	B101	✓	✓	952
21	B103			
22	B108	✓	✓	952
23	B114	✓	✓	952
24	B121	✓	✓	952
25	B127	✓	✓	952
26	B129	✓	✓	952
27	B135			
28	B138			
29	B152	✓	✓	952

30	B159			
31	B473	✓	✓	952
32	B474			
33	B476			
34	B507			
35	B542	✓	✓	952
36	B565			
37	B618	✓	✓	952
38	B672	✓	✓	952
39	B716	✓	✓	952
40	B831	✓	✓	952
41	B873			
42	B895	✓	✓	952
43	B896			
44	B898	✓	✓	952
45	5	✓	✓	B883
46	8			
47	10	✓	✓	B883
48	17			
49	24	✓	✓	B883
50	25	✓	✓	B883
51	26	✓	✓	B883
52	34			
53	62			
54	67			
55	99	✓	✓	B883
56	115			
57	131	✓	✓	B883
58	148	✓	✓	B883
59	152	✓	✓	B883
60	153	✓	✓	B883
61	157	✓	✓	B883
62	159			
63	160			

64	186	✓	✓	B883
65	192			
66	236	✓	✓	B883
67	244	✓	✓	B883
68	248	✓	✓	B883
69	249	✓	✓	B883
70	250	✓	✓	B883
71	253	✓	✓	B883
72	254	✓	✓	B883
73	255	✓	✓	B883
74	257	✓	✓	B883
75	258	✓	✓	B883
76	260	✓	✓	B883
77	261	✓	✓	B883
78	262			
79	263	✓	✓	B883
80	264	✓	✓	B883
81	267	✓	✓	B883
82	301	✓	✓	B883
83	308	✓	✓	B883
84	316	✓	✓	B883
85	317	✓	✓	B883
86	321	✓	✓	B883
87	321	✓	✓	B883
88	327	✓	✓	B883
89	330	✓	✓	B883
90	1102	✓	✓	B883
91	1103	✓	✓	B883
92	1103	✓	✓	B883
93	1105	✓	✓	B883
94	1106	✓	✓	B883
95	1109	✓	✓	B883
96	1115	✓	✓	B883

Annexure VIII
Lambing of crop 2 (F1)

Sr.No.	Lambing date	Sex of Lamb	Tag No.	Dame No.	Sire No.	Remarks
1	2/15/2014	M	2045	248	B883	
2	2/15/2014	M	2046	b58	952	
3	2/16/2014	M	2047	257	B883	
4	2/16/2014	F	1157	B79	952	
5	2/18/2014	M	2048	B63	952	
6	2/18/2014	F	1158	B618	952	
7	2/18/2014	F	1159	10	B883	weak, Died within 24 hr
8	2/18/2014	F	1160	10	B883	
9	2/19/2014	F	1161	B129	952	
10	2/19/2014	F	1162	B129	952	
11	2/20/2014	M	2049	99	B883	
12	2/20/2014	M	2051	B672	952	
13	2/20/2014	M	2052	B672	952	
14	2/21/2014	M	2053	254	B883	
15	2/21/2014	M	2054	153	B883	
16	2/21/2014	M	2055	153	B883	
17	2/22/2014	F	1163	25	B883	
18	2/23/2014	M	2056	B20	952	
19	2/24/2014	M	2057	253	B883	
20	2/25/2014	M	2058	152	B883	
21	2/25/2014	M	2059	B898	952	
22	2/25/2014	M	2060	330	B883	
23	2/26/2014	M	2061	157	B883	
24	2/27/2014	M	2062	B101	952	
25	2/27/2014	M	2063	B101	952	
26	2/28/2014	F	1164	B127	952	
27	2/28/2014	M	2064	B127	952	
28	3/1/2014	F	1165	131	B883	
29	3/1/2014	F	1166	B542	952	
30	3/1/2014	F	1167	B542	952	
31	3/2/2014	M	2065	249	B883	
32	3/2/2014	M	2066	264	B883	
33	3/3/2014	M	2067	B46	952	

