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Use of the *FecB* (Booroola) gene in sheep-breeding programs

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Use of the *FecB* (Booroola) gene in sheep-breeding programs

Proceedings of the Helen Newton Turner Memorial International Workshop held in Pune, Maharashtra, India, 10–12 November 2008

Editors: S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta

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GPO Box 1571, Canberra ACT 2601, Australia
Telephone: 61 2 6217 0500
aciar@aciar.gov.au

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Cover: Mr B.V. Nimbkar and two traditional smallholder sheep owners (Mr Namdeo Madane and Mr Bhagunath Kokare) with a NARI Suwarna ewe and its twin lambs. Photo: Pradip Ghalsasi.

Foreword

This workshop has its origins in two successful Australian Centre for International Agricultural Research (ACIAR) projects on meat sheep development in Maharashtra, India, that ran between 1998 and 2008. These projects aimed to increase sheep meat production by traditional smallholder sheep owners in an environment where the coarse wool produced by local sheep had lost most or all of its value. Smallholders are a socially and economically disadvantaged group in rural India and boosting their income and status was an important goal. The Indian partners on these projects had established that the low reproductive rate of sheep on the Deccan Plateau was a major limitation on productivity for meat production. They had identified the highly fecund Garole sheep from West Bengal, which regularly produce twins and some triplets, as a potential means of genetically improving the reproductive component of productivity.

Under the ACIAR projects, it was established that the basis of fecundity in the Garole is the *FecB* (Booroola) gene mutation. This was originally described in Australia by Helen Newton Turner, in whose honour this workshop is named, and attributed by her to early importation of Bengal sheep. Using direct DNA tests for the presence of the mutation, the project team set about introducing (introgressing) the gene into an experimental flock comprising local Deccani sheep and their crosses with promising local meat breeds. This work suggested that the mutation conferred useful increases in fecundity and meat production efficiency. During 2003–08 this was confirmed in 26 collaborating smallholder sheep owners' flocks in which the biological and economic impact of introduction of the mutation was assessed.

Use of the *FecB* gene is one of the few examples where DNA technologies have been shown to clearly benefit practical breeding programs. ACIAR is therefore pleased to be a major sponsor of this, only the second, international workshop dedicated to the *FecB* (Booroola) gene.

The first workshop on 'The Booroola Merino' was held in Armidale (New South Wales, Australia) in 1980. The first paper in those proceedings was written by Dr Helen Newton Turner on the 'Origins of the CSIRO Booroola' and included the postulate about the Bengali origin of the gene. Between these two workshops dedicated to *FecB*, another two workshops on major genes in sheep were held in Toulouse, France, in 1990 and 2003, at which advances in understanding of *FecB* were also presented. Major developments over the 28 years since the first workshop include the discovery of the single gene origin of the fecundity effect, improved understanding of the physiological basis of the effect and the reproductive and economic consequences of this, and the development of methods for accurate genotyping of animals at the *FecB* locus, which culminated in the development of a direct DNA test. The *FecB* gene has spread from the Booroola Merino to some 40

breeds of sheep worldwide. It is now also spreading from the Garole, Hu and Han breeds in India and China, from one of which it probably originated.

The workshop was attended by approximately 100 delegates and speakers from 13 countries and 9 Indian states. Invited speakers were largely drawn from researchers from around the world involved in major projects on the introgression of the *FecB* gene. The aim was that these proceedings would summarise our current understanding of the *FecB* mutation, with special emphasis on the biological and economic consequences of its use in new breeds for commercial reasons. These proceedings are testament to the achievement of this goal. They also include a summary of a panel discussion on recommendations and strategies for a wider introgression of the *FecB* gene into the Indian sheep population.

These proceedings will be an invaluable resource for those involved in commercial use of the *FecB* mutation in any country. ACIAR is proud to have been centrally involved in much of the research reported at the workshop, and in its sponsorship and publication. The ACIAR Indian projects have provided some of the clearest evidence of successful commercial use of the *FecB* gene, and it can be expected that this will contribute to a substantial improvement in sheep productivity and smallholder sheep owners' incomes in India.

Nick Austin
Chief Executive Officer
ACIAR

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Abbreviations

AGBU	Animal Genetics and Breeding Unit
AI	artificial insemination
BC	backcross
BL	Booroola Leicester
BLUP	best linear unbiased prediction
BM	Booroola Merino
BMP	bone morphogenetic protein
BMPR	bone morphogenetic protein receptor
BMP-6	bone morphogenetic protein-6
CL	corpora lutea
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSWRI	Central Sheep and Wool Research Institute
DM	dry matter
EBV	estimated breeding value
EE	ewe efficiency
EPE	ewe productivity efficiency
F1	first cross
FecB	gene notation—*Fec* for fecundity and *B* for Booroola
$FecB^B$	the *FecB* allele promoting higher fecundity at this locus
$FecB^+$	the *FecB* wild-type allele
FF	$FecB^{BB}$ genotype
F+	$FecB^{B+}$ genotype
++	$FecB^{++}$ genotype
FIG-α	factor in the germline alpha
FL	Finnish Landrace
FSH	follicle stimulating hormone
GC	granulosa cell
GDF-9	growth differentiation factor-9
GMM	German Mutton Merino
Gn	gonadotrophin
GnRH	gonadotropin-releasing hormone
HJ	St Croix hair sheep (H) × JTT (J)
HMJ	HJ × MJ
IC	intercross
IOR	induced ovulation rate
ITT	Indonesian Thin Tail
JTT	Javanese Thin Tail
LB/L	lambs born per lambing
LH	lutenising hormone
LS	litter size
MA	Mérinos d'Arles
MAI	marker-assisted introgression
MARC	Meat Animal Research Center (Nebraska)
MAS	marker-assisted selection
MHJ	MJ × HJ
MJ	Mouton Charollais (M) × JTT (J)
mRNA	messenger ribonucleic acid
NARI	Nimbkar Agricultural Research Institute
NCL	National Chemical Laboratory (Pune, India)
NLW	number of lambs weaned
NOBOX	newborn ovary homeobox (gene)
OR	ovulation rate
PCR–RFLP	polymerase chain reaction–restriction fragment length polymorphism
PT	pregnancy toxaemia
QTL	quantitative trait locus
SE(M)	standard error (of the mean)
SL	sustainable livelihoods
STT	Sumatran Thin Tail
TC	theca cell
UTR	untranslated region

Acknowledgments

We are grateful to ACIAR and the Australian Academy of Technological Sciences (ATSE; International Science Linkages – Science Academies Program) for their major financial support for the workshop. We are also grateful for the financial support from two Indian Government institutions, namely the Department of Biotechnology and the Department of Science and Technology. Furthermore, we acknowledge financial support from the National Bank for Agriculture and Rural Development, Nimbkar Seeds Private Limited, the Nimbkar Agricultural Research Institute (NARI) and a number of other small businesses and individuals who contributed.

During the workshop we were assisted by many volunteers from Dr Gupta's team at the National Chemical Laboratory in Pune, and this organisation also kindly provided their facilities to host the workshop. We also thank the team at NARI, Phaltan, for hosting us during the workshop, and Ms Ilona Schmidt for her work on development and maintenance of the workshop website.

We especially thank Dr John Copland who, for many years as Research Program Manager for ACIAR, provided dedicated support and encouragement to project teams in India and Australia.

Most importantly we wish to acknowledge the work of Dr Helen Newton Turner (1908–95), one of Australia's foremost geneticists involved with sheep breeding and in whose honour these proceedings are named.

Helen Newton Turner, a remarkable scientist: the story of her indirect contribution to the Indian sheep industry

Bonbehari V. Nimbkar[1]

This workshop has been called the Helen Newton Turner Memorial International Workshop on 'Use of the *FecB* (Booroola) gene in sheep-breeding programs'. Her name is familiar to the fraternity of scientists worldwide who have been involved in the improvement of this valuable animal. She was born in 1908 in Sydney, Australia, and died in 1995. We are fortunate that she left behind her travel memoirs in a 380-page volume entitled 'And yonder lies', which consists of travel notes from 1939 to 1982. There is hardly a country in the world that kept sheep that she did not visit. She was called to almost every part of the world as a consultant to improve the quantity and quality of wool. She was called because of her direct and significant contribution to the growth and wellbeing of the Australian wool industry. Although it was substantial, she, being a modest woman, would have said, 'It wasn't me, it was my team'.

In her travel memoirs she gives vivid descriptions of the lands and people that she visited and met, and comments on the sheep industries of those countries. The second chapter of her memoirs, entitled 'Why sheep?', describes in a nutshell the development of sheep over the centuries.

She mentions that she realised the importance of prolificacy to the Australian sheep industry and the fact that it had probably come from Bengal sheep. She has also described her efforts at tracing the origin of the Booroola gene, which probably came from two importations of Bengal sheep into Australia in the late 18th century.

After I realised the importance of prolificacy in the development of the sheep industry and her role as a sheep geneticist, I wrote to her in 1991 for advice as to where I could obtain a source of prolificacy for introduction into India. I had earlier visited Cyprus and Israel looking for a possible breed for this purpose, but the Chios and Awassi breeds were not recommended as suitable for India. Helen Newton Turner replied promptly to my letter, in which I had also asked her why she had not recommended prolificacy to the Indian Council of Agricultural Research during one of her four visits to India between 1954 and 1981. In her indignant reply she said she certainly had, and asked me to refer to her article in the Indian Journal of Animal Genetics and Breeding [1(1)] of January 1979.

[1] Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India

In this letter she also suggested that I look for Bengal sheep, which she thought was the source of the Booroola gene for prolificacy in the Booroola Merino. Following her advice, I sent Dr P.M. Ghalsasi to West Bengal in 1992, where he located the prolific Garole and brought back to Phaltan two ewes and two rams. When I wrote and told this to Dr Turner she was delighted, and asked a colleague who happened to be in India at that time to visit Phaltan and have a look. This resulted in ACIAR project AS1/1994/022, which gave rise to a subsequent project (AH/2002/038) and ultimately this workshop.

What was not mentioned, but which I knew, was that the sheep breeders in India were obsessed with the idea of developing a fine wool industry based on an Indian Merino. They have only just realised the futility of this effort after 60 years of failure in trying to achieve this. I am happy to report that they are at last concentrating on meat production, and in this effort I am confident that the *FecB* gene will play a vital role.

Overview—workshop background, objectives and summary

S.W. Walkden-Brown[1], J.H.J. van der Werf[1], C. Nimbkar[2] and V.S. Gupta[3]

Background

The idea of holding this workshop arose during the 2007 project coordination meeting for ACIAR project AH/2002/038 'Improved productivity, profitability and sustainability of sheep production in Maharashtra through genetically enhanced prolificacy, growth and parasite resistance', and was enthusiastically embraced by all those present. We felt it would be the culmination of more than a decade of research in India on improved meat sheep production supported by ACIAR.

This work had a strong focus on genetic improvement of reproductive rate, and was initiated in 1993 at the Nimbkar Agricultural Research Institute (NARI) at Phaltan, near Pune, with the purchase of prolific Garole[4] sheep from the Sunderbans regions of West Bengal. This purchase had come about following correspondence between Mr Bon Nimbkar, the founder of NARI, and Dr Helen Newton Turner, the celebrated Australian sheep geneticist and advocate of genetic improvement of reproductive rate in sheep, which had commenced in 1991. Dr Newton Turner was unable to visit NARI at the time of the Garole purchase, but a colleague working on an ACIAR livestock project in India at the time was able to do so. From this start, with some seed funding from ACIAR to investigate sources of Garole sheep and involvement of molecular geneticists in Dr P.K. Ranjekar's group from the National Chemical Laboratory (NCL) in Pune, a new project was born.

That project, ACIAR AS1/1994/022 'Prolific worm-resistant meat sheep for Maharashtra, India', commenced funding in 1998, shortly before Douglas Gray took up a new position and passed the project leadership on to Dr Steve Walkden-Brown at the University of New England, Armidale, Australia. Between 1998 and 2008 there was an unbroken period of ACIAR support for the work through an extension to the original project, allowing a new project (AH/2002/038) with an extension of funding to be set up. During this period it was established that:

- the prolificacy of the Garole breed was due to the *FecB* mutation, which appeared to be a fixed trait in the breed
- the Garole and its first-cross offspring were not suitable for the local sheep-raising system
- the Garole and its crosses exhibited strong genetic resistance to gastrointestinal helminthosis
- crossbred ewes carrying the *FecB* mutation had increased prolificacy, but increases in ovulation rate (OR) and litter size (LS) were generally lower than reported elsewhere. The increases appeared manageable under traditional management practices and of economic benefit to smallholder sheep owners.

It is this final outcome, based on extensive work with participating sheep owners since 2003, that was the major stimulus for holding this workshop. The other important consideration was the enormous growth in knowledge about *FecB* since the only other major scientific meeting dedicated to it, the original workshop on 'The Booroola Merino' held

[1] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia
[2] Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
[3] Division of Biochemical Sciences, National Chemical Laboratory, Pune, Maharashtra, 411 008, India
[4] In these proceedings sheep breed names are capitalised for consistency and according to convention.

in Armidale (New South Wales, Australia) in 1980. The intervening 28 years had seen a considerable evolution in our understanding of, and attitude to, *FecB*. The major phases in this evolution, which overlapped considerably in time, are summarised as:

- initial enthusiasm and expectation of practical application, mainly in developed countries
- lack of widespread use by industry in these countries due to excessive LS and lack of practical methods for accurately genotyping animals at the *FecB* locus
- focus instead on basic research into the mode of action of *FecB*, culminating in identification of the causative mutation and resultant direct DNA test in 2001
- subsequent use of direct genotyping to reveal the presence of *FecB* in native sheep breeds in India and China, which clearly pre-dates its appearance in the Australian Booroola Merino. In some of these breeds *FecB* appears to be a fixed trait with apparent utility
- successful introgression of *FecB* into non-fecund Deccani sheep in India using genotype-assisted selection. This has not resulted in the excessive LS experienced elsewhere and is associated with positive effects on biological and economic efficiency.

Some of the earlier developments over this 28-year period were reported at the 2nd International Workshop on Major Genes for Reproduction in Sheep a decade later (1990) in Toulouse, France, and at the International Workshop on Major Genes and QTL in Sheep and Goats in 2003, again held at Toulouse. However, it was now timely to hold a second international workshop dedicated to *FecB*. Naming it in honour of Dr Helen Newton Turner is testament to her central role in its discovery in Australia, and her role, with Mr Bon Nimbkar, in initiating the Indian research that culminated in this workshop.

Objectives and workshop structure

Pune was chosen as the workshop location as it is close to the ACIAR research project site at NARI, and NCL, a project partner, offered its excellent auditorium and guesthouse to hold the workshop. Pune sits on the Deccan Plateau, an area where some 20 million Deccani sheep in the three states of Maharashtra, Karnataka and Andhra Pradesh play an integral role in the agricultural systems.

The specific objectives set for the workshop were to:

- review current knowledge of *FecB* and its worldwide application in sheep breeding
- present the key results of ACIAR projects related to *FecB* in India during 1998–2008
- assist Indian Government policymakers to formulate policy regarding the wider dissemination of *FecB* in the national flock
- consider the implications of the workshop findings for countries other than India.

The workshop was organised into four invited speaker sessions, a poster session for short proffered papers and a final panel discussion on 'The policy implications for wider dissemination in India of sheep containing the *FecB* gene'.

On the second day of the workshop participants travelled 110 km from Pune to Phaltan to visit the NARI research site and the flock of a collaborating smallholder sheep owner, Mr Dattatray Sopan Pisal, into which *FecB* carrier rams had been used since 2003. There are now more than 25 *FecB* carrier ewes in this flock and Mr Pisal has retained two *FecB* carrier rams born in his flock for use as breeding rams.

Invited papers summary

The invited speaker sessions covered the spectrum from the scientific aspects of the mechanism of action of the gene to the mechanics, experience, constraints and advantages of its practical use. These sessions were:

1. Background and history of the *FecB* mutation (2 papers)
2. Physiological aspects of the *FecB* gene mechanism (4 papers)
3. Case studies on introgression of *FecB* in local breeds (8 papers)
4. The way forward—introgressing *FecB* in the wider population (5 papers).

Session 1: Background and history of the *FecB* mutation

The paper by Dr George Davis provides a comprehensive review of the origin, distribution, use and management of the *FecB* mutation. It details the initial discovery in Australia; the subsequent period of intense research in Australia and New Zealand; the wide dissemination to other developed countries; the almost simultaneous discovery of the *FecB*

mutation in New Zealand, France and the UK in 2001; and the exciting studies into the origin of the mutation that this discovery enabled. *FecB* is present only in a small number of the world's prolific sheep breeds, and in only two breeds—the Indian Garole and Chinese Hu—is it fixed in the homozygous state. The mutation is likely to have originated in one of these breeds, or possibly in both independently. Interest in the use of *FecB* has seen a major shift to Asia, where several crossbreeding and introgression efforts have been in progress for some time. The paper makes an additional important contribution to the workshop by clearly identifying the main determinants of success and failure in the use of *FecB*.

The results of a questionnaire survey on management practices, production and current status of Garole sheep are presented in the paper by Prof. Subhransu Pan and Prof. A.K. Sahoo. The survey was conducted over a year in 60 randomly selected villages in the breeding tract, covering about 2,600 sheep farmers and their 10,000 sheep. The paper provides a comprehensive description of the breed and its management in its native region. Mean LS ranged from 1.63 at first parity to 1.94 at third parity. Garole sheep numbers stopped increasing in the decade 1994–2003 and the breed may now be under threat, probably due to loss of grazing resources. Interestingly, the word Garole is a colloquial Bengali term for 'stupid'. The earlessness observed in the Garole (and the Hu—discussed in the Davis paper) is noted and sheep exhibiting this trait have a special name of their own.

Session 2: Physiological aspects of the *FecB* gene mechanism

The paper by Prof. Bruce Campbell and colleagues reviews current understanding of the mechanism of action of the *FecB* mutation. The *FecB* mutation is a single point mutation in the intracellular domain of the bone morphogenic protein (BMP) receptor 1B gene (BMPR1B). In practice this leads to precocious maturation of ovarian follicles and deregulation of normal follicular selection processes. It is now clear that the increase in fecundity associated with *FecB* is not due to increased FSH secretion, but to increased sensitivity to the actions of FSH mediated by intra-follicular factors, with a major role for those associated with the BMP. These may be augmenters (e.g. BMP-6) or attenuators (e.g. BMP-15, AMH) of gonadotrophic action. Complete understanding of the mechanisms involved remains elusive.

Dr Varsha Pardeshi and colleagues discuss aspects of the genetic and environmental modulation of *FecB* gene expression. The approach taken is to review the variation in OR and LS associated with *FecB*. They also examine the potential role of environmental factors, maternal effects and genetic influences such as background breed effects, the presence of other quantitative trait loci (QTL), or secondary non-reproductive effects of the BMPR1B mutation. The authors conclude that at least some of the observed variation in expression may be associated with genetic factors such as modifier genes or novel mutations within the BMPR1B mutation.

Dr Neal Fogarty's paper on environmental modulation of *FecB* expression reviews the effects of *FecB* on production traits in a range of genetic comparisons, environments and production systems. The author concludes that the effect of *FecB* on reproduction traits following introgression into many breeds is remarkably consistent, with each copy increasing OR by 1.1–2.0. LS increased by 0.5–1.3 with the first copy and by little more with the second copy, apart from Chinese and Indian breeds in which a further increase of about half of the initial effect was observed with the second copy. Nutritional effects on OR do not differ between *FecB* carriers and non-carriers. There is scope for using reduced nutrition at mating to reduce OR in *FecB* carriers, and increased nutrition at later stages to augment lamb weight and survival in carriers.

The prenatal and postnatal consequences for foetal physiology of multiple ovulation and large LS are reviewed by Dr Geoff Hinch. LSs greater than 2 present a significant physiological challenge in sheep and the resultant increases in embryo loss and later foetal mortality are very difficult to alter. This is probably due to the limited number of uterine attachment sites for the placenta. Adverse effects carry over into lamb birth weight and survival, and even the best nutritional strategies can only partly ameliorate them. This paper clearly demonstrates the practical limits of dealing with multiple birth lambs, particularly under natural grazing conditions.

Session 3: Case studies on introgression of *FecB* in local breeds

In this session there were eight papers that discussed the biological and economic conse-

quences of the presence or introgression of *FecB* in different breeds from different countries. As reported in these papers, the experience to date in Australia and the USA has largely been negative; in France and Indonesia there is some cause for optimism; while in India, China and Israel the experience has largely been positive.

The paper by Dr Chanda Nimbkar and colleagues details the generally positive consequences of *FecB* introgression into Deccani sheep from the Garole since 1998. In crossbred Deccani sheep (> 75% Deccani) at NARI one copy of *FecB* resulted in an increase in OR from 1.1 to 2.1, and in LS from 1 (808 ewes) to 1.58 (447 ewes), slightly more than the 1.42 (167 ewes) observed in collaborating smallholder flocks. Based on a limited number of observations, a second copy of the gene increased LS at NARI to 1.65 (45 ewes), suggesting that dramatic increases in LS are unlikely in homozygous ewes. In smallholder flocks, LSs at 3 months were 1.2 and 0.9 for carrier and non-carrier ewes, respectively, producing an increase in gross margin of 37–50% for *FecB*-carrier ewes.

The experience with introgression in the Australian Merino sheep, as reported by Prof. Steve Walkden-Brown and colleagues, provides a sobering contrast. Despite being present in Australia for more than 200 years, the *FecB* gene has had a negligible impact on sheep production at a commercial level and is unpopular, due largely to excessive LS and subsequent lamb mortality. The authors cite recent studies in which the three *FecB* genotypes can be compared in animals of the same genetic and environmental background in a commercial environment. They suggest that the heterozygous carrier produces sufficient additional lambs weaned (26%) to be economically viable (albeit with excessive lamb mortality), but that the homozygote is commercially disastrous, exhibiting significantly lower conception rates and lamb survival rates despite similar LS as the heterozygote. These effects appear to be due to the excessive OR of the homozygote.

