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Final report

project

Improved productivity, profitability and sustainability of sheep production in Maharashtra, India, through genetically enhanced prolificacy, growth and parasite resistance

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1 Acknowledgments

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2 Executive summary

Broadly this project aimed to improve sheep meat production and rural incomes in the large Indian State of Maharashtra. The target local sheep breed, the Deccani, is by far the dominant sheep breed in India, comprising 31% of India's 61.5 million sheep. The project built on the findings of ACIAR Project AS1/94/022 that had established that low prolificacy was a major constraint on the productivity of sheep in Maharashtra and that genetic improvement of prolificacy using the Booroola fecundity gene (*FecB*) found in Garole sheep was likely to be successful. This project had also established that the Garole sheep exhibited significant genetic resistance to gastrointestinal nematode parasitism.

Project AH/2002/038, with extensions, ran from 2003 to 2008 and involved collaboration between three lead institutions: the Nimbkar Agricultural Research Institute (NARI), an NGO in Phaltanin rural Maharashtra, The National Chemical Laboratory (NCL), a CSIR institute in Pune, Maharashtra and the University of New England (UNE), Armidale, NSW Australia. The main thrust of the project was to test, under normal shepherding 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 objectives were a) to develop and multiply at NARI promising sheep genotypes for testing in shepherds' flocks, b) test their performance in shepherds' flocks in the Phaltan region, and to develop appropriate management technologies, c) to investigate the regulation of expression of *FecB* in Indian and Australian breeds of sheep d) develop extension and genetic models for the dissemination of proven genotypes within and beyond the local project area.

At NARI a complex breeding program was successfully implemented using artificial insemination, full pedigree recording, determination of estimated breeding values and mate selection based on TGRM® software. The program was designed to allow valid breed and *FecB* genotype comparisons. Animals were genotyped for *FecB* by DNA test at NCL and NARI at a young age to identify those carrying no copy of the gene (WW), one copy (BW) or two copies (BB). At the end of the project there were 45 BB rams and 123 BB ewes and 35 BW rams and 322 BW ewes at NARI. Distribution of *FecB* carrier ewes, rams and semen to 26 participating shepherds' flocks commenced in 2003 and by the end of the project these flocks contained 13 BB and 240 BWadult ewes. Shepherds exhibited resistance to the undesirable features of the Garole breed in first cross progeny with the Deccani, but backcrossed ewes and rams (25% Garole or less) had greater acceptability.

It was shown that one copy of the *FecB*gene increased litter size from 1.03 to 1.58 in the NARI flock and from 1.03 to 1.35 in the shepherds' flocks, a moderate and manageable increase. Two copies of the *FecB* geneincreased litter size to 1.65at NARI, a similar increase to that seen with one copy. By sale age of 3 months surviving litter sizes at NARI and smallholders respectively were 0.95 each for WW ewes, 1.35 and 1.21 for BW ewes and 1.34 for BB ewes(NARI only). Overall BW ewes produced 27% greater weight of 3-month lamb than non-carriers, and twin-bearing ewes produced 42% more. Economic analysis revealed that twin bearing ewes had a gross margin 30% greater than that of single bearing ewes.

In commercial Merino sheep in Australia, *FecB* conferred higher increases in litter size than in India, and under the extensive management system used there,lamb mortality limited the utility of *FecB* carrier ewes. While the heterozygote exhibited superior overall productivity, homozygous ewes had significantly depressed fertility and lamb survival. This homozygote problemhas not been seen to date in India, but will need to be monitored.

In India it is recommended that wider introgression of the *FecB* gene continue. To maximise the success of introgression, *FecB*-carrier animals to be disseminated into local flocks should have a similar phenotype as the local breed and be selected and superior for other economically important traits. As introgression is a process requiring at least

three generations of backcrossing, it will need excellent institutional infrastructure including a network and extension program among local sheep owners.

The project concluded with a successful International Workshop on using the *FecB*gene in sheep breeding programs which is Proceedings 133 in the ACIAR Proceedings Series.

3 Background

The ACIAR-funded project AS1/94/022 'Prolific, Worm-Resistant Meat Sheep for Maharashtra, India' was implemented from 1998 to 2000 and extended for a further two years up to 2002. The main objective of the project was to address two important constraints to the economic viability of sheep rearing in India and Australia - low reproduction rate and production losses due to parasitism. The first constraint applies more to India and the second one equally to both countries. The project AH/2002/038 intended to build directly on the outputs of Project AS1/1994/022, consolidate them, prove their feasibility and profitability under commercial sheep rearing conditions and extend them to the wider shepherd community who belong to the poorer sections of the rural communities in Maharashtra.

One of the major outputs of project AS1/94/022 was the discovery of the Booroola fecundity gene (*FecB*) in the Garole sheep in which its action appeared to be damped relative to that in European breeds of sheep into which it has been introgressed. The Garole was also shown to have considerable genetic resistance to infection with *Haemonchus contortus*, the predominant gastro-intestinal nematode parasite of sheep and goats in the tropics and sub-tropics. The broad objective of the new project was to multiply the most promising genotypes carrying the *FecB* gene, test their performance in shepherds' flocks in the Phaltan region and form an opinion about, and policies for, the wider introgression of *FecB* into the sheep population. While the overall aim was to develop and evaluate highly productive meat sheep genotypes for Maharashtra, the key challenge was to determine the expression of the *FecB* gene in the new composite breed and in Deccani sheep to which it had been introgressed. The *FecB* gene has proved unsuccessful in many countries, including Australia, due to the induction of extreme litter sizes and concomitant high lamb mortality, particularly for homozygous ewes. It was important to demonstrate that this was not the case for local sheep in the Maharashtra environment.

The technically correct notation for sheep carrying no copy of the *FecB* gene is $FecB^{++}$, one copy $FecB^{B+}$, and two copies $FecB^{BB}$. However for ease of understanding in a report of this nature we have chosen to use the notation WW, BW and BB respectively, with 'W' denoting the wild type allele and 'B' the Booroola allele that confers prolificacy.

Project justification: In India, there is urgent need to increase meat production to increase the per capita consumption of meat to alleviate malnutrition, especially among women and children. Sheep meat is important in maintaining an adequate supply of protein in India because there are no religious taboos associated with the consumption of sheep meat. There are 61 million sheep in India of which over 3 million are in the state of Maharashtra. About 0.5 million of these are in an area within a 100-km radius of Phaltan where the proposed project will be located in Maharashtra. In the semi-arid areas of Maharashtra sheep rearing is the traditional occupation of 85,000 families of mainly the 'Dhangar' but also other communities, and the Deccani is the local breed of sheep. Sheep production is the most adaptable, and often the only potentially viable economic activity in these semi-arid land ecosystems. Sheep are kept predominantly for meat and manure production with wool being a minor product.

At the country consultations held by ACIAR in New Delhi in 2001, consensus was achieved on several over-arching issues, including the need to address increased demand for livestock products arising from increased incomes, to raise small holder farmers' incomes and to improve nutrition through agricultural diversification. The project addressed the following two priority areas identified at the consultations in India.

1. Molecular strategies for improving selection in livestock breeding
2. Sustainable control of parasitic diseases in sheep

Sheep have a generally low reproductive rate as a species. Individual ewes usually give birth to 1 lamb and rarely more than 2, and have an interval between lambings of 8-12 months. A 1990 study of sheep production in Maharashtra identified low reproductive rate as the factor most responsible for the low productivity of traditional sheep production systems in that state (DAH, 1992; Rath, 1992). Project AS1/1994/022 confirmed this and established that the local Deccani sheep of Maharashtra usually produce only single lambs and have a mean litter size of 1.02 and lambing interval of 10 months. Reproductive rate is also particularly low in the Australian Merino. The efficiency and profitability of both wool and meat production, particularly the latter, is very sensitive to reproductive rate (Turner, 1976). During project AS1/1994/022 it was established under shepherd conditions that twin bearing ewes weaned 50% more lamb than ewes producing single lambs.The Garole breed of sheep is perhaps the only prolific sheep breed in India. It however, has a number of undesirable traits associated mainly with extremely small body size with adult ewes weighing about 14 kg. The basis of prolificacy of the Garole breed has been shown to be primarily a single mutation, the Booroola or *FecB* mutation (Davis*et al.* 2002). A direct gene test for this mutation was developed in 2001 (Wilson*et al.* 2001) and the availability of this greatly facilitates the introgression of *FecB* into the Deccani and other breeds.The results of early crossbreeding experiments on Project AS1/1994/022 established that increases in litter size in Garole crosses with Deccani and Bannur sheep carrying the *FecB*gene appeared to be manageable and were likely to be beneficial (Nimbkar *et al*., 2003a).

Gastro-intestinal parasitism is also recognised as a major constraint to sheep production in both India and Australia (Sanyal, 1996). The problem of parasitism in sheep is compounded by the practice of communal grazing and lack of grazing management in the local sheep production system in Maharashtra. This pastoral practice increases greatly the probability of drenched sheep becoming rapidly reinfected from pasture contamination. Many shepherds are illiterate and for this reason incorrect doses of dewormers are often administered. Such inadvertent errors are likely to reduce the efficacy of treatments and hasten the development of anthelmintic resistance. Genetic resistance to gastro-intestinal nematode infection has a moderate heritability (Gray, 1995) and the Garole sheep breed was shown in Project AS1/1994/022 to exhibit significant resistance to worm infection (Nimbkar *et al*., 2003b). Therefore there are good options for improving gastrointestinal parasite control in India by animal breeding as well as by improved diagnosis and control practices.

The proposed principal beneficiaries of the project were the shepherd communities in Maharashtra with benefits taking the form of increased profits from available grazing land by higher turnoff rates of sheep for market per stock unit. Consumers will start to see benefits in the longer term once the outputs of the project have disseminated deeply into the sheep population leading to an increase in market supplies. Indian and Australian scientists were also seen as significant beneficiaries of the project, benefiting from interaction with each other, experience of new environments and production systems.

Some of the proposed outcomes were likely to be relevant to Australian sheep producers with the project leading to a re-evaluation of the potential of the *FecB* gene to lift reproductive performance in Australian sheep without the excessive litter sizes and lamb mortality that have invariably been associated with its use in Merino sheep in the past.

4 Objectives

In both the initial project and the project extension (2006-07)there were four objectives. Objective A and B varied little, Objective D varied significantly and Objective C changed completely between the initial project and the project extension,

4.1 Objectives of the initial project (2003-2005)

Objective A. Production of appropriate genotypes for testing in shepherds' flocks

To produce sufficient homozygote carrier (BB), heterozygote carrier (BW) and non-carrier (WW) ewes and rams of both a new composite breed and the Deccani breed for testing in shepherds' flocks.

Objective B. Testing of improved genotypes in shepherds' flocks and development of appropriate management technologies

To undertake extensive performance testing on-station and in shepherds' flocks to compare the productivity and profitability of the composite and the fecund Deccani (Deccani BB, BW) with that of the traditional Deccani.

Objective C. Regulation of expression of FecB in Indian and Australian breeds of sheep

To investigate the regulation of expression of *FecB* in different genetic backgrounds with varying proportions of Garole genes from 12.5% to 100% and also lines of Garoles selected divergently for litter size. In Australia to examine environmental regulation of *FecB* expression.

Objective D. Development of extension and genetic models for the dissemination of proven genotypes

Communication, extension systems and dissemination of project results. Formal design of optimal introgression methods for successful genotypes into the wider Maharashtra sheep population.

4.2 Objectives of the project extension (2006-2007)

Objective A. Ongoing production of appropriate genotypes for testing in shepherds' flocks

Emphasis will be on generation of maximal numbers of homozygous lambs with low Garole breed content (<25%) while accommodating the maintenance of genetic diversity in the nucleus flock.

Objective B. Complete the measurement of performance of carrier rams and progeny by dissemination of improved FecB carrier rams and semen into shepherds' flocks.

Rams or semen from *FecB* carrier rams will continue to be used in the 20 existing participating shepherd flocks and will also be introduced into 10 new shepherds' flocks.

Objective C. Evaluate the socio-economic benefit of the FecB dissemination program

An independent formal evaluation of the dissemination and extension program from 2003-2006 will be conducted using conventional survey methodology and a participatory

techniques approach. This will identify critical issues for the wider dissemination strategy to follow this project and will facilitate the development of specific recommendations for future dissemination activity.

Objective D: Devise a strategy and mechanisms for dissemination of the FecB mutation beyond the current project area.

Based on the results of Objective C and other information, a clear strategy for dissemination of the *FecB* mutation in improved genotypes in other regions of India will be formulated.

5 Methodology

Animal work in India was carried out at the Rajale, Wadjal and Dhuldeo farms of the Nimbkar Agricultural Research Institute (NARI) (18^0 N 74^0 E) and in 26 sheep flocks belonging to smallholders within a radius of 30 km from Phaltan. Phaltan is situated on the Deccan plateau and has a dry monsoonal climate. The average annual rainfall is 525 mm and 90% of it falls from June to October. Laboratory work in India was carried out at NARI or in Dr Vidya Gupta's laboratory the National Chemical Laboratory, Pune, Maharashtra. Animal work in Australia was carried out on David Wolfenden's property "Allandale" at Rand between Wagga Wagga and Albury in Southern NSW.

5.1 Ewe management at NARI (Objective A)

The Deccani ewes at NARI and their crosses were herded for grazing in one or more flocks and housed in open-sided sheds at night. The ewes were kept at NARI's Lundy farm at Rajale. They were grazed on crop residues, weeds and fallow plots on neighbouring farmers' land, in return for sheep manure, similar to the method followed by local shepherds. They were therefore exposed to seasonal excesses and shortages of forage but were given a small quantity of supplementation. Supplementary feed of 200 gm per ewe was given from 2002 onwards to all ewes available for breeding for two months at breeding and to pregnant ewes from two weeks before lambing to about three months after lambing. Ewes stayed in the same flock from breeding through to lambing and weaning of lambs. Ewes were weighed every month except in the last three months of pregnancy. Purebred Garole ewes were managed separately - stall-fed partially or completely.

5.2 Sheep breeding at NARI (Objective A)

Cervical artificial insemination (AI) with fresh semen was the method of breeding adopted, initially because of the small size of Garole rams relative to Deccani and Bannur ewes. Later, it was found feasible to use AI to maintain accurate pedigrees because the breeding plan became complex with rams and ewes of many breeds and crossbred types and the large number of rams used at every mating. Twenty to 40 breeding rams were used for 200-300 ewes at every mating. One AI program was carried out each year except 2004, 2005, 2007 and 2008 when inseminations were carried out twice each year. Each AI program lasted for a period of two oestrus cycles (35-40 days). Breeding rams were kept stall-fed partially or completely at Wadjal farm and were taken to Lundy farm, Rajale for about five weeks twice a year for breeding. The same rams were also sent to various shepherds' flocks for breeding at other times of the year.

Ewes were inseminated with fresh, diluted semen once at natural oestrus (detected by vasectomized rams). The composition of the semen diluent was based on one suggested by Evans and Maxwell (1987) and modified for local conditions. It was a synthetic diluent containing Tris buffer, D Glucose, citric acid and the antibiotics Benzyl penicillin and Streptomycin sulphate. Ewes that returned to oestrus after insemination were inseminated again up to a total of three times during the designated period. Some ewes were naturally hand-mated to rams if the rams were too shy for semen collection or sometimes if a ram had only one ewe allotted to it out of the ewes on oestrus on a particular day. In total, six per cent of the matings (244 out of 3771) were done by natural service (NS).

Ewes were scanned ultrasonically (with an Aloka scanner SSD500 3.5 Mhz with a convex abdominal probe) 41-66 days after the inseminations and the number of fetuses was recorded. Both artificial insemination and ultrasonic scanning were done by Pradip Ghalsasi. He alsocollected blood samples of Garole ewes for Objective C, measured ovulation rate laparoscopically and vasectomised the rams used for detecting oestrus.