34	3/4/2014	M	2069	B716	952	
35	3/5/2014	F	1168	250	B883	
36	3/5/2014	F	1169	26	B883	
37	3/6/2014	F	1170	260	B883	
38	3/6/2014	M	2070	5	B883	
39	3/7/2014	M	2071	B26	952	
40	3/7/2014	F	1171	B114	952	
41	3/8/2014	F	1172	236	B883	
42	3/8/2014	M	2072	236	B883	weak, Died within 24 hr
43	3/10/2014	M	2073	B108	952	
44	3/11/2014	M	2074	267	B883	
45	3/12/2014	F	1173	24	B883	
46	3/12/2014	F	1174	148	B883	
47	3/12/2014	F	1175	255	B883	
48	3/14/2014	M	2075	B895	952	
49	3/17/2014	F	1176	B896	952	
50	3/18/2014	F	1177	1106	B883	
51	3/18/2014	M	2076	1102	B883	
52	3/18/2014	M	2077	1105	B883	
53	3/20/2014	M	2078	321	B883	
54	3/20/2014	F	1178	321	B883	
55	4/11/2014	F	1179	186	B883	
56	4/11/2014	M	2079	B73	952	
57	4/11/2014	F	1180	308	B883	
58	4/12/2014	M	2080	B76	952	
59	4/12/2014	M	2081	B121	952	
60	4/12/2014	F	1181	258	B883	
61	4/13/2014	F	1182	B152	952	
62	4/13/2014	F	1183	B831	952	
63	4/14/2014	M	2082	B473	952	
64	4/15/2014	F	1184	B59	952	
65	4/15/2014	M	2083	244	B883	
66	4/17/2014	M	2084	261	B883	
67	4/17/2014	M	2085	263	B883	
68	4/18/2014	M	2086	327	B883	
69	4/18/2014	M	2087	B81	952	
70	4/19/2014	M	2088	316	B883	
71	4/19/2014	F	1185	1103	B883	
72	4/19/2014	F	1186	1103	B883	
73	4/20/2014	M	2089	301	B883	
74	4/21/2014	F	1187	1115	B883	

75	4/23/2014	F	1188	1109	B883	
76	4/23/2014	F	1189	317	B883	

Annexure IX

Genotypes of crop 2 (F1) of parent generation for BMPR1B and BMP15

Sr. No.	Tag No.	Sex	BMPR1B			BMP15		
			BB	B+	++	BB	B+	++
1	2059	M			++			++
2	1176	F			++			++
3	2075	M			++			++
4	1183	F		B+				++
5	2087	M			++			++
6	1157	F		B+				++
7	2080	M			++			++
8	2079	M			++			++
9	2069	M			++			++
10	2051	M			++			++
11	2052	M			++			++
12	2048	M			++			++
13	1158	F			++			++
14	1184	F		B+				++
15	2046	M			++			++
16	1166	F		B+				++
17	1167	F		B+				++
18	2082	M		B+				++
19	2067	M			++			++
20	2071	M		B+				++
21	2056	M			++			++
22	1182	F			++			++
23	1161	F			++			++
24	1162	F		B+				++
25	1164	F			++			++
26	2064	M			++			++
27	2081	M		B+				++
28	1171	F			++			++
29	2073	M		B+				++
30	2062	M		B+				++
31	2063	M		B+				++
32	1187	F		B+				++
33	1188	F		B+				++
34	1177	F		B+				++

35	2077	M		B+				++
36	1185	F		B+				++
37	1186	F		B+				++
38	2076	M		B+				++
39	2060	M		B+				++
40	2086	M		B+				++
41	2078	M		B+				++
42	1178	F		B+				++
43	1189	F		B+				++
44	2088	M		B+				++
45	1180	F		B+				++
46	2089	M		B+				++
47	2074	M		B+				++
48	2066	M		B+				++
49	2085	M		B+				++
50	2084	M		B+				++
51	1170	F	BB					++
52	1181	F		B+				++
53	2047	M		B+				++
54	1175	F		B+				++
55	2053	M		B+				++
56	2057	M		B+				++
57	1168	F		B+				++
58	2065	M		B+				++
59	2083	M		B+				++
60	1172	F		B+				++
61	2072	M		B+				++
62	1179	F		B+				++
63	2061	M		B+				++
64	2054	M		B+				++
65	2055	M		B+				++
66	2058	M		B+				++
67	1174	F		B+				++
68	1165	F		B+				++
69	2049	M		B+				++
70	1169	F		B+				++
71	1163	F		B+				++
72	1173	F		B+				++
73	1159	F		B+				++
74	1160	F		B+			B+	
75	2070	M	BB					++

Annexure X

Breeding status of parental generation for crop 3 (F1)

a.) Group 1

RAM B883

Sr. No	Tag No.	DOB	Birth Status	Service date
1	248	16.02.11	S	10/4/2014
2	249	17.02.11	S	
3	251	18.02.11	S	9/15/2014
4	254	19.02.11	S	
5	255	20.02.11	S	9/25/2014
6	258	22.02.11	T	10/18/2014
7	262	26.02.11	T	9/27/2014
8	263	27.02.11	S	9/16/2014
9	264	28.02.11	S	
10	265	28.02.11	S	9/30/2014
11	267	01.03.11	S	9/21/2014
12	273	07.03.12	T	10/17/2014
13	281	29.02.12	T	10/5/2014
14	289	15.08.11	T	9/23/2014
15	290	15.08.11	T	
16	301	25.12.12	T	10/13/2014
17	308	26.02.12	T	9/15/2014
18	311	28.02.12	T	11/4/2014
19	316	05.03.12	T	
20	321	08.03.12	S	9/17/2014
21	325	10.03.12	S	
22	326	11.03.12	T	10/2/2014
23	327	09.03.12	T	9/30/2014
24	330	18.03.12	T	9/17/2014