Dr Amrit Lal Arora and colleagues describe the second significant *FecB* introgression project in India—that into the Malpura sheep of Rajasthan, again from the Garole. In Malpura crosses in an institutional flock, LSs at birth were 1.01, 1.71 and 1.83, respectively, with 0 (69 ewes), 1 (187 ewes) or 2 (12 ewes) copies of *FecB*, which translates into LSs at 6 months of age of 0.84, 1.36 and 1.33 respectively. These values are similar to but somewhat higher than those observed at NARI. The negative effects of the tiny Garole breed on body weight are evident in the first-cross animals and it is desirable to undertake further backcrossing with the Malpura to reduce this effect. This report is limited to data from an institutional flock.

The experience of *FecB* introgression into Awassi and Assaf sheep in Israel, commencing in 1986, is reported in comprehensive detail by Dr Elisha Gootwine. Apart from central institutional flocks, rams of both breeds carrying *FecB* (Afec in the author's terminology) have been quite widely disseminated to industry. The improved genotypes have been most successful to date in fully intensive dairy and non-dairy production systems with artificial rearing of lambs. As in the Australian experience, the heterozygote is more productive than the homozygote carrier due to higher lamb survival despite similar LS. Typical LSs of the Awassi are 1.31, 1.90 and 1.92 for 0, 1 and 2 copies of *FecB* respectively; equivalent values for the Assaf are 1.68, 2.40 and 2.55 respectively. *FecB* is generally associated with reduced birth weight, growth and milk production but remains economically viable for intensive systems.

The paper by Dr David Notter and colleagues on the biological and economic consequences of the *FecB* mutation in the USA reveals very limited uptake and a generally negative experience. Early studies following the introduction of the Booroola Merino showed that increases in OR and LS were roughly additive to the underlying polygenic effect in the crossbred ewes under observation, but weaning rates were not improved and growth of lambs was inferior. With an abundance of sources of polygenic fecundity in the USA, it appears that the level of prolificacy conferred by *FecB* is excessive relative to that desired by producers.

Dr Ismeth Inounu and Ms Atien Priyanti paint a more positive picture of *FecB* in Indonesian Thin Tail (ITT) sheep in Java, Indonesia, than that reported under the extensive production systems in the USA and Australia, but with some caveats (as was the experience in Israel). The Indonesian production system is semi-intensive, more like that in India. Under Indonesian conditions when nutrition is good, ewes carrying one copy of *FecB* exhibit the highest gross margin, followed by homozygotes then non-carriers. Under conditions of poor nutrition there was no economic benefit of *FecB* at all. Typical LSs of the ITT sheep are 1.22,

2.02 and 2.5 for 0, 1 and 2 copies of *FecB*, respectively, with respective lamb survival to 90 days being 84.3%, 71.9% and 59.2%.

The paper by Ms Hua Guohua and Prof. Yang Liguo, on the biological and economic consequences of the *FecB* mutation in Chinese breeds of sheep, comprehensively reviews the gene and allele frequency of *FecB* in Chinese sheep breeds and summarises the LS data available. *FecB* is almost fixed in the Hu breed and is present in some other prolific Chinese breeds, including the Small Tail Han, Cele, Duolang and the prolific strain of the Chinese Merino. There are suggestions that other major genes for fecundity may also be segregating in the Hu and Small Tail Han breeds. *FecB* is relatively widespread in Chinese sheep and there has been intense research activity into it since the advent of the direct DNA test for it. Reported LSs from the various studies generally exceed 2 for heterozygote carriers (1.92–3.53) and are somewhat higher (in the range 2.47–3.67) in the homozygote.

The final paper in the session, by Dr Jacques Teyssier and colleagues, reports on the biological and economic consequences of introgressing the *FecB* mutation into the French Mérinos d'Arles sheep in France. Introgression into this breed was a formal process that commenced in 1983. As is the case in Australia and the USA, widespread uptake of *FecB* by industry has been low. This paper reports on datasets collected from both an institutional farm and a commercial producer. There was a clear economic advantage for *FecB* heterozygote ewes based on consistent production of 50–65% additional lambs for sale or replacement. However, under the French system the homozygote carrier is undesirable, and comparatively complex breeding systems are required to use only the heterozygote.

Clear themes emerging from this session include the following:

- *FecB* carriers perform better in intensive and semi-intensive production systems where individual ewe and lamb care and nutrition can be provided. Success under fully extensive grazing systems without housing is unlikely.
- In no case was the homozygous carrier ewe considered superior to the heterozygote ewe. In India the two may not differ greatly, and in Israel the homozygote continues to confer advantage over the non-carrier but is inferior to the heterozygote. Results from Australia and Israel showed that the reproductive performance of homozygous ewes was inferior to that of heterozygous ewes due to lower fertility and/or lamb survival at equivalent litter size. These effects are likely to be the consequence of greater embryonic and foetal competition and losses in utero due to a greater disparity between ovulation rate and uterine capacity in homozygous ewes. In Israel there is also evidence of direct negative effects of the homozygous state in lambs, and direct effects in ewes have also been postulated.

Session 4: The way forward—introgression of *FecB* in the wider population

The paper by Prof. Julius van der Werf describes the genetic aspects of introducing a major gene from a donor breed into a commercial recipient breed. The efficiency of introgression is markedly influenced by merit of the donor population relative to that of the commercial population—overall merit, not just merit for the trait being introgressed. Generally, several generations of backcrossing will be required before intercrossing to maximise the merit of the intercross. It is generally most profitable to cross females from backcross generations with males from the recipient breed. Marker-assisted selection (MAS) can significantly improve the efficiency of an introgression program, particularly for traits that are difficult to measure, such as *FecB* genotype.

Dr Andrew Swan reviews the economics of LS in meat sheep. Economic analysis of sheep meat production systems generally shows that LS has a significant impact on profitability; however, this comes at the expense of higher costs associated with increased feed requirements for ewes during pregnancy and lactation and for finishing larger numbers of lambs. Poorer lamb survival in large litters also has an impact on costs. For these reasons the economic value for LS should be determined from a realistic bioeconomic model that accounts for the relationship between LS, feed cost and lamb survival in addition to other economically important traits. This is particularly important when evaluating the importance of LS in harsh environments.

The potential pathways and strategies for wider use of the *FecB* gene in Maharashtra State and other parts of India are discussed by Dr Chanda Nimbkar and colleagues. Results in NARI's and smallholder sheep owners' flocks indicate that it would be worthwhile to introgress the *FecB* gene into Deccani sheep more widely in other parts of Maharashtra State as

well as into other suitable Indian sheep breeds. 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 superior for key economically important traits. Introgression is a process requiring at least three generations of backcrossing and would require excellent institutional infrastructure, including a network and extension program among local sheep owners in the surrounding region. The steps to be followed in a successful introgression are described.

The paper of Dr Julian Prior and colleagues reports on smallholder sheep owners' views on the value and management of Deccani crossbred *FecB*-carrier ewes with a higher twinning percentage, and the implications for a future introgression extension program. The results of a socioeconomic survey of collaborating shepherds in the ACIAR sheep projects are presented. The survey of the 25 case study sheep owners suggests that their view of the introduction of twinning lambs into their flocks was generally positive. There was universal recognition among the sheep owners of the need for supplementary feeding and additional management. Twin lambs were not associated with perceptions of increased risk. There was, however, some resistance to the Garole phenotype of the *FecB*-carrier rams. Areas of further research required are described.

Dr Karen Marshall and colleagues introduce the sustainable livelihoods framework as a useful tool in translating animal-breeding research into livelihood improvements for the world's rural poor. This framework recognises the interacting components of assets, activities, vulnerability context, institutional context and livelihood outcomes. It provides a way of thinking about livelihoods and prompts users to ask the right questions in the design and implementation of potential interventions. While the framework is well recognised and used by researchers and development organisations supporting agricultural endeavours such as cropping, the same does not hold for animal breeding. It is proposed that the framework can be similarly used for animal breeding, and that its application will lead to a greater proportion of animal-breeding development interventions being successful, both in terms of impact and sustainability.

Clear themes emerging from this session include the following:

- Introgression strategies need to be carefully planned, taking into account the closeness in merit (or desirability) between the source and target populations and the benefits of MAS. Attempts to disseminate animals prior to sufficient backcrosses to the target population are likely to be counterproductive.
- The economics of increasing reproductive rate for meat production is complex, and simplistic analysis should be avoided.
- A great deal has been learned from the experience of introgressing *FecB* into Deccani sheep by NARI and this experience should be utilised. The positive perception of *FecB* by Indian smallholder sheep owners represents a very rare example of a successful marker-assisted introgression of a livestock production trait.
- The socioeconomic aspects of introgressing a novel sheep genotype are also important and complex. The papers in this session make important recommendations in this regard.

Poster papers summary

Nine one-page poster papers, presented in a dedicated session at the workshop, are included in the proceedings.

Dr Albert Visscher from the Netherlands presented data on the effect of the Booroola gene on meat production efficiency in Texel sheep. Heterozygote ewes provided an economic benefit of about € 50 per ewe due largely to increased litter size. Progeny showed some improved muscle traits but were also significantly fatter.

Dr Moh'd Faruque outlined the distribution, morphology and genotypes of Garole sheep in Bangladesh, where there is a small population of some 40,000 in the extreme south-western corner. Preliminary studies show that the Garole reaches puberty earlier, has more twin lambs, and shows greater resistance to internal parasites and greater tolerance of saline drinking water than other Bangladeshi sheep breeds.

Dr Elzbieta Martyniuk outlined the experience with use of Booroola sheep in Poland. The crossbreeding of Polish Merino (PM) ewes with Booroola (B) rams resulted in a substantial increase in the ovulation rate and litter size in F1 ewes. However, despite encouraging results obtained in the two-stage crossbreeding of PM with B and Suffolk sires, the Booroola sheep did not gain popularity in Poland. In the context of prime lamb production for export, the lower body weight and very poor conformation were responsible for rejection of the breed.

Dr Ran Vir Singh from the Indian Veterinary Research Institute, Uttar Pradesh, India, reported on an investigation into genetic polymorphism of the *FecB* gene in Garole and Muzaffarnagari sheep carrying the mutation. An A-G transition was observed in Garole sheep and a C-A transition in both Garole and Muzaffarnagari sheep. On phylogenetic analysis, it was found that the *FecB* gene in Garole sheep showed a very high percentage of identity with that of other sheep and the Muzaffarnagari breed.

Dr Singh also provided a preliminary report on detection of the *FecB* gene in Muzaffarnagari sheep. Nine of 32 ewes were found to carry the gene, with no increase in litter size, which remained at 1.00. He acknowledged the need for this unexpected finding to be confirmed by genotyping at several laboratories and determination of ovulation rates or litter sizes of genotyped ewes over several parities.

Dr Satish Kumar of the Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India, reported identification of the Booroola mutation in Kendrapada sheep of Orissa, India. This breed, which is found about 400 km from the Sunderbans of West Bengal (home tract of the Garole) produces 62.8% twins and 2.3% triplets. Of 65 sheep tested, 27 were homozygous for *FecB* (BB), 30 were heterozygous (B+) and 8 were non-carriers (++). As the Kendrapada is much larger in size than the Garole (23–27 kg mature weight), it provides an attractive alternative source of the *FecB* gene.

Prof. Steve Walkden-Brown presented results of an experiment in Australia on the use of nutritional restriction at mating time to dampen the reproductive performance of *FecB*-carrier Merino ewes over 2 years. Both conception rate and ovulation rate were reduced by the feeding restriction (R). Litter size was also reduced by R when expressed per ewe scanned, but not per ewe in lamb. Lamb losses between scanning and weaning were significantly lower in the R treatment, in line with the objective of the experiment. However, the marked reduction in conception rate seen in ewes in the R treatment makes this approach unsatisfactory.

Dr Ramanuj Banerjee from Kolkata, India, presented data on the presence of the *FecB* mutation in three Indian breeds of sheep, including the Garole in and away from its native tract. The mutation was found in the Garole and Shahabadi but not the Muzaffarnagari breed. The higher percentage of mutant homozygotes in the Garole population than the Shahabadi suggests an onward transmission of $FecB^B$ from Garole to Shahabadi, both breeds being found in close geographical proximity. Increased mean litter size in heterozygote Muzaffarnagari × Garole F_1 crossbreds (mean litter size 1.42) is a further demonstration of the potential to increase reproductive rate by the introduction of *FecB* to breeds of low fecundity.

Dr Pradip Ghalsasi of the Nimbkar Agricultural Research Institute, Maharashtra, India, presented the results of a socioeconomic study of smallholder sheep rearers in Phaltan Taluka, Dist. Satara, Maharashtra, India. This survey was conducted in 2001–03 as a prelude to initial distribution of *FecB*-carrier sheep to sheep owners' flocks. The survey of about 100 sheep-owner families revealed low literacy and socioeconomic status, with the major factors preventing improvement of their economic conditions being continuous reduction of grazing areas, high incidence of disease among the animals and lack of marketing facilities. The survey also revealed that sheep owners valued prolificacy as a trait in their sheep and had established methods for dealing with multiple-birth lambs.

Panel discussion summary

At the end of the workshop there was a 2-hour panel discussion on the policy implications for wider dissemination in India of sheep containing the *FecB* gene arising from the ACIAR projects.

This gave broad support for the view that it is beneficial to increase fecundity in most meat sheep production systems, with the value of the benefit depending on the environment and management system. It was agreed that the benefit is likely to be highest where the current level of fecundity is low (around 100% lambing rate), and that an increase of up to 160% in lambing rate is likely to be the most profitable. It is also likely to be of more benefit in settled farming than migratory systems and in areas of reasonable feed supply. Dissemination of appropriate genotypes is critical, and the objective should be the efficient introgression into existing major breeds via backcrossing rather than the creation of new breeds or dissemination at a very early stage of backcrossing.

The ACIAR project experience with collaborating smallholder sheep owners indicates that heterozygous genotype is advantageous under flock owners' conditions, so introgression of *FecB* as a

means of increasing reproductive rate is feasible. It was recognised that the homozygous *FecB* carrier genotype has been undesirable in many cases, but preliminary results at NARI indicate that this is not the case to any significant extent in the Deccani crosses. However, there is clearly a need to confirm this as further evidence becomes available.

It was agreed that the uncertainty about the homozygous genotype should not be an impediment to commencing wider dissemination of *FecB* using carrier rams. It was also recognised that sufficient momentum for this idea had already gathered and that it would be difficult to stop. The consensus was that the dissemination/introgression process is unlikely to be the subject of an overarching all-India project but will be the result of disparate initiatives all over India.

We hope that these proceedings will be a valuable reference tool for those involved in this process.

Session 1:
Background and history of the *FecB* mutation

The Booroola gene: origin, distribution, use and management of the *FecB* mutation

G.H. Davis[1]

Abstract

This paper reviews evidence that the *FecB* mutation identified in Booroola sheep in 2001 originated from the Garole breed in West Bengal. *FecB* has now spread to 48 breeds and composites in 19 countries, mainly through the Booroola Merino. Breeding programs for using *FecB* in commercial flocks and the implications of increased litter sizes are discussed. It is concluded that, for most management systems, access to DNA testing in ram breeding flocks will be necessary to effectively use *FecB*.

Origin

The *FecB* mutation was first identified in Booroola Merino (BM) sheep originating from Australia, but recent DNA marker technology has revealed that its origins can be traced back to sheep in Asia. In order to clarify the Asian origins, it is perhaps useful to first examine the BM. The BM was named by Dr Helen Newton Turner after the property 'Booroola', located at Cooma in New South Wales, Australia, which is where brothers Jack and Dick Seears had identified a line of highly prolific Merinos descending from a ewe in the main flock that had triplets. In 1958 they donated a ram to the Commonwealth Scientific and Industrial Research Organisation (CSIRO), and in the same year CSIRO purchased 12 ewes born as triplets or quadruplets (Turner 1978). The purchased ewes were selected by Dr Newton Turner, in whose honour this workshop is being held. From this foundation, plus a second ram donated in 1959 and a further purchase of 91 ewes in 1965, CSIRO embarked on a research program studying the genetics of the BM. The mean litter size (LS) of the Booroola flock over the 3 years 1977–79 was 2.29, compared with 1.22 for the control (Piper and Bindon 1982).

At a workshop in Armidale, Australia, in 1980, Dr Newton Turner speculated that the high prolificacy of the BM could be traced back to either the Bengal or Cape sheep imported into Australia in the late 18th century (Turner 1982). Her meticulous research through historical records of the Australian Stud Merino Flock Register revealed that the Egelabra strain of Merinos to which the BM belongs could be traced back to the flock of Samuel Marsden, who was one of the early owners of large flocks of both Bengal and Cape sheep. The first shipment of 12 of the so-called Bengal sheep arrived in Australia from Calcutta in 1792, followed by an illegal shipment of 100 in 1793. Early descriptions of these diminutive sheep fit the phenotype of the Garole breed (Ghalsasi and Nimbkar 1993) from the coastal belt of the swampy Sundarbans delta of West Bengal near Calcutta.

A BM research program was also established at the Tara Hills High Country Research Station in New Zealand following the donation of three rams from CSIRO in 1972. In 1979 ovulation rates (ORs) of BM progeny in commercial flocks in New Zealand revealed that some rams were not passing on the high prolificacy trait to their daughters (Kelly

[1] Invermay Agricultural Centre, Private Bag, Mosgiel, New Zealand; george.davis@agresearch.co.nz

et al. 1980). At the 1980 Armidale workshop Drs Laurie Piper and Bernie Bindon proposed that the high prolificacy of the BM might be due, in part, to a single major gene or closely linked group of minor genes (Piper and Bindon 1982), which would explain why some Booroola rams did not pass high prolificacy to their progeny. It would also explain how the Seears brothers had maintained a prolific flock, where they had only selected for prolificacy on the dam side. The findings of Piper and Bindon (1982) were based on the frequency of triplet litters among CSIRO's 14 foundation ewes and their 19 daughters. Subsequent analysis of the records of OR and LS of 459 Booroola ewes at the Tara Hills High Country Research Station provided very strong evidence of a segregating major gene that had an additive effect on OR and a partially dominant effect on LS (Davis et al. 1982). Segregation criteria of at least one record of OR ≥ 3 and at least one record of OR ≥ 5 were proposed to identify heterozygous and homozygous ewes respectively. These phenotypic criteria were widely used until accurate genotyping was possible following the identification of the *FecB* mutation in 2001 and the establishment of a commercial DNA testing service by the *Genomnz*™ laboratory at Mosgiel, New Zealand.

Following wide trans-Tasman consultation, the gene was initially named *fecundity* and the symbol *F* was assigned to the locus and allele (Davis et al. 1982). Later, when it became apparent that there were other alleles with major effects on fecundity in sheep, the locus became known as *Fec* and the allele was renamed *Booroola*, with the symbol changed to *FecB* (Lauvergne et al. 1996).

The almost simultaneous discovery of the *FecB* mutation in New Zealand (Wilson et al. 2001), France (Mulsant et al. 2001) and the United Kingdom (Souza et al. 2001) provided the tool for the controlled use of *FecB* in breeding programs through a commercial DNA test. The point mutation (Q249R) is in the BMP-1B receptor on chromosome 6. The availability of a DNA test also provided the means to further investigate Dr Newton Turner's theory about the origins of prolificacy in the BM. DNA extracted from a few drops of blood could be used to genotype an animal for *FecB* and, as the test was based on a direct marker, no pedigree information was needed. DNA was collected from Garole sheep and the tests confirmed the presence of the *FecB* mutation (Davis et al. 2002). The high frequency of the mutation among the sheep tested suggested that it was fixed (i.e. all sheep were homozygous carriers of *FecB*) in some Garole populations. Bradford and Inounu (1996) had suggested that *FecB* might also be segregating in prolific Javanese Thin Tail (JTT) sheep. Accordingly, samples from these sheep were also tested. The results showed that JTT sheep were a mixture of heterozygous and homozygous carriers of *FecB* (Davis et al. 2002). These findings support the theory that *FecB* was introduced into Australian flocks in the 1790s from Garole sheep imported from India, and subsequent crossbreeding with Merino sheep ultimately led to the formation of the BM. It is also probable that JTT sheep inherited *FecB* directly from the Garole breed, as Bradford and Inounu (1996) have suggested that JTT sheep probably originated from the India/Bangladesh region.

A further study of prolific sheep revealed that *FecB* was also present in two Chinese sheep breeds (Davis et al. 2006). All of the Hu sheep sampled were homozygous carriers of *FecB*, whereas all three *FecB* genotypes were present in the Small Tail Han sheep. The Hu and Han both descended from Mongolian Fat Tail sheep that were brought to Zhejiang and Jiangsu in eastern China by migrants from northern China during the 960–1279 AD Sung dynasty (Ch'ang 1979). The phenotypes of the Garole sheep of India and the Hu sheep of China are quite different (Davis et al. 2006), and it is not known whether they share a common ancestor or whether *FecB* arose from separate mutation events in the two breeds. For example, adult Hu sheep typically weigh 32–44 kg, have a small fat tail and white fleece, and both sexes are polled; whereas adult Garole sheep weigh 11–14 kg, have a short thin tail, and males are horned. The fleece colour of Garole sheep ranges from white to various shades of brown, with some sheep having distinct patches of white and brown. Interestingly, Garole, Hu and Javanese sheep are all reported to occasionally have an earless phenotype (Mason 1980a, b; Bose et al. 1999), which may also indicate common ancestry.

Recent studies of mitochondrial DNA have identified two widely distributed lineages among the world's domestic sheep population (haplotypes A and B) plus a less common haplotype C that has been observed in Turkey and China. Samples from Garole sheep have revealed only haplotype A (Meadows et al. 2005), whereas the Hu breed has haplotypes A, B and C (Chen et al. 2006). These results from very

small samples (8 per breed) suggest that the maternal lines in these two breeds are quite distinct. However, if introgression of *FecB* was through males, which is more likely, the differences in mitochondrial DNA between Garole and Hu sheep do not preclude the possibility of introgression from one breed to the other. How *FecB* became fixed in Garole and Hu sheep without either OR measurements or DNA marker tests is a cause for wonder. Selection for fecundity based on LS would be unlikely to eliminate heterozygous ewes from a population because of the overlapping LS phenotypes of heterozygous and homozygous ewes. In addition to the four breeds in which *FecB* has been identified, there is no evidence of *FecB* in any samples from a further 25 of the world's highly prolific breeds or strains of sheep (Davis et al. 2002, 2006).