5.3 Lamb management at NARI (Objective A)

Lambs were retained in the sheep housing for the first month of life being provided with water and supplementary feed. Suckling occurred in the evening when the ewes returned from grazing. Lambs of ewes which did not secrete sufficient milk were cross-fostered for the first 3 months to ewes with ample milk or those which had aborted or otherwise lost their lambs. Some lambs were accepted by some foster mothers as though they were their own. Most cross-fostered lambs were suckled to several ewes and the same lambs were not suckled to the same ewes every day. The proportion of cross-fostered lambs was about 8%. All lambs were given supplementary feed from 2003 onwards, receiving 50 to 75 g each of groundnut cake and cotton seed cake from about two months of age to weaning at about 15 kg body weight (approximately 3 months of age). Lambs were weighed on electronic weighing scales at birth and every 15 days thereafter up to six months of age and every month after that.

Sheep maintenance, breeding and lamb management were carried out by NARI's shepherds, farm labourers and farm supervisors under the guidance of Drs. Pradip Ghalsasi and Chanda Nimbkar. From 2003 to 2007, Pradip Ghalsasi carried out most of the inseminations while the supervisors did ram semen collection. Thereafter, the farm supervisors, trained by Pradip, started to do most of the inseminations also. The supervisors were Mr. Ashok Magar, Rupchand Khanvilkar, Kanhaiya Chavan, Shyam Kulkarni, Malhari Dhembare, Datta Mulik and Dilip Bhandari. Lamb blood sample collections on FTA paper for genotyping were done mainly by Pradip Ghalsasi with the help of supervisors. Faecal samples of sheep were analyzed for worm burden assessment by Padmaja Ghalsasi.

5.4 Genotyping at the *FecB* locus (Objective A)

All lambs that were likely to be *FecB* carriers were genotyped by collecting blood samples at about two weeks of age on FTA paper (Whatman Bioscience, UK). Following DNA extraction from the paper the forced PCR-RFLP direct DNA test described by Wilson *et al*. (2001) and modified as described by Pardeshi *et al*. (2005) was used to determine *FecB* genotype. The test method is so designed that the PCR products of the *FecB* carrier animals contain the AvaII enzyme restriction site, whilst products from non carriers lack this site. The primers amplify a 140 base pair (bp) band. After digestion with the restriction enzyme AvaII the homozygous (BB) animals have a 110 bp band, heterozygous (BW) animals have two bands 140 and 110 bp, and the non-carrier (WW) animals have a 140 bp band. The bands were separated by electrophoresis on a 3.0% Metaphor agarose gel and visualized with ethidium bromide.

Control samples of all three genotypes and a negative control without DNA were included in each batch of samples on a 96-well PCR plate. The samples that did not amplify or where there were doubts about the genotype were re-tested until a confirmed result was obtained. Blood samples were freshly collected for re-testing in some cases. Blood samples of some lambs whose genotypes were known from their pedigree were also tested for confirmation.

In 2003-04, genotyping was carried out by Ms. Varsha Pardeshi at the National Chemical Laboratory (NCL), Pune. From 2005 onwards, genotyping was carried out jointly by Ms. Varsha Pardeshi of NCL and Ms. Padmaja Ghalsasi of NARI at NCL.

Genetic diversity analysis of the Garole breed using microsatellite markers was done by Ms. Varsha Pardeshi at NCL.

5.5 Selection of animals (Objective A)

Initially, all *FecB* carrier crossbred ewes carrying one or two alleles of *FecB* and all homozygous *FecB* carrier rams were maintained and used for breeding if they were

healthy and could reproduce normally. Heterozygous *FecB* carrier rams were selected on body weight. Ewes heterozygous for *FecB* were subjected to selection from 2003, based on an index of estimated breeding values of weight and reproductive traits. Culling of homozygous *FecB* carrier rams and ewes for weight was started in 2006. From 2007, another important selection criterion was added - a 'phenotype and conformation score' based on the preferences of the local smallholder sheep owners. Selection of animals was done jointly by Pradip Ghalsasi and Chanda Nimbkar with the aid of farm supervisors and shepherds.

5.6 Allocation of rams to ewes (Objective A)

The mate selection approach (Kinghorn *et al*., 2002) was implemented for allotting rams to ewes using the Total Genetic Resource Management Program (TGRM^{TM}) (X'Prime Pty Ltd., 2005). An index of Estimated Breeding Values (EBV) for reproductive and weight traits was used while accounting for the *FecB* genotypic value at the level of the progeny so as to maximize genetic merit, increase frequency of the *FecB*^{B} allele and control inbreeding. TGRM runs were done by Chanda Nimbkar. X'Prime Pty made available the software on their server for the use of the project free of charge.

5.7 Data recording, computer entry, analysis and preparation of reports and manuscripts (Objective A)

1. Primary data recording was done by NARI's farm supervisors in registers. Data entry into the specially designed data base was done by Chanda Nimbkar. She also did all the data analysis and preparation of reports and manuscripts.

5.8 Work in collaborating shepherd flocks (Objective B)

1. Identification of shepherds, drawing up contracts with them, selection of ewes to be sent to shepherd flocks and placement of ewes in different flocks were done jointly by Pradip Ghalsasi, Chanda Nimbkar and Kanhaiya Chavan. Ear tagging of all animals in selected shepherds' flocks and initial data recording were done by Pradip Ghalsasi and Kanhaiya Chavan with the help of assistants from NARI.
2. Weekly visits to shepherd flocks, monitoring, data recording, monthly weighing of ewes and lambs, collection of faecal samples, treatment of sick animals, blood sample collection of lambs that needed to be genotyped for *FecB*, working with shepherds to develop technologies to manage multiple births were done by Kanhaiya Chavan with the help of other supervisors from NARI. Shyam Kulkarni helped him in these tasks from 2006.
3. Data entry into the specially designed database for shepherds' flocks was done by Madhukar Nalawade until Oct 2006 and by Pradip Ghalsasi, Kanhaiya Chavan and Shyam Kulkarni after that. NARI got the database designed (using SQL and dotnet) from a software design firm in Pune called Dataman.

5.9 Shepherd Training (Objective B)

1. Training programs for shepherds were conducted by Pradip Ghalsasi with the help of Kanhaiya Chavan, Shyam Kulkarni and other NARI supervisors.

5.10 Garole DNA analysis to study genetic regulation of *FecB* expression (Objective C)

1. This was carried out at NCL by Ms. Varsha Pardeshi and Dr. Vidya Gupta. To test the hypothesis that variation in *FecB* expression was due to another mutation within the BMPR-IB gene eight primer pairs spanning the whole cDNA sequence were designed. They were used on DNA from 20 Garole sheep (10high EBV and 10 low EBV for OR) and the amplified products analyzed for size and sequence variationand comparedwith the available sequence of the BMPR-IB gene.
2. To test the hypothesis that variation was due to sequence variation in the region flanking the BMPR-IB gene, primers were designed to amplify markers in the flanking region (JL2, JL26, JL36 and JP27). The same Garole samples were then analysed using these markers and the allelic variation at the marker sites examined.

5.11 Regulation of *FecB* expression in Australian Merino sheep (Objective C)

1. Initial genotyping of ewes at Allandale was carried out by UNE BRurSci Honours student Rachel Flanigan, David Wolfenden and Jill Maddox (UMelb). David and Rachel collected the blood samples and Rachel performed the DNA extractions and *FecB* RFLP-PCR tests (Wilson *et al*., 2001) at UNE and in Jill Maddox's lab in Melbourne. Methods are detailed in Flanigan (2004). Briefly, blood was collected onto Whatman FTA paper, air-dried and stored prior to DNA extraction. After PCR and AvaII digestion the products were separated by electrophoresis using a polyacrylamide gel. Products were labelled with 33P and visualised on BioMax MR® film (Kodak X-ray film) with a 24 hr exposure time.
2. A similar procedure was used to genotype ewes in the UNE Booroola flock with Rachel Flanigan and Steve Walkden-Brown collecting the blood samples.
3. Nutritional studies at Allandale were designed by Steve Walkden-Brown and David Wolfenden. Hormone implant studies were designed by Jim McFarlane at UNE in consultation with David Wolfenden and Steve Walkden-Brown. All implementation of studies was carried out by David Wolfenden who implemented the treatments, conducted the laparoscopies and ultrasound scanning for litter size, and collated the raw data in spreadsheets. Data were sent to Steve Walkden-Brown for final collation, analysis and write up.

The endocrinology of *FecB* in Garole sheep was investigated by a NARI-UNE team comprising Pradip Ghalsasi and Chanda Nimbkar at NARI and Dr Jim McFarlane at UNE. Resource animals came from the NARI Garole flock and the UNE Booroola flock (Ex CSIRO Booroola flock). In the Merino study 5 WW, 12 BB without oestradiol implants and 12 BB with implants were blood sampled over 3 oestrus cycles. Ovulation rate was determined by laparoscopy 5-7 days after the ewe was marked by a teaser ram. The Garole study utilised blood samples from 5 homozygous and 5 heterozygous Garoles collected daily over an estrous cycle with ovulation rate detected by laparoscopy. Hormone assays were carried out in Dr McFarlane's lab at UNE.

5.12 Socio-economic analysis of the *FecB* dissemination program (New objective C)

A draft survey questionnaire was prepared in consultation with two agricultural extension specialists, Mr. Julian Prior from the University of New England, Australia and Dr. D.V. Rangnekar from Ahmedabad, India for interviewing the shepherd households where *FecB* carrier rams/ewes were introduced. The questionnaire was modified by Chanda Nimbkar and Pradip Ghalsasi together with Kanhaiya Chavan and Shyam Kulkarni and was

translated into Marathi. Interviews of shepherds were conducted by Kanhaiya Chavan and Shyam Kulkarni. Answers given by shepherds in Marathi were tabulated in English in Excel files by Ms. Madhuri Joshi who was hired temporarily and Chanda Nimbkar and were sent to Mr. Prior for analysis and writing up.

5.13 *FecB* dissemination strategies within Maharashtra and beyond (Objective D).

1. This has been driven primarily by discussions between Steve Walkden-Brown, Chanda Nimbkar and Julius Van Der Werf, with input also from Pradip Ghalsasi and B.V. Nimbkar. An initial discussion paper was prepared by Steve Walkden-Brown and this has evolved into a paper on *FecB* introgression prepared by Chanda Nimbkar and presented at the HNT *FecB* workshop in November 2008.dissemination. Spreadsheetsmodelling various aspects of introgression have been prepared by Steve Walkden-Brown and Julius Van Der Werf.

6 Achievements against activities and outputs/milestones

This table lists activities and outputs for the project AH/2002/038 (P), its extension during 2006-07 (E) and its unfunded extension for 2008 (UE))

Objective A (PC): Production of appropriate genotypes for testing in shepherds' flocks

The output of Objective B in the initial project was:

Sufficient numbers of ewes of the Deccani breed and the composite breed with 0, 1 or 2 copies of the FecB gene. It is conservatively estimated that in 2005, there will be 100 adult ewes each of Deccani and composite breeds with one copy of FecB and <25% Garole genes and 50 ewes with two copies of FecB available for breeding (Appendix C1-2)

Achievement against this output can be assessed from achievements against activities milestones in the table below.

PC = partner country, A = Australia

no.	activity	outputs/ milestones	completion date	comments
A.1 (P) PC	Estimation of breeding values, ranking of animals and making selection and mating decisions	Mating lists for the A.I. program, continuous improvement of breeding values through accurate selection of rams and ewes	Carried out every year from 2003 and ongoing	Achieved. However the criteria for ranking animals changed over the years depending on shepherds' preferences.
A.2 (P) PC	Breeding program to generate BW ewes of the composite and Deccani breeds with <25% Garole genes, using Artificial Insemination (A.I.) with fresh, diluted semen	Production of BW ewes. Sufficient numbers of ewes of the Deccani breed and the composite breed with 0, 1 or 2 copies of the *FecB* gene. It is conservatively estimated that in 2005, there will be 100 adult ewes each of Deccani and composite breeds with one copy of *FecB* and <25% Garole genes and 50 ewes with two copies of *FecB* available for breeding	31 December 2005	Partial achievement. In Dec 2005 there were 111 BW and 14 BBewes Table 1 below gives the number of *FecB* carrier ewes available for breeding in March 2009.
A.3 (P) PC	Multiple ovulation and embryo transfer (MOET) to maximise numbers of BB genotype	Production of BB ewes	15 July 2004	Not achieved. A MOET program was implemented but was not successful and turned out to be a setback rather than impetus. Table 1 below gives the number of BB ewes produced through natural breeding.
A.4 (P) PC	Lambing, pedigree recording and monthly measurement of lamb weights, recording sick lambs, deaths and postmortem findings, data analysis, *FecB* genotyping of all progeny likely to be carriers of the gene	Completed database of all lambs produced	Carried out at every lambing from 2003 and ongoing	Achieved. An up to date database is complete with entries of all lambs born up to September 2008. All lambs have been genotyped whose *FecB* genotypes were not apparent from their parents' genotypes.

no.	activity	outputs/ milestones	completion date	comments
A.5 (P) PC	Grazing / feeding management of the breeding ewes, rams and lambs and data management		Carried out at every lambing from 2003 and ongoing	NARI maintains 600 ewes conditions similar to those under which local shepherds rear sheep. They are reared on about 10 hectares of pasture and on the fields of farmers in exchange for manure. NARI has been able to reduce lamb mortality up to 6 months from around 20% in 2003 to <5% in 2008, due to better management practices.
A.1 (E) PC	Continued breeding and selection in the fecund Deccani and fecund composite nucleus flock at NARI (500 breeding ewes including 45 BB ewes and 50 rams in total)	Annual output of 630 weaned lambs (315 males, 315 females) Continuous genetic improvement in selection index traits	Annual	Partially achieved only. Lamb numbers were 434, 153, 487,486 and 461 in 2003, 2004, 2005, 2006 and 2007 respectively. Genetic improvement through selection achieved.
A.2 (E) PC	DNA testing at NCL to determine *FecB* status of animals	*FecB* genotypes of lambs (about 700) whose genotypes cannot be determined from their pedigrees	Annual	Achieved. Now there are only three animals at NARI whose genotypes are still uncertain.
A.3 (E) PC	Estimate effect of one and two copies of *FecB* on reproductive performance and profitability at NARI	Reliable estimates of effect of one and two copies of *FecB* independent of breed composition and other fixed effects	2008	Largely achieved. Effect of 1 copy estimated with >800 records, 2 copies from 78 records. The live litter size at birth of Deccani and Deccani crossbred WW, BW and BB ewes was 1.03, 1.58 and 1.65, thus indicating an almost dominant effect of the mutation.
A.4 (E) PC	Estimate breeding values and estimate genetic trend in selection index traits over the last five years (2000-2005)	Information on how much genetic progress has been achieved	2008	Breeding values were estimated and used for selection but estimation of genetic trend was not achieved or attempted.
A.5 (E) PC/A	Publish results in peer-reviewed scientific journals	At least one journal paper based on Objective A	2008	Partially achieved. A manuscript is in preparation for submission to 'Small Ruminant Research'.
A.6 (E) PC	Maintain a nucleus Garole flock (40 ewes and 10 rams)	Genetic improvement in body size and growth rate of pure Garole animals		Achieved. Selection is being carried out based on growth rate.

Table 1. Selected ewes and rams of the three *FecB* genotypes available for breeding at NARI at the beginning and end of the project.

	Homozygous BB	Heterozygous BW			Non-carrier WW	Total
Garole	≤ 25%	50%	≤ 25%	Total	≤ 25%	

proportion												
	Rams	Ewes	Rams	Ewes	Rams	Ewes	Rams	Ewes	Rams	Ewes	Rams	Ewes
2003 (start of project)	-	2	5	65	4	51	9	116	7	375	16	493
2008 (end of Project) *	45	123	-	5	35	322	35	327	13	150	93	600

*The ewes and rams available in 2008 are selected for growth rate, reproductive performance and a phenotype score.