b.) Group 2

RAM 975

Sr. No	Tag No.	DOB	Birth Status	Service date
1	B20	2/19/2011	T	9/17/2014
2	B26	2/20/2011	T	
3	B 54	26/2/2011	T	9/19/2014
4	B 62	27/2/2011	T	
5	B 72	2/28/2011	T	9/30/2014
6	B 73	28/2/2011	T	
7	B 76	28/2/2011	T	9/15/2014
8	B 79	3/1/2011	T	9/25/2014
9	B 81	2/3/2011	T	
10	B 85	3/3/2011	T	10/6/2014
11	B 88	3/4/2011	T	9/27/2014
12	B 91	3/5/2011	T	
13	B 95	3/6/2011	T	10/13/2014
14	B 101	9/3/2011	T	
15	B 103	3/10/2011	T	9/28/2014
16	B 108	3/11/2011	S	10/20/2014
17	B 114	3/3/2011	T	10/4/2014
18	B 127	15/3/2011	T	
19	B 129	3/16/2011	T	10/15/2014
20	B 135	17/3/2011	T	10/18/2014
21	B 138	3/18/2011	T	9/21/2014
22	B 152	21/3/2011	T	
23	B159	6/24/2011	T	10/3/2014
24	B177	6/30/2011	T	10/2/2014
25	B895	2/5/2011	T	10/7/2014
26	B 896	2/5/2011	T	10/11/2014

Annexure XI
Lambing of crop 3 (F1)

Sr. No.	Lambing date	Sex of Lamb	Tag No.	Dame No.	Sire No.	Remarks
1	10-03-15	M	2091	316	B883	
2	10-03-15	M	2092	327	B883	
3	11-03-15	M	2093	251	B883	
4	11-03-15	M	2094	B95	975	
5	11-03-15	M	2095	289	B883	
6	11-03-15	F	1190	289	B883	
7	12-03-15	F	1191	B177	975	
8	12-03-15	M	2096	B177	975	
9	14-03-15	F	1192	B127	975	
10	14-03-15	M	2097	264	B883	
11	14-03-15	M	2098	B108	975	
12	14-03-15	F	1193	249	B883	
13	15-03-15	F	1194	290	B883	
14	15-03-15	F	1195	290	B883	
15	15-03-15	F	1196	B152	975	
16	15-03-15	M	2099	B152	975	
17	16-03-15	F	1197	273	B883	
18	16-03-15	F	1198	273	B883	
19	17-03-15	M	2100	267	B883	
20	18-03-15	F	1199	263	B883	
21	20-03-15	M	2101	B62	975	
22	22-03-15	F	1201	B159	975	
23	22-03-15	F	1202	B895	975	
24	22-03-15	F	1203	B76	975	
25	23-03-15	M	2102	311	B883	
26	25-03-15	M	2103	B85	975	
27	15-04-15	M	2108	B91	975	
28	15-04-15	M	2109	B91	975	
29	01-07-15	M	2112	248	B883	
30	04-08-15	M	2114	B135	975	

Annexure XII

Genotypes of crop 3 (F1) of parent generation for BMPR1B and BMP15

Sr. No.	Tag No.	Sex	BMPR1B			BMP15		
			BB	B+	++	BB	B+	++
1	2091	M		B+				✓
2	2092	M		B+				✓
3	2093	M		B+				✓
4	2094	M			++			✓
5	2095	M		B+				✓
6	1190	F		B+				✓
7	1191	F		B+				✓
8	2096	M		B+				✓
9	1192	F			++			✓
10	2097	M		B+				✓
11	2098	M			++			✓
12	1193	F		B+				✓
13	1194	F		B+				✓
14	1195	F		B+				✓
15	1196	F		B+				✓
16	2099	M			++			✓
17	1197	F		B+				✓
18	1198	F		B+				✓
19	2100	M		B+				✓
20	1199	F		B+				✓
21	2101	M			++			✓
22	1201	F		B+				✓
23	1202	F			++			✓
24	1203	F		B+				✓
25	2102	M		B+				✓
26	2103	M			++			✓
27	2108	M			++			✓
28	2109	M		B+				✓
29	2112	M		B+				✓
30	2114	M			++			✓