Distribution

Based on the evidence for a major gene for prolificacy in BMs, there has been strong international interest in using these sheep in crossbreeding programs. By 1994 BMs, and hence *FecB*, had been exported as live animals, embryos or semen from Australia and New Zealand to France, Canada, Israel, South Africa, Uruguay, USA, UK, Germany, Hungary, Poland, Czechoslovakia and Spain (Thimonier et al. 1991; Veress 1996). These exports pre-dated DNA marker tests for *FecB*, but most were either progeny-tested rams assigned a *FecB* genotype using the segregation criteria proposed by Davis et al. (1982) or their offspring.

FecB is currently known to occur in at least 48 sheep breeds in 19 countries. From Garole sheep in India it is being bred into the Deccani, Bannur, Awassi, Malpura and Avikalin breeds. The Garole is also the most likely source of *FecB* in JTT sheep in Indonesia and the BM in Australia (Davis et al. 2002). In China *FecB* is fixed in the Hu breed, which is the source of *FecB* in the Chinese Merino prolific meat strain and also probably the source of *FecB* in the prolific Small Tail Han breed. Most of the spread of *FecB* to other breeds worldwide has resulted from crossbreeding with the BM during the last 30 years, using either rams or artificial insemination. The appendix to this paper lists the breeds into which *FecB* has been introgressed from the BM.

Use

Crossbreeding between Hu and Chinese Merino sheep began in 1981 and has established the Chinese Merino prolific meat strain (25% Hu and 75% Chinese Merino). This strain was developed before it was known that the Hu breed carried *FecB*—recent DNA tests have confirmed that *FecB* is segregating in this strain (Guan et al. 2007). Garole sheep in India were crossed with the Deccani breed in 1996 and the F1 backcrossed to the Deccani in 2000 (Nimbkar et al. 2007). Since 2001 the DNA marker test has been used to maintain *FecB* in the backcrossed Deccani sheep. A breeding program crossing Garole and Malpura sheep commenced in India in 1997 (Sharma et al. 2004) and recent tests have confirmed the presence of *FecB* in 74% of the crossbred sheep (Kumar et al. 2006). The Deccani introgression program aimed to backcross to the Deccani to maintain attributes of the Deccani that are well suited to the semi-arid Deccan plateau in Maharashtra. The Malpura program in the hot semi-arid environment of Rajasthan has involved interbreeding the first cross to establish a prolific strain comprised of 50% Garole and 50% Malpura genes. Garole sheep have also been crossbred with Avikalin sheep in India, using artificial insemination (Naqvi et al. 2002). Some of the crossbreeding programs have eventually resulted in new strains of sheep carrying *FecB*. For example, the Afec–Awassi and Afec–Assaf milking sheep in Israel were derived from the BM, and after repeated backcrossing have only 3% or less Merino genes (Gootwine et al. 2008). A composite comprised of the Garole, Deccani, Bannur and Awassi breeds carrying *FecB* is also under development in India (Nimbkar et al. 2007).

FecB is an autosomal gene located on chromosome 6 and follows simple Mendelian heritance. Where the *FecB* mutation is fixed in a population, such as the Garole or Hu, there is no need for DNA marker testing because all sheep are homozygous carriers. However, *FecB* is increasingly being introgressed into other breeds, and in these breeding programs there is a requirement to use DNA marker testing to identify heterozygous individuals at each generation for further outcrossing. In the final stage of introgression, where heterozygous $FecB^{B}/FecB^{+}$ rams are bred with heterozygous $FecB^{B}/FecB^{+}$ ewes, the DNA marker tests identify homozygous

$FecB^B/FecB^B$ progeny of both sexes in order to fix *FecB* in the breed or composite of choice.

Where the aim is to establish a prolific flock of heterozygous ewes, the simplest system is to mate homozygous rams across non-carrier ewes and retain the daughters. As all daughters will be heterozygous, there is no need to DNA test them. A key feature of this system is the requirement for continued access to homozygous rams and non-carrier ewes, from which heterozygous progeny are bred.

In addition to its use in lamb production systems, *FecB* has also been a very useful model for studies of reproductive physiology. These studies were reviewed by McNatty et al. (2005), and a key finding has been that the oocyte has an important role in the control of OR. McNatty et al. (2005) concluded that research using *FecB* and other mutations with major effects on OR in sheep is likely to lead to new therapeutic reagents for regulating fertility in other mammalian species.

Management

The impact of *FecB* on LS can vary according to breed. Reports in the literature range from Garole sheep homozygous for *FecB* with a mean LS of 1.98 (Kumar et al. 2006) to Chinese Merino prolific meat strain sheep homozygous for *FecB* with a mean LS of 2.84 (Guan et al. 2007). Large variations in LS, ranging from 2.07 to 3.03, have also been observed between years in an 8-year study of Romneys homozygous for *FecB* (Farquhar et al. 2006). There is evidence that the effect of *FecB* on prolificacy is multiplicative (Davis et al. 1999; Gootwine et al. 1993, 2008). This exacerbates the problem of hyper-prolificacy, where *FecB* is introgressed into breeds or environments where non-carrier ewes already have quite high prolificacy.

At extreme levels of OR there may be no benefit from *FecB* on numbers of lambs weaned. For example, in a Romney flock where homozygous $FecB^B/FecB^B$ ewes had a mean OR of 6.24, their LS was 2.34 and barrenness levels averaged 16.4% (Farquhar et al. 2006). Barrenness levels rose steeply at ORs of 8 and higher. In contrast, the heterozygous $FecB^B/FecB^+$ ewes had an OR of 4.05 but an LS of 2.44 and barrenness of 7.6%. The very high LS of Romney sheep carrying one or two copies of *FecB,* with associated increases in lamb mortality and slower lamb growth rates, has resulted in negligible uptake of *FecB* in New Zealand's pasture-based sheep industry, where artificial rearing is uneconomic.

The successful use of *FecB* is likely to involve introgression into breeds where carrier ewes have manageable prolificacy and are well adapted to the local environment. The introgression of *FecB* from the Garole to the Deccani breed in India is an example of this approach. The Garole lacks size and has very high lamb mortality under Maharashtra's farming conditions, whereas the Deccani is very well adapted to this environment (Nimbkar et al. 2002). Deccani crossbred $FecB^B/FecB^+$ ewes weaned 1.36 lambs per ewe, which is an extra 0.41 lambs compared with $FecB^+/FecB^+$ ewes (Nimbkar et al. 2007). This contrasts with a survey of BM crossbred sheep carrying *FecB* in seven countries, which concluded that *FecB* has the potential to increase lamb production, but that high lamb mortality and reduced lamb growth rates frequently resulted in quite small increases in productivity (Davis et al. 1991). However, most of the crossbreds in this study were half Merino, and productivity would be expected to improve as backcrossing reduced the Merino genes in the *FecB* carriers. A novel use of *FecB* is in intensively managed milking sheep in Israel, where the Afec–Awassi and Afec–Assaf ewes are allowed to raise no more than two lambs (Gootwine et al. 2006, 2008). Surplus lambs are artificially reared and the lambs that are left on their mothers are weaned early, at 5.5 weeks of age. This system works well because in Israel it is profitable to artificially rear lambs.

The two long-established breeds that are homozygous carriers of *FecB*, the Garole and the Hu, are each well adapted to hot and humid environments. The Garole is native to the Sundarbans marshlands of the Ganges delta in West Bengal, where they are farmed for meat (Ghalsasi and Nimbkar 1993; Banerjee and Banerjee 2000). They are typically managed in flocks of fewer than 10 sheep which graze the edges of paddocks and roads, and are housed at night. The Garole is a hardy breed that is very well adapted to wet conditions and will stand knee-deep in water and continue grazing in the rain. Apart from during the monsoon season, no supplements are fed. The use of embryo transfer to conserve *FecB* in the small population of Garole sheep has been investigated in India (Naqvi et al. 2006). Thirty-six transferable embryos were obtained from seven super-ovulated Garole ewes

but, following transfer into Awassi × Malpura ewes, only 33% of the transferred embryos resulted in full-term lambs.

The Hu sheep are farmed in the Zhejiang and Jiangsu provinces of China, where intensive cropping is carried out and where there is practically no grazing land. They have been described as a barnyard breed (Ch'ang 1979) because they have no opportunity to graze. They are kept indoors in the semi-dark all year round and all feed is carried to the sheep house. High prolificacy has been an important economic trait for Hu sheep because the main commercial product is pelts from lambs slaughtered at 2–28 days of age.

Where *FecB* is being introgressed into a flock, care should be taken to ensure that none of the other major genes known to increase prolificacy are present because interactions among the genes can lead to hyper-prolificacy. Due to the multiplicative effect, ewes heterozygous for *FecB* and also carrying one copy of $FecX^I$ (Inverdale) had a mean OR of 4.36, which was 1.35 higher than those heterozygous for *FecB* but not carrying $FecX^I$ (Davis et al. 1999). More recently, 10 1.5-year-old ewes heterozygous for both *FecB* and $FecX^I$ had a mean OR of 7.40 (G.H. Davis, unpublished data). The combined effect of three major genes on OR was studied in four mixed-age ewes each carrying copies of three mutations affecting OR—*FecB*, $FecX^I$ and $FecX2^W$ (Davis et al. 2008). ORs were measured 12 times and the mean was 8.5, with individual ewes ranging from 7.6 to 11.0.

The possibility of a second segregating major gene for prolificacy in Booroola sheep was raised by Farquhar et al. (2006) to explain very large increases in OR over a 20-year period in a Booroola flock. This could possibly explain an anomalous BM sire among the early importations to New Zealand reported by Davis et al. (1982). More recently, progeny OR differences of 0.7 in the $FecB^B/FecB^+$ offspring of two half-sib $FecB^B/FecB^B$ rams from the same flock were also noted to be consistent with a second segregating gene (Juengel et al. 2008). A progeny test program is underway at the Invermay Agricultural Centre in New Zealand to test the second-gene hypothesis.

The effect of *FecB* on prolificacy across many breeds is well documented but there is increasing evidence that *FecB* may be associated with a negative effect on growth rate, independent of birth rank and rearing rank effects. Reduced lamb growth associated with *FecB* has been reported by Walling et al. (2000), Gootwine et al. (2006) and Kumar et al. (2008). The effect on lamb weights at about 3 months of age was in the range 0.5–1.4 kg, although the management implications of an effect of this magnitude on growth are likely to be less than those that arise from increased multiple births from *FecB*-carrier ewes.

DNA testing is a key tool in the introgression of *FecB* to other breeds. However, the widespread use of *FecB* in different breeds and synthetics is likely to require fixation of the gene so that, eventually, homozygous flocks of $FecB^B/FecB^B$ sheep can be maintained without the need for the inconvenience and expense of ongoing DNA testing, in the same manner in which the high prolificacy of Garole and Hu sheep has been retained for many centuries.

References

Anderson J.M.L., Boyd J.S., Harvey M.J. and Waterhouse A. 1997. The effect of litter size, age of ewe and Booroola gene (Fec-B) on the birth and placental characteristics of Texel ewes. Proceedings British Society of Animal Science 179.

Banerjee S. and Banerjee S. 2000. Garole sheep of Bengal. Asian Livestock 24(3), 19–21.

Bose S., Duttagupta R. and Maitra D.N. 1999. Phenotypic characteristics and management practices of Bengal sheep. Indian Journal Animal Production Management 15(1), 18–22.

Boulton M.I., Haley C.S., Springbett A.J. and Webb R. 1995. The effect of the Booroola (FecB) gene on peripheral FSH concentrations and ovulation rates during oestrus, seasonal anoestrus and on FSH concentrations following ovariectomy in Scottish Blackface ewes. Journal of Reproduction and Fertility 103, 199–207.

Bradford G.E. and Inounu I. 1996. Prolific breeds of Indonesia. Pp. 137–145 in 'Prolific sheep ' ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Bunge R., Thomas D.L. and Nash T.G. 1995. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: lamb production of F1 adult ewes. Journal of Animal Science 73, 1602–1608.

Ch'ang T.S. 1979. Livestock production in China with particular reference to sheep. Wool Technology and Sheep Breeding 27, 19–28.

Chen S.Y., Duan Z.Y., Sha T., Xiangyu J., Wu S.F. and Zhang Y.P. 2006. Origin, genetic diversity, and population structure of Chinese domestic sheep. Gene 376, 216–223.

Cristian H.K. 1994. Study on the performance of the Booroola race under intensive production system: reproductive efficiency, survival, growth and carcass characteristics. AGRIS record CL9600493.

Davis G.H. 1991. Booroola gene research in New Zealand. Pp. 15–17 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway S.M., Lumsden B.M., Hanrahan J.P., Mullen M., Mao X.Z., Wang G.L., Zhao Z.S., Zeng Y.Q., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranz J.J. and Notter, D.R. 2006. Investigation of the Booroola (FecB) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92, 87–96.

Davis G.H., Dodds K.G. and Bruce G.D. 1999. Combined effect of the Inverdale and Booroola prolificacy genes on ovulation rate in sheep. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 13, 74–77.

Davis G.H., Elsen J.M., Bodin L., Fahmy M., Castonguay F., Gootwine E., Bor A., Braw-Tal R., Greef J.C., Lengyel A., Paszthy G. and Cummins, L. 1991. A comparison of the production from Booroola and local breed sheep in different countries. Pp. 315–323 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomiquee (INRA): Paris, France.

Davis G.H., Galloway S.M., O'Connell A.R., Farquhar P.A., McNatty K.P. and Juengel J.L. 2008. Hyper-prolific ewes carrying copies of three major genes: a model for studying genes controlling ovulation rate. Biology of Reproduction, Special Issue: Abstract 244, 110.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson, T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Davis G.H. and Meyer H.H. 1983. What Booroolas have to offer. Proceedings of Ruakura Farmers Conference 35, 21–23.

Davis G.H., Montgomery G.W., Allison A.J., Kelly R.W. and Bray A.R. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Decuypere E., Onagbesan O.M., Michels H., Beerlandt G., Peeters R., Bister J.L. and Paquay R. 2004. Gene-specific pituitary gland responsiveness of ovariectomized FecB or FecC carrier and non-carrier ewe crosses with German Mutton Merino, Texel and Suffolk breeds to LHRH before and after oestradiol or progesterone treatments. Small Ruminant Research 51, 7–22.

Driancourt M.A., Gauld I.K., Terqui M. and Webb, R. 1986. Variations in patterns of follicle development in prolific breeds of sheep. Journal of Reproduction and Fertility 78, 565–575.

Fahmy M.H. 1996. Spread of prolific sheep: the Americas. Pp 228–233 in. 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Fahmy M.H. and Castonguay F. 1991. Research and commercialization of the Booroola gene in Canada. Pp. 19–26 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Farquhar P.A., Dodds K.G. and Davis G.H. 2006. Introgression of the Booroola mutation (FecB) leads to hyper-prolificacy in a Romney sheep flock. Proceedings 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, 13–18 August 2006, CD-ROM communication no. 04-33.

Fernandez-Abella D. 1991. The Booroola sheep in Uruguay. Pp. 27–29 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Fernandez-Abella D., Cognie Y., Thimonier J., Seck M. and Blanc M.R. 2005. Effects of the FecB gene on birth weight, postnatal growth rate and puberty in Booroola × Merinos d'Arles ewe lambs. Animal Research 54, 283–288.

Ghalsasi P.M. and Nimbkar B.V. 1993. The 'Garole' – microsheep of Bengal, India. Animal Genetic Resource Information, UN Environmental Program, Bulletin 12, 73–79. FAO: Rome.

Gootwine E., Braw-Tal R., Shalhevet D., Bor A. and Zenou A. 1993. Reproductive performance of Assaf and Booroola–Assaf crossbred ewes and its association with plasma FSH levels and induced ovulation rate measured at prepuberty. Animal Reproduction Science 31, 69–81.

Gootwine E., Reicher S. and Rozov A. 2008. Prolificacy and lamb survival at birth in Awassi and Assaf sheep carrying the FecB (Booroola) mutation. Animal Reproduction Science 108, 402–411.

Gootwine E., Rozov A., Bor A. and Reicher S. 2006. Carrying the *FecB* (Booroola) mutation is associated with lower birth weight and slower post-weaning growth rate for lambs, as well as a lighter mature bodyweight for ewes. Reproduction Fertility and Development 18, 433–437.

Guan F., Liu S.R., Shi G.Q. and Yang L.G. 2007. Polymorphism of FecB gene in nine sheep breeds or

strains and its effects on litter size, lamb growth and development. Animal Reproduction Science 99, 44–52.

Haley C.S. 1991. The Booroola gene in the UK. Origins, research and development. Pp. 33–38 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Hinch G.N., Kelly R.W., Davis G.H., Owens J.L. and Crosbie S.F. 1985. Factors affecting lamb birth weights from high fecundity Booroola ewes. Animal Reproduction Science 8, 53–60.

Juengel J.L., Proctor L.E., Farquhar P.A. and Davis G.H. 2008. Association among ovulation rate, serum concentrations of FSH, response to exogenous FSH and fertility in ewes heterozygous for the Booroola gene (FecB). Reproduction in Domestic Animals 43 (suppl. 3), 79.

Kaulfuss K.H., Suss R. and Rossler H.J. 2004. The effect of the Booroola FecB gene on the reproductive performance of the German Mutton Merino and the German Blackhead Mutton breeds of sheep. Tieraerztliche Umschau 59, 711–717.

Kelly R.W., Davis G.H. and Allison A.J. 1980. Productive changes in longwool breeds in New Zealand following crossbreeding with Booroola-type rams. Proceedings Australian Society of Animal Production 13, 2413–2416.

Kleemann D.O., Walker S.K., Walkley J.R.W., Ponzoni R.W., Smith D.H., Grimson R.J. and Seamark R.F. 1991. Effect of pre-mating nutrition on reproductive performance of Booroola Merino × South Australian Merino ewes. Animal Reproduction Science 26, 269–280.

Klewiec J., Martyniuk E. and Gabryszuk M. 2001. Effect of different shares of the Booroola genotype on growth rate and reproduction performance of crosses with Polish Merino. Animal Science Papers and Reports 19, 123–130.

Klewiec J., Martyniuk E., Gabryszuk M. and Baranowski A. 2004. Ovulation rate and prolificacy in Booroola × Olkuska crossbred ewes. Animal Science Papers and Reports 22, 325–333.

Kumar S., Kolte A.P., Mishra A.K., Arora A.L. and Singh V.K. 2006. Identification of the FecB mutation in Garole × Malpura sheep and its effect on litter size. Small Ruminant Research 64, 305–310.

Kumar S., Mishra A.K., Kolte A.P., Arora A.L., Singh D. and Singh V.K. 2008. Effects of the Booroola (FecB) genotypes on growth performance, ewe's productivity efficiency and litter size in Garole × Malpura sheep. Animal Reproduction Science 105, 319–331.

Lauvergne J.J., Dolling C.H.S. and Renieri C. (eds) 1996. Mendelian inheritance in sheep. University of Camerino Press: Camerino.

McNatty K.P., Galloway S.M., Wilson T., Smith P., Hudson N.L., O'Connell A., Bibby A.H., Heath D.A., Davis G.H., Hanrahan J.P. and Juengel J.L. 2005. Physiological effects of major genes affecting ovulation rate in sheep. Genetics Selection Evolution 37 (suppl. 1), S25–S38.

Mason I.L. 1980a. Hu-yang breed of China. Pp. 90–91 in: 'Prolific tropical sheep', ed. by I.L. Mason. FAO Animal Production and Health Paper 17. FAO: Rome.

Mason I.L. 1980b. Prolific sheep in Java. Pp. 65–76 in 'Prolific tropical sheep', ed. by I.L. Mason. FAO Animal Production and Health Paper 17. FAO: Rome.

Meadows J.R.S., Kantanen K.L.J., Tapio M., Sipos W., Pardeshi V., Gupta V., Calvo J.H., Whan V., Norris B. and Kijas J.W. 2005. Mitochondrial sequence reveals high levels of gene flow between breeds of domestic sheep from Asia and Europe. Journal of Heredity 96, 494–501.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y. and Elsen J.M., 2001. Mutation in bone morphogenetic protein receptor-1B is associated with increased ovulation rate in Booroola Merino ewes. Proceedings National Academy of Science USA 98, 5104–5109.

Naqvi S.M.K., Joshi A., Gulyani R., Kumar D., Kolte A.P., Kumar S., Maurya V.P., Saha S., Mittal J.P. and Singh V.K. 2006. Production of prolific micro-sheep by embryo transfer into large non-prolific sheep. Veterinary Record 159, 522–526.

Naqvi S.M.K., Maurya V.P., Joshi A., Sharma R.C. and Mittal J.P. 2002. Production of crossbred lambs through artificial insemination of non-prolific medium size Malpura and Avikalin ewes using fresh diluted semen of prolific micro size Garole rams. Asian–Australasian Journal of Animal Science 15, 633–636.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Walkden-Brown S.W., Maddox J.F., Gupta V.S., Pardeshi V.C., Ghalsasi P.P. and van der Werf J.H.J. 2007. Reproductive performance of Indian crossbred Deccani ewes carrying the FecB mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 430–433.

Nimbkar C., Ghalsasi P.M., Walkden-Brown S.W. and Kahn L.P. 2002. Breeding program for the genetic improvement of Deccani sheep of Maharashtra, India. Proceedings 7th World Congress on Genetics Applied to Livestock Production, Montpellier, 19–23 August 2002, CD-ROM communication no. 25-11.

Nowak Z. and Charon K.M. 2001. Identification of fecundity gene (FecB) carriers using microsatellite markers and its effect on sheep weight. Journal of Applied Genetics 42, 49–57.

Piper L.R. and Bindon B.M. 1982. The Booroola Merino and the performance of medium non-peppin crosses at Armidale. Pp. 9–19 in ‘The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24-25 August 1980’, ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Piper L.R., Bindon B.M., Davis G.H. and Elsen J.M. 1988. Control of litter size: major genes and industry utilization of the Booroola F gene. In ‘Proceedings 3rd World Congress on Sheep and Beef Cattle Breeding, Paris’, 2, 589–609.