Local Maharashtrian shepherds do not like the physical features of the Garole such as small size, wide forehead and horns. Therefore the project had the difficult task of using the available animals to produce fast a large number of heterozygous and homozygous rams and ewes with <25% Garole proportion. *FecB* carrier ewes therefore had to be mated to *FecB* carrier rams. They were more likely to be related because they were the progeny of only a few heterozygous rams with <25% Garole proportion. Also, in order to introduce the phenotypic characteristics traditionally preferred by shepherds, ewes needed to be mated to phenotypically superior non-carrier rams of the Deccani or Madgyal breed. The Madgyal is a non-prolific hair sheep breed from southern Maharashtra that is tall and faster growing than the Deccani. Shepherds in Maharashtra have introduced breeding rams of the Madgyal breed in their flocks for about the last 10 years. We placed more emphasis on producing more *FecB* carrier animals with the inevitable consequence that local shepherds were not happy with the phenotype of most of the *FecB* carrier rams. This situation improved slightly in the last two years of the project by using purchased non-carrier superior rams. Now some participating shepherds have retained *FecB* carrier rams of their desired phenotype born in their own flocks for breeding.

Objective B (PC): Testing of improved genotypes in shepherds' flocks and development of appropriate management technologies.

Outputs of Objective B in the initial project were:

1. Knowledge of comparative performance of ewes with and without the FecB gene and their lambs, in shepherds' flocks. This will help to determine the feasibility of shepherds having prolific ewes in their flocks.
2. Information on appropriate technologies to manage multiple-born lambs in shepherds' flocks
3. Information on the comparative performance of the NARI composite sheep and the fecund Deccani under shepherds' conditions.
4. Information on economic value of various production traits, eg reproduction meat and parasite resistance.

Outputs of this objective in the project extension were:

- B.1 Approximately 25 ewes/ram mated or inseminated each year. Increase in gene frequency of *FecB* mutation in shepherds' flocks. Detailed animal performance and financial records maintained for shepherds'flocks. Comparison of performance of carrier and non-carrier ewes.
- B.2 Dissemination into distant flocks and testing of *FecB* carrier rams in a wider range of environments. Approximately 25 ewes/ram mated or inseminated each year.

- B.3 One journal paper based on Objective B
- B.4 Wider spread of knowledge of the benefits of the project among potential beneficiaries 15 farmer workshops resulting in better awareness among shepherds on ways of managing their flocks more efficiently and profitably
- B.5 Funding to continue generation and dissemination of genetic improvement

Achievement against these outputs can be readily assessed from achievements against activities and milestones in the table below.

no.	activity	milestones	completion date	comments
B.1 (P) PC	Identification of shepherds, drawing up contracts, selection of ewes to be sent out, placement of ewes in different flocks	Identification of at least 10 shepherds willing and able to participate in the project	Activity completed in 2004. Output (1) achieved in 2006.	Achieved. In 2003 and 2004 48 BW NARI Suwarna (fecund Deccani) ewes, 40 WW ewes consisting of only Deccani and Garole breed proportions, 12 BW NARI Composite ewes and 20 WW Composite ewes (consisting of Awassi breed additionally) were introduced into 14 smallholder flocks.
B.2 (P) PC	Eartagging of all animals in selected flocks, initial data recording		2004	Achieved
B.3 (P) PC	Monitoring of BW, BB and Deccani (WW) ewes in shepherds' flocks, data collection, genotyping of all progeny of BW ewes	Data on performance of different ewe genotypes and their progeny in shepherds' flocks	2005	Achieved. All progeny of BW ewes have been genotyped. See below table for discussion on genotype comparisons.
B.4 (P) PC	Working with shepherds to develop appropriate technologies to manage multiple births in their sheep	Achieve good lamb survival among lambs born as multiples	2005	Partially achieved. By the end of 2005 lamb mortality of 236 lambs in shepherds' flocks was 26% and 18% for *FecB* carriers and non-carriers respectively.
B.5 (P) PC	Extension activities in participating shepherds' flocks	Raised awareness of shepherds about management of sheep health and multiple births		Achieved. Eg. For B.4 a CD about the care and management of pregnant and lactating ewes and single, twin and triplet lambs was produced and shown widely to smallholder flock owners. Low cost feeders were also designed to feed young lambs.

no.	activity	milestones	completion date	comments
B.1 (E) PC	Introduction of *FecB* carrier rams/ semen into 20 existing participating shepherd flocks with intensive monitoring.	At least 450 ewes mated/inseminated out of which at least 70% will conceive	2007	Partly achieved. By the end of 2007 there were13 intensively monitored flocks with 900 ewes. In that year 18 BB rams were introduced to the flocks for 35 to 90 days and then brought back to NARI as the same rams were used for breeding in NARI's flock.
B.2 (E) PC	Introduction of *FecB* carrier rams into 10 new shepherd flocks with less intensive monitoring	At least 250 ewes mated in shepherd flocks	2007	Achieved. Up to 2007 18 BB and 8 BW rams were introduced into 19 less intensively monitored flocks containing about 1000 ewes. During 2008, 11 BB and 3 BW rams were introduced into 11 flocks. Seven of these rams have spent close to a year in these flocks.
B.3 (E) PC	Compilation and analysis of flock performance data collected since 2003	One paper based on results of introgression in shepherds' flocks submitted to appropriate journal	2007	Partly achieved. Data have been analysed and a paper written for the Helen Newton Turner Memorial International Workshop held in November 2008. It needs to be developed into a journal paper.
B.4 (E) PC	Dissemination of project objectives and results to shepherds, researchers and relevant State government personnel.	15 farmer workshops resulting in better awareness among shepherds on ways of managing their flocks more efficiently and profitably Presentations at district coordination meetings of govt. clinics One workshop at NARI for animal scientists from agricultural universities in Maharashtra	2006, 2007	Largely achieved. Ten one-day training workshops held for shepherds in 2007. A workshop for around 500 shepherds held on 8 Nov. 2006 inaugurated by the Secretary, Animal Husbandry, Maharashtra Govt. Visitors to NARI's farms are given information on the results of the project. NARI staff attend livestock shows in and outside the State and publicize the project. Additionally 16 BB and 22 BW rams have been sold to sheep owners, State governments and NGOs in Maharashtra, Andhra Pradesh, Tamilnadu, Rajasthan and Jammu and Kashmir.
B.5 (E) PC	Prepare a case for Indian Government support of future work.	Funding proposal to Indian Govt. agency prepared and lodged.	2007	Achieved late. Funding for three years from March 2009 has been obtained from the Department of Biotechnology, Ministry of Science and Technology, Govt. of India to continue genetic improvement in the nucleus flock at NARI, to establish at NARI the DNA test to detect the *FecB* mutation, to disseminate *FecB* carrier rams into shepherds' flocks and monitor the performance of their progeny and to develop appropriate management techniques for ewes with twins in shepherds' flock conditions.

PC = partner country, A = Australia

Further comments relating to the outputs of Objective B are provided below.

Out of the 60 BW and 60 WW ewes introduced into shepherds' flocks, 23 BW and 24 WW ewes are still present and producing in those flocks. Seven of these BW ewes and two WW ewes were purchased by these shepherds from NARI.

Arising from the ram and AI programs in shepherds' flocks there are now 13 BB and 240 BW adult ewes present in shepherds' flocks. These were born in the flocks.

Comparison between NARI composite and fecund Deccani sheep under shepherds' conditions could not be made. This was because more than one NARI ram was usually present in each flock, making it difficult to identify the correct sire of the lambs born. These rams had different breed compositions due to which the breed compositions of their progeny could not be determined.

Regarding the economic value of various production traits in shepherds' flocks, the comparative economic values of the traits in the breeding objective were estimated to be: Fertility 1.0, Live litter size 11.8, Weight at 3 months 4.1 and Weight at 6 months 0.3.

The comparative performance of carrier and non-carrier ewes in shepherd's flocks at the end of 2007, and comparative performance with NARI animals is shown in Table 2. In shepherds' flocks ewes with one copy of *FecB* had an extra 0.42 lambs/ewe lambing, compared with and 0.55 at NARI. By 3 months of age the advantage for carrier ewes 0.2 and 0.37 respectively.

Table 2. Comparative performance of *FecB* carrier and non-carrier ewes in shepherd's flocks and NARI 2002-2007 (number of records in parenthesis).

Trait	Shepherds' flocks		NARI	
	WW	BW	WW	BW
Litter size at birth per ewe lambed	1.02	1.44	1.02	1.57
Litter size at 3 months per ewe lambed	0.88 (3636)	1.08 (431)	0.96 (808)	1.33 (532)

Objective C: (PC and Australia) Regulation of expression of FecB in Indian and Australian breeds of sheep

Outputs of Objective C in the initial project were:

- Variability in expression of *FecB* in terms of FSH and other hormone concentrations, litter sizes, lamb survival and lamb growth rates in *BB* and *BW* ewes of different background genotypes defined viz. Garole (G), 25% G-75% Deccani (D), 12.5% G-87.5% D, 25% G-25% Awassi-50% D, Merino.
- Selection lines for divergent litter size in BB Garole ewes established.
- Phenotypic and genetic relationships between *FecB* and other production traits investigated eg meat production and parasite resistance.
- Achievement against these outputs can be readily assessed from achievements against activities and milestones in the table below and from the summary below the table.

no.	activity	milestones	completion date	comments
C.1 (P) PC	Statistical (BLUP) analysis of ovulation rates and litter sizes of homozygous and heterozygous pure and crossbred Garole ewes	Information on extent of genetic variation in litter size within *BB* and *BW* genotypes and the existence of regulating genes	2004	Achieved. Breeding values for litter size were estimated for Garole ewes and rams. Ewes were classified into two groups – low and high EBV for litter size. Blood samples of 10 Garole ewes and 5 rams having extreme EBVs were supplied to NCL.

no.	activity	milestones	completion date	comments
C.2 (P) A	Genotyping of Booroola Merino ewes in Australia and data analysis	Information on associations between *FecB*genotype and reproductive and other productive traits in Booroola Merino ewes	2004	Achieved. 460 ewes were genotyped for *FecB* from "Allandale" a commercial Merino property in Southern NSW. All 13 rams and 54 ewes in the UNE flock were genotyped and confirmed to be homozygous. Reproduction and production data from Allandale were analyzed and published. Subsequent to this a further 2 years of work has been done.
C.3 (P) PC	Establish divergent selection lines for prolificacy within the BB genotype of Garole sheep	Information on inheritance of litter size and its response to selection in opposing directions	2004	Not achieved. It was decided at the first coordination meeting not to set up the divergent selection lines because of insufficient number of Garole ewes with low litter size.
C.4 (P) PC/ A	Microsatellite marker study of region flanking the *FecB* locus in crossbred genotypes in India and Australia	Information on extent of variation in marker haplotypes of markers located on chromosome 6 in Garole and Booroola Merino sheep	2005	Partially achieved. In India two approaches were taken and appropriate methods developed at NCL. One was to look for an additional mutation within the BMPR-1B gene (location of the *FecB* mutation) while the other was to look at variation in the flanking region of the gene. Point mutations within the gene and marker polymorphism in the flanking region were detected but not able to be clearly associated with variation in litter size. In Australia it was agreed not to pursue this work at the 2004 project coordination meeting since the work in C2 had not revealed major adverse production consequences for carrying the *FecB* gene.
C.5 (P) PC/ A	Comparative reproductive endocrinology of *FecB* carriers of different breeds in India and Australia	Comparison of the physiological basis for recorded ovulation rates and litter sizes in Garole and Booroola Merino sheep	2005	Achieved. Plasma samples from 5 BB and 5 BW Garole ewes taken throughout the oestrus cycle were sent to UNE (Dr Jim McFarlane) and assayed for a range of reproductive and metabolic hormones and the data analysed. Garole sheep has lower progesterone profiles. Full report in 2005 Annual report.

PC = partner country, A = Australia

With regards the original outputs, no clear genetic or endocrinological markers for variation in *FecB* expression were revealed and no divergent selection lines were set up in the Garole flocks. On the other hand genetic analysis of the 3 *FecB* genotypes in Australia has revealed that there are no deleterious associations between the *FecB* allele and wool and growth traits other than those secondary to changes in litter size. However, a deleterious effect on conception rate and lamb viability was observed in homozygous carrier ewes. To date there is no evidence of this in the Indian flocks.

New Objective C (Project Extension): (PC and Australia) Evaluate the socio-economic benefit of the *FecB* dissemination program.

Outputs of Objective C in the project extension were:

- Information on the perceived impact of new genotypes, suitability of extension methods used, barriers to adoption of new genotypes and/or extension messages, and information needs of shepherds

- Specific recommendations about future introgression and extension methods
- Achievement against these outputs can be readily assessed from achievements against activities and milestones in the table below and from the summary below the table.

no.	activity	milestones	completion date	comments
C.1 (E) PC/ A	Carry out an independent survey of shepherd households where *FecB* carrier rams/semen and/or ewes were introduced and publish results	One paper submitted to appropriate journal by end of project	2007	Partially achieved. Surveys were conducted with 23 of the 26 shepherds participating in the project. Shepherds' response to twin lambs was positive in general and they viewed twin lambs as more profitable than single lambs. They felt that survival and adequate growth of twin lambs depended on availability of supplementary feeding and management. A paper based on analysis of survey results was written and presented in the Helen Newton Turner Workshop in November 2008.
C.2 (E) PC/ A	Develop specific recommendations about future introgression and extension methods	Recommendations for future introgression	2007	Partially achieved. Delays due to delays in analysing survey results and time taken to reach consensus on key points. A paper on future introgression and extension methods written and presented in the Helen Newton Turner Workshop in November 2008.

PC = partner country, A = Australia

Significant progress occurred in both these activities during the unfunded extension, spurred by the need to produce papers for the HNT workshop. For C.1 language differences greatly slowed implementation and analysis. For C.2 the final version of the HNT workshop paper should be consulted. In brief targets for introgression should major sheep breeds of India, particularly meat and dual-purpose breeds in areas where feed restrictions are not severe and migrations are not long and arduous. *FecB* should be introduced from a source as similar as possible to the target breed and a classical backcrossing program should be used at large institutional flocks (>500 ewes) to generate animals with 87.5% target breed carrying the *FecB* gene. Dissemination prior to sufficient back-crossing is a major risk as shepherds are very conservative about the appearance of their animals. After a very brief period of dissemination of heterozygous rams, homozygote rams will predominate. The main extension messages need to centre on the nutritional management of ewes and lambs in flocks with significant multiple births.

Objective D: (PC/Australia) Development of extension and genetic models for the dissemination of proven genotypes

Outputs of Objective D in the initial project were:

- Formal predictive model for the introgression of superior genotypes in India and productive consequences of such introgression.
- Formal recommendations on associated optimal extension and information dissemination methods to be used.
- Communication of the results of the project widely – within and outside Maharashtra State and India
- Achievement against these outputs can be readily assessed from achievements against activities and milestones in the table below and from the summary below the table.

no.	activity	milestones	completion date	comments
D.1 (P) PC/A	Design appropriate extension strategy and methods for dissemination of relevant information arising from the project	Development of appropriate strategies for dissemination	2005	Partly achieved. Early strategies formulated based on NARI experience viz. Disseminate via rams not ewes Ensure 12.5% or less Garole blood Extension messages centred on nutrition and health care.
D.2 (P) PC/A	Model the introgression of the *FecB* gene into the Indian sheep population	Estimates of the speed of introgression of the *FecB* gene under different scenarios and optimal pathways for introgression	2005	Partly achieved. A spreadsheet model of the proportion of $FecB^{B}$ alleles in the population over time following distribution of homozygous rams was developed. A separate spreadsheet model was then developed to model the consequences of different levels of ram genotyping on the penetration of *FecB* in the Indian sheep population and the economic benefits. This was used in application for the project extension.