Annexure XIII

Breeding status of F1 generation for F2

a.) Group 1

RAM 2030

Sr. No.	Animal ID	DOB	Birth type	Service date
1	1118	20/2/2013	S	
2	1122	12/10/2012	S	21-Oct-14
3	1123	13/10/2012	T	28-Oct-14
4	1124	13/10/2012	T	21-Oct-14
5	1125	18/10/2012	S	
6	1126	8/10/2012	T	1-Nov-14
7	1127	8/10/2012	T	10-Feb-15
8	1131	14/10/2012	T	22-Oct-14
9	1135	4/10/2012	S	26-Sep-14
10	1138	7/11/2012	T	
11	1141	6/10/2012	T	27-Oct-14
12	1143	10/10/2012	S	

b.) Group 2

RAM 2016

Sr. No.	Animal ID	DOB	Birth type	Service date
1	1119	3/10/2012	T	
2	1120	1/10/2012	S	3-Oct-2014
3	1121	5/10/2012	S	
4	1129	13/10/2012	S	26-Oct-2014
5	1132	18/10/2012	S	16-Jan-15
6	1133	16/10/2012	T	2-Nov-2014
7	1134	16/10/2012	T	5-Oct-14
8	1137	7/11/2012	S	
9	1140	7/11/2012	S	29-Oct-2014
10	1144	8/11/2012	S	1-Nov-2014
11	1148	2/9/2013	S	1-Sep-2014

c.) Group 3
RAM 2015

Sr. No.	Animal ID	DOB	Birth type	Service date
1	1102	7/13/2012	S	25-Oct-14
2	1103	7/19/2102	T	25-Oct-14
3	1105	8/27/2012	S	
4	1106	8/27/2012	S	28-Sep-14
5	1107	9/10/2012	T	11-Feb-15

d.) Group 4
RAM 2037

Sr. No.	Animal ID	DOB	Birth type	Service date
1	1108	15-Nov-12	S	
2	1109	24-Nov-12	S	1-Nov-14
3	1110	12-Dec-12	S	2-Nov-14
4	1111	15-Dec-12	T	5-Oct-14
5	1112	15-Dec-12	T	5-Oct-14
6	1114	25-Dec-12	T	14-Oct-14
7	1115	13-Jan-13	T	28-Sep-14
8	1116	13-Jan-13	T	4-Oct-14
9	1164	28-Feb-14		1-Jan-15

Annexure XIV
Lambing of F1 to produce F2

Sr. No.	Lambing date	Sex of Lamb	Tag No.	Dame No.	Sire No.	Remarks
1	01-03-15	M	2090	1148	2030	
2	21-03-15	F	1200	1106	2015	
3	26-03-15	M	2104	1116	2037	
4	26-03-15	F	1204	1116	2037	
5	30-03-15	M	2105	1115	2037	
6	30-03-15	F	1205	1135	2030	
7	01-04-15	F	1206	1112	2037	
8	03-04-15	F	1207	1120	2016	
9	04-04-15	F	1208	1111	2037	
10	04-04-15	F	1209	1111	2037	
11	04-04-15	M	-	1134	2016	Still birth
12	04-04-15	M	-	1134	2016	Still birth
13	05-04-15	M	2106	1114	2037	
14	05-04-15	M	2107	1114	2037	
15	01-07-15	M	2110	1164	2037	
16	01-07-15	M	2111	1164	2037	
17	11-07-15	F	1210	1132	2016	
18	01-08-15	M	2113	1127	2030	
19	04-08-15	F	1211	1107	2015	

Annexure XV
Genotypes of F2

Sr. No.	DOB	Sex	Animal ID	Sample	DNA	PCR	BB	B+	++
1	01-03-15	M	2090	✓	✓	✓		B+	
2	21-03-15	F	1200	✓	✓	✓		B+	
3	26-03-15	M	2104	✓	✓	✓	BB		
4	26-03-15	F	1204	✓	✓	✓		B+	
5	30-03-15	M	2105	✓	✓	✓		B+	
6	30-03-15	F	1205	✓	✓	✓			++
7	01-04-15	F	1206	✓	✓	✓		B+	
8	03-04-15	F	1207	✓	✓	✓			++
9	04-04-15	F	1208	✓	✓	✓		B+	
10	04-04-15	F	1209	✓	✓	✓		B+	
11	05-04-15	M	2106	✓	✓	✓		B+	
12	05-04-15	M	2107	✓	✓	✓	BB		
13	01-07-15	M	2110	✓	✓	✓			++
14	01-07-15	M	2111	✓	✓	✓		B+	
15	11-07-15	F	1210	✓	✓	✓			++
16	01-08-15	M	2113	✓	✓	✓			++
17	04-08-15	F	1211	✓	✓	✓			++