Sharma R.C., Arora A.L., Mishra A.K., Kumar S. and Singh V.K. 2004. Breeding prolific Garole with Malpura sheep for increasing reproductive efficiency in semi-arid tropics of India. Asian–Australasian Journal of Animal Science 17, 737–742.

Southey B.R., Thomas D.L., Gottfredson R.G. and Zelinsky R.D. 2002. Ewe productivity of Booroola–Rambouillet crossbred sheep during early stages of the introgression of the FecB allele into a Rambouillet population. Livestock Production Science 75, 33–44.

Souza C.J.H., Chagas L.M. and Moraes J.C.F. 1995. The influence of the prolificacy gene FecB on the reproductive biology of 3/4 Romney Marsh × 1/4 Merino Booroola ewes. Revista Brasileira de Genetica 18, 121–124.

Souza C.J., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. Journal of Endocrinology 169, R1–6.

Thimonier J., Davis G.H., Fahmy M.H., Castonguay F., Fernandez-Abella D., Greef J.C., Hofmeyr J.H., Gootwine E., Bor A., Braw-Tal R., Haley C.S., Klewiec J., Gabryszuka M., Slowak M., Piper L.R., Bindon B.M., Veress L., Lengyel A., Paszthy G., Horn P., Visscher A.H., Wassmuth R. and Young L.D. 1991. The F gene in the world: use and research objectives. Pp. 3–13 in ‘Major genes for reproduction in sheep’, ed by J.M. Elsen, L. Bodin and J. Thimonier. L’Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Turner H.N. 1978. Selection for reproduction rate in Australian Merino sheep: direct responses. Australian Journal of Agricultural Research 29, 327–350.

Turner H.N. 1982. Origins of the CSIRO Booroola. Pp. 1–7 in ‘'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24-25 August 1980’, ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Veress L. 1996. Spread of prolific sheep: Europe. Pp. 218–227 in ‘Prolific sheep’, ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Veress L., Horek F., Komlosi T., Javor A. and Magyar K. 1988. Breeding possibilities of Booroola Merino in East-Europe. Journal of Agricultural Science Finland 60, 591–596.

Visscher A.H., Dijkstra M., Lord E.A., Suess R., Roesler H.J., Heylen K. and Veerkamp R.F. 2000. Maternal and lamb carrier effects of the Booroola gene on food intake, growth and carcass quality of male lambs. Animal Science (Penicuik) 71, 209–217.

Walling G.A., Dodds K.G., Galloway S.M., Beattie A.E., Lord E.A., Lumsden J.M., Montgomery G.W. and McEwan J.C. 2000. The consequences of carrying the Booroola fecundity (FecB) gene on sheep liveweight. Proceedings British Society Animal Science, p.43.

Wassmuth R., Glahn-Luft B., Erhardt G., Paszthy G., Hiendleder S., Lewalski H., Herlinger D., Eisler P., Schmahl G., Sonnen A. and Krogmeier D. 1991. Booroola F-gene activity report. Pp. 53–55 in ‘Major genes for reproduction in sheep’, ed. by J.M. Elsen, L. Bodin and J. Thimonier. L’Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Wilson T., Wu Xi-Yang, Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O’Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Young L.D. 1991. The Booroola gene in USA. Pp. 57–59 in ‘Major genes for reproduction in sheep’, ed. by J.M. Elsen, L .Bodin and J. Thimonier. L’Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Appendix

Distribution of *FecB* from the Booroola Merino into other sheep breeds

Breed	Country	Reference(s)
Assaf	Israel	Gootwine et al. (2008)
Awassi	Israel	Gootwine et al. (2008)
BLM (Border Leicester–Merino)	Australia	Piper et al. (1988)
Border Leicester	Australia, New Zealand	Davis and Meyer (1983); Piper et al. (1988)
Borderdale	Australia	Piper et al. (1988)
Cheviot	UK	Haley (1991)
Columbia	USA, Canada	Fahmy and Castonguay (1991); Young (1991)
Coopworth	New Zealand, Canada	Davis and Meyer (1983); Fahmy and Castonguay (1991)
Corriedale	New Zealand, Poland, Uruguay	Davis and Meyer (1983); Fernandez-Abella (1991); Nowak and Charon (2001)
Czechish Merino	Czechoslovakia	Veress et al. (1988)
DLS (Dorset–Leicester–Suffolk)	Canada	Fahmy and Castonguay (1991)
Dohne Merino	South Africa	Davis et al. (1991)
Dorset Horn	Australia, Canada, UK	Piper et al. (1988); Haley (1991); Fahmy (1996)
Finn	Canada, USA	Fahmy and Castonguay (1991); Young (1991)
German Blackhead Mutton	Germany	Kaulfuss et al. (2004)
German Mountain	Germany	Wassmuth et al. (1991)
German Mutton Merino	Germany, Belgium	Decuypere et al. (2004); Kaulfuss et al. (2004)
Hungarian Merino	Hungary	Veress (1996)
Hyfer (Dorset–Merino)	Australia	Piper et al. (1988)
Ile de France	UK	Haley (1991)
Merino	Australia, New Zealand, Chile, Uruguay	Piper and Bindon (1982); Hinch et al. (1985); Fernandez-Abella (1991); Cristian (1994)
Merinoland	Germany	Wassmuth et al. (1991)
Merinos d'Arles	France	Fernandez-Abella et al. (2005)
Olkuska	Poland	Klewiec et al. (2004)
Perendale	New Zealand	Davis and Meyer (1983)
Polish Merino	Poland	Klewiec et al. (2001)
Poll Dorset	New Zealand	Davis (1991)
Polwarth	Uruguay	Fernandez-Abella (1991)
Polypay	Canada	Fahmy and Castonguay (1991)
Rambouillet	USA, Canada	Fahmy and Castonguay (1991); Southey et al. (2002)
Romanov	France, Canada	Driancourt et al. (1986); Fahmy and Castonguay (1991)

Breed	Country	Reference(s)
Romney	New Zealand, UK, Brazil	Hinch et al. (1985); Haley (1991); Souza et al. (1995)
Scottish Blackface	UK	Boulton et al. (1995)
South Australian Merino	Australia	Kleemann et al. (1991)
Suffolk	UK, USA, Chile, Canada	Haley (1991); Fahmy and Castonguay (1991); Cristian (1994); Bunge et al. (1995)
Targhee	USA	Bunge et al. (1995)
Texel	Netherlands, Belgium, UK	Anderson et al. (1997); Visscher et al. (2000); Decuypere et al. (2004)
Welsh Mountain	UK	Haley (1991)
Western Whiteface	USA	Young (1991)

The Garole sheep: history, management, production and current status[1]

S. Pan[2] and A.K. Sahoo[2]

Abstract

This study was conducted using a survey to record the history, management practices, production and current status of Garole sheep in the core breeding tract, located in West Bengal. A stratified two-stage sampling design was followed using three different survey questionnaires in 60 villages randomly identified as representing the breeding tract. The survey was conducted over a 1-year period on 9,984 sheep and 2,666 sheep farmers. Little is known about the origin of the Garole breed of sheep. The Garole is valued for its production of mutton, skin and manure; there is no practice of shearing the sheep although they produce coarse hairy fibre with more than 85% medullation. Garole sheep are managed using either free or tethered grazing, with minimum housing provided to the animals, and there is no practice of flock migration. The breed appears to be fairly tolerant to diseases, particularly considering their living conditions. The total population in the breeding tract is 273,500 as per the 2003 census, but the population growth rate has decreased sharply over the last three decades. Vigorous steps need to be initiated immediately to save the Garole sheep, the original source of the *FecB* gene, from extinction.

Introduction

Improved animal genetic resources with versatile characteristics are necessary to meet growing human demand. Eastern India in general and West Bengal in particular are considered unsuitable for sheep production because of the hot, humid climate and heavy rainfall. However, a small sheep, locally known as 'Garole', is the predominant livestock species in the swampy Sundarbans delta of West Bengal. The area is low lying and marshy, highly saline under tidal influence and prone to cyclones. The breed has escaped scientific attention until recently, possibly because of the remoteness of the area and its difficult access. The breed is not mentioned in the lists of sheep breeds documented by either Mason (1980) or Acharya (1982). However, Turner (1982) reported that some very prolific sheep from Bengal were brought to Australia during 1792 and 1793, and these may be the source of the prolific strain of Merino known as Booroola. In 1792, 10 Bengal ewes and 2 rams arrived in Australia from Calcutta, and a further shipment of about 100 Bengal sheep arrived the following year. The Rev. Samuel Marsden was the early flock owner of Bengal sheep (Davis et al. 2002). Bradford and Inounu (1996) suggested that the thin-tailed sheep of Java probably also originated from the India–Bangladesh region of Asia. 'The Booroola Merino (BM) breed was developed last century by Samuel Marsden, from Merinos, Cape sheep and the Bengal breed. Studies have shown that the superfertility was genetic and that it came from a single gene or a closely linked set of genes' (Doyle 1991). The discovery of the *FecB* mutation in Garole sheep strongly supports the theory that the Booroola gene was introduced into Australia through Garole sheep

[1] This paper is largely an extract from Pan and Sahoo (2003)

[2] West Bengal University of Animal and Fishery Sciences, 37 K.B. Sarani, Kolkata-700 037, India; span28@rediffmail.com

(Davis et al. 2002). Kar and Prasad (1992) wrote about prolific and disease-resistant Garole as the emerging miniature sheep breed of the Sundarbans delta. They claimed that the sheep has adapted to the highly humid and cyclone-prone riverine conditions over the centuries.

Ghalsasi and Nimbkar (1993) and Ghalsasi et al. (1994) published some information about the sheep and their production system. Subsequently, Bose and Maitra (1995), Singh and Bohra (1996), Bose et al. (1999a, b) and Sahana et al. (2001) reported on different physical, phenotypic and production traits of Garole sheep. However, all these reports are based on small samples and are limited to small pockets of the breeding tract. The alarming fact is that, in recent years, the growth rate of the population has recorded a sharp decline despite the prolificacy being as high as 170–225%. This is mostly due to a lack of scientific care and management, leading to high morbidity and mortality, and the absence of any effort to develop or conserve this outstanding category of genetic resource. Garole sheep production is facing a constant threat from the gradual shrinkage of grazing land and other feed resources. Many resource-poor farmers these days prefer singleton-producing ewes to prolific ewes that produce malnourished, non-viable triplets or quadruplets. Proper usage of this breed is destined to open new horizons for sheep farmers, with the fecundity gene used to improve the prolificacy of other breeds. It is even believed that Garole sheep are going to have an important role to play in understanding and improving mammalian fertility.

Methodology

This study was conducted through field surveys following a stratified two-stage sampling design. Sixty villages were randomly identified, evenly spaced throughout the breeding tract, which was determined through extensive field visit and sample study. Villages constituted the first unit and houses within the villages the second unit. All sheep and sheep farmers of the identified villages were included in the study. Accordingly, 9,984 sheep and 2,666 sheep farmers constituted the sample size. Three sets of questionnaires were used, as follows:

Questionnaire 1: Householder, management practices, flock information and reproduction (recorded once)

Questionnaire 2: Feeding and grazing, body weights and measurements, reproduction, health and disposal (repeated every 3 months for 1 year)

Questionnaire 3: Slaughter data recorded by the authors and their team of workers.

Daily climatic data over 10 years were collected from three observatories located in the breeding tract. Relevant information on topography, land-use pattern and vegetation was collected from concerned government institutions and departments.

Breeding tract

The core breeding tract of Garole sheep is located between 21°32' to 22°40' N and 88°05' to 89°00' E (Figure 1). It comprises parts of two districts, namely North and South 24 Parganas. The Hoogly River forms the western, the Bay of Bengal the southern, the Ichamati–Kalindi–Raimangal river system the eastern, and the outskirts of greater Kolkata suburb the northern boundaries. The region is crisscrossed by tidal rivers and creeks that form 104 islands, of which 54 have human habitation and Garole sheep. The total area of the breeding tract is estimated as 6,210 sq. km. The topography is generally flat, but many of the islands are below sea level and are occasionally flooded during monsoon and high tide. Embankments provide some protection.

Relatively fewer Garole sheep are found in the western *char* (a newly formed land mass) area of the Hoogly River and even fewer within 5–10 km further west. In that area Chotanagpuri-type sheep are predominant. The Garole breed is also equally prevalent in the Sundarbans area of Bangladesh (Anon. 2004). The concentration of Garole sheep varies from location to location in the breeding tract. There appears to be an inverse relationship between density of Garole sheep and intensity of crop production. The concentration increases gradually in remote locations and on islands closer to the bay (Figure 2), where the prospect of crop cultivation is less due to scarce irrigation, the occasional cyclone and inundation by saline water and flooding. There are two identifiable seasons, namely cold moist (November to February) and hot humid (March to October) in the breeding tract. According to Lee (1953), two other seasons, namely warm humid and warm wet, could also be identified. The tract experiences moderate to heavy rainfall from May to October due to south-west monsoons. Average annual rainfall was found to be 1,802.7 mm.

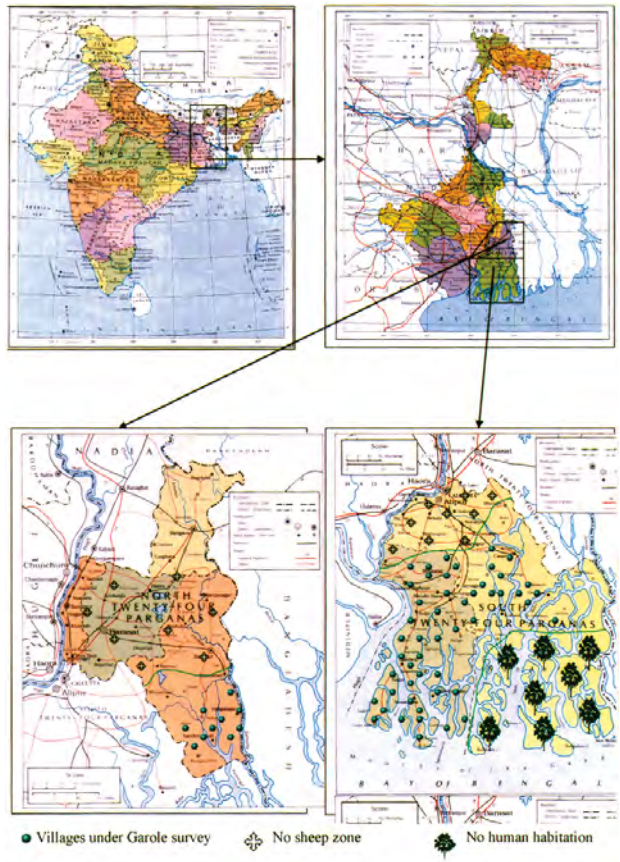

Figure 1. Location of core area of the Garole breeding tract

Breed name and synonyms

The breed is locally known as Garole, a word used by farmers to indicate not only the breed but also 'sheep' in general. 'Garole', a colloquial Bengali word, means stupid. The Garole sheep exhibits typical ovine behaviour and habits, namely innocence, an apparently foolish attitude, getting panicky at the slightest disturbance and huddling together. Farmers often call the breed 'Bheda', which means sheep in Bengali as this is the only breed of sheep known to the local farmers. Garole with rudimentary or short ears are also common and are known as 'Meda', a colloquial Bengali word meaning 'earless'.

Origin of the breed

Little is known about the origin of the Garole breed of sheep. Possibly, sheep from other parts of the continent were moved into the area by their owners when they first inhabited this forest area. The owners put greater emphasis on animal production, as the area was poorly suitable for crop production. The present day sheep owners are descendent from the armies and other associates of feudal lords, zaminders and landholders, as evident from their title/caste (Table 1). They are either Hindu (69.6%) or Muslim (29.7%) by religion. Their primary professions include cultivation (53.8%), daily labour (28.7%), small business (6.6%), fishing (6.1%), service (1.2%) and miscellaneous (3.7%). Landholding size is very small, with 31% of the sheep owners sampled possessing less than 1 acre of land, used mainly for vegetable (81.0%) and paddy (17.8%) production, and 34.9% being landless.

These animals appear to be well adapted to local conditions and have acquired many versatile characteristics through evolution. Their size has reduced,

possibly in an effort to thrive in the hot, humid climate and on a low plane of nutrition. Higher prolificacy may be a natural effort to compensate for the higher mortality due to diseases and harsh climate. The geoclimatic condition of the area appears to be hostile for sheep production by conventional notions of suitable conditions for sheep rearing.

Table 1. Titles of Garole farmers

Title/caste	Meaning
Laskar/Naskar	Soldier/seaman
Piyada	Foot soldier
Paik	Peasant militia
Gayen	Court singer
Khansamah	Royal cook
Senapati	Commander in chief
Mollah	Mohammedan priest
Dalapati	Leader

Utility of the breed

The early inhabitants of the breeding tract possibly considered sheep as the most useful enterprise as the area was not suitable for crop production. Sheep, with their capacity to endure long migration and their ability to survive in a harsh climate and poor feed regime, were more suitable than other domestic species. The Garole breed is valued for its production of mutton, skin and manure. There is no practice of shearing the sheep although they produce coarse hairy fibre with more than 85% medullation. There is no marketing outlet for the fibre so it is of no commercial value to the farmers. A few animals are shorn to give them some relief during summer months, to keep them clean and, occasionally, to control ectoparasites. Milk yield is low and is often found to be insufficient for triplet- and quadruplet-born lambs, leading to lamb mortality if not supplemented with rice gruel. The breed has lost its ability to wander long distances and is now managed within a 1-km radius of farmers' residences. The faeces and urine collected from night shelters is good fertiliser with a high nitrogen value. Resource-poor farmers prefer Garole sheep to the equally prolific Bengal goat, although the relative market price of the former is less. The Garole's ability to survive on scarce resources and their disease resistance make them more attractive. During the rainy season almost all conventional pastures, except those on roadsides, become submerged. At this time feed becomes even scarcer for the sheep, and all stock except for a few breeding animals are sold at this time at a low market price.

Population and flock composition

The total population of Garole sheep in the region was 273,500 according to the latest census conducted in 2003. Thus, the concentration is 44 sheep per square kilometre. There is concern that the population growth rate over the last three decades has declined sharply. The annual population growth

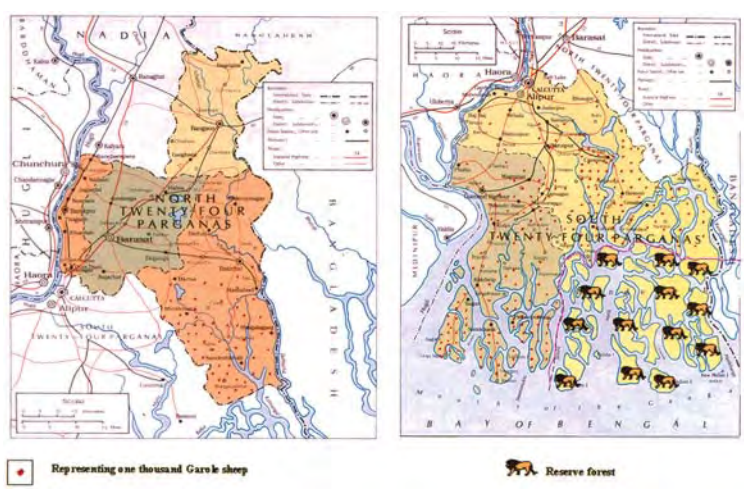

Figure 2. Concentrations of Garole sheep in different locations of the breeding tract

rates during 1972–84, 1984–94 and 1994–2003 were 8.34%, 2.84% and 0.3% respectively. Opposing market forces, lack of state service support and steady shrinkage of natural resources are the major reasons behind the decline in the growth rate. The government launched a conservation program in 2004 but its magnitude and pace are insufficient to reverse the trend.

Farmers manage Garole sheep in flocks of varying sizes. Most of the flocks have four or fewer sheep, and 56% of the total animals are managed under flocks of this size. Only 9% of the sheep are reared in flocks of eight or more animals. This is mostly due to the decreasing availability of natural pasture resulting from the gradual expansion of cash-crop production. Natural pasture is almost unavailable from July to September due to waterlogging (rain and high tide), and farmers prefer to dispose of their stock just before the rainy season to avoid a crisis. Age-wise compositional analysis of the Garole population reveals that lambs up to 3 months of age constitute only 13% of the flock (Table 2). This is mostly due to high mortality observed in this age group. Animals belonging to the 12.1–24-month age group are the most common, at 24% of the flock. Beyond this age, the number reduces because of marketing. A sex-wise analysis further reveals that the reduction due to marketing of male animals begins after 6 months of age. From a maximum of 8% at 3.1–6 months of age, the proportion of males reduces sharply and reaches a minimum of 2% of the flock among the 24 months or more age group. These males are retained for breeding purposes. Farmers always need to retain females as breeding stock for production of replacement stock. Consequently, the market value of females for slaughter is less than their value as prolific breeding stock. The relative ratios of males to females in the flock vary according to age group. Male animals constitute 27.1% of the flock irrespective of age. The male: female ratio is almost 50:50 up to 6 months of age, but as males reach marketing age their numbers decrease sharply. Farmers often prefer to castrate the male lambs to be reared specially for meat purposes. The study revealed that 31% of males in the population are castrated at an average age of 2.6 months. Local non-qualified people perform 94.3% of the castrations using a crude open method.

Garole sheep versus other farm animal species

Sheep farmers often rear other livestock (cattle, goat, fowl and duck) under a classical composite farming system. Rearing of pigs is an uncommon practice in the area. Only 11% of those farmers interviewed were found to rear sheep only, whereas 42% reared two other species in addition to sheep. Possibly because they rely heavily on livestock production, and also to avoid uncertainty, 15% of Garole farmers maintain all four of the abovementioned livestock species as well as sheep.

Housing management

Garole sheep are managed using either free or tethered grazing, with no practice of flock migration. Even under free grazing the flocks are restricted within a 1 square kilometre area of the owner's residence. The sheep are provided with only night shelter by 72% of farmers, with the remainder providing both day and night housing. This second group farms in the area with a higher intensity of cash-crop production where the scope for grazing is limited. The farmers practise partial stall-feeding, and only 0.1% of farmers have no arrangement for housing their animals. Garole sheep are often housed with other domestic species managed by the farmers (35%) or even within their bedroom (7%). Farmers with prior knowledge of the expected date of lambing (15%) confine the pregnant ewe nearing lambing until 7–10 days post lambing, depending on the season. Ewes and lambs are provided with paddy straw bedding and cold draught protection during winter.