*Revised Objective D in the Project Extension: (PC/Australia)*Devise a strategy and mechanisms for dissemination of the *FecB* mutation beyond the current project area.

no.	Output/activity	milestones	completion date	comments
D.1 (E) PC/A	Recommend a strategy for wider *FecB* introgression in India	Recommendation as to desirability of wider introgression of *FecB* made with optimum strategy for doing so.	2007	Achieved. At the HNT workshop it was agreed that wider introgression is desirable. A paper on 'Potential introgression pathways and strategies for wider utilization of the *FecB* gene in Maharashtra State and other parts of India' was written for the workshop and outlines key introgression strategies.
D.2 (E) PC/A	Recommend supporting extension messages and activity	Recommendations on supporting extension methodology prepared.	2007	Largely achieved. While not summarised in a single document, the key extension messages emerging from the NARI experience and the Shepherds' survey are well covered in the Introgression and Shepherds' survey papers at the HNT workshop. NARI has an extensive array of extension material.
D.3 (E) PC/A	Develop a spreadsheet model to simulate the speed and consequences of introgression under different conditions	Spreadsheet model developed.	2007	Partially achieved. Apart from the two models existing at the start of the project extension a 3rd spreadsheet model has been developed to model genotype and breed flows, and numbers of animals disseminated in the first 10 years of an institutional backcrossing flock using the classical introgression method.

The Helen Newton Turner Memorial Workshop held in November 2008 was a great stimulus to Objective D (and revised Objective C) with 4 directly relevant papers being presented with several others having a less direct bearing on the issues involved. The panel discussion was also focused on the issue of wider introgression of *FecB*.

Key elements of the recommended introgression strategy and supporting extension requirements include:

1. Several generations of backcrossing (to 87.5% target breed) should take place before dissemination occurs, to limit shepherd resistance to new phenotypes. This also provides and opportunity to maximize the genetic attributes of the new genotype by selecting superior rams of the target breed for the backcrossing process.
2. Regarding the requirement for improved management (particularly nutrition) of the new genotypes we conclude the following:
3. Improved management and associated extension effort are required to optimise returns from improved genotypes but are not a pre-requisite for successful introgression. Slow introgression of the improved genotypes via ram introduction in normal years is unlikely to precipitate a problem with twin lamb management, even in the absence of supplemental feeding. By the time significant numbers of multiple births occur most shepherds will have adapted to twin rearing methods. Note that 2%

of Decani sheep have twins each year (~ approx 200,000 pairs of Deccani twins/year) and project surveys had shown that Deccani shepherds value these sheep and their lambs.

4. It is important to emphasise to shepherd's that the disseminated rams or semen themselves will not increase the twinning rate of ewes. Increases will only be seen in their progeny, and in the case of heterozygous rams, only a proportion of these.
5. With improved nutritional management the question arises as to whether this should apply to the whole flock, or just the improved genotypes (*FecB* carriers). As a general rule, targeting twin bearing ewes in the last third of pregnancy and twin born lambs in the first 3 months of life for special treatment will maximize benefits. Shepherd's intimate knowledge of their animals will generally enable this slective application of feeding to occur.
6. What nutritional supplements should be used? As the price of concentrates increases, the utility of high quality forages for this purpose should be considered. The outstanding success of lucerne in the Pisal brothers' project collaborating flock suggests that in irrigated country where it can be grown, it is the forage of choice, being high in protein, low in structural carbohydrate and consequently highly digestible for sheep. Furthermore in a mixed farming system the lamb/sheep refusals can be fed to cattle and buffalo.Lucerne hay has been used successfully at NARI as a nutritional supplement and whole maise grain is used routinely.

In non-irrigated areas the problem is more challenging and C4 tropical grasses and leguminous forages such as those used at NARI should have a role. However these forages are typically harvested and fed to sheep at an advanced stage of maturity with low digestibility making them unsuitable as forages for supporting growth and production for reproducing ewes or young growing lambs. Research into the optimum use of such forages for sheep, as opposed to cattle/buffalo/goats should be a high priority. Ensiling the forages at the appropriate stage of growth appears to be promising as a fodder conservation practice as has been demonstrated at NARI.At NARI, *Leucaena leucocephala* leaves are used as a high protein supplement for young lambs routinely and effectively.

7 Key results and discussion

The detail of most of the key results arising from the project is captured in publications prior to and including those presented at the Helen Newton Turner Memorial Workshop on 10-12 November 2008 (Walkden-Brown *et al*., 2009a).

7.1 Breeding objectives in Deccani sheep

Traits influencing profit of a Deccani sheep flock in a smallholder production system were identified (Nimbkar, 2006; Tables 3 and 4). Litter size had a positive economic value after accounting appropriately for feed cost, indicating its importance for the profitability of the Deccani sheep production system. However, the economic value of litter size was highly sensitive to assumptions about lamb survival.The economic value of one and two copies of the *FecB*gene was found to be positive although it reduced with an increase in average (base) litter size of the flock. The actual economic benefits measured in participating shepherds flocks are discussed in Section III below.

Table 3. Economic values (E.V.) per ewe per year for a unit change in traits in the breeding objective, economic weights after accounting for differential trait expressions at the discount rate of 10% and after rescaling. (Source: Nimbkar 2006).

Trait name	Unit	E.V. based on cost of estimated ME intake	E.V. converted to economic weights[1]	Economic weights after rescaling[1,2]
Ewe traits		US cents	US cents	US cents
Age at first lambing	days	-0.19	-0.14	-0.15
Ewe survival	percent	30.06	22.93	24.24
Fertility	percent	14.94	11.40	9.74
Litter size	lambs	1130.40	819.54	728.69
Lambing interval	days	-6.17	-4.47	-4.34
Lamb survival	percent	30.12	21.84	21.10
Cull ewe weight	Kg	6.47	4.93	4.93
Ewe daily feed intake	MJ/d	-100.74	-76.87	-71.07
Greasy fleece weight	gm	0.02	0.02	0.02
Worm resistance	$\sqrt[3]{epg}$	-7.78	-5.93	-5.93
Lamb traits				
Weight at 3.5 months	Kg	91.18	91.18	91.18
Weight at 7 months	Kg	18.38	6.54	6.54
Sale lamb daily feed intake	MJ/d	-36.90	-36.90	-20.2
Replacement lamb daily feed intake	MJ/d	-22.70	-8.10	-7.9
One copy of *FecB* gene	lambs	542.59	393.38	349.78
Two copies of *FecB* gene	lambs	881.71	639.24	568.38

[1]These values are discounted at 10% and reflect differential expression of traits.

[2]Economic weights were rescaled to a constant flock feed intake by changing the number of [b]reeding ewes.

Table 4. Genetic standard deviation (σA = hσP) of each trait in the breeding objective using economic values based on estimated cost of ME intake.(Source: Nimbkar 2006).

Trait name	Units	Mean	Phenotypic standard deviation (σP)	Heritability (h2)	Genetic standard deviation (σA = hσP)	
			Physical units		Physical units	Value (US cents)
Ewe traits						
AFL	days	540	93	0.07	24.61	-4.7
ESUR	percent	95	11	0.07	0.03	0.9
FERT	percent	95	5	0.08	0.01	0.2
LS	lambs	1.02	0.14	0.10	0.04	45.2
LINT	days	300	29	0.04	5.80	-35.8
LSUR	percent	Singles:0.93 Twins:0.87	0.15	0.07	0.04	1.2
EWT	Kg	31	4.5	0.42	2.92	18.9
EFI	MJ/d	6.1-12.5	0.6	0.13	0.22	-22.2
GFW	gm	500	100	0.50	70.71	1.4
FWEC	$\sqrt[3]{epg}$	10.6	7.1	0.27	3.69	-28.7
					Lamb traits	
WWT	Kg	Males:15 Females:13	2.0	0.21	0.92	83.9
PWT	Kg	Males:26.9 Females:21.6	3.0	0.30	1.64	30.1
SLFI	MJ/d	2.5-4.7	0.5	0.06	0.12	-4.4
RLFI	MJ/d	7.0-9.6	0.5	0.08	0.14	-3.2

AFL=age at first lambing, ESUR=ewe survival, FERT=fertility, LS=litter size, LINT=lambing interval, LSUR=lamb survival, EWT=cull ewe weight,

EFI=ewe daily feed intake, WWT=weight at 3.5 months, PWT=weight at 7 months,

SLFI=sale lamb daily feed intake, RLFI=replacement lamb daily feed intake

7.2 Influence of the *FecB* mutation on ewe reproductive performance in India

*Effects on ovulation rate.*The expression of the *FecB* mutation in terms of its influence on ovulation rate (OR) was moderate in Garole ewes and their crosses. One copy of $FecB^B$ led to an increase in OR from 1.1 to 2.0 in ¼ Garole ewes. The second copy of *FecB* increased OR by a further 1.3 (Nimbkar *et al*., 2003a). This is a much smaller effect than the literature reports of an increase in OR of 1.5 and 3 with one and two copies of $FecB^B$ respectively (Davis 2004). This result led to a reassessment of the utility of *FecB* in increasing ewe productivity.

Before the DNA test to detect the *FecB* mutation became available only ewes with at least one litter size record or one OR record greater than or equal to three were classified as

heterozygous *FecB* carriers. However, only 11 of 113 (9.7%) maiden *BW* ewes in this study had at least one OR record of three and none had any record greater than three while 15 of 69 second parity BW ewes (21.7%) had at least one OR record of three and one had a record of four which was the maximum (unpublished data). It is thus possible that the criterion used in earlier studies could have biased the genotype effect upward.

Effects on live litter size at birth. One copy of *FecB*^{B}led to an increase in live litter size at birth per ewe lambed from 1.0 to 1.6 in the NARI flock and from 1.0 to 1.4 in smallholder flocks (Table 5). This means that only about 60% and 40% respectively of the heterozygous ewes in NARI and smallholder flocks gave birth to twins at a time and the rest gave birth to singles. Less than 5% of the litters of *BW* ewes were triplets. This is a manageable increase under field conditions in India because of the system of management involving close personal attention to the flock by the owner at all times. This increase in litter size translates into an increase compared to non-carriers, of 0.4 and 0.3 respectively in litter size at three months per ewe lambed in NARI's and smallholders' flocks (Table 5, Nimbkar C*et al*., 2009a). There is a large demand for lambs and tender lambs fetch a high price. Therefore the increase of 0.3-0.4 lambs per ewe is large enough to lead to an increase in the smallholder shepherds' income and profit.

Table 5. Least squares means and standard errors for ewe's *FecB* genotype for Deccani and crossbred ewes in NARI and shepherds' flocks (Source: Nimbkar C*et al.*, 2009a).

Traits per ewe lambing with at least one live lamb	Flock	Ewe Genotype		
		WW	**BW**	**BB**
Live litter size at birth	NARI	1.03 ± 0.01[a] (1632)	1.58 ± 0.02[b] (806)	1.65 ± 0.09[c] (45)
	Smallholder	1.03 ± 0.01[a] (2465)	1.42 ± 0.02[b] (325)	few records available
Live litter size at 3 months	NARI	0.95 ± 0.01[a] (1632)	1.35 ± 0.02[b] (806)	1.34 ± 0.10[b] (45)
	Smallholder	0.95 ± 0.01[a] (2406)	1.21 ± 0.02[b] (321)	few records available
Total weight of 3-month old lamb	NARI	10.63 ± 0.24[a] (1326)	13.52 ± 0.26[b] (739)	12.93 ± 1.08[b] (41)
	Smallholder	13.91 ± 0.10[a] (1385)	14.96 ± 0.27[b] (186)	few records available

[a,b] Least squares means with different superscripts within a row are significantly different.

*analysis of unpublished data

Twin-bearing ewes in both NARI and smallholder flocks weaned 0.8 more lambs than single-bearing ewes.

The percentage of abortions and stillbirths was 6, 10 and 11% for non-carrier, heterozygous and homozygous ewes respectively in the NARI flock. This indicated that the level of reproductive wastage in *FecB* carrier ewes was limited enough to maintain their advantage over non-carrier ewes.

A second copy of *FecB*^{B} led to an increase of 0.07 in live litter size at birth per ewe lambed. This indicates a partially dominant or almost a dominant effect of *FecB* on live litter size at birth. About 10% of the litters of homozygous ewes were triplets or quadruplets up to the fourth parity. Results for parities>4 are not yet available.

Effects on live litter size at 3 months. Litter size at 3 months per ewe inseminated in NARI's flock was 0.59, 0.89 and 1.02 for *WW*, *BW* and *BB* ewes respectively, indicating a clear advantage of *FecB* carrier ewes.

*Effect of ewe genetic background.*Variation in the proportions of Garole, Deccani, Bannur and Awassi breeds in the breed composition of a ewe did not have a significant influence on ovulation rate or litter size of the ewe. The higher prolificacy of the Garole was found to be solely due to the *FecB* mutation.

An increase in the proportion of Garole breed in the dam and the lamb led to a decrease in lamb survival, weight at birth and at three months among crossbred lambs. The Awassi breed had a positive maternal effect on lamb weight and survival. The Bannur breed had a significant negative effect on litter size per eweconceived, lamb survival and lamb weight. It would therefore be desirable to reduce the proportion of Garole and Bannur breeds in further generations while retaining the *FecB*gene (Nimbkar, 2006). The *FecB*genotype of the lambor the dam did not significantly influence lamb survival or weight adversely.

Effects on litter size distribution. Forty-seven and 58% of the lambings of *BW* ewes in NARI and shepherds' flocks respectively were singles, 49 and 40% twins and 4 and 2% were triplets. The difference between the proportions of single, twin and triplet births in NARI's and smallholders' flocks was significant ($P = 0.005$ with a χ^2 test).

The significantly higher proportion of twin and triplet births among *BW* ewes in NARI's flock compared to smallholder flocks could be attributed to the extra feed given to NARI's ewes for about two months during breeding, starting 3 weeks before the commencement of breeding. A supplement of 50 gm maize grain and 50 gm mixed, pelleted concentrate with 16-18% protein per head was given to NARI's ewes. Additionally, breeding was controlled in NARI's flock and the lambing interval was generally more than one year while in shepherds' flocks, ewes were mated by the rams in the flock whenever they exhibited oestrus. It is therefore likely that NARI's ewes were in better body condition at breeding than the ewes in smallholder flocks. The better body condition at breeding, however, seems to have led to an increased litter size in only *BW* ewes. This indicates that the benefit in increased litter size from extra feeding was only evident in *FecB*carrier ewes.

*Effects on total weight of lamb at 3 months.*The total weight of 3-month old lamb per ewe lambed (that gave birth to at least one live lamb) in the NARI flock was 10.6 kg for non-carrier ewes, 13.5 kg for heterozygous ewes and 12.9 kg for homozygous ewes. In the shepherds' flocks non-carrier ewes produced 13.9 kg weight of 3-month old lamb while heterozygous ewes produced 15.0 kg (Table 5).

The average 6-month weight of 72 ewe lambs born in August 2008 in the NARI flock selected for weight and a phenotype score was 22.5 kg for singles and 21.1 kg for twins (unpublished data). The gross margin for twin-bearing ewes therefore worked out to almost twice that of single-bearing ewes, including the feeding, grazing and veterinary costs of lambs for six months and of ewes for eight months.

Shepherds were found to use innovative strategies to care for lambs born as twins to ewes which they thought would not be able to rear both lambs. One of the strategies was to take one lamb away at birth and suckle it to goat does in their flock whose kids had been weaned.

7.3 Results of the socio-economic survey conducted in shepherds' flocks where the *FecB* mutation was introduced:

The results of this study are detailed in the paper by Prior *et al*. (2009). The response of shepherds to twin lambs was generally positive. This reinforced the findings of the wider survey of 87 shepherd families in 2001-02 prior to the introduction of fecund genotypes (Nimbkar B *et al*., 2009). Shepherds recognised that additional management inputs would be necessary with increased lamb numbers, but perceived that there were net benefits

accruing in terms of increased profits. There was no clear perceived increased risk associated with twin lambs so long as supplementary feeding of lambs was undertaken. These generally positive views were reinforced by the stated intention of all shepherds, bar one, to increase their *FecB* carrier ewe numbers (Prior *et al*., 2009).

The gross margin per WW breeding ewe was found to be Rs. 450 to Rs. 800 per year in shepherds' flocks. Only one of the flocks had data from a reasonable number of BW breeding ewes i.e. 8, 41 and 50 ewes in the years 2005 to 2008 respectively. The number of WW breeding ewes in that flock was 98, 89 and 74 respectively in the three years. In this flock, the gross margin per BW breeding ewe was Rs.150 to Rs.300 higher than WW ewes. This amounted to an increase in gross margin of between 37 and 50%. The lamb mortality was 31, 24 and 19% among lambs of BW ewes and 14, 18 and 24% among lambs of WW ewes in the three years. Despite the higher mortality, BW ewes weaned a higher number of lambs per ewe than WW ewes which led to the higher gross margin. Twin-born lambs were sold at about Rs.100 less on average than single-born lambs and they were two to four weeks older at sale than single-born lambs.

There was a strong perception among shepherds that the smaller Garole-type *FecB* carrier rams were inferior to the larger framed and 'better looking' Deccani-type rams. However, some shepherds mentioned that the later *FecB* carrier rams were superior in appearance to the earlier version. This is an indication of the efforts being made at NARI to breed larger bodied *FecB* carrier rams that more closely resemble the phenotype of local Deccani and Madgyal types which the shepherds prefer.

The fact that increased twinning rates require that shepherds adopt a higher level of management and higher cost feeding strategies, means that ongoing extension support for participating shepherds must be a priority. Case studies of shepherds who have been successful and profitable in integrating the *FecB* carrier animals and associated management strategies into their flocks will provide useful demonstration value for potential adopters.

It is important that a *FecB* extension program is targeted at those groups of shepherds who are likely to benefit from gene introgression and who can take advantage of increased lamb numbers. Shepherds who are likely to benefit include those with irrigation, access to extra labour, and who are more settled and less nomadic.

It is essential that extension agents understand the informal information networks that exist in the shepherd community, and take advantage of these information networks in the extension strategies they utilise.

7.4 Studies on the *FecB* mutation in commercial Merino sheep in Australia

These studies took place on a single commercial property at near Rand between Wagga Wagga and Albury in Southern NSW. This is a commercial fine wool (19.5 µm) Merino operation with approximately 3500 breeding ewes into which Booroola Merino rams had been introduced in 1982, 1985 and 1991. In 2002 an experimental flock was established to enable valid comparisons of the 3 *FecB* genotypes in a common commercial environment and to enable comparisons with the data emerging from India on the project.

The work had the following broad aims:

1. Documenting reproductive performance in ewes of the 3 *FecB* genotypes as determined by a direct gene test, for the first time in Australia
2. Investigating the effects of the $FecB^B$ allele on production traits
3. Investigating environmental means of moderating the reproductive effects of the $FecB^B$ allele

Results for 2002-2004 are reported by Flanigan (2004), those for 2002-2006 by Walkden-Brown *et al.* (2007) and those for 2006-2007 by Walkden-Brown *et al.* (2009b).

*Effect of FecB on reproductive traits.*OR in ewes with 0, 1 and 2 copies of the B allele was 1.27, 2.48 and 3.86 respectively (Figure 1, Walkden-Brown *et al*., 2009). This compares with values of 1.1, 2.0 and 3.3 in Garole x Deccani crosses and homozygous pure Garole ewes in India (Nimbkar *et al*., 2003a) The increases in OR of 1.21 and 1.38 with the 1st and 2ndcopies respectively areslightly higher than observed in India, and slightly lower than most other published estimates in the Merino.

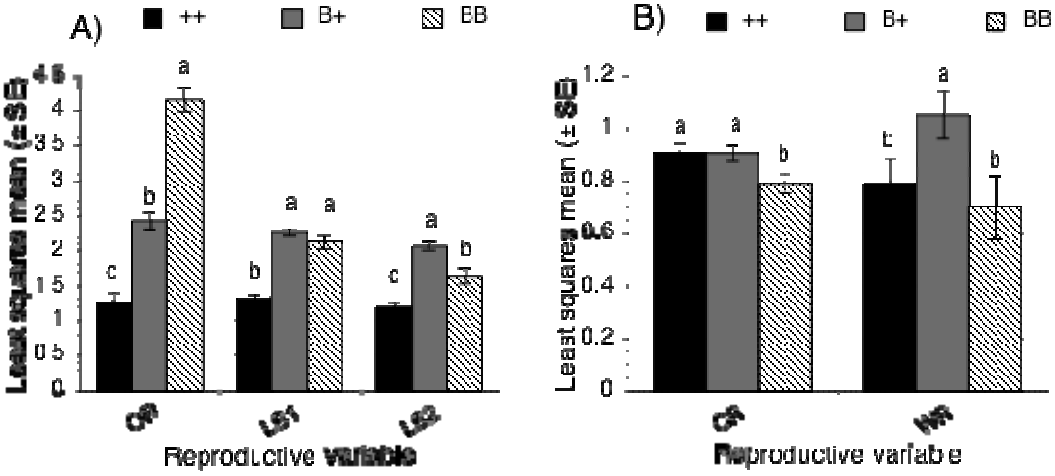

Figure 1. Least squares means (±) for the genotype effect (adjusted for year and age) for the effect of *FecB* genotype on ovulation rate on the cycle of conception (OR), ultrasound scanned litter size per pregnant ewe (LS1), ultrasound scanned litter size per ewe scanned (LS2), conception rate (CR - ewes pregnant/ewes mated) and weaning rate (WR - lambs weaned/ewes scanned). Data are for commercial Merino ewes for lambings 2004-2006 with 599 records in total. OR was only measured in 2006. Source Walkden-Brown *et al*. (2009b).

Conception rate was significantly reduced in BB commercial Merino ewes by 6-16% relative to heterozygous carriers and non-carriers. A significant but much smaller negative effect of *FecB* on fertility can be seen in the data of Piper and Bindon (1982).

Scanned litter size per pregnant ewe was 1.22, 2.19 and 2.06 in ewes with 0, 1 and 2 copies of the B allele respectively (Figure 1) demonstrating complete dominance of the B allele for this trait despite its additive effect on OR. The comparative values for live litter size at birth in the NARI crossbred flock are 1.03, 1.58 and 1.65 (Nimbkar C*et al*., 2009a) also demonstrating dominance, but being considerably lower. The increase in live litter size between 0 and 1 copy of the B allele in shepherds' flocks is even lower, increasing from 1.03 to 1.42 (Nimbkar C *et al*., 2009a).

In the Australian study lamb survivalfrom scanning to weaning was also significantly lower in BB ewes than BW and WWewes being 0.53, 0.67 and 0.76 respectively (Walkden-Brown *et al*. 2009). This, coupled with the significantly lower conception rate (Figure 1) resulted in significantly fewer of lambs weaned/ewe joined for BB ewes (0.77) than BW (1.05). In fact BB ewes weaned no more lambs than WW ewes (0.77 and 0.79 respectively, Walkden-Brown *et al*. 2009).

This significant "homozygote penalty" is not readily apparent in the older literature, partly because initial studies rarely compared the 3 *FecB* genotypes in the same genetic background and few studies reported conception rate or lamb survival which are the two critical variables influencing this.More recent publications tend to lend support to the homozygote penalty observations. Farquhar *et al*. (2006) showed that in Romney sheep BB ewes had significantly higher rates of barrenness than BW or WW ewes, being 16.4,

7.6 and 4.1% respectively. Gootwine *et al*. (2009) reported 7% lower lamb survival in BB than BW ewes in both Awassi and Assaf crosses despite similar total litter size and numerically lower live litter size. However as there was not a significant interaction between *FecB* genotype and litter size for lamb survival they ascribed the negative effect of *FecB* to litter size alone.An earlier publication from the same group (Gootwine *et al*., 2006) showed that lambs born to BB ewes were significantly lighter at birth and at maturity than lambs born to BW or WW ewes, despite including litter size in the statistical model. As yet there is no evidence of a BB homozygote penalty in the Indian part of the project, presumably due to the lower OR and LS observed there. However an ongoing watch needs to be kept on this.

*Effect of FecB on other production traits.*When adjusted for the effects of birth type and year, there was no significant effect of *FecB* genotype on any of the ewe production variables (weaning weight, hogget weight, greasy fleece weight and mean fibre diameter) although there was a strong trend towards higher mean fibre diameter in ewes carrying the *FecB* gene. The lack of an effect on productivity is consistent with several earlier studies but contrasts with the report of adverse effects on productivity associated with *FecB* in Assaf ewes (Gootwine *et al*. 2006).

7.5 Breed comparison of resistance to gastro-intestinal nematodes

Garole sheep were shown to have greater genetic resistance than Deccani, Bannur and Awassi breeds to infection with gastro-intestinal (GI) nematodes, in particular *Haemonchus contortus*,the predominant nematode parasite of sheep and goats in the tropics and sub-tropics. This was shown in crossbred lambs where Garole sired lambs were significantly more resistant than Deccani sired lambs to natural infection with GI nematodes as well as to an artificial challenge with *H. contortus*, as evident from their significantly lower FEC post infection. Bannur sired lambs appeared to have nematode resistance characteristics intermediate between the Garole sired and Deccani sired lambs (Nimbkar *et al*., 2003b). It was later also confirmed using purebred Garole rams grazed together with crossbred rams (Ghalsasi *et al*., 2009). Increase in Garole proportion led to a reduction in FEC and an increase in PCV (Nimbkar, 2006). An increment of 0.25 in Garole proportion was found to cause FEC after an artificial challenge to reduce by 1341 epg while another increment of 0.25 led to another decrease of 980 epg. Corresponding reductions in FEC after natural infection in ewes were 140 epg and additional 126 epg. These results indicated a polygenic basis of resistance to GIN in the Garole. The *FecB* gene did not have any influence on FEC or PCV (Nimbkar, 2006).

These findings are important considering the widespread and increasing resistance to anthelmintics among worms in countries in many parts of the world as well as on some institutional farms in India.

7.6 Comparative reproductive endocrinology of *FecB* carriers of different breeds in India and Australia

There have been a number of studies into the endocrinology of the Booroola Merino with the majority showing that the overall concentration of FSH is higher in Booroola ewes than in Merino ewes (McNatty & Henderson 1987; Xia *et al* 2003). The apparent expression of the Booroola gene seems to be significantly different between the Garole and Merino breeds. The Garole ewe has a mean ovulation rate of approximately 3.5 while the BB Merinos at UNE have a mean ovulation rate of approximately 6.8. While there are some differences in ovulation rate reported in the literature this data was obtained prior to the identification of the BMPR-1B mutation and hence identification of the geneotype was based on ovulation rate. This methodology would result in a considerable number of heterozygous animals being included in the studies. The flock managed at UNE has been

screened and all are homozygous for the Booroola mutation. Previous work by us has shown that if a 3cm 17β-estradiol implant is put under the skin to Booroola Merinos prior to the commencement of the joining estrous cycle the ovulation rate can be reduced to about 3.5. While lower doses result in higher ovulation rates, a higher dose results in ovarian failure and not a lower ovulation rate. The estradiol implant is likely to have its effect by negative feedback effects on the pituitary suppressing FSH production. This has led us to hypothesize that the high ovulation rate is partly due to intra-ovarian effects and by an increased production of FSH from the pituitary, both modulated by the BMPR-1B receptor. The ligands responsible for signaling through this receptor remain unknown but are likely to be BMP-2, 4 and 7 either individually or in combination as all three are present in the ovary and pituitary (Erickson *et al* 2003; Faure *et al* 2005; Huang *et al* 2001). The ovulation rate of the Garole ewe was similar to that of the estradiol suppressed Booroola ewe hence the aim of the current research project was to compare the FSH profiles of the Garole with that of the Merino and Booroola ewes at UNE.

A group of 15 normal Merinos, 12 Booorolas without capsules and 12 Booroolas with capsules were monitored over 3 cycles. The day of estrous was determined using rams harnessed with marking crayons. The ewes were run in open paddocks and blood samples were taken daily over the 2nd cycle. Ovulation rate was determined by laparoscopy 5-7 days after the ewe was marked by the ram.

Blood samples from 5 homozygous and 5 heterozygous Garoles were collected daily over an estrous cycle and the ovulation rate detected by laparoscopy. Plasma samples were assayed for progesterone, FSH and LH.

A comparison of ovulation rates between the 5 groups is shown in Figure 2. The mean ovulation rate of Merino ewes was 1.5 ± 0.2 (mean ± se), Booroola ewes 6.5 ± 0.5, and Booroola ewes with an implant 5.0 ± 0.4. The reduction in ovulation rate with the implant was significant but less than expected. The average ovulation rate in the Garoles was 3.6 and in the heterozygous Garoles was 2.

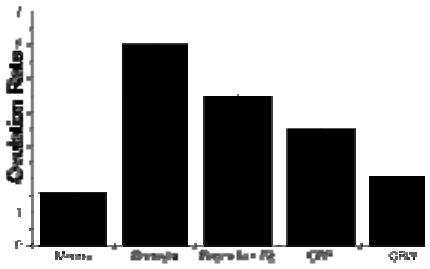

Figure 2: A comparison of ovulation rate in Merino WW, Merino BB, Merino BB + E2, Garole (GBB) and heterozygous Garole (GBW) ewes.

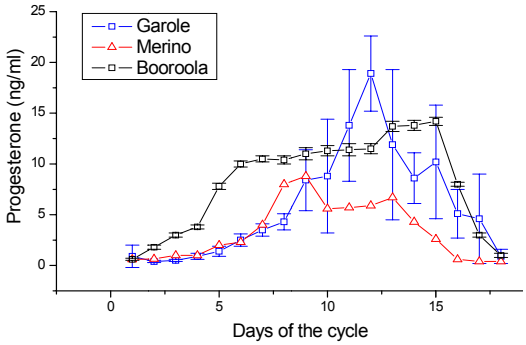

Figure 3: Progesterone profiles for Merino, Booroola and Garole ewes over an oestrous cycle.

The progesterone profiles from the Merino, Booroola and Garole ewes are shown in figure 3. There were as expected major differences between the Merino and Booroola ewes. Progesterone in Booroola tends to rise several days before it does in Merinos and to higher concentrations with complete regression of the corpus luteum occurring a few days later than Merinos. Although there was considerable variation between individuals

progesterone in the Garoles did not show the early rise as seen in the Booroola but did rise to similar concentrations and declined in a similar manner.

The average concentration of LH during the surge and the early and mid luteal phase of the cycle is shown in figure 4. The mean LH during the LH surge was significantly different between all three groups with the Merino having the highest and the Garole the lowest. Interestingly while the Booroola had significantly higher LH concentrations across the rest of the cycle the Merino and Garole were not different from each other. The FSH concentrations around ovulation, day 3 and day 13 are shown in figure 4. The concentration of FSH during the LH surge was significantly higher in Merino than the Booroola which were higher than the Garole ewes. However FSH was highest around day 3 in the Booroola ewe, with the Merino and Garole not different from each other. In all groups FSH was significantly higher on day 3 compared to day 13.

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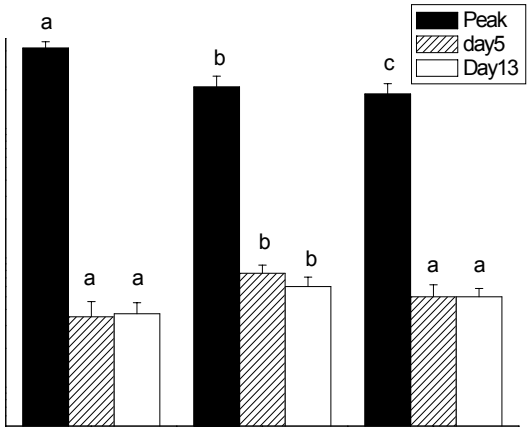

Figure 4: Mean LH concentrations during the LH surge (peak), days 3-5 and 11-15 from Merino Booroola and Garole ewes

The high ovulation rate found in the estradiol treated Booroola ewes was unexpected. However the capsules were made from a new batch of silastic tubing and it is possible that the delivered dose was less than in our previous experiments. The Merino, untreated Booroola and Garole ewes gave ovulation rates as expected.