Table 2. Composition of surveyed flocks (%) by sex and age

Sex	Age group (months)				
	0–3	3.1–6	6.1–12	12.1–24	> 24
Male	6.1	8.3	7.4	3.6	1.7
Female	7.4	9.0	13.7	20.8	21.8
Total	13.5	17.4	21.2	24.4	23.5

Housing design and materials

Garole farmers use varieties of locally available cheap materials and housing design with all efforts to keep the housing cost as low as possible. They use paddy straw (90%), earthen tiles (8%), tree leaves (1%) and corrugated galvanised iron sheets (1%) as roofing materials. Mud floor and walls, being the cheapest, are most commonly used (96.6%). Bamboo, wood, paddy straw, tree leaves, iron sheets and jute bags are used for fabrication of doors and windows. Farmers usually don't make any in-house fixed feeding (manger) and drinking (water trough) arrangements, but use bamboo baskets (85%), earthen pots (8%) or simply a gunny bag sheet (7%) for offering fodder to the animals. All these items are moveable and fodder is offered either within the Garole house or in the courtyard. Often the fodder is offered in a common manger along with that for other domestic species such as goats and cattle.

The majority of Garole sheep drink accumulated rainwater while grazing. The animals are even found to drink saline water for several days, as there are limited sources of fresh water on many of the islands. All the rivers, creeks, bogs and ditches are fed with tidal saline water. However, 84% of farmers regularly offer drinking water to the animals at the end of the day on their return from grazing. Iron or plastic buckets (65%), earthen pots (22%), kettles (12%) and miscellaneous items (1%) are used for supplying drinking water.

The mosquito menace is high in the Garole breeding tract, being located in coastal waterlogged areas. To give respite to their animals, 19.1% of farmers use mosquito nets at night. In addition, 3.2% of Garole farmers provide a night lamp in the shed, which helps to keep the mosquito menace low and the animals more contented.

Feeding management

Garole sheep are managed both by grazing and stall-feeding. No animal is managed exclusively on stall-feeding but 16% of animals are managed exclusively on grazing, with the rest being on both grazing and stall-feeding. Feeding management practices vary marginally over the seasons. Animals reared exclusively on grazing are allowed to graze either in two shifts (morning and evening) to safeguard them from midday heat, or throughout the day. On average, 96% of those animals managed exclusively on grazing grazed throughout the day. This value is highest (99%) during the rainy season and lowest (95%) during summer. The total duration of grazing per day (10.4 ± 0.05 hours) is almost the same in the different seasons. Animals allowed two shifts for grazing also grazed for 8–11 hours in different seasons—marginally lower during summer and highest during the rainy season. The breed is accustomed to graze during rain, and in waterlogged marshy areas their body may be submerged up to the neck.

Shepherds often accompany the grazing sheep (48%) to protect them and the standing crops. Tethered grazing is also common to prevent the sheep from grazing on standing crops as well as to minimise the loss of sheep due to straying. A maximum of 27% of sheep are tethered-grazed during the rainy season when the cash-crop coverage is at a maximum, and this declines to 24% in winter and 22% in summer. The average length of the tethering rope is 3.5 metres.

Animals graze on natural pastures comprising harvested crop fields, boundaries of crops, roadsides, banks of irrigation channels, waterways, rivers and water bodies, and low land during ebb tides. Freshly grown grass stubbles on low-lying areas exposed during ebb tides are found to be the most attractive to Garole sheep.

Farmers practise supplementary feeding of Garole sheep depending upon need and the availability of supplementary feed. More supplementary feeding is provided during summer when the quality of natural pasture is low, and during the rainy season when grazing is limited due to heavy rain, submerged pasture or unharvested crops. Harvested grass, weeds, tree leaves, dry grass, paddy straw and other crop residues (as concentrate) are items of supplementary feeding. Common tree leaves used for feeding are ficus (*Ficus bengalensis*), babool (*Acacia arabica*), mango (*Mangifera indica*), banana and various mangrove species. Harvested grass is most popular and used by 44% of farmers, followed by tree leaves (31%), dry fodder (13%), miscellaneous items (11%) and concentrate (1%). The quantity of different items fed varies from 1.32 ± 0.03 kg to 2.15 ± 0.25 kg per animal per day. Supplementary feeding more than once per day is uncommon. None of the Garole farmers purchase any supplementary feed items, such as oil cakes, concentrate or mineral mixture, for their animals.

Physical traits

The Garole sheep is a small-sized breed with a low-set body (Figure 3). The head is straight and well set but is a little higher than the body and appears triangular from the front. The nose bridge is straight and the muzzle is small and pale cream or black in colour. The eyes are black and well set in the long face, and the neck is long and fine and heavier in males. The legs are thin with black hooves. The chest and abdomen are barrel-like and heavier posteriorly in females. The back is straight. The udder is not well developed, even during lactation. The teats are small and placed on the ventro-lateral angle of the udder. The scrotum in adults is large.

Garole sheep possess both pure and mixed coat colour. Pure colours are white, grey, black and brown. Mixed colours are formed by the combination of any two of the pure colours. Two colour mixes (grey–brown and white–brown) have not been observed. The distribution of the different coat colours in the population is white (28%), grey (48%), black (12%), brown (4%), white–black (3%), white–grey (1%), black–grey (4%) and black–brown (<1%). The prevalence of different coat colours, either pure or mixed, varies from zone to zone. It appears that variation in coat colour in the population is due to different ratios of black:white genes. Lambs with jet-black coat colour at birth slowly change to grey after 3 months of age. The breed has hairy, non-lustrous, straight fibre with more than 86% medullation. The average length and diameter of the fibre at 12 months of age are 4.99 ± 0.06 cm and 53.02 ± 0.56 μm respectively. No seasonal variation in coat colours or canary colouration has been noticed. The head, face, belly and legs are bare. There is no practice of shearing the animals, so farmers are losing income from this resource due to a lack of awareness and marketing channels.

Up to 24 months of age, 60% of males and 97% of females are polled. The proportion of straight horn is higher than that of curved when the length of horn is less than 5 cm. But the proportion of curved horn increases when the horn grows beyond 5 cm. Horns belonging to both sexes of every age group are whitish grey in colour. Wide variation is observed in both the size and type of ear of Garole sheep. Ears are either erect or pendulous, and no differences are observed according to sex. Ear size ranges from 3.1 to 10 cm among 70% of the population. Very small (rudimentary) ears are noticed among 10% of animals, whereas 20% possess large ears (more than 10 cm). The breed possesses a short tail, unlike most other breeds of sheep, and no sexual dimorphism in tail characters has been noticed. The tail is very short (up to 5 cm) among 5% of the animals and medium in length (5.1–10 cm) in 66%. The proportion of animals with tails more than 15 cm is negligible. The breed possesses neither wattles nor a beard.

Body size in different ages of both sexes has been quantified in terms of body weight. Body weights and different body measurements are presented in Tables 3a, 3b and 4 respectively.

Reproduction

Males reach puberty at 8.3 ± 0.05 months, with a range of 5–12 months according to the farmers interviewed. Only 1.8% of males in the population were found to have some form of morphological testicular abnormality. Males exhibit uniform libido year round. Semen volume per ejaculate varies from 0.3 mL to 0.95 mL, with an average of 0.523 ± 0.02 mL. The colour of semen is milky white to creamy white, and the most common consistency is ‘moderately thick’ to ‘thick’. Mass activity lies between 3 to 5, with an average of

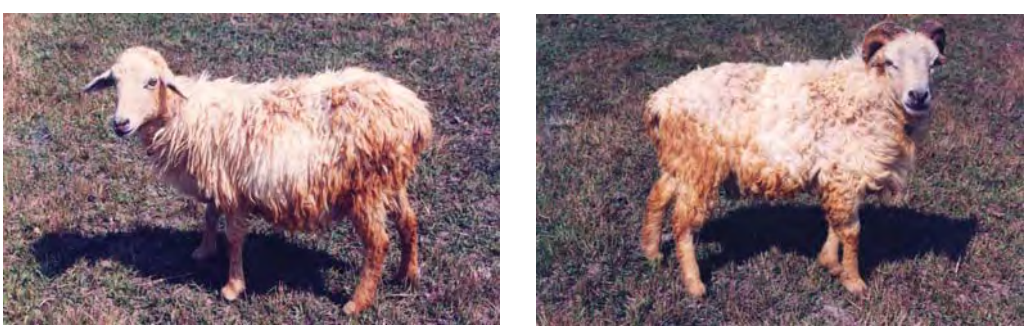

Figure 3. Photographs of Garole ewe (left) and Garole ram (right)

4.3 ± 0.113 (1–5 scale), and the pH of freshly collected semen ranges from 6.5 to 7.00. The average number of spermatozoa (in millions per mL) is 3,570 ± 146.62, with a range of 2,100 to 5,430. The dimensions of spermatozoa are presented in Table 5. Both flock and pen systems of mating are in vogue in the breeding tract. However, 93.8% animals are flock mated during community grazing.

Mean values of different female reproductive parameters are given in Table 6. Mean values of oestrous duration, oestrous interval and gestation length are found to be higher in comparison to those of other sheep breeds, and hence need validation under farm conditions. Females also do not exhibit any seasonality in reproduction. The proportions of animals lambed during summer, the rainy season and winter are 29.64%, 34.37% and 35.98% respectively. Lifetime lambing performance analysis of Garole sheep revealed a maximum of 28% of animals in the second lambing in the surveyed population (Table 7). This value is only 0.4% for animals in the tenth lambing. Farmers usually start selling female animals after their second lambing, to meet family needs. The average litter size is highest in the third lambing (1.94), and the sex ratio of lambs shows a marginally higher proportion of females. The incidence of twinning is highest (66%), followed by singletons (22%), triplets (11%) and quadruplets (< 1%).

Table 3a. Body weights (kg) of Garole sheep at different ages (mean ± SE)

Sex	Age group (months)				
	Up to 1	2–3	4–5	6–8	12
Male	1.94 ± 0.07 (186)	4.17 ± 0.11 (422)	6.47 ± 0.15 (412)	8.68 ± 0.11 (405)	10.88 ± 0.14 (266)
Female (not pregnant or nursing)	1.89 ± 0.07 (226)	4.07 ± 0.09 (751)	6.45 ± 0.15 (738)	8.08 ± 0.11 (781)	10.37 ± 0.14 (843)

Note: Figures in parentheses indicate the number of observations in each category.

Table 3b. Body weights (kg) of pregnant or nursing female Garole sheep at different ages (mean ± SE)

	Age group (months)	
	8–11	12
Pregnant	10.15 ± 0.4 (1,099)	12.29 ± 0.17 (2,133)
Nursing	6.75 ± 0.25 (512)	8.61 ± 1.46 (505)

Note: Figures in parentheses indicate the number of observations in each category.

Table 4. Body measurements (cm) at different ages (mean ± SE)

Parameter	Sex	Age group (months)				
		Up to 1	2–3	4–5	6–8	11–13
Chest girth	Male	29.3 ± 0.5 (186)	40.6 ± 0.4 (422)	49.7 ± 0.5 (412)	58.9 ± 0.3 (405)	65.4 ± 0.4 (266)
	Female	28.4 ± 0.5 (226)	39.9 ± 0.4 (751)	48.8 ± 0.5 (738)	58.0 ± 0.3 (2,392)	65.2 ± 0.3 (3,481)
Height at withers	Male	23.3 ± 0.5	32.5 ± 0.2	39.7 ± 0.2	42.3 ± 0.1	49.9 ± 0.5
	Female	22.9 ± 0.5	32.0 ± 0.2	39.4 ± 0.2	42.2 ± 0.1	48.7 ± 0.2
Body length	Male	22.5 ± 0.3	26.8 ± 0.2	37.2 ± 0.2	42.7 ± 0.1	53.5 ± 0.5
	Female	22.3 ± 0.3	25.9 ± 0.2	37.1 ± 0.2	42.4 ± 0.1	53.0 ± 0.3

Note: Figures in parentheses indicate the number of observations in each category

Carcass characteristics

Carcass characteristics of Garole sheep were recorded on the animals slaughtered in the region. Slaughtered male animals are divided into two age groups, namely up to 12 months and 13–24 months. The only females that are slaughtered are more than 24 months old, when they have passed the prime age of reproduction. The optimum age of slaughter is 12 months at a slaughter weight of 12 kg. Males reach their mature body size at 12 months of age; the gain in body weight subsequent to 12 months is due to fat deposition and therefore is not economical. The dressing percentage is highest (53.5 ± 0.2%) in animals up to 12 months of age (Table 8), and is lowest (50.8 ± 0.4%) in females. Skin weight percentage is lowest (10.5 ± 3.4%) in younger males and highest (16.8 ± 1.2%) in females, due to the different ages at slaughter. Mutton from females fetches lower market prices because of inferior quality.

Carcass compositions for male and female Garole sheep have been evaluated according to proximate composition, pH, waterholding capacity and fibre diameter of *longissimus dorsi* muscle. The findings are presented in Table 9. Although muscle fibre diameter is marginally finer in the female, the overall quality of mutton from females is inferior due to the much higher age at slaughter.

Table 5. Dimensions of Garole ram spermatozoa

Dimension	Mean ± SE	Range	CV[a]
Head length (μm)	8.79 ± 0.07	7.69–9.82	23.42
Head breadth (μm)	5.41 ± 0.04	5–6.15	22.91
Mid-piece length (μm)	15.39 ± 0.13	14–17	23.27
Tail length (μm)	47.04 ± 0.34	44.62–51.79	22.18
Total length of spermatozoa	71.38 ± 0.42	66.81–76.79	16.59
Head shape (length:breadth)	1.64 ± 0.02	1.34–1.85	34.39

[a] CV = coefficient of variation

Table 6. Female reproductive parameters of Garole sheep

Age at 1st heat (months)	Oestrus duration (hours)	Oestrus interval (days)	No. of services/ conception	Gestation length (days)	Service period (days)	Lambing interval (days)
8.5 ± 0.03 (3,649)	42.3 ± 0.20 (3,159)	18.5 ± 0.09 (2,350)	1.50 ± 0.01 (2,713)	157.1 ± 0.23 (3,562)	26.8 ± 0.19 (3,452)	189.2 ± 0.38 (3,339)

Note: Figures in parentheses indicate the number of observations in each category

Table 7. Lifetime lambing performance of Garole sheep

Lambing number	Number of observations	Breeding ewes in surveyed population (%)	Number of lambs per lambing	Sex ratio of lambs (M : F)	Ewe life expectancy (expected no. of lambings)
1	2,000	24.4	1.63	46.2 : 53.7	3.10
2	2,304	28.1	1.87	48.1 : 51.9	1.69
3	1,740	21.2	1.94	47.1 : 52.9	1.23
4	1,065	12.9	1.86	48.6 : 51.4	1.02
5	513	6.3	1.79	43.5 : 56.5	1.12
6	271	3.3	1.76	44.8 : 55.2	1.13
7	145	1.8	1.75	47.1 : 52.9	1.12
8	83	1.0	1.67	48.9 : 51.1	0.96
9	47	0.6	1.65	41.6 : 58.4	0.70
10	33	0.4	1.69	48.3 : 51.7	0.00

Table 8. Carcass characteristics of Garole sheep (mean ± SE)

Sex	Age (months)	Slaughter body weight (kg)	Carcass weight (kg)	Dressing percentage	Skin length (cm)	Skin width (cm)	Skin area (cm^2)	Skin weight (kg)	Skin weight (%)
Male	< 12 (57)	12.25 ± 2.5	6.61 ± 0.1	53.5 ± 0.2	65.3 ± 8.67	59.7 ± 9.8	4048.3 ± 91.7	1.2 ± 0.2	10.5 ± 3.4
Female	13–24 (75)	16.87 ± 1.21	8.66 ± 0.2	51.7 ± 0.3	84.1 ± 4.99	63.2 ± 8.9	5474.1 ± 92.7	2.3 ± 0.3	12.0 ± 2.1
	> 24 (42)	13.04 ± 0.42	6.59 ± 0.2	50.8 ± 0.4	62.14 ± 7.71	36.1 ± 8.1	2433.1 ± 75.1	1.8 ± 0.2	16.8 ± 1.2

Note: Figures in parentheses indicate the number of observations in each category

Table 9. Carcass composition of Garole sheep (mean ± SE)

Sex	Moisture (%)	Protein (%)	Ether extract (%)	Total ash (%)	pH	Waterholding capacity (mL/100 gm)	Muscle fibre diameter (μm)
Male	70.7 ± 0.1	17.3 ± 0.05	10.7 ± 0.1	1.1 ± 0.02	6.0 ± 0.02	31.8 ± 0.7	15.6 ± 0.04
Female	70.1 ± 0.1	17.1 ± 0.08	11.5 ± 0.1	1.1 ± 0.02	6.1 ± 0.02	28.2 ± 0.4	15.4 ± 0.1

Morbidity and mortality

Garole sheep appear to be fairly tolerant to diseases, particularly considering their living conditions. Morbidity and mortality rates for lambs up to 1 month of age (38.45% and 30.8% respectively) are the highest in all the age groups. The rates reduce with age. At more than 8 months of age only 15% of animals were identified as sick and 6.3% died. Overall morbidity and mortality rates, irrespective of age group (21.3% and 14.7% respectively), are lowest during winter among all age groups, with females appearing to suffer more than males. The incidence of gastrointestinal tract problems is highest (55%), followed by miscellaneous cases (21%). Incidences of abortion, repeat breeding and placenta retention are 8%, 9% and 3% respectively. Faecal samples of Garole sheep collected randomly from the field were screened to evaluate parasitic load; every sample was found to be positive for intestinal parasitic infection. Different parasites identified were *Strongyloides* sp., *Moniezia* sp. and *Eimeria* sp. (coccidia). However, no trematodal infection was recorded.

Disposal of live animals

Farmers usually dispose of animals by two main modes, namely the sale of live animals, and the slaughter and sale of mutton and skin. Sales of live animals account for 89% of animals disposed of, and slaughter and sale accounts for 10%. Only 0.1% are used for home consumption. Middlemen routinely visit farmers' houses for the purchase of animals. Farmers dispose of 97% of animals through middlemen, and animals are often booked with part advance payment. Only in 3% of cases do farmers take their animals to local markets to sell to meet financial needs in the family. For slaughter and sale, the owner normally books prospective buyers for the mutton in the village or locality and then the animals are slaughtered. This practice is more common during the festival season. Farmers also often need to sacrifice animals for family consumption to meet social obligations. In any case, skins of the slaughtered animals are sold to middlemen from different tanneries in Calcutta. Animals of up to 6, 6.1–12 and more than 12 months of age account for 9%, 37% and 54%, respectively, of the total number of disposed animals. The proportion of male animals disposed of is much higher than that of females for the first two age groups because females are retained for breeding purposes. Neighbouring farmers purchase some of the surplus young females for breeding. The ratio of male to female animals disposed of at the oldest age group is 50:50, as the farmers do not like to retain older female stock past their peak breeding efficiency. The body weight of females disposed of is marginally lighter compared to their male counterparts in every age group. The sale price is also different for different sexes in each age group, with males always fetching a higher price than females. Sale prices of females of the oldest age group are lowest as these animals produce the poorest quality meat and their breeding capacity is on the wane.

Task ahead

The original source of the *FecB* gene, Garole sheep, are now facing tremendous problems of survivability in their natural habitat. A sharp decline in population growth rate is one of the prominent indicators. The present trend suggests that the breed will not be able to maintain its population size within the next 5 years. The core breeding habitat is fast receding towards the south. The overall situation is alarming and calls for immediate attention from scientists, administrators and other stakeholders. The Government of India has launched a conservation program on Garole sheep, which is being implemented by the Government of West Bengal. Under the program an island called 'Machranga', located in the core area of the breeding tract, is being developed as an ideal site for Garole breeding and improvement, in a similar way to the conservation of Jersey cattle on Jersey Island. The program also includes training of Garole farmers in scientific management and promotion of the valuable genetic resource. Although the approach is a positive step, the magnitude and complexity of the forces acting against the survivability of Garole sheep suggest that much has to be done immediately to save this genetic resource from extinction.

References

Acharya R.M. 1982. Sheep and goat breeds of India. FAO Animal Production and Health Paper 30. FAO: Rome.

Anon. 2004. Animal genetic resources of Bangladesh: first report on the state of the world's Animal Genetic Resources (AnGR). Bangladesh Livestock Research

Institute (BLRI). The Government of the People's Republic of Bangladesh.

Bose S., Duttagupta R. and Maitra D.N. 1999a. Phenotypic characteristics and management practices of Bengal sheep. Indian Journal of Animal Production and Management 15, 18–22.

Bose S., Duttagupta R. and Maitra D.N. 1999b. Reproductive performance of Bengal sheep in Sundarbans. Indian Journal of Animal Production and Management 15, 157–160.

Bose S. and Maitra D.N. 1995. Bengal breed of sheep in the 'Sundarbans'. Asian Livestock 20(2), 16–17.

Bradford G.E. and Inounu I. 1996. Prolific breeds of Indonesia. Pp. 137–145 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Doyle J. 1991. Jean Fleming sheep scientist. At: < http://nzsm.webcentre.co.nz/article1543.htm>.

Ghalsasi P.M. and Nimbkar B.V. 1993. The 'Garole' – microsheep of Bengal, India. Animal Genetic Resources Information 12, 73–79. FAO.

Ghalsasi P.M., Nimbkar C. and Gray G.D. 1994. Garole prolific microsheep of West Bengal, India. Proceedings of 5th World Congress of Genetics Applied to Livestock Production, Guelph, 20, 456–459.

Kar K. and Prasad C. 1992. Technological interventions for promotion of small ruminants for resource poor farmers in rainfed areas. Pp. 953–969 in 'Recent advances in goat production. Proceedings of the 5th International Conference on Goats', ed. by R.R. Lokeshwar. Indian Council of Agricultural Research: New Delhi.

Lee D.H.K. 1953. Manual of field studies on the heat tolerance of domestic animals. P. 49 in 'Improvement of livestock production in warm climate', ed. by R.E. McDowell. FAO Development Paper No. 38. W.H. Freeman and Company: San Francisco.

Mason I.L. 1980. A world dictionary of livestock breeds type and varieties. CAB International: Wallingford, Oxon, UK

Pan S. and Sahoo A.K. 2003. Garole sheep. West Bengal University of Animal and Fishery Science.

Sahana G., Gupta S.C. and Nivsarkar A.E. 2001. Garole: the prolific sheep of India. Animal Genetic Resources Information 31, 55–64.

Singh R.N. and Bohra S.D.J. 1996. Garole sheep: a prolific Bengal breed of sheep (locally known as Garole). Indian Journal of Small Ruminants 2, 38–42.

Turner H.N. 1982. Origins of the CSIRO Boroola. Pp. 1–7 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24-25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Session 2:
Physiological aspects of the *FecB* gene mechanism

The mechanism of action of the *FecB* (Booroola) mutation

B.K. Campbell[1,2], P. Marsters[1] and D.T. Baird[3]

Abstract

It is 50 years since the Commonwealth Scientific and Industrial Research Organization (CSIRO) acquired the Booroola flock of highly prolific Merino sheep. Over this time, major advances have been made in discovering the mechanisms of action of the *FecB* mutation. It is now clear that the increase in prolificacy resulting from this mutation is not due to an increase in the circulating FSH concentrations, but rather to an increased sensitivity to FSH mediated by the action of intra-follicular local factors. The identity of these factors is still uncertain, but the results to date are consistent with a major role for the bone morphogenetic protein system in modulating proliferative and differentiative responses of both granulosa and theca cells to gonadotrophic stimulation, and may entail both increased actions of stimulators (e.g. BMP-6) and decreased actions of inhibitors (e.g. BMP-15, AMH) of gonadotrophic actions. Such a mechanism, in which multiple intra-follicular regulatory pathways are affected, would explain the profound effect of the *FecB* mutation in inducing precocious maturation of ovarian follicles, and hence deregulating the normal follicle selection mechanisms operating in this species. However, more evidence is needed to support this hypothesis.

Introduction

It is 50 years since CSIRO identified and acquired a flock of remarkably prolific sheep, which had up to 10 lambs per pregnancy, from a commercial sheep property called 'Booroola' near Cooma, New South Wales (Bindon 1984). This increase in lambing rate was subsequently found to be due to an increase in the number of eggs ovulated each cycle (ovulation rate; OR). The gene responsible segregated as a single gene, or a closely linked series of genes, with the heterozygote having an OR intermediate (designated F+ with OR 3–4) between the homozygote (designated FF with ovulation rate > 4) and the wild type (designated ++ with ovulation rate 1–2) (Davis et al. 1982; Bindon 1984).[4] Since then, the mechanisms resulting in this extraordinary increase have been the subject of intensive research in many laboratories around the world investigating the control of ovarian function. A major advance in this regard came in 2001 when it was demonstrated by three groups, including our own, that the increase in fecundity in Booroola ewes was associated with a single point mutation in the intracellular domain of one of the receptors for bone morphogenetic proteins (BMPR1B) (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001). However, rather than solving a mystery, this discovery opened new avenues of research as the BMP system had not formerly been recognised as a major system

[1] School of Human Development, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom

[2] Corresponding author; Bruce.Campbell@nottingham.ac.uk

[3] Centre for Reproductive Biology, University of Edinburgh, EH16 4SA, United Kingdom

[4] FF, F+ and ++ are an alternative notation for *FecB* genotypes, equivalent to $FecB^{BB}$, $FecB^{B+}$ and $FecB^{++}$, respectively, used in other papers in these proceedings.

regulating ovarian function. The objective of this review is to provide an overview of our current understanding of the possible mechanisms underlying the changes in ovarian function observed in sheep carrying the *FecB* mutation.

The control of ovarian follicle development in monovulatory species

Ovarian folliculogenesis is a lengthy and intricately regulated process marked by dramatic proliferation and precisely orchestrated differentiation of both the somatic and germ cell elements. The ovarian follicle is the fundamental developmental unit of the mammalian ovary, consisting of a germ cell (oocyte) in close association with somatic cells (granulosa cells; GC). Primordial follicles represent the source from which follicles will be recruited for growth throughout life, and the paired ovaries of an individual contain around 100,000–250,000 of these follicles at birth (Campbell et al. 1995; Webb et al. 1999). Once follicles have been initiated to grow, the GCs proliferate to form multilaminar structures (preantral follicles), which subsequently form a fluid-filled space (antrum), a well-differentiated theca layer and the ability to respond to the pituitary gonadotrophins (FSH and LH) at a diameter of around 250 μm. Further development of these so-called gonadotrophin-responsive (Gn-responsive) follicles past a diameter of 2–4 mm in most species relies on the provision of adequate levels (threshold) of FSH, and these larger antral follicles are termed Gn-dependent. The transition from the Gn-responsive to the Gn-dependent phase is associated with widespread atresia (50–70% for follicles over 1 mm) so that the vast majority (>99%) of follicles fail to ovulate (Turnbull et al. 1977).

Although the latter stages of follicle development are primarily regulated by the pituitary gonadotrophins (FSH and LH), there is now strong evidence that this process relies heavily on complex actions and interactions between locally produced hormones and growth factors (Knight and Glister 2003; Webb and Campbell 2007). These systems include the insulin/IGF system (Webb et al. 1999), the inhibin/activin system (Campbell and Baird 2001; Campbell et al. 2003b) and the bone morphogenetic system (Souza et al. 2001, 2002; Campbell et al. 2006). In addition, recent studies also suggest that the oocyte, rather than being purely a passenger within the follicle, secretes numerous factors that affect follicle development and ovarian function. Known oocyte-secreted factors include growth differentiation factor-9 (GDF-9) (Juengel et al. 2004), bone morphogenetic protein-6 (BMP-6) (Knight and Glister 2003; Campbell et al. 2006) and BMP-15 (Galloway et al. 2000), as well as factor in the germline alpha (FIG-α) (Huntriss et al. 2002), NOBOX (Huntriss et al. 2006) and kit receptor (Driancourt et al. 2000). Gap junction-mediated communication between the oocyte and the surrounding somatic cells is essential for the coordinated development of both cell types, and this link is maintained throughout follicle growth, during which time somatic cells provide the oocyte with metabolic substrates and meiosis-arresting signals (Themmen 2005).

The orderly, stage-specific expression of these somatic- and oocyte-derived factors at the correct time, or 'intrafollicular cascade', is thought to be essential for the development of the follicle to an ovulatory size, the production of an ovulatory signal and the release of a fully developmentally competent oocyte in response to that signal. During the gonadotrophin responsive and dependent stages of follicle development, it has been postulated that local factors regulate the sensitivity of follicular somatic cells to gonadotrophins and are therefore considered to be central to the mechanism of follicle selection and the control of OR. Our mechanistic models of the control of follicle selection (Figure 1A) therefore postulate that these factors act to either attenuate or augment the stimulatory actions of gonadotrophins on follicle development, so that the fate of individual follicles relies on the balance between these conflicting local actions (Campbell et al. 1995; Webb and Campbell 2007). From this model it is therefore possible to postulate an increase in OR through either an increase in circulating gonadotrophin concentrations, an increase in the activity of augmentors of gonadotrophic actions or a decrease in the activity of attenuators of gonadotrophic actions (Figure 1B).

Booroola phenotype

Early work on the Booroola animals (Bindon 1984; Bindon et al. 1985) was hampered by the fact that the only phenotype expressed was OR and number of offspring in the female, but early studies on the ovarian physiology of *FecB*-gene carriers in

Merinos suggested that they had smaller preovulatory follicles with fewer GCs and correspondingly smaller corpora lutea (CL) than control Merinos (Scaramuzzi et al. 1981; Baird et al. 1982). These initial observations were confirmed and extended by an elegant and comprehensive series of experiments by McNatty and colleagues in New Zealand. Using Romney Marsh ewes carrying the gene, they showed that small antral follicles matured precociously in gene carriers, becoming oestrogenic and developing LH receptors on the membrana granulosa at diameters of 2.5–3.5 mm compared to 4–6 mm in non gene carriers (Henderson et al. 1985; McNatty et al. 1985, 1986a, b, c). More recently, we have extended these observations by showing that expression of messenger ribonucleic acid (mRNA) for both cytochrome P450 aromatase and the $ß_A$-subunit of inhibin/activin can be detected in much smaller follicles in *FecB*-gene carriers compared to controls (Figure 2).

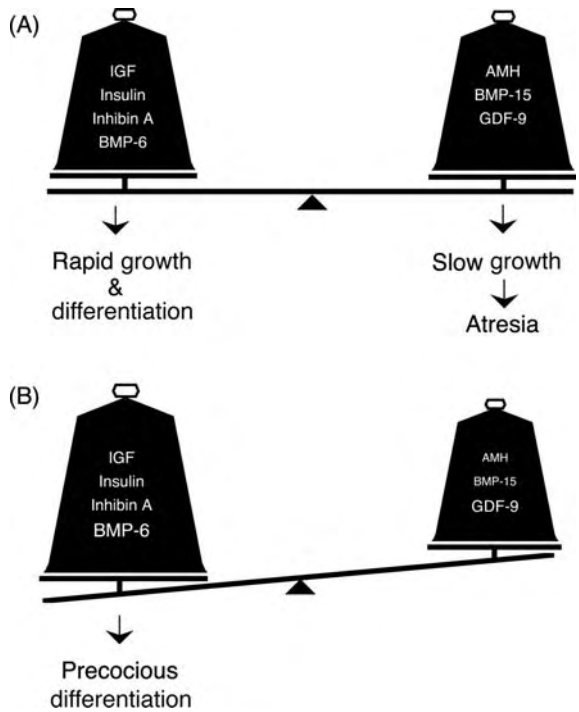

Figure 1. (A) Simplified model illustrating the concept that the fate of each Gn-dependent follicle relies on the intra-follicular balance between factors that augment the action of gonadotrophins to stimulate follicular growth and differentiation and those that attenuate the action of gonadotrophins and which will slow growth and ultimately lead to atresia of the follicle. (B) Hypothesis that the precocious differentiation of ovulatory follicles in *FecB* mutants may result from either the increased activity of augmentors of gonadotrophins (e.g. BMP-6), the decreased activity of attenuators of gonadotrophins (e.g. AMH or BMP-15) or the combined action of both systems. Each of these factors has been shown to be capable of signalling through the BMPR1B (ALK6).

Examination of the kinetics of folliculogenesis in *FecB*-gene carriers during the preovulatory period has been attempted using follicular dissection (McNatty et al. 1985, 1986b), ink labelling (Driancourt et al. 1985) and ultrasound imaging in ewes with ovarian autotransplants (Souza et al. 1997). These studies collectively suggest that the OR differences of the *FecB*-gene carriers are not due to differences in the total number of antral follicles but to an extended recruitment period together with a low incidence of atresia, resulting in the ovulation of a large number of small ovulatory follicles. The smaller size of these ovulatory follicles explains the observation that ovarian oestradiol (Baird et al. 1982; McNatty et al. 1986b; Souza et al. 1997), androgen (Souza et al. 1997) and inhibin A (Souza et al. 1997) secretion during the preovulatory period, and luteal progesterone concentrations (Bindon 1984; McNatty et al. 1985), do not differ among animals with two, one or no copies of the *FecB* gene. In fact, McNatty has calculated that the total population of GCs in oestrogenic follicles is identical in the different genotypes (McNatty et al. 1985).

In addition to ovarian effects, the development of many organs is retarded in the foetus in *FecB* mutants (McNatty and Henderson 1987), suggesting that the gene product is likely to be important in embryonic development. Differences in heart girth and chest width have also been reported in Chinese lambs carrying the mutation (Guan et al. 2006), but in the adult animals of our flock in Edinburgh (Scottish Blackface Merino cross) the only difference observed was slightly lighter adrenal glands in gene carriers (Souza and Baird 2004). These developmental effects, however, may be confounded by the effect of litter size (LS) on birth weight.

Molecular basis of Booroola mutation

In 2001 it was demonstrated by three groups (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001) that the increase in fecundity in Booroola ewes was associated with a single point mutation in the intracellular domain of one of the receptors for BMPs (BMPR1B-ALK6). The fecundity gene is situated on chromosome 6 in a locus corresponding to chromosome 4 in the human, and subsequent work has shown that the same mutation occurs in other prolific breeds of sheep including the Garole, Javanese, Hu and Han (Davis et al. 2002, 2006). At around the same time, other spontaneous mutations

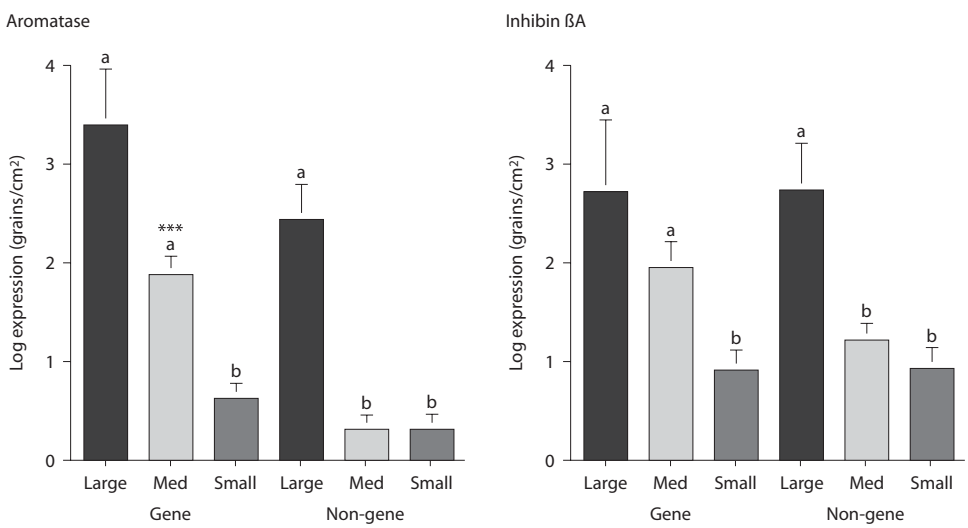

Figure 2. Expression of mRNA, determined by in-situ hybridisation, for P450-aromatase and inhibin-βA sub-unit in the granulosa cells of antral follicles of different sizes in Scottish Blackface Merino cross ewes with and without the *FecB* mutation. Note increased levels of expression of these markers of differentiation at smaller follicle sizes in ewes carrying the *FecB* mutation. Values are means ± SEM, and different letters indicate statistically significant differences ($P < 0.05$). *** $P < 0.001$

Source: B.K. Campbell, L. Harkness and D.T. Baird (unpublished observations).

which led to alterations in OR in sheep were identified, and these were shown to involve ligands rather than receptors of the transforming growth factor beta (TGFβ) superfamily. Thus, in Inverdale (*FecX*^{I}) and Hanna (*FecX*^{H}) ewes, separate point mutations were identified in the bone morphogenetic protein (BMP-15) gene on the X chromosome corresponding to sites in the mature peptide coding region of the BMP-15 growth factor (Galloway et al. 2000).

A remarkable characteristic of these mutations is that those which are heterozygous for the *FecX*^{I} or *FecX*^{H} mutation have higher than normal ORs and LSs, whereas the homozygotes are sterile (Davis et al. 2001). Similarly, in Cambridge and Belclare ewes, mutations in both BMP-15 and the closely related GDF-9 led to marked increases in OR (Hanrahan et al. 2004). TGF-β superfamily members signal via a heteromeric receptor complex consisting of a type 1 and a type 2 receptor serine/threonine kinase. Upon ligand binding, the type 2 receptor recruits the non-ligand binding type 1 receptor into the complex, resulting in phosphorylation of signalling pathway effector proteins called Smads (Rey et al. 2003). At present the identity of the ligands that signal through the BMPR1B receptor in the sheep ovary are uncertain. Studies using non-ovarian cell types and lines have demonstrated that there are a limited number of ligands in the TGF-β family which, after binding to a type 2 receptor, use BMPR1B. These include BMPs 2, 4, 6, 7, 15 and AMH (Shi and Massague 2003; Miyazono et al. 2005). In contrast, it appears likely that GDF-9 uses TGFBR1 (ALK5) as its type 1 receptor (Mazerbourg et al. 2004). A similar promiscuity is also evident in potential type II receptors. Although the type 2 AMH receptor (AMH R2) is unique and does not bind with other TGF-β superfamily members, BMPs 2, 4, 6, 7, 15 and GDF-9 can use BMPR11 as their type 2 receptor (Massague 2000; Juengel et al. 2004; Mazerbourg et al. 2004).

We have demonstrated expression of both BMPR1B and BMPR1A type 1 receptors and the type 2 BMP receptors in ovarian somatic cells in sheep across all stages of folliculogenesis (Souza et al. 2002). We and others have also demonstrated specific expression of BMP-6, BMP-15, GDF-9 and AMH (Figure 3) in either the oocyte or somatic cells of ovarian follicles in this species (Juengel et al. 2004; Campbell et al. 2006). AMH R2 expression has been described in the sheep ovary (McNatty et al. 2007) and we have observed responses of sheep GCs to AMH in vitro (Campbell et al. 2005). It therefore appears that all components of this regulatory system are expressed in the sheep ovary.

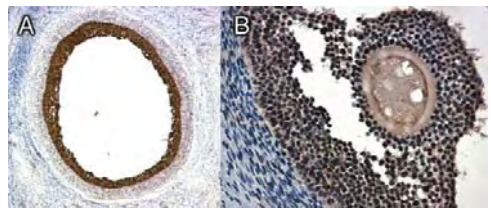

Figure 3. Small healthy antral sheep follicles showing specific abundant immunoactivity for AMH in the mural granulosa cells layer (A,B) and cumulus and oocyte (B).
Source: B.K. Campbell and A. Skinner (unpublished)

Mechanisms of action

Gonadotrophic effects

While precocious development of preovulatory follicles appears to explain the increase in prolificacy in *FecB*-gene carriers, the mechanism behind this early maturation is unclear. As FSH is the primary hormone controlling follicular growth and development, it has been suggested that the *FecB* mutation may act by either increasing the release of FSH from the pituitary or increasing the sensitivity of follicular cells to FSH within the ovary. Studies designed to test these hypotheses have provided evidence to support both. Initially, it was reported that the concentrations of FSH were similar in both carriers and non-carriers of the *FecB* gene (Bindon et al. 1985), but the development of improved FSH assays saw the publication of a number of papers supporting the hypothesis that elevated jugular venous FSH concentrations are associated with the *FecB* gene (Bindon 1984; McNatty and Henderson 1987). However, the fact that a number of authors have reported no consistent association between *FecB* and FSH (Boulton et al. 1995; Souza et al. 1997) casts some doubt on the causality of this relationship. Equally, equivocal results have been obtained from studies that have examined this question using hypophysectomised (Fry et al. 1988) or hypothalamic-pituitary disconnected and gonadotropin-releasing hormone (GnRH)-agonist

suppressed ewes (Hudson et al. 1999) stimulated with exogenous gonadotrophins. The study of Fry et al. (1988) showed a continued difference in OR in *FecB*-gene carriers stimulated with pregnant mare serum gonadotrophin (PMSG), but the study of Hudson et al. (1999) concluded that the *FecB* gene acts at both a pituitary and ovarian level to stimulate OR. In what we believe was a key experiment, FSH and LH were infused in physiological amounts that closely simulated those found in the intact animal into ewes of both genotypes which had been rendered hypogonadotrophic by the administration of a potent GnRH-antagonist (Campbell et al. 2003a). In this study the difference in OR and the characteristic phenotype of smaller ovulatory follicles and CL was retained, providing strong evidence that the *FecB* mutation acts at the level of the ovary to modulate gonadotrophic responsiveness (Figure 4).

Altered sensitivity of somatic cells to gonadotrophins

Testing the hypothesis that ovarian follicular cells are more sensitive to gonadotrophic stimuli in *FecB*-gene carriers has been complicated by the precocious maturation of ovulatory follicles in gene carriers, as it is difficult to find a satisfactory comparative basis between genotypes. The existing evidence shows that smaller (2.0–4.5 mm) follicles from *FecB*-gene carriers are more sensitive (in terms of cAMP production) to LH and FSH and have higher aromatase activity than similar sized follicles from control ewes. In contrast, theca cell (TC) and GC LH binding characteristics and thecal LH-stimulated androstenedione production have not been found to differ according to genotype (McNatty, Henderson et al. 1985; McNatty et al. 1986b; McNatty et al. 1986c). Finally, using physiological serum-free culture systems that allow gonadotrophin-dependent induction of cellular differentiation in vitro, we have been able to show that both GCs and TCs from non-differentiated small follicles of less than 1 mm in diameter from *FecB*-gene carriers are more sensitive in terms of gonadotrophin-induced oestradiol production than similar sized or medium-sized (1–3 mm) follicles from non-gene carriers (Campbell et al. 2006). Thus, the available evidence suggests that the *FecB* mutation is acting at an ovarian level to modulate the sensitivity of somatic cells to gonadotrophic stimulation.

Local actions of potential ligands of the BMPR1B in ovarian somatic cells

As discussed above, the BMPR1B receptor interacts with a number of local factors which may act to augment or attenuate the actions of pituitary gonadotrophins. Cell culture studies in sheep have shown that BMPs 2, 4 and 6 result in an increase in FSH-induced estradiol production (Campbell et al. 2006) and a decrease in FSH-induced progesterone production (Shimasaki et al. 1999), by GCs. In contrast, both AMH and BMP-15 act as inhibitors of FSH-induced estradiol production by GCs in sheep (Campbell et al. 2003a) and other species (Otsuka et al. 2001). In terms of TC function, high doses of BMP 2, 4 and 6 (5–50 ng/mL) inhibited LH-stimulated androstenedione (A4) production by TCs, whereas lower doses (0.005–0.05 ng/mL) stimulated TC proliferation and total androstenedione production (Campbell et al. 2006). Inhibitory effects of AMH, GDF-9 and BMP-15 on thecal steroidogenesis have also been observed but only at very high doses (Campbell et al. 2005). Of these ligands, unequivocal evidence indicating both mRNA and protein expression in ovarian cell types in sheep has been observed for BMP-6 (oocyte, GC: Campbell et al. 2006; Juengel et al. 2006), AMH (GC: Figure 3), GDF-9 (oocyte: Juengel et al. 2004)) and BMP-15 (oocyte: Juengel et al. 2004).