The progesterone profiles of Booroola and Merino ewes were similar to that reported previously (Xia *et al* 2003), however interestingly the profile from the Garole ewes was not similar to the Booroola profile. The rise in progesterone was similar to that seen in the Merino but reached higher concentrations and remained elevated similar to the Booroola. It has been suggested that the follicles in ewes expressing the Booroola gene mature and ovulate earlier than the wild type explaining the early rise in progesterone. However in the case of the Garoles this does not seem to have occurred. The late fall and slow decline at the end of the luteal phase is perhaps due to the larger number of declining corpus lutea.

Differences in LH concentrations seen between the 3 groups while significant may not accurately represent the true picture of total LH released around ovulation. The release of LH occurs over a narrow window and frequent blood samples around this time are

required to accurately map and estimate LH released. However it is clear that the Booroola has higher concentrations of LH throughout the cycle compared to either the Garole or the Merino.

The rise in FSH just prior to ovulation is much less dramatic than LH hence the data shown in Figure 4 are likely to more closely represent actual amounts of FSH released although this data does not take into account the width of the FSH peak which we have reported previously is much broader in the Booroola than in the Merino (Xia *et al* 2003). Of particular interest is the concentration seen in the Garole which is about half that seen in the Merino. This breed also does not show elevated FSH in the early stage of the luteal phase as seen in the Booroola.

In summary the Garole does not share the same features of the Booroola. In many ways it appears to have aspects which are in common with either the Merino or the Booroola particularly in respect to progesterone secretion. The small numbers of sheep examined particularly in the Garole group suggest caution but it is likely that the reduced FSH is cause of the lower ovulation rate seen in this breed compared to the Booroola. While comparison with the Merino is instructive more reliable information would be gained by examining the endocrine profile of the wild type Garole not possessing the mutant BMPR-1B gene. Never the less it would appear the expression of the mutant BMPR-1B has different outcomes in the Garole compared to the Booroola. This may be due to another closely linked mutation in either breed or perhaps because of different genetic backgrounds influencing the behaviour of the mutation.

7.7 Selection and mating scheme for the nucleus flock at NARI

The detailed background to this area of work can be found in Nimbkar (2006). The breeding program at NARI faced a number of complex decisions because of the trade-offsbetween maximizing polygenic merit, maximizing frequency of the major gene alleleand controlling inbreeding. Many of these also apply to optimal strategies for wider introgression of *FecB* in India. The Total Genetic Resource Management (TGRM^{TM})program was used to explore these trade-offs and to find out the best selection and matingstrategy for use at NARI. The breeding program under consideration here needed to achieve a fairly rapid increase in frequency as it had to be able to start disseminating*FecB*homozygous rams and at least heterozygous (if not homozygous) ewes todemonstrate its impact. This had to be balanced against the opportunity of obtaininggreater long-term response by slowing the increase in frequency of the favourableallele. By using the TGRM program, it was possible to optimize gain in polygenic genetic merit while increasing the frequency of the *FecB*^{B}allele and the homozygous genotype and keeping the coancestry limited.

7.8 Potential introgression pathways and strategies for wider utilization of the *FecB* gene in India.

The results of the project work on this topic can be found in the papers of Nimbkar C *et al*., (2009b) and Van der Werf (2009). The efficiency of the introgression process can be derived from the merit of the introgression population versus that of the commercial population at a certain time following the commencement of the program. The relative merit depends not only on the effect of the major gene and the genetic difference between the donor breed and the commercial breed, but also on the rate of genetic gain in the commercial breed, and the genetic lag of the introgressed breed. Generally, several generations of backcrossing are required to recover the recipient genome. The efficiency of marker-assisted introgression is compared to introgression without markers and this difference can be small for traits that are easy to measure, but is larger for reproduction traits as in the case of Booroola.

The project team identified 29 Indian Breeds of sheep fo which introgression of the *FecB* would be appropriate. These breeds have an estimated combined population of 37 million, with the Deccani (18.8 million), Marwari (5.0 million) and Nellore (1.6 million) being the major breeds. For most situations, where the target breed and the source breed of *FecB* are not very alike, at least 3 generations of backcrossing to the target breed is recommended prior to dissemination. This provides carrier animals with at least 87.5% target breed which are unlikely to encounter resistance from the relevant shepherd population. The team modelled the implementation of such a backcrossing system using a nucleus flock of 1000 ewes. The number of heterozygote (BW) and homozygote (BB) carrier rams and ewes dispersed over time from the initiation of the flock is shown in Figure 5. Assumptions for the model are as follows (Nimbkar C*et al*., 2009b):

- Adult ewes have a conception rate of 0.9 and lamb weaning rate (per ewe lambing) of 0.9, 1.3 and 1.3 for WW, BW and BB ewes respectively.
- Maiden ewes have a conception rate of 0.8 and lamb weaning rate (per ewe lambing) of 0.8, 1.1 and 1.1 for WW, BW and BB respectively.
- Adult survival 90% per year
- Ewes are mated every 10 months.
- Maiden ewes are mated first at 15 months of age.
- Only animals with 87.5% target breed are disseminated.
- Culling rate of 30% applied to BW and BB rams prior to dissemination.

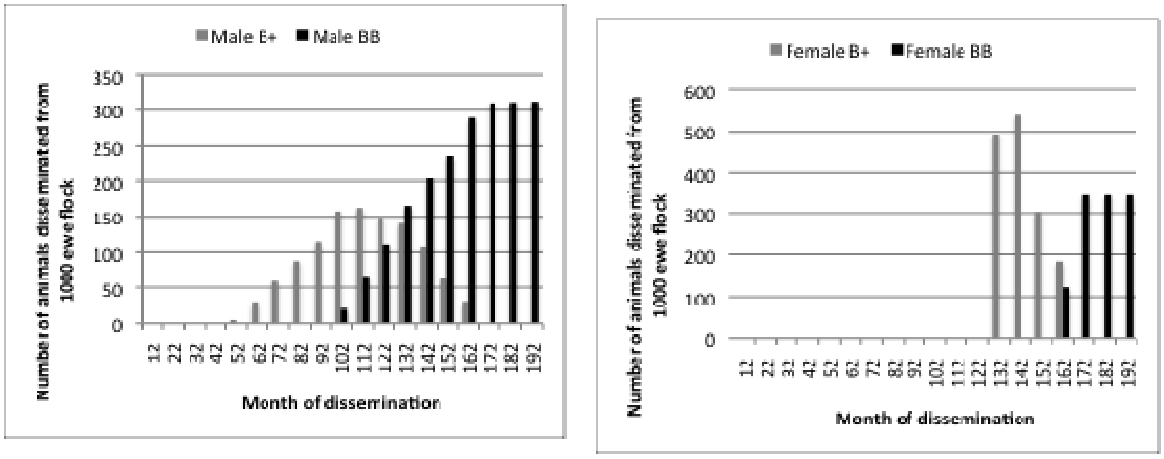

Figure 5. Dissemination of *FecB* carrier animals from a 1000 ewe institutional flock following a backcrossing program. Projected numbers of male (left panel) and female (right panel) surplus 12-month-old animals with 87.5% of target breed and carrying the *FecB* mutation disseminated to shepherds. Model assumptions are in the text. Month of dissemination is measured in months from the 1st mating in the flock. (Source: Nimbkar C *et al*., 2009b).

8 Impacts

8.1 Scientific impacts – now and in 5 years

8.1.1 International FecB workshop.

The major scientific impact arising from the project has been the holding of the Helen Newton Turner Memorial International Workshop on using the *FecB* (Boooroola) gene in sheep breeding programs at NCL, Pune on 10-12 November 2008. This represented only the 3rd international meeting on fecundity genes in sheep, following on from:

- The Booroola Merino: a workshop held at Armidale, N.S.W. 24 August 1980
- Major genes for reproduction in sheep. 2nd International Workshop Toulouse July 16-18, 1990

The workshop proceedings have been published by ACIAR as proceedings 33 (Walkden-Brown *et al*., 2009a). At the workshop 28 papers were presented comprising 19 invited review papers and 9 submitted short contributions. The last session of the workshop comprised a panel discussion on "The policy implications for wider dissemination in India of sheep containing the *FecB* gene arising from the ACIAR projects". All papers were peer reviewed. A printed set of workshop papers (224 pages) was provided to delegates at the workshop.

The workshop was attended by 83 registered delegates from 15 countries including 17 invited speakers. It provided a comprehensive scientific overview of our state of understanding of the *FecB* gene in sheep and its practical application around the world. The workshop did not form part of this project, being separately funded by:

- The Australian Centre for International Agricultural Research
- The Australian Academy of Technological Sciences and Engineering (ATSE) International Science Linkages – Science Academies Programme (ISL-SAP)
- Department of Science and Technology (Government of India)
- Department of Biotechnology (Government of India)
- Nimbkar Agricultural Research Institute (NARI), NCL and the University of New England (UNE).

Being the first meeting dedicated to *FecB* research in 18 years there was a great deal of new material to integrate, particularly that arising from ACIAR project AH/02/38. Major new findings and discussion points included:

- The direct DNA test for the *FecB* mutation is robust and widely used around the world to genotype animals at an early age for this trait. This greatly facilitates its adoption.
- The extent of the distribution of *FecB* mutation in Chinese sheep and the level of interest and publications on this. Litter size in carriers tends to be mostly in the upper half of published values, in contrast to the more modest litter sizes seen in the Deccani sheep in Maharashtra. The gene appears to be or have been fixed in the Hu sheep, much like the Garole in India.
- A similarly high level of interest and activity in India with a wide geographical spread.

- Clear evidence of the failure of sheep carrying the *FecB* mutation to be commercially successful under extensive grazing conditions (eg. Australia and USA). Under very intensive housed conditions the mutation is economically advantageous in Israel. Indications are that under intermediate conditions it may also be successful, provided nutrition is adequate. Examples include the closely supervised grazing conditions with night housing seen in India and Indonesia.
- Negative effects of the *FecB* mutation on lamb survival, independent of litter size, were reported from Australia and Israel. This effect is not evident in India to date, although Indian scientists are aware that they will need to evaluate this as numbers of homozygous carrier ewes increase.
- In some countries (eg. NZ, China) the *FecB* appears to be present with other fecundity genes, both known and postulated. Where major deviations from expected expression are observed this possibility should be taken into account.
- Emphasis should be on rapid and efficient introgression of the *FecB* mutation into suitable breeds of sheep by systematic backcrossing, rather than the generation of new breeds or strains at intermediate steps of introgression.

8.1.2 The project as an example of best practice.

Project AH/02/38 is increasingly cited in scientific and development publications as the only successful genetic improvement program using new developments in biotechnology for the benefit of smallholder sheep keepers. Marshall *et al*. (2009) stated that the project should serve as a model for others considering the implementation of marker assisted introgression (MAI) and that MAI is unlikely to succeed unless similar conditions are met. The example of this project was used recently in a position paper for the Gates Foundation for developing a strategy to invest in livestock genetic improvement for poor farmers in sub-Saharan Africa.

A case study of this project was also included recently in a FAO Technical Paper “Current status of application of animal biotechnologies in developing countries”. This document is associated with the FAO international technical conference on Agricultural Biotechnologies in Developing Countries (ABDC-10) that takes place in Guadalajara, Mexico from 1 to 4 March 2010 (see http://www.fao.org/biotech/abdc/ for more details). Dr. Chanda Nimbkar has been invited to present the case study in the session on the successful use of biotechnologies in the livestock sector in developing countries. This presentation will be one of only 2-3 presentations from other parts of the world in this session.

The close involvement with the local shepherding community at all stages of the project and the careful evaluation of new genotypes under typical shepherding conditions in addition to the main research site are seen as key elements of its success.

NARI and NCL were awarded the Council for Scientific and Industrial Research (CSIR), Government of India Award for ‘Science and Technology Innovations for Rural Development – 2007’ (jointly with another institution) for “Use of the FecB gene in Deccani breed of sheep to increase lamb production and thereby the incomes of shepherds”. It was awarded by Dr. Manmohan Singh, the Prime Minister of India, on 20 December 2008 in New Delhi.

8.1.3 New research project.

NARI, in collaboration with National Bureau for Animal Genetic Resources, Karnal, Haryana, India has succeeded in obtaining the large research grantdetailed below:

Funding source	Project title	Chief investigators	Funding
Department of Biotechnology, Govt. of	Increasing profitability of sheep production by genetic improvement	Nimbkar Agricultural Research Institute:	2009-2012 Rs.76.32 lakh

India	using the *FecB* (Booroola) mutation and improved management	Dr. Chanda Nimbkar National Bureau for Animal Genetic Resources, Karnal, Haryana, India: Dr. Bishnu P. Mishra	(~A$190,800)

The project will enable NARI to continue *FecB* introgression work by establishing the DNA test for *FecB* detection there and enabling NARI to continue working on cost effective management techniques for ewes and lambs under shepherds' flock conditions. The role of the National Bureau of Animal Genetic Resources is to analyse expression profile of candidate/regulatory genes associated with fecundity in the different genotypes under different nutritional status (farm vs field).

8.2 Capacity impacts – now and in 5 years

The Animal Husbandry Division of NARI has established its credibility within India and internationally as a research institution with excellent contacts among small-holder shepherds. This has become possible because of the ACIAR project AH/2002/038. NARI-AHD is also now recognized as a centre for sheep and goat parasitological investigations. Other organizations bring faecal samples to NARI for analysis rather than taking them to veterinary colleges.

- The recent research grant awarded to NARI from the GOI (see above) is evidence of its research credibility. This funding will give NARI the opportunity to
- Produce *FecB* carrier rams of the phenotype desired by local shepherds
- Carry out a financial analysis of cost-effective management and supplementary feeding of twin lambs.
- Continue extension and dissemination in shepherds' flocks while building on the lessons learnt from AH/02/38.

In recognition of the excellent science carried out by NARI and NCL on this project the project team at these institutions received CSIR Award of Innovation in Science and Technology for Rural Development (CAIRD) 2008 as noted in 8.1 above. Part of award money to NCL was donated to the NCL research foundation support the "Best Research Paper Award in Biological Sciences".

Ms. Chanda Nimbkar, project leader at NARI from 2006, obtained a PhD degree in Animal Breeding from the University of New England, Australia in 2006 with the help of an ACIAR John Allwright Fellowship. As a result, she can now be a principal investigator on a research project and apply for funding from Indian government and other agencies. Dr Nimbkar was recently successful in developing and obtaining a large research grant entitled "Increasing profitability of sheep production by genetic improvement using the *FecB* (Booroola) mutation and improved management" Ministry of Science and Technology, Government of India (See Section 8.1 3).

As a result of Dr. Chanda Nimbkar's enhanced knowledge and skills as a consequence of her degree and the experience gained by her while implementing the project, she was appointed as a member of the governing body of the Indian Council of Agricultural Research, the apex public sector body in India for coordinating, directing, funding and promoting agricultural research, education and extension. She can thus participate in policy development in that capacity.

Ms. Varsha Pardeshi of NCL, pursued her PhD studies based on the project while working on the project and received her PhD in 2009. Her thesis was titled "A study towards genetic diversity and genetic basis of prolificacy in important sheep breeds of India" in December 2008.

In 2008 Varsha Pardeshi received the Keerti Sangoram Award for the best student of the year sponsored by NCL Research Foundation (2008).

Almost all the staff at NARI who worked on the project have developed their skills and capacities in different areas and they are using these skills in areas outside the scope of the project. For example, Ms. Padmaja Ghalsasi learnt parasitology techniques in the project and she now uses these to measure faecal worm egg counts of sheep and goats belonging to local farmers. She has now learnt on her own using techniques described in the literature how to carry out faecal egg counts to assess liver fluke and paramphistome infections. Padmaja Ghalsasi also learnt at NCL the methodology of all steps of carrying out the DNA test for the *FecB* mutation so that now the proportion of non-amplified samples is less than 10%.

NARI extension staff Mr. K.M. Chavan and Mr. Shyam Kulkarni have learnt a lot about effective extension methods and they are now using these to form 'Shepherds' Clubs' under a scheme of the National Bank for Agriculture and Rural Development. The first such club has been formed and is functioning well. The scheme is for 'Farmers' clubs' so this might be the first shepherds' club formed under it.