Effect of *FecB* mutation in BMPR1B on cellular responsiveness

Fabre et al. (2003), using a reporter construct approach in which wild-type and *FecB* (Q249R) mutant BMPR1B were transfected into human kidney cells, showed that the *FecB* transfected cells exhibited an increase in basal luciferase activity (relative to the wild type) but had an attenuated response to stimulation with BMP-4. We have extended these observations with alternate cell types and ligands to show that the *FecB* mutation leads to an attenuation in response to stimulation of both HEK-293 and HEP-G2 cells with both BMP-4 (Figure 5) and BMP-6. No data are currently available on the effect of the mutation on the responsiveness of cells to BMP-15 or AMH, but the data currently available from the in-vitro transfection of human cell lines suggests that the *FecB* mutation acts to attenuate intracellular signalling of the BMPR1B.

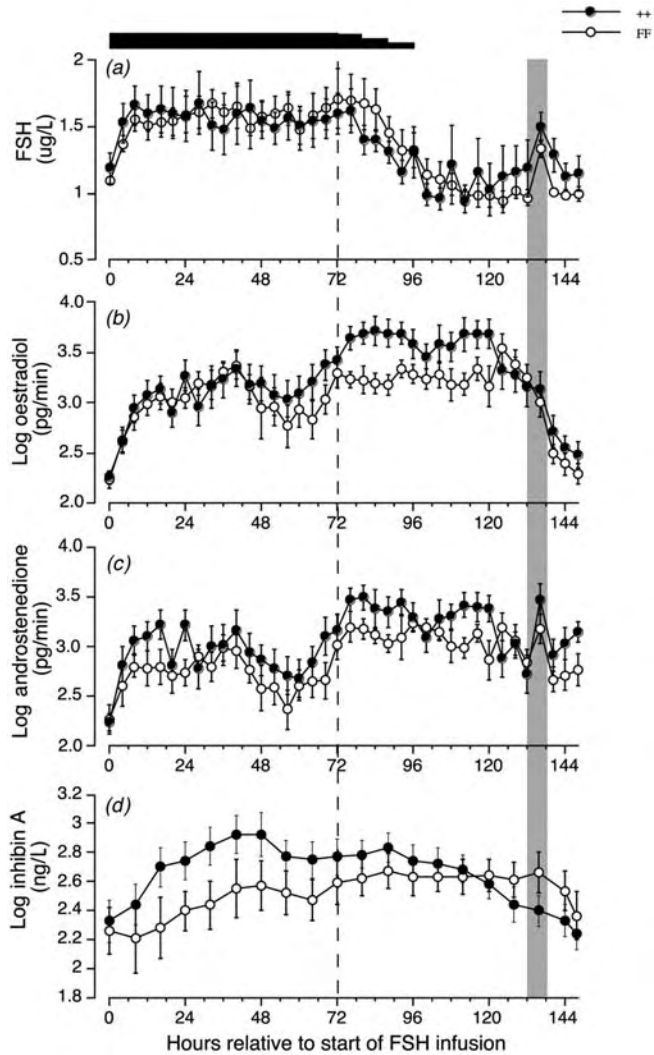

Figure 4. Jugular venous FSH and ovarian secretion of oestradiol, androstenedione and inhibin A in wild-type (closed symbols; ++) and ewes bearing the *FecB* mutation (open symbols; FF) following FSH infusion and across the artificial follicular phase. The solid horizontal bar indicates the period and level of FSH infusion, and the vertical hatched bar indicates the period of the artificial 'LH surge'. The dashed line indicates the time of sponge withdrawal. Following ovulation, the genotypic difference was retained, with gene carriers having more preovulatory follicles / corpora lutea (3.8 ± 0.3) of a small diameter than non-gene carriers (1.7 ± 0.3; $P < 0.05$). Values are means ± SEM.
Source: reproduced from Campbell et al. (2003a).

This observation, however, is at odds with some of the findings of cell culture experiments conducted in our (Campbell et al. 2006) and other (Fabre et al. 2003) laboratories using ovarian somatic cells from ewes with and without the *FecB* mutation. In the presence of low concentrations (0.1 ng/mL) of IGF-1, the maximum increase in the production of E2 and inhibin A by GCs from *FecB* ewes in response to BMP-6 was observed at doses that were 3–10-fold lower (3–10 ng/mL) than from wild-type ewes (30 ng/mL) (Campbell et al. 2006). Conversely, low doses of BMP-6 stimulated proliferation of TCs from wild-type but not *FecB* ewes (Figure 6).

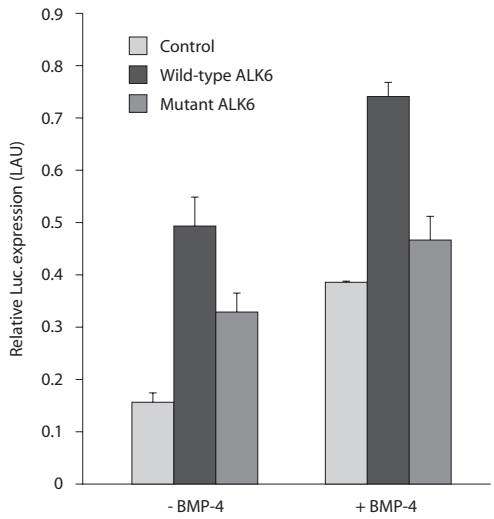

Figure 5. Luciferase expression from BMP-responsive element (BRE)-firefly. Luciferase reporter constructs were determined relative to luciferase expression from co-transfected Sea pansy luciferase reporter constructs 6 hours after transfection into Hep G2 cells cultured either in the presence of or the absence of 10 ng/mL BMP-4. The luciferase reporters were either transfected alone (control) or further co-transfected with BMPR2 and either BMPR1B wild type (Wild-type ALK6) or BMPR1B *FecB* mutant (Mutant ALK6) expression constructs. Results represent the means ± SEM of at least three separate experiments.
Source: P. Marsters, L. Guo and B.K. Campbell (unpublished).

Similarly, recent data have suggested that the depressive effects of AMH, BMP-15 and GDF-9 on FSH-stimulated GC differentiation are blunted in *FecB* mutants compared to wild-type animals (B.K. Campbell, S. Shimasaki and D.T. Baird, unpub-

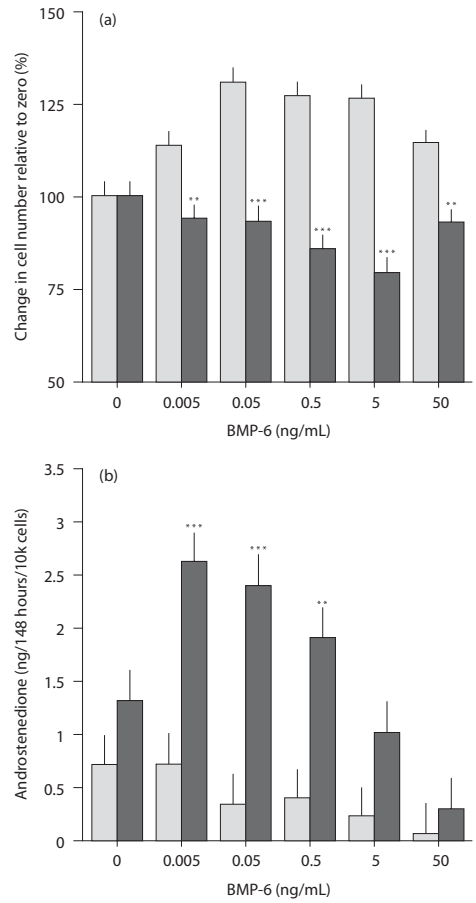

Figure 6. Effect of *FecB* mutation on response of theca cells to BMPs. These data show cell number (a) and androstenedione (b) production by theca cells isolated from small antral follicles of Fec^{++} (lighter column) and $FecB^{FF}$ (darker column) animals and cultured under serum-free conditions for 144 hours in the presence of increasing doses of BMP-6. Values are means ± SEM, and asterisks denote significant difference between genotypes at the same dose—$^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
Source: reproduced from Campbell et al. (2006).

lished). Thus, it appears likely that the effects of the *FecB* mutation may be cell and ligand specific, and more work is required to elucidate exact mechanisms. At present, however, the available evidence supports a model whereby gonadotrophic actions are amplified through either increased activation of an augmentor (BMP-6), decreased activation of attenuators (BMP-15, AMH) or a combination of these two mechanisms (Figure 1B).

Conclusion

Since its discovery, nearly 30 years ago, major advances have been made in elucidating the mechanisms of action of the *FecB* mutation, and these studies have been central to our understanding of the mechanism of follicle selection in all monovulatory species. It is now clear that the increase in prolificacy resulting from this mutation is not due to an increase in the circulating FSH concentrations, but rather to an increased sensitivity to FSH mediated by the action of intra-follicular local factors. The identity of these factors is still uncertain, but the results to date are consistent with a major role for the BMP system in modulating proliferative and differentiative responses of both GCs and TCs to gonadotrophic stimulation, and may entail both increased actions of augmentors (e.g. BMP-6) and decreased actions of attenuators (e.g. BMP-15, AMH) of gonadotrophic actions. Such a mechanism, in which multiple intra-follicular regulatory pathways are affected, would explain the profound effect of the *FecB* mutation in inducing precocious maturation of ovarian follicles, and hence deregulating the normal follicle selection mechanisms operating in this species. However, more work is required to confirm this speculative hypothesis.

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References

Baird D.T., Ralph M.M., Seamark R.F., Amato F. and Bindon B.M. 1982. Pre-ovulatory follicular activity and estrogen secretion of high (Booroola) and low fecundity Merino ewes. Proceedings of the Australian Society of Reproductive Biology 14, 83.

Bindon B. 1984. Reproductive biology of the Booroola Merino sheep. Australian Journal of Biological Sciences 37, 163–189.

Bindon D., Piper L., Cummins L., O'Shea T., Hillard M., Findlay J. and Robertson D. 1985. Reproductive endocrinology of prolific sheep: studies of the Booroola Merino. Pp. 217–235 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Boulton M., Haley C., Springbett A. and Webb R. 1995. The effect of the Booroola (FecB) gene on peripheral FSH concentrations and ovulation rates during oestrus, seasonal anoestrus and on FSH concentrations following ovariectomy in Scottish Blackface ewes. Journal of Reproduction and Fertility 103, 199–207.

Campbell B.K. and Baird D.T. 2001. Inhibin A is a follicle stimulating hormone-responsive marker of granulosa cell differentiation, which has both autocrine and paracrine actions in sheep. Journal of Endocrinology 169, 333–345.

Campbell B.K., Baird D.T., Souza C.J. and Webb R. 2003a. The FecB (Booroola) gene acts at the ovary: in vivo evidence. Reproduction 126, 101–111.

Campbell B.K., Scaramuzzi R. and Webb R. 1995. Control of antral follicle development and selection in sheep and cattle. Journal of Reproduction and Fertility (suppl. 49), 335–350.

Campbell B.K., Sharma S., Shimasaki S. and Baird D.T. 2005. Effect of AMH, BMP-15 and GDF-9 on FSH-induced differentiation of sheep granulosa cells. Biology of Reproduction Abstracts 2005: 241.

Campbell B.K. and Souza C. et al. 2003b. Domestic ruminants as models for the elucidation of the mechanisms controlling ovarian follicle development in humans. Reproduction Supplement 61, 429–443.

Campbell B.K., Souza C.J., Skinner A.J., Webb R. and Baird D.T. 2006. Enhanced response of granulosa and theca cells from sheep carriers of the FecB mutation in vitro to gonadotropins and bone morphogenetic protein-2, -4, and -6. Endocrinology 147, 1608–1620.

Davis G.H., Balakrishnan L. et al. 2006. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92, 87–96.

Davis G.H., Dodds K.G., Wheeler R. and Jay N.P. 2001. Evidence that an imprinted gene on the X chromosome increases ovulation rate in sheep. Biology of Reproduction 64, 216–221.

Davis G.H., Galloway S.M. et al. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Davis G.H., Montgomery G.W., Allison A.J., Kelly R.W. and Bray A. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Driancourt M., Cahill L. and Bindon B. 1985. Ovarian follicular populations and preovulatory enlargement in Booroola and Merino ewes. Journal of Reproduction and Fertility 73, 93–107.

Driancourt M.A., Reynaud K., Cortvrindt R. and Smitz J. 2000. Roles of KIT and KIT LIGAND in ovarian function. Reviews of Reproduction 5, 143–152.

Fabre S., Pierre A., Pisselet C., Mulsant P., Lecerf F., Pohl J., Monget P. and Monniaux D. 2003. The Booroola mutation in sheep is associated with an alteration of the bone morphogenetic protein receptor-IB functionality. Journal of Endocrinology 177, 435–444.

Fry R.C., Clarke I.J., Cummins J.T., Bindon B.M., Piper L.R. and Cahill L.P. 1988. Induction of ovulation in chronically hypophysectomized Booroola ewes. Journal of Reproduction and Fertility 82, 711–715.

Galloway S.M., McNatty K.P. et al. 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nature Genetics 25, 279–283.

Guan F., Liu S.R., Shi G.Q., Ai J.T., Mao D.G. and Yang L.G. 2006. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Yi Chuan Xue Bao 33, 117–124.

Hanrahan J.P., Gregan S.M., Mulsant P., Mullen M., Davis G.H., Powell R. and Galloway S.M. 2004. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biology of Reproduction 70, 900–909.

Henderson K., Kieboom L., McNatty K., Lun S. and Heath D. 1985. Gonadotrophin-stimulated cyclic AMP production by granulosa cells from Booroola × Romney ewes with and without a fecundity gene. Journal of Reproduction and Fertility 75, 111–120.

Hudson N., O'Connell A., Shaw L., Clarke I. and McNatty K. 1999. Effect of exogenous FSH on ovulation rate in homozygous carriers or noncarriers of the Booroola FecB gene after hypothalamic-pituitary disconnection or after treatment with a GnRH agonist. Domestic Animal Endocrinology 16, 69–80.

Huntriss J., Gosden R., Hinkins M., Oliver B., Miller D., Rutherford A.J. and Picton H.M. 2002. Isolation, characterization and expression of the human Factor In the Germline alpha (FIGLA) gene in ovarian follicles and oocytes. Molecular Human Reproduction 8, 1087–1095.

Huntriss J., Hinkins M. and Picton H.M. 2006. cDNA cloning and expression of the human NOBOX gene in oocytes and ovarian follicles. Molecular Human Reproduction 12, 283–289.

Juengel J.L., Bodensteiner K.J. et al. 2004. Physiology of GDF9 and BMP15 signalling molecules. Animal Reproduction Science 82–83, 447–460.

Juengel J.L., Reader K.L., Bibby A.H., Lun S., Ross I., Haydon L.J. and McNatty K.P. 2006. The role of bone morphogenetic proteins 2, 4, 6 and 7 during ovarian follicular development in sheep: contrast to rat. Reproduction 131, 501–513.

Knight P.G. and Glister C. 2003. Local roles of TGF-beta superfamily members in the control of ovarian follicle development. Animal Reproduction Science 78, 165–183.

McNatty K. and Henderson K. et al. 1985. Ovarian activity in Booroola × Romney ewes which have a major gene influencing their ovulation rate. Journal of Reproduction and Fertility 73, 109–120.

McNatty K. and Henderson K. 1987. Gonadotrophins, fecundity genes and ovarian follicular function. Journal of Steroid Biochemistry 27, 365–373.

McNatty K., Kieboom L., McDiarmid J., Heath D. and Lun S. 1986a. Adenosine cyclic 3',5'-monophosphate and steroid production by small ovarian follicles from Booroola ewes with and without a fecundity gene. Journal of Reproduction and Fertility 76, 471–480.

McNatty K., Lun S., Heath D., Ball K., Smith P., Hudson N., McDiarmid J., Gibb M. and Henderson K. 1986b. Differences in ovarian activity between Booroola × Merino ewes which were homozygous, heterozygous and non-carriers of a major gene influencing their ovulation rate. Journal of Reproduction and Fertility 77, 193–205.

McNatty K., O'Keefe L., Henderson K., Heath D. and Lun S. 1986c. 125I-labelled hCG binding characteristics in theca interna and other tissues from Romney ewes and from Booroola × Romney ewes with and without a major gene influencing their ovulation rate. Journal of Reproduction and Fertility 77, 477–488.

McNatty K., Reader K., Smith P., Heath D. and Juengel J. 2007. Control of ovarian follicular development to the gonadotrophin-dependent phase: a 2006 perspective. In 'Reproduction in domestic ruminants VI', ed. by J.L. Juengel, J.F. Murray and M.F. Smith. Society for Reproduction and Fertility 64.

Massague J. 2000. How cells read TGF-beta signals. Nature Reviews: Molecular Cell Biology 1, 169–178.

Mazerbourg S., Klein C., Roh J., Kaivo-Oja N., Mottershead D.G., Korchynskyi O., Ritvos O. and Hsueh A.J. 2004. Growth differentiation factor-9 signaling is mediated by the type I receptor, activin receptor-like kinase 5. Molecular Endocrinology 18, 653–665.

Miyazono K., Maeda S. and Imamura T. 2005. BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. Cytokine Growth Factor Reviews 16, 251–263.

Mulsant P., Lecerf F. et al. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences 98, 5104–5109.

Otsuka F., Yamamoto S., Erickson G.F. and Shimasaki S. 2001. Bone morphogenetic protein-15 inhibits follicle-stimulating hormone (FSH) action by suppressing FSH receptor expression. Journal of Biology and Chemistry 276, 11387–11392.

Rey R., Lukas-Croisier C., Lasala C. and Bedecarras P. 2003. AMH/MIS: what we know already about the gene, the protein and its regulation. Molecular Cell Endocrinology 211, 21–31.

Scaramuzzi R., Turnbull K., Downing J. and Bindon B. 1981. Luteal size and function in the Booroola merino. Proceedings of the Australian Society for Reproductive Biology 13, 77.

Shi Y. and Massague J. 2003. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113, 685–700.

Shimasaki S., Zachow R.J., Li D., Kim H., Iemura S., Ueno N., Sampath K., Chang R.J. and Erickson G.F. 1999. A functional bone morphogenetic protein system in the ovary. Proceedings of the National Academy of Sciences USA 96, 7282–7287.

Souza C.J. and Baird D. 2004. The Booroola (FecB) mutation is associated with smaller adrenal glands in young adult ewes. Reproductive Biomedicine Online 8, 414–418.

Souza C.J., Campbell B.K., McNeilly A.S. and Baird D.T. 2002. Effect of bone morphogenetic protein 2 (BMP2) on oestradiol and inhibin A production by sheep granulosa cells, and localization of BMP receptors in the ovary by immunohistochemistry. Reproduction 123, 363–369.

Souza C.J., Campbell B.K., Webb R. and Baird D.T. 1997. Secretion of inhibin A and follicular dynamics throughout the estrous cycle in the sheep with and without the Booroola gene (FecB). Endocrinology 138, 5333–5340.

Souza C.J., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. Journal of Endocrinology 169, R1–6.

Themmen A.P. 2005. Anti-Mullerian hormone: its role in follicular growth initiation and survival and as an ovarian reserve marker. Journal of the National Cancer Institute. Monographs 34,18–21.

Turnbull K., Braden A. and Mattner P. 1977. The pattern of follicular growth and atresia in the ovine ovary. Australian Journal of Biological Sciences 30, 229–241.

Webb R. and Campbell B. 2007. Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. Reproduction in Domestic Ruminants VI. Reproduction Supplement 64, 141–164.

Webb R., Campbell B., Garverick H., Gong J., Gutierrez C. and Armstrong D. 1999. Molecular mechanisms regulating follicular recruitment and selection. Journal of Reproduction and Fertility Supplement 53, 33–48.

Wilson T and Wu X et al. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Genetic modulation of the *FecB* gene expression

V.C. Pardeshi[1], J.M. Maddox[2], N.Y. Kadoo[1] and V.S. Gupta[1,3]

Abstract

Improving the lambing percentage in sheep could be one of the key factors in increasing farm profitability. Major genes for production traits provide opportunities for large and rapid increases in the efficiency of sheep production. The first major gene for prolificacy identified in sheep was the Booroola (*FecB*) gene in Australia, which is additive for ovulation rate and partially dominant for litter size. The *FecB* locus is located in the region of ovine chromosome 6, which shows synteny to human chromosome 4. Recent discoveries have revealed that the high prolificacy in Booroola sheep is the result of a mutation (*FecB*) in the bone morphogenetic protein receptor 1B (BMPR1B) gene. This discovery led to the development of the DNA test that enabled researchers to screen the mutation in other prolific breeds. Besides Booroola, the gene has been reported to be present in the Garole, Javanese, Hu and Small Tail Han sheep breeds. Although the Garole breed from India has been proposed as the ancestral breed, the origin of this mutation remains unknown. The expression of the gene varies in different breeds, resulting in different levels of prolificacy. Such differences observed in fecundity could be due to various factors, namely environmental conditions, ewe parity, background breed, selection, maternal nutrition and presence of other genes. Besides the increasing effects on ovulation rate and litter size, some negative effects on body mass, milking ability and high prenatal mortality have also been found in animals carrying this mutation. In general, it has been indicated that the gene might work well in moderate-sized breeds. Besides body size, uterine capacity (the ability to bear healthy triplets), mothering ability and milking ability of the sheep are important criteria for a commercially viable breed containing the Booroola gene.