In the Phaltan taluka in the project region, some 26 shepherds have worked closely with the project and developed new skills in sheep management and data recording as a consequence of this. Understanding and interest in the *FecB* has spread rapidly from this nucleus of producers.

The International Booroola Workshop organized by the project in November 2008 helped in the capacity building of all project related personnel at NARI and NCL. This was in the scientific and organization related aspects. This workshop would have had a significant capacity building component regarding understanding of the *FecB* mutation within India and internationally.

In Australia the project has supported the only Booroola studies based on DNA genotyping for the mutation. The UNE collaborators developed significant skills in genotyping and analysis doing this work in collaboration with Dr Jill Maddox at the University of Melbourne. The understanding of the *FecB* gene and its actions in Merino sheep in Australia has been substantially improved by this work.

In 5 years time it is likely that the enhanced capacity at NARI will have been retained and flow on capacity impacts initiated by this project will have grown. NARI has already secured funding for ongoing work from 2009-2012 from the Government of India (GOI). In May 2009 the GOI Animal Husbandry Dept. approved a scheme for the "Integrated Development of Small Ruminants" and it has a provision for setting up three 'Biotech centres for fecundity genes' and a financial allocation of Rs.15 crore. It states that 'The advantages of rearing sheep and goat of breeds having high fecundity are well established. Efforts will be made to exploit high fecundity breeds. Under the biotechnology centres, programs will be undertaken to attain flock strength of 500 animals which are homozygous for the high fecundity genes.'" NARI will be an applicant for setting up one of those centres.

NARI have also submitted a collaborative project proposal to the Indo-Australia Biotechnology Fund together with Scientists from CSIRO Livestock Industries. The project is about developing an integrated parasite management system including a multivalent recombinant vaccine and will look at the effects of the vaccine on resistant (Garole crosses) and susceptible (Awassi crosses) breed types.

8.3 Community impacts – now and in 5 years

A major community impact of this long-term project (11 years including the preceding project AS1/1994/022) is the increased awareness of sheep rearing as a profitable livelihood option available even to people not belonging to the 'Dhangar' community of

traditional shepherds and the enhanced status of this occupation as a result. The case that can be cited in proof is that of six farmers from Latay village who bought flocks of sheep to diversify their agricultural activities and earn more income. They started rearing sheep in preference to rearing dairy cows. A shepherd in whose flock the project had conducted a trial in 2003 to 'assess the utility of anthelmintics in increasing shepherds' incomes', told the farmers from Latay about NARI and the project and that they could approach NARI for support. NARI supplied *FecB* carrier breeding rams to these new flocks and included them in the project as 'less intensively monitored' flocks.

The project supplied six *FecB* carrier rams in 2005 to the NGO ‘Gram’ in Nizamabad district of Andhra Pradesh. Gram released these rams into shepherds’ flocks. Mr. N. Samson, Director of Gram visited NARI a couple of times and participated in the Booroola workshop in November 2008. He “developed a keen interest in small ruminants as a means of rural development” after visiting NARI and prepared a project proposal for “Productivity enhancement to strengthen livestock based livelihoods”. This proposal was sanctioned for funding by Sir Dorabji Tata Trust in March 2009. Gram proposes to promote *FecB* as a part of the new project and wishes to enter into an institutional arrangement with NARI for this.

As dissemination of *FecB* carrier sheep gathers pace, the community benefits from this can be expected to grow, so in 5 years time we can expect them to be larger. In 5 years an additional 500 or so carrier rams can be expected to have been disseminated from NARI alone, and the benefits of the gene spread widely beyond the local NARI region. As an indication of the demand for carrier animals, in late 2009 NARI sold 100 inseminated heterozygous carrier ewes for Rs.2,800 each and 20 non-carrier ewes for Rs.3,000 each (with 1-month old lambs) to a group of 12 shepherds who are members of the shepherds’ club mentioned above. They obtained bank loans as club members to purchase the ewes from NARI.

8.3.1 Economic impacts

The biological and economic impact of introducing the *FecB* gene into participating shepherds’ flocks has is detailed in NimbkarC *et al*. (2009a) from which the following is extracted. The income and expenditure from 16 smallholder flocks from 1 Feb 2004 to 31 March 2008 were compiled and analyzed. Income from sheep rearing included lamb sale proceeds and a notional income for lambs that were retained, sale proceeds of manure and wool and sale proceeds of adult ewes and rams. Items of expenditure included labour charges for flock supervision (actual charges if help was hired or notional charges if family labour was used), purchase of fodder, grazing and concentrates, veterinary treatment of sheep and shearing expenses.

The gross margin per WW breeding ewe was found to be Rs. 450 to Rs. 800 per year. Only one of the flocks had data from a reasonable number of BW breeding ewes i.e. 8, 41 and 50 ewes in the years 2005 to 2008 respectively. The number of WW breeding ewes in that flock was 98, 89 and 74 respectively in the three years. In this flock, the gross margin per BW breeding ewe was Rs.150 to Rs.300 higher than WW ewes. This amounted to an increase in gross margin of between 37 and 50%. The lamb mortality was 31, 24 and 19% among lambs of BW ewes and 14, 18 and 24% among lambs of WW ewes in the three years. Despite the higher mortality, BW ewes weaned a higher number of lambs per ewe than WW ewes which led to the higher gross margin. Twin-born lambs were sold at about Rs.100 less on average than single-born lambs and they were two to four weeks older at sale than single-born lambs.

The gross margin per twin-bearing ewe in smallholder flocks was 30% higher than that of single-bearing ewes. There was a small amount of extra expenditure made on supplementary feeding to twin-bearing ewes and their lambs and they weaned 0.8 more lambs than single-bearing ewes (Nimbkar C *et al*., 2009).

Based on the findings above some broad indication of the economic impact of *FecB* introgression can be estimated. Using a value of Rs 250/year benefit from having the *FecB* gene, for every 10% of the target population of 37 million sheep (22.2 million ewes) that *FecB* is successfully introgressed to an annual economic benefit of Rs 550 million can be expected (approx $13.9 million Australian).

Some more personal local examples of economic impact are provided below.

1. The shepherd Dattatraya Tarate who has the largest number of *FecB* carrier ewes in his flock, has built a big new open-sided shed with a galvanized iron roof for his sheep. Previously, the sheep used to be kept in a rope enclosure without a roof. He also bought 4 acres land.
2. Participating shepherds Ashok Pisal and Ganesh Pisal also bought 3 acres and 1/8 acre land respectively.
3. Participating shepherds Eknath and Dattatray Pisal sent two of their children for higher education; one for a Masters in Social Work and one for a course in Automobile Engineering. Eknath Pisal also bought a tractor with the help of a bank loan.

These impacts were seen largely because of

1. Increased income from sheep rearing due to increased number of lambs for sale. This in turn was due to increase in flock size (owing to increased twinning, NARI convincing shepherds to retain all *FecB* carrier ewes and better lamb management)
2. Reduced mortality due to NARI's timely preventive and emergency interventions.

8.3.2 Social impacts

The social benefits arsing from this project include:

1. The project supported regional and rural development in India with the bulk of the project funds being directed to a rural NGO as the main collaborator in the project.
2. The economic benefits listed above and the focus of the project on sheep has resulted in an elevation in status of sheep rearing from that of very low status, at least in the local project area. This is detailed in the introduction to Section 8.
3. Sheep with high fecundity are not well suited to very long migrations. It is possible that the returns available from high fecundity sheep in more settled mixed farming systems such as those investigated in this project, will assist the in the reduction of transhumance as a lifestyle, with the social problems (particularly children's education) associated with it.

Project ACIAR AS1/1994/022 preceding this conducted an extensive socio-economic survey of sheep smallholders that is reported in summary by Nimbkar B *et al*. (2009c). During the current project, a socio-economic survey of the participating shepherds was undertaken and is reported by Prior *et al*. (2009). This survey showed (as did the earlier one) that the sheep owners' responses to twin lambs were positive. Twin lambs were viewed as more profitable than single lambs, with the main disadvantage cited as the need to undertake supplementary feeding and management to ensure adequate growth rates and survival of twin lambs. Recommendations made as a result of the survey include the need for further financial analysis of cost effective supplementary feeding and management of twin lamb flocks; the development of phenotypes for *FecB*-carrier rams that are more acceptable to local sheep owners; and the need for an education and extension program to support sheep owners in their adoption of the new technology.

8.3.3 Environmental impacts

Overall the project outcomes are unlikely to have major environmental impacts.

The environmental impact of sheep rearing under the project, both at NARI and in shepherds' flocks, was generally positive. The positive impact is mainly on weed reduction and enhancement of soil fertility due to deposition of manure and urine. The year 2007 was a year of above average rainfall. It started raining early in the season and the rainfall was well-distributed from June to November. As a result, there was tremendous growth of grass on cultivated lands before any crops could be sown. Grazing sheep on such lands turned out to be a 'win win' solution to this problem and all flocks in the area had continuous 'invitations' to graze throughout the rainy season.

Because of the enhanced reproductive rate of ewes carrying the *FecB* gene, their biological efficiency at converting pasture into saleable meat is generally higher, meaning that for a given level of meat production, the grazing pressure required to produce it is reduced in flocks carrying the*FecB*mutation. This can help limit overgrazing, but only to the extent that ewe numbers are reduced to compensate for the increased efficiency.

There is also a potential environmental benefit from the project if sheep numbers are maintained or increased at the expense of goats. In recent decades the goat population in India has increased greatly, while the sheep population has not. There are many reasons for this, but the higher fecundity of goats is one of them. To the extent that the project assists with maintaining sheep numbers at the expense of goats, it is likely to have an environmental benefit as sheep are generally accepted as less destructive of soil and vegetation cover than goats.

8.4 Communication and dissemination activities

The Helen Newton Turner Memorial International Workshop on using the *FecB*(Boooroola) gene in sheep breeding programs was held at the National Chemical Laboratory (NCL), Pune, India on 10-12 November 2008.

The workshop was attended by 83 registered delegates from 15 countries including 17 invited speakers. It provided a comprehensive scientific overview of our state of understanding of the *FecB* gene in sheep and its practical application around the world.

The workshop was co-sponsored by:

1. The Australian Centre for International Agricultural Research
2. The Australian Academy of Technological Sciences and Engineering (ATSE) International Science Linkages – *Science Academies Programme* (ISL-SAP)
3. Department of Science and Technology (Government of India)
4. Department of Biotechnology (Government of India)
5. Nimbkar Agricultural Research Institute (NARI), NCL and the University of New England (UNE).

Income from registration fees of delegates totalled over $4,000 and was the 3rd highest source of income for the workshop after the ATSE and ACIAR sponsorship.

Workshop and post workshop details can be found on the workshop web site (http://www.une.edu.au/ers/hnt-workshop.php). At the workshop 28 papers were presented comprising 19 invited review papers and 9 submitted short contributions. The last session of the workshop comprised a panel discussion on *"The policy implications for wider dissemination in India of sheep containing the FecB gene arising from the ACIAR projects"*. All papers were peer reviewed and have been published by ACIAR as Proceedings 133(Walkden-Brown *et al*., 2009). A printed set of workshop papers was also provided to delegates at the workshop.

Project Coordination meetings were held in India on 4-7/2/2003, 20-23/7/2004, 14-17/11/2005 and 11-16/2/2007. All meetings were held at NARI, Phaltan, Maharashtra and generally had invitees from various levels of Government in addition to the Indian and

Australian collaborators on the projects. Detailed reviews of project findings and planning for future stages of the project occurred at these meetings. Because of the attendance of government representatives, and the publicity activity associated with the meetings, they provided a significant avenue for communication and dissemination of project outcomes.

A workshop on “Use of the *FecB* gene to increase productivity of sheep in India and Bluetongue disease of sheep” was organized on 8 November 2006 for shepherds in and around Phaltan. Around 500 shepherds attended the workshop. Ms. Leena Mehendale, Principal Secretary, Animal Husbandry and Dairy Development Department of Government of Maharashtra inaugurated the workshop. Some funding was provided by the National Bank for Agriculture and Rural Development (NABARD).

The following one-day training programs were also conducted for smallholder shepherds by Dr. Ghalsasi, Mr. K. Chavan and Mr. S. Kulkarni of NARI. In the two programs conducted on NARI's Lundy farm, shepherds were shown the management of young lambs at NARI. In all the other programs, the concepts that sheep can have twin lambs due to the *FecB* gene and how this can increase profits were explained to the participating shepherds. They were shown the CD prepared by NARI about the management of twin lambs and their questions were answered. About ten of the participants later visited NARI's Wadjal farm to see the NARI Suwarna breeding rams.

Date (2007)	Place	Participants	Comments
9 June	Modnimb, Dist. Solapur	76	
10 July	NARI's Lundy farm, Rajale	21	
13 July	Bhavenagar, Tal. Koregaon, Dist. Satara	300	Organized by Maharashtra Sheep and Goat Corporation
23 July	NARI's Lundy farm, Rajale	19	
30 July	Salape, Tal. Phaltan	60	Organized by Maharashtra Sheep and Goat Corporation
10 Sept	Salape, Tal. Phaltan	49	
27 Sept	Ahmednagar, Dist. Ahmednagar	20	Organized by Bosco Gramin Vikas Kendra
2 Oct	Ahire, Tal. Khandala,	19	
26 Dec	Warwand, Tal. Daund, Dist. Pune	18	
	Total	582	

NARI held a training workshop on 31 October 2007 on “First aid treatment in sheep" for shepherd couples (62 men and women) participating in the project. An illustrated Marathi booklet ‘First aid treatment in goats and sheep’, prepared by NARI was released at the time. Dr. P.M. Ghalsasi gave information to the participants about common ailments in sheep, their symptoms and treatment. Each participant was given a copy of the booklet and a first aid kit with medicines and other supplies. Some funding for the workshop was obtained from the Maharashtra govt's ‘Agricultural Technology Management Agency’.

A one-day training and consultancy program was conducted at NARI for the members of BAIF Development Research Foundation, Karnataka on 19 November 2007. Dr. Ghalsasi gave them a presentation on ‘Sheep and goat improvement initiatives at NARI’. Mr. B.V. Nimbkar and Dr. Chanda Nimbkar discussed with them the potential for sheep development and what initiatives are likely to succeed.

Chanda Nimbkar delivered a lecture in Marathi on “Sheep: way to increase the income from this neglected resource” (durlaxit mendhya: utpanavadhicha marg) on 27 May 2006 in a lecture series organized by Appropriate Rural Technology Institute, Phaltan, India.

Dr. Pradip Ghalsasi gave a power point presentation on 'Use of the *FecB* gene to increase sheep productivity' to all District Deputy Commissioners and District Animal Husbandry Officers of the Government of Maharashtra, whose meeting was organized by the Commissioner, Animal Husbandry, Maharashtra State at Central Building, Pune, India on 11 October 2006.

The Animal Husbandry Division (AHD) of NARI participated in the Yashawant Agricultural, Business and Livestock Exhibition held at Karad, Maharashtra, India on 24-28 November 2006. Some *FecB* carrier rams were exhibited by AHD. Mr. K. M. Chavan gave information about the importance of these rams to visitors at the stall. Dr. Pradip Ghalsasi gave a power point presentation on 'Use of the *FecB* gene in sheep to increase productivity' on 28 November 2006.

8.4.1 Conferences attended and presentations made:

*Scientific meetings.*As can be seen from the publications list, Project Scientists have made presentations on project findings at the following scientific meetings:

1. 2003. 15th Conference of the Association for the Advancement of Animal Breeding and Genetics, Melbourne, Australia. (Chanda Nimbkar)
2. 2003. FAO/IAEA international symposium on applications of gene-based technologies for improving animal production and health in developing countries Vienna, Austria, 6–10 October 2003. (Chanda Nimbkar)
3. 2005. FAO Symposium on "Integrated Animal Parasite Management: From Academic Interest to Reality" held at Indira Gandhi Agricultural University, Durg, Chhattisgarh, India. December 8, 2005. (Chanda Nimbkar)
4. 2006. International Conference on Plant Genomics & Biotechnology: Challenges & Opportunities. Raipur, India October 26-28 (Varsha Pardeshi)
5. 2006, Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, 13-18 August, 2006 Belo Horizonte, Brazil (Chanda Nimbkar)
6. 2006. XVII National Congress of Veterinary Parasitology and National symposium. Rajiv Gandhi College of Veterinary and Animal Sciences, Kurumbapet, Puducherry, India. November 15-17, 2006. (Pradip Ghalsasi)
7. 2006. National Seminar of the Indian Society for Sheep and Goat Production and Utilization (ISSGPU) on "Innovations and Recent Advances in Reproduction for Augmenting Small Ruminant Production". Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India. 28-30 December 2006 (Pradip Ghalsasi)
8. 2006. National Workshop-cum-Seminar of Indian Society for Sheep and Goat Production and Utilization held at Central Institute for Research on Goats, Makhdoom, India. March 4-5, 2006 (Chanda Nimbkar)
9. 2006. National Seminar of the Indian Society for Sheep and Goat Production and Utilization (ISSGPU) on "Emerging diseases of small ruminants and their containment under WTO regime". Central Institute for Research on Goats. Makhdoom, UP, India. February 3-5, 2007. (Pradip Ghalsasi)
10. 2006. Symposium on National Biodiversity and Ecosystem Information Infrastructure: Challenges and Potential. Pune, India, January 30- February 2. (Varsha Pardeshi, Vidya Gupta)
11. 2006. International Society of Animal Genetics (ISAG). Porto Seguro, Brazil on August 20-25**.** (James Kijas)
12. 2007. 17th Conference of the Association for the Advancement of Animal Breeding and Genetics,Armidale, Australia. (Chanda Nimbkar, Steve Walkden-Brown)

13. 2007. Scientific Forum of First International Technical Conference on Animal Genetic Resources, Interlaken, Switzerland. 1-7 September 2007. (Chanda Nimbkar)
14. 2008. Use of the *FecB* (Booroola) gene in sheep-breeding programs. Helen Newton Turner Memorial International Workshop. Pune, India. 10-12 November 2008. (Chanda Nimbkar, Bon Nimbkar, Pradip Ghalsasi, Vidya Gupta, Julius Van Der Werf, Geoff Hinch, Steve Walkden-Brown)
15. 2009. XIX National Congress of Veterinary Parasitology and National Symposium on "National impact of parasitic diseases on livestock health and production". Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. 3-5 February 2009. (Pradip Ghalsasi, Padmaja Ghalsasi)

8.4.2 Other meetings, seminars and workshops.

1. Pradip Ghalsasi attended the State Level Workshop on Sheep Breeding Policy for Andhra Pradesh and presented a paper "Sheep genetic improvement initiatives at Nimbkar Agricultural Research Institute (NARI), Phaltan, Maharashtra". August 2006.
2. Dr. Chanda Nimbkar presented a paper "Genetic improvement of Deccani sheep for increased profit." at the seminar on the "Sustainable Use and Conservation of the Deccani Sheep (Meat and Wool)" held by Anthra, Hyderabad, India. 20-22 February 2007.
3. Mr. B. V. Nimbkar, Dr. Chanda Nimbkar and Dr. P. M. Ghalsasi along with two other staff members visited Krantisinh Nana Patil Veterinary College, Shirval, Tal. Khandala on 7 March 2007. Dr. Ghalsasi gave a presentation to the college staff about the research and extension work carried out under the ACIAR project at NARI.
4. Dr. P. M. Ghalsasi, Ms. Padmaja Ghalsasi, Mr. K. M. Chavan and Mr. Shyam Kulkarni attended a workshop on 'Vaccination and deworming of sheep' held by Animal Husbandry Dept of Satara Zilla Parishad, Maharashtra and Punyashlok Ahilyadevi Maharashtra Sheep and Goat Development Corporation Ltd. held at Bhavenagar, Tal. Koregaon, Dist. Satara on 13 July 2007. Dr. Ghalsasi gave a presentation on "NARI's activities in shepherds' flock, 'NARI Suwarna' sheep and PPR and bluetongue diseases in sheep".
5. Dr. Chanda Nimbkar gave a presentation on "Pastoralists oriented work" carried out at NARI and chaired a session on 'Breeds and breed improvement' at a meeting on "Pastoralism and rangeland conservation" organized by the Ford Foundation at Ahmedabad. 9-10 July 2007.
6. On an invitation from the FAO, Dr. Chanda Nimbkar presented the paper 'Sustainable use and genetic improvement' in the Scientific Forum at the First International Technical Conference on Animal Genetic Resources for Food and Agriculture at Interlaken, Switzerland 3-7 September 2007.
7. Dr. Chanda Nimbkar participated in a panel discussion on "Making animal breeding work for the poor amidst the excitement of the genetic revolution" at the John Vercoe Memorial Conference "Animal breeding for poverty alleviation – harnessing new science for greater impact" held at Nairobi, Kenya by the International Livestock Research Institute on 8-9 November 2007.
8. Dr. P. M. Ghalsasi gave two presentations on the 'Use of the *FecB* gene to increase sheep productivity' at the National Animal Genetic Resources Centre of Uganda and at Makerere University in Kampala during his visit to Uganda. This visit was sponsored by Dr. Lorna Brown, Wales, UK to help her with oestrus synchronization and artificial insemination work under 'The Village Goat Improvement Program' from 9-14 December 2007.

9. Ms Varsha Pardeshipresented a paper on “Unravelling genetic diversity of valuable Indian ovine breeds”. Biochemical Sciences division evaluation, National Chemical Laboratory, Pune. January 24, 2008.
10. Dr Vidya Gupta presented a paper on “Searching and saving genes for sustainable agricultural productivity; A case study”. International forum on conservation and stewardship of agricultural biodiversity in an era of climate change at MSSRF, Chennai, India, August 7-9, 2009.
11. Dr Vidya Gupta presented a paper on “Searching and saving genes for sustainable livelihood of a socio-economically poor group of India”. Symposium on Emerging Trends in Agricultural Biotechnology at College of Agricultural Biotechnology, Loni, January 29, 2009
12. Ms Varsha Pardeshi presented a poster on “Genetically improved prolific sheep enhances income of Indian shepherds”. Poster presentation for the Technologies for Rural development at National Chemical Laboratory, Pune, India. 2009.
13. Dr Vidya Gupta presented a paper on “Role of prolificacy gene in sheep genetic improvement”. Jaydeep Naik and Sunil Newaskar memorial lecture at Abasaheb Garware College, Pune March 26, 20

8.4.3 Dissemination of *FecB* carrier breeding rams

Apart from the rams sent into local shepherds’ flocks for breeding, sixteen BB and twenty-twoBW rams were sold to individual sheep keepers, NGOs and governments between July 2004 and March 2009. Notable among the buyers were:

- 5 BB rams sold to the Veterinary Science University in Kashmir
- 2 BB and 1 BW rams sold to the Sheep Husbandry Dept., Govt of Jammu and Kashmir
- 19 carrier rams sold to sheep farmers and NGOs in Karnataka, Andhra Pradesh and Tamilnadu States
- 8 carrier rams purchased by sheep farmers in Maharashtra

A questionnaire will be prepared and sent to these buyers to find out how widely the rams have been used and how many progeny have been born.

9 Conclusions and recommendations

9.1 Conclusions

The major conclusions that can be drawn from the project are as follows:

1. The main traits of economic importance for sheep productivity in Maharashtra are litter size, lamb survival and growth to weaning. Wool has virtually no value and may be a liability in some instances as it has to be removed and discarded.
2. Traditional shepherds have strong views about what constitutes a “desirable” animal. These views are not fixed and change over time as evidenced by “fashions” for different sub-types of Deccani sheep. These views must be taken into account in any introgression program as attempts to disseminate sheep with unsuitable phenotypes will meet resistance.
3. When surveyed, shepherds consider twin-bearing ewes more valuable than normal ewes, and have generally positive views about the introduction of *FecB* into their flocks. Most reservations relate to the phenotype of introduced sheep rather than their prolificacy, although it is acknowledged that twin-bearing ewes require more inputs than single-bearing ewes. Shepherds have their own management strategies for dealing with twin born lambs, and are also receptive to new strategies.
4. Detailed measurements made in the flocks of 26 collaborating shepherds showed that the introgression of the *FecB* gene into the flocks resulted in moderate increases in litter size at birth (extra 0.39 lamb in BW ewes) and 3 months (extra 0.26 lamb in BW ewes). Total lamb weight was increased by 7.5% and could be expected to increase further with increased adoption of management strategies for multiple births. In one flock with the largest number of *FecB* carrier ewes, economic analysis indicated an increase in gross margin of 37 to 50% per ewe carrying *FecB*. Across the smallholder flocks the gross margin per twin-bearing ewe was 30% higher than that of single-bearing ewes.
5. Initial data on BB ewes at NARI (45 records) suggest that their performance is very similar to that of BW ewes and problems of excessive litter size and lamb mortality are unlikely to occur.
6. The modest increases in reproductive rate observed in *FecB* carrying ewes at NARI and in smallholder flocks contrast with findings in some overseas countries. Project studies on the Australian Merino revealed higher litter sizes and mortality rates than those seen in India, and significant problems with fertility and lamb survival in the BB ewe. The performance of BB ewes at NARI and in smallholder flocks in India will need to be monitored to determine whether this problem occurs to any extent in India also.
7. The basis for the differences in litter size observed in *FecB* carrier sheep in India and Australia is not clear. It is likely to involve different levels of underlying (genetic) fecundity and also possibly differences in nutritional status of ewes at the time of mating. Other mechanisms may be involved and these warrant investigation.
8. Genotyping animals for *FecB* status using the direct DNA test is an efficient and practical method for use in India, as demonstrated by the project. Given the low level of natural twinning in Deccani sheep, repeat twinning would also be an adequate phenotypic marker for *FecB* in this population.
9. On the basis of items 3-5 above, significant improvements in the productivity of sheep in Maharashtra could be expected following introgression of the *FecB* gene more widely into the Deccani sheep population. The same may be true for other low fecundity sheep in India (the majority of sheep breeds in India).

9.2 Recommendations

1. Wider introgression of the *FecB* gene into Indian breeds of sheep should be supported by the Government of India, subject to the conditions below:
2. The targets for introgression should be major sheep breeds of India, particularly meat and dual-purpose breeds in areas where feed restrictions are not severe and migrations are not long and arduous.
3. *FecB* should be introduced from a source as similar as possible to the target breed. To achieve this a classical backcrossing program should be used at large institutional flocks (>500 ewes) to generate animals with 87.5% target breed carrying the *FecB* gene. Dissemination prior to sufficient back-crossing is a major risk as shepherds may reject the resultant phenotypes. The temptation to form new “breeds” at various stages of introgression should be resisted. Significant selection pressure can be obtained in generations 2 and 3 of the backcrossing program by selecting target breed rams of high genetic merit for the backcrossing.
4. Dissemination should predominantly be by carrier rams. After a brief period of dissemination of heterozygotes, homozygotes will predominate.
5. The dissemination should be accompanied by an extension program aimed at boosting lamb survival and growth. This will need to centre on the nutritional and health management of ewes and lambs in flocks with significant multiple births.
6. Introgression should only continue while there remains no evidence of marked adverse effects in the homozygote carrier ewe.
7. The performance of homozygous carrier ewes deserves special attention. NARI, as the holder of the largest number of BB ewes with a high Deccani proportion should be supported to carry out a full comparison of performance of the BB relative to BW and WW ewes, with at least 500 BB records. Performance should be measured in both the NARI flock and shepherds flocks.
8. Research into the optimum nutritional and health management of twin bearing ewes and their progeny should be supported. 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.
9. Research into the underlying basis of differences in reproductive expression of the *FecB* trait in sheep in different countries should be supported.
10. ACIAR should conduct follow up impact studies on this project at 5 and 10 years post-project.

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10.2 List of publications produced by project

Publications are listed in ascending chronological order sorted by author within year.

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Ghalsasi, P.P., Ghalsasi, P.M. and Nimbkar, C. (2009). Pure Garole rams have superior worm resistance compared to crosses comprising Deccani, Bannur, Garole and Awassi breeds in Phaltan, Maharashtra. In Proceedings of the XIX National Congress of Veterinary Parasitology and National Symposium on “National impact of parasitic diseases on livestock health and production”. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. 3-5 February 2009. p. 31.

10.2.6 Popular articles (English)

Nimbkar, C. (2002). Gains from Garole - the 'wonder' sheep of West Bengal. Partners in Research for Development. ACIAR. 15:31-36.

Ghalsasi, P.M. (2005). Fecundity B gene in shepherds’ flocks to increase productivity. News Bulletin of the Bombay Veterinary College Alumni Association, Mumbai.

MacDonald, Whitney (2005). Sheep genes go full circle. Partners in Research for Development. ACIAR. October 2005 p7.

Nimbkar, C. (2007) Harnessing genetics to increase sheep production on the Deccan plateau. ACIAR South Asia Newsletter July 2007, 1-3.

Penfold, Kellie (2008) Twin genes to help India lift meat production. Partners in Research for Development. July-October 2008. ACIAR. pp.18-19.

There have also been several stories about the project developed by the UNE Publicity office. These have featured as headline stories on the UNE homepage and have usually been followed up in the press.

10.2.7 Popular articles (Marathi)

Nimbkar, B.V. (2005). Sheep and goat rearing for increasing meat production (Masotpadan wadhisathi sheli-mendhi palan) published in a Special supplement of a local newspaper for farmers. 12 October 2005. This article was later reprinted in several other Marathi weeklies and magazines.

‘Sheep rearing – a profitable enterprise’ (‘mendhipalan ek phaydeshir udyog’). (2007). ‘Annadata’ magazine. February 2007. pp. 26-27

Gadre, Amit. (2007). A series of Marathi articles on the new strain of *FecB* carrier Deccani sheep with higher prolificacy called ‘NARI Suwarna’ developed under the ACIAR funded project. “Agrowon” daily agricultural newspaper of Sakal papers 26-29 November 2007.

Nimbkar, C. (2008). A new path for the prosperity of farmers – use of science and technology for goat and sheep rearing. ‘Balirajachya unnaticha nawa marg’. Special supplement, Newspaper ‘Lokmat’. 15 May 2008. p.14.

Nimbkar, B.V. (2008). An assessment of goat and sheep rearing in Maharashtra. ‘Maharashtratil sheli va mendhi palan vyavasayacha sarvankash adhava’. Silver Jubilee issue of magazine ‘Shwetkranti’. Maharashtra Pashusamvardhak Sanghatana. Shrirampur, Maharashtra. Pp. 79-87.

Kulkarni, Shyam (2009). Sheep rearing boosted financial gain. (Mendhi palanane dili arthik sath). Success story of Shri Changdeo Deokate. Agrowon Daily newspaper. 16 February 2009. p.9.

Nimbkar, C. (2009). New direction for goat and sheep rearing. 'Sheli-mendhi palanas navi disha'. Daily newspaper 'Sakal'. Special issue on Phaltan taluka 'Towards development'. P. 21 and 23.

10.2.8 Booklets published by NARI

A short film (CD) in Marathi and English on "Sheep Rearing: Increasing profits with twin lambs" was prepared by NARI in November 2005. This has been distributed widely.

A booklet "Harnessing genetics to increase meat production from sheep". This was prepared to give information about the discovery of the *FecB* gene, its introduction into Deccani sheep and achievements of ACIAR projects AS1/94/22 and AH/02/38. This booklet has been distributed widely in India and abroad.

The Marathi version of this booklet published in 2004 was reprinted in several leading magazines for farmers.

Bluetongue disease in sheep and goats. 2006. Marathi booklet released at the time of the Seminar of shepherds held at Nimbkar Agricultural Research Institute, Animal Husbandry Division, Phaltan on 8 November 2006. 13 p.

First aid treatment in goats and sheep. 2007. An illustrated Marathi booklet.