Introduction

Most of the domestic sheep breeds of the world are reported to deliver only one or two lambs at each lambing. The increase in production associated with litter size (LS) is controlled by both genetic and environmental factors. Since the heritability of LS is low in sheep, attempts were made to discover the gene(s) controlling ovulation rate (OR) and thus LS. The OR analysis of the first recorded highly prolific breed, 'Booroola Merino' (BM) of Australia, provided strong evidence for the presence of a single gene governing OR (Davis et al. 1982). In 1993 the first DNA marker test for the Booroola gene was developed and two microsatellite markers, OarAE101 and OarHH55, were found to be linked to the *FecB* locus (Montogomery et al. 1993). This locus is situated in the region of ovine chromosome 6, which shows synteny to human chromosome 4q22-23 (Montogomery et al. 1994), which also contains the bone morphogenetic protein receptor (BMPR1B) gene (Wilson et al. 2001). A mutation in BMPR1B could have physiological importance in triggering the phenotype consistently observed in the Booroola animals as it maps within the critical region for the locus (Wilson et al. 2001), and segregation of the polymorphism is consistent with

[1] Division of Biochemical Sciences, National Chemical Laboratory, Pune, Maharashtra, 411 008, India
[2] School of Veterinary Science, Centre for Animal Biotechnology, University of Melbourne, Victoria 3010, Australia
[3] Corresponding author: vs.gupta@ncl.res.in

phenotypic differences (Wilson et al. 2001). The BMPR1B is a member of the transforming growth factor superfamily beta (TGF-β) proteins. The members of this superfamily are multifunctional proteins that regulate growth and differentiation of many cell types. In 2001 three independent laboratories simultaneously identified the non-conservative point mutation (Q249R) in the intracellular kinase-signalling domain of the BMPR1B gene responsible for the Booroola phenotype (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001). Q249R is the A→G transition at position 746 (GenBank Acc. No. AF357007), substituting the glutamine present in the wild-type BMPR1B protein with an arginine. This discovery helped to develop a DNA test to screen the mutation in other prolific breeds of sheep without prior knowledge of the pedigree (Wilson et al. 2001).

The mechanism of action of the mutated gene has not yet been fully understood. It was known to result in ovulation of a higher number of follicles with smaller diameter and fewer granulosa cells than the wild type, thus speeding the differentiation of ovulatory follicles (Mulsant et al. 2001). The predicted amino acid substitution is located close to the L45 loop, which is critical in determining the specific SMAD protein (evolutionarily conserved proteins identified as mediators of transcriptional activation by members of the TGF-β superfamily of cytokines, including TGF-β, activins and BMP; upon activation, these proteins directly translocate to the nucleus, where they may activate transcription). In $FecB^{B+}$-carrier ewes, Q249R substitution would impair the inhibitory effect of BMPR1B on granulosa cell steroidogenesis, leading to their advanced differentiation and an advanced maturation of follicles (Mulsant et al. 2001). Souza et al. (2001) also found another point mutation at position 1113 (C→A); however, this mutation did not change the coding amino acid. Two substitutions in the 3' untranslated region (UTR) were also found (Lehman et al. 2003), without any effect on prolificacy. However, it is known that the mutations in the 3' or 5' UTR region play an important role in the regulation and expression of the gene (Cazzola and Skoda 2000; Mazumder et al. 2003). This paper describes the origin of the *FecB* gene and the different expressions of the gene in various sheep breeds and the factors affecting them.

Garole, a source of the *FecB* mutation?

In 1982 Dr Helen Newton Turner suggested that the Garole sheep from Sunderbans, West Bengal, India, could be the source of the *FecB* mutation in highly prolific BMs. Later, in 1996, Piper and Bindon reported the inclusion of the prolific Bengal sheep in an early Australian BM flock. Davis et al. (2002) detected the *FecB* mutation in Garole and Indonesian Javanese sheep, thereby confirming the hypothesis that the *FecB* gene in the Garole is the same as that reported in Australian BMs. As the same mutation was also found in the Javanese sheep, it was hypothesised that the presence of *FecB* in BM and Javanese sheep could probably be traced back to Garole sheep of India (Davis et al. 2002, 2006). Later, the *FecB* mutation was also reported in the Chinese Hu and Small Tail Han sheep breeds (Davis et al. 2006). Recent research confirmed that the *FecB* mutation is fixed in populations of Garole and Hu sheep but is segregating in the Javanese, BM and Han breeds (Davis et al. 2002, 2006). It is suggested that the mutation in the Garole and Hu breeds either could be due to two separate mutation events or these breeds could have a common ancestor.

The Garole is a micro-sheep (11–14 kg adult weight) with a short tail and light brown fleece. The females of this breed are polled and males are horned. On the contrary, the Hu breed from Jiangsu and Zhejiang provinces of China is large (32–44 kg), having a small fat tail with a triangular fat deposit near the base. The fleece is white and both the sexes are polled. However, despite the marked phenotypic differences between Garole and Hu sheep, both breeds are reported to have some individuals with an earless phenotype (Mason 1980a; Bose et al. 1999), suggesting that these breeds might be distantly related. The earless phenotype is also present in Javanese Garut sheep (Mason 1980b), reported to have the *FecB* gene segregating in the population (Davis et al. 2002). It is thus quite possible that transportation of the animals carrying *FecB* might have occurred through the ancient silk route (from Kolkata to Shanghai), resulting in the introduction of the gene in China. On the other hand, Chang (1979) reported that both the Hu and the Han breeds descended from Mongolian sheep. Records suggest that traders brought Mongolian sheep to Zhejiang, Jiangsu, Hebei, Henan and Shandong provinces as early as the 5th century AD (Feng et al. 1996), and Hu (Zhejiang

and Jiangsu) and Han (Hebei, Henan and Shandong) sheep are presently concentrated in these provinces. However, narration by George Bogle (Markham 1986) and Dorji et al. (2003) reported the presence of sheep resembling the Garole (prolific breed) in the Tibet–Bhutan border region, suggesting that Garole sheep might have originated in Tibet. The sheep were brought by Tibetan traders and traded in the plains of Bengal in the pre-colonial era. Hence, due to these conflicting hypotheses, the source of the *FecB* mutation still remains unknown.

Expression of the *FecB* gene in various prolific sheep breeds

The concept of breeding for increased fertility has been accepted and adapted in ruminant breeding. Increasing prolificacy in sheep via selection within the available breeds is a very slow process, and maximum improvement of 1% per year in lambing could be achieved provided the selection is solely for this trait. The *FecB* gene presented a unique and exciting opportunity to enhance the reproductive performance of the sheep that fit the environment well, while maintaining other traits important to the adaptability and marketability of that flock. Being controlled by a single gene (chromosome 6q23-31), the *FecB* phenotype is not diluted when transferred to other non-prolific breeds. The *FecB* mutation is additive for OR and partially dominant for LS, with one copy of the gene increasing OR by 1.3–1.6 and two copies by 2.7–3.0 (Davis 2004). Similarly, LS is increased by 0.9–1.2 in ewes carrying a single copy of the gene and 1.1–1.7 in ewes with two copies (Piper et al. 1985; Dodds et al. 1991).

The discovery of the *FecB* mutation led to the development of a commercial DNA test, and prompted researchers to screen other prolific sheep breeds to determine whether the same mutation is also responsible for their high prolificacy. A number of prolific breeds throughout the world were screened. Besides Garole and BM, the mutated gene was found to be present in Javanese (Indonesia: Davis et al. 2002), Small Tail Han (China: Liu et al. 2003; Wang G.L. et al. 2003; Jia et al. 2005; Yan et al. 2005; Davis et al. 2006) and Chinese Hu sheep (Wang G.L. et al.; Wang Q.G. et al. 2003, 2005; Yan et al. 2005; Davis et al. 2006; Guan et al. 2006).

The *FecJ* gene was the major gene segregating in Javanese Thin Tail (JTT) sheep and had a smaller additive effect on LS and OR than that of the *FecB* gene (Roberts 2000). The effect of one copy of *FecJ* on OR in JTT sheep was about 0.8 (Bradford et al. 1991), which was only half that reported for the *FecB* in other breeds by Piper et al. (1985). The mean LSs of the homozygous carriers of the *FecB* and *FecJ* were comparable, with BM having a mean LS of 2.59 (Piper and Bindon 1996) and JTT a mean of 2.83 (Bradford et al. 1991), although the difference between the corresponding ORs of BM (5.65) and JTT (2.92) was large. Davis et al. (2002) subsequently found that the *FecJ* gene has the same mutation as *FecB* and its effects were lower in the JTT breed.

Chinese Hu sheep were reported to have a mean LS of about 2.1 (individual litters range from 1 to 8) (Feng et al. 1996; Yue 1996) and 2.61 (Tu 1989; Wang et al. 1990), and had the ability to lamb twice per year. The presence of a single BB genotype suggested fixing of the mutation in the Hu population. Small Tail Han sheep from China are also highly prolific, averaging 2.47 lambs born per ewe lambing (Feng et al. 1996). The Garole is a prolific sheep breed from the hot and humid coastal region of Sunderbans of West Bengal, having a mean LS of 2.27 in the native tract with 7.3% single births, 65.45% twins, 21.8% triplets and 5.45% quadruplets (Ghalsasi and Nimbkar 1993). Although the Garole breed is proposed as the ancestral sheep breed for the *FecB* gene mutation (Davis et al. 2002), it has a lower mean LS (2.30; Davis et al. 2002). Many homozygous carriers in the Garole breed showed a maximum LS of twins (Davis et al. 2002), whereas some of the heterozygous ewes produced even triplets and/or quadruplets (Kumar et al. 2006), indicating that LS criterion is not consistent for determining the status of the *FecB* mutation in Garole ewes.

The observed differences in the fecundity of the prolific breeds carrying the *FecB* mutation probably could be due to environmental conditions, ewe parity, selection, maternal nutrition and breed background, and these are discussed below.

Environmental conditions

The varied expression of the *FecB* gene in different breeds could be due to the differential expression of the gene in different environments or to interaction between the genotype and the environment. Whereas the effect of the *FecB* gene in the BM showed a similar increase in OR (1.54) and LS (0.6) in different environments (Davis et al. 1991), in the

Garole and Hu sheep breeds the gene expression varied between locations. The reported mean LS of Garole from the Sunderbans of West Bengal, the breed's native tract, was 2.27 (Ghalsasi and Nimbkar 1993), which reduced to 1.74 and 1.68–1.87 in the semi-arid climate of the Deccan Plateau of Maharashtra (Nimbkar et al. 1998) and in Rajasthan (Sharma et al. 1999, 2001), respectively. The Hu is the predominant breed in Suzhou, Shanghai and Dongshan regions of China, where it is well adapted to the hot and humid environment. Average LS (2.12) at first lambing from the Hu at the Natural Source Conservative Region was found to be significantly higher than that from the other two regions sampled (Shanghai 1.78 and Suzhou 1.90; $P < 0.05$) (Guan et al. 2006). These findings indicate that either the *FecB* gene itself, or some other gene(s) or quantitative trait locus (QTL) modulating *FecB* gene expression in Garole and Hu sheep, are in turn being modulated by the environmental factors.

Ewe parity

Bathei (1994) studied the Iranian fat-tailed Mehraban breed of sheep and reported that ewe productivity increases as parity proceeds. These observations indicate the possibility of the role of sexual maturity regulatory genes in modulation of OR and/or LS with parity.

Young and Dickerson (1991) reported that BM-sired ewes were more prolific at the second and third parity. Liu et al. (2003) reported the mean LS of BB, B+ and ++ genotype Small Tail Han ewes as 2.47, 2.05 and 1.50 in primiparous sheep; and 3.17, 2.55 and 1.67 in multiparous sheep, respectively. Similar results were obtained for Garole × Deccani / Bannur ewes. The effect of one copy of the *FecB* gene on LS in 25% Garole ewes was 0.52, 0.61 and 1.03 for the first, second and third parity, respectively. The effect appeared to increase with parity (Nimbkar et al. 2003). However, in Garole × Malpura ewes one copy of the *FecB* mutation increased LS by 0.93, 0.78, 1.2 and 0.8 in the first, second, third and fourth parity, respectively (Kumar et al. 2006), indicating that the ewe's productivity increased up to the third parity and decreased thereafter.

Breed effect

To date, the *FecB* gene has been or is being introduced into a range of different sheep breeds in several countries in Africa, Asia, America, Europe, Oceania etc., including Merino of different strains and prolific breeds such as the Finnsheep and Romanov (Davis et al. 1991; Thimonier et al. 1991). Assessment of performance of the Booroola-crossed flocks in different countries showed that the carriers of the prolificacy mutation had higher ewe productivity. Shulze et al. (2003) reported that the introgression of the *FecB* allele in Rambouillet sheep increased OR by 1.18 for B+ ewes, whereas Southey et al. (2002) reported an increase in OR of 1.54. In back-crosses of BM and Mérinos d'Arles from France with different genotypic classes, the differences between B+ and ++ ewes for OR ranged from 0.92 to 1.72, with an average difference of 1.2 (Bodin et al. 1991). The mean LS of BM-crossed Awassi and BM-crossed Assaf increased by 0.66 lambs born per ewe lambing (Gootwine et al. 2001). Similarly, results from New Zealand using either the Romney (Davis and Hinch 1985; Davis et al. 1991) or Coopworth (Piper et al. 1988), or Mérinos d'Arles from France (Bodin et al. 1991; Elsen et al. 1994), as the recipient ewes produced 0.6 more ova and 0.3 more lambs. Teyssier et al. (1998) reported an increase of 0.9 lambs born per ewe lambing due to the presence of the *FecB* allele in Mérinos d'Arles-cross ewes. These effects of the *FecB* allele on prolificacy were slightly lower than those estimated by Piper et al. (1985), but were similar to those from the worldwide summary reported by Davis et al. (1991) of several local breeds compared to BM × local breed crossbreds (mostly 50% and 25% BM). The Chinese Merino prolific meat strain is also reported to have lower OR (2.83) than the BM.

Nimbkar et al. (2002) suggested that the effect of the Garole *FecB* gene varies according to the breed into which the gene is introgressed. In Garole crossbred animals, for example Garole × Malpura, Garole × Deccani and Garole × Bannur, introduction of one copy of the *FecB* allele showed an increase of 0.7 (Nimbkar et al. 2003; Kumar et al. 2006, 2007) in the mean LS, which was lower than that estimated for Booroola crosses (0.9–1.2 lambs per ewe). In Garole × Malpura ewes some heterozygous (B+) individuals produced a single lamb even after 2–4 parturitions (Kumar et al. 2007). Similarly, JTT sheep from Indonesia and Hu and Small Tail Han sheep from China also showed lower mean LS (2.5, 2.09, 2.47, respectively) compared to the BM (Davis et al. 2002; Liu et al. 2003; Wang Q.G. et al. 2003). The above findings suggest that the reduction in fecundity or LS might have been affected by the

background genotype of the recipient breed. The genes of the recipient breed and/or the prolificacy environment in the recipient breed might play an important role in *FecB* gene expression.

Other gene(s) or QTL

Davis et al. (2006) observed that one non-carrier Han ewe had consistently large litters (four sets of triplets, one set of quadruplets and one set of quintuplets), suggesting that other genes besides those reported to date might also be present in Small Tail Han sheep, causing high prolificacy. Recent studies in Han sheep of the ovine melatonin receptor 1a gene (MTNR1A), located on ovine chromosome 26, showed an association between a polymorphism at nucleotide position 604 of exon 2 and prolificacy (Chu et al. 2003). Three types of genotypes were observed: AA (290 bp, 290 bp), AB (290 bp, 267 bp / 23 bp) and BB (267 bp / 23 bp, 267 bp / 23 bp). The AA genotype had a mean LS that was 1.06 and 0.94 higher than the AB and BB averages, respectively. The effect in adult ewes (second parity) was large. Furthermore, Chu et al. (2007) reported another prolificacy mutation BMP-15 ($FecX^G$) in Small Tail Han sheep. Ewes carrying mutations in both the BMPR1B and BMP-15 genes had greater LSs than those with either mutation alone. The interaction between the *FecB* and $FecX^G$ mutations appears to be multiplicative in those animals which were heterozygous for both the Booroola and Inverdale mutations and had ORs greater than the increase expected for an additive effect alone (Davis et al. 1999). However, the effect of the BMPR1B mutation was observed to be greater than that of the BMP-15 gene mutation on LS.

Sheep husbandry practices and flock management

Although all breeds carrying the *FecB* mutation are highly prolific, there are differences in the husbandry systems in which they have evolved. This could have affected the process whereby a spontaneous mutation leading to large litters was either retained or lost. The breeds in which the *FecB* mutation is found (Garole, Hu, Small Tail Han and Javanese) were mainly reared for meat production (Inounu et al. 1984; Ghalsasi and Nimbkar 1993), and increasing the prolificacy was a boon to sheep economics; hence, it is quite possible that all the mutations increasing the prolificacy were indirectly selected in these sheep breeds. For Garole and Hu sheep, the aim of breeding was high prolificacy, while maintaining high-quality lamb skins for Hu sheep only, further allowing the selection and retention of prolific animals. Strict selection of Hu sheep at the Dongshan, which is one of their original sources (Geng et al. 2002), showed significant improvement in LS (2.256 to 2.567, $P < 0.05$; Wang et al. 1998). In the BM, selection by the Seears brothers for highly prolific animals allowed the survival of the mutation in this breed (Turner 1982). In Garole and Hu sheep, husbandry practices played an important role in maintaining the prolificacy gene mutation and the mutation is fixed in these populations.

Nutrition

The expression of *FecB* in the Indonesian thin-tailed flock was shown to be modulated by nutrition and husbandry practices. High levels of supplementary feeding over several months showed higher OR, lower embryo survival and a small increase in LS (Roberts 2000). The relatively low nutritional value of the tropical forages (Devendra 1992, 2000) available to ewes thus suggests it to be another possible reason for the observed lower prolificacy of the Asian breeds (Garole, Javanese, Hu and Small Tail Han) compared to BM and its crosses.

Uterine capacity

Regulation of foetal development in sheep depends on interactions between the intrinsic capacity of the foetus for growth and the maternal environment. Nevertheless, the trend of decreasing embryo survival rates with increasing ORs was detected in many prolific breeds, reflecting decreased marginal response of uterine efficiency with increased numbers of embryos (Meyer 1985). Gootwine (2005) suggested that the *FecB* allele might be involved in the control of uterine capacity and uterine function. Meyer and Piper (1992) and Meyer et al. (1994) showed that the BM had a higher uterine capacity than the other Australian Merino strains, particularly at the higher ORs. Apart from possessing the fecundity gene responsible for increasing OR, the high incidence of multiple births in the Garole sheep (Ghalsasi and Nimbkar 1993) might be possible because of its small maternal size environment, which can also sustain the survival and development of multiple embryos up to parturition.

Secondary effects of the BMPR1B gene mutation

Many studies have shown that the *FecB* gene plays a role in reproductive endocrinology (Smith et al. 1993) and organ development. It affects the size of specific organs like adrenal glands (Souza and Baird 2004), the development of specific tissues like fats and muscles (Visscher et al. 2000) and body mass (Smith et al. 1993), as well as ovary development and LS. However, a few side effects, such as negative effects on foetal growth and development and body mass during gestation and higher perinatal mortality, have also been reported. It has been reported that the *FecB* allele significantly affected the effective productive efficiency of Garole × Deccani / Malpura ewes at all ages, and heterozygous ewes weaned high litter weights compared to homozygous and wild-type ewes (Nimbkar et al. 2003; Kumar et al. 2007). The exact mechanism of action of the *FecB* mutation on growth and development has not been clearly understood. Souza et al. (2001) hypothesised that the *FecB* mutation might have partial deactivation of the BMPR1B receptor. The reduced body weight and growth rate in Booroola lambs carrying the *FecB* mutation might be associated with impaired skeletal development. However, such an observation (direct effect) is not found in other sheep populations of very different histories, or in other species with known action of BMPR1B mutation. It has also been reported that the FecB allele increased sperm concentration in carrier breeds compared to non-carriers (Kumar et al. 2007), while an adverse effect on milk production in the Awassi and Assaf breeds has also been reported (Gootwine et al. 2001).

These secondary effects could be due to the fact that the gene (BMPR1B) is a member of the TGF-β superfamily, which is known to play an important role during embryogenesis in development of skeletal growth and organ formation of vertebrates (Shimasaki et al. 1999). In humans and mice it has been shown to affect body weight and reproductive traits (Baur et al. 2000; Yi et al. 2000, 2001; Demirhan et al. 2005).

Conclusion

The mean OR (5.7) and LS (2.6) of the BM are quite high compared to other breeds carrying the *FecB* mutation. This difference might be due to various factors such as environmental variation, background breed, nutrition or selection. Or it may be due to other genetic factors such as modifier genes or QTL or novel mutations within the BMPR1B gene that individually or together modulate expression of the *FecB* trait in these sheep breeds. Studies of genetic variation at the whole genome scale as well as at the *FecB* gene locus within the Garole as well as the Hu breeds might help to throw light on this issue. Such information will be useful in determining the causes of variation in gene expression when a particular gene is transferred from one breed to another, and also to take remedial measures to ensure optimum expression of the introgressed gene.

References

Bathei S.S. 1994. Influence of ewes age on reproductive performance of Iranian fat tailed Mehraban breed of sheep. World Review of Animal Production 29, 55–60.

Baur S.T., Mai J.J. and Dymecki S.M. 2000. Combinatorial signaling through BMP receptor IB and GDF5: shaping of the distal mouse limb and the genetics of distal limb diversity. Development 127, 605–619.

Bodin L., Cornu C., Elsen J. M., Molenat G. and Thimonier J. 1991. The effects of Booroola genotype on some traits in a Mérino d'Arles flock. Pp. 371–379 in 'Major genes for reproduction in sheep.' ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Bose S., Dutta-Gupta R. and Maitra D.N. 1999. Phenotypic characteristics and management practices of Bengal sheep. Indian Journal of Animal Production and Management 15(1), 18–22.

Bradford G.E., Inounu I., Iniguez L.C., Tiesnamurti B. and Thomas D.L. 1991. The prolificacy gene of Javanese sheep. Pp. 67–73 in 'Major genes for reproduction in sheep.' ed. by J.M. Elsen, L. Bodin and J. Thimonier. Colloque No. 57, L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Cazzola M. and Skoda R.C. 2000. Translational pathophysiology: a novel molecular mechanism of human disease. Blood 95, 3280–3288.

Chang T.S. 1979. Livestock production in China with particular reference to sheep**.** Wool Technology in Sheep Breeds 27(11), 19–28.

Chu M.X., Ji C.L. and Chen G.H. 2003. Association between PCR–RFLP of melatonin receptor 1a gene and high prolificacy in small tail Han sheep. Asian–Australian Journal of Animal Science 16, 1701–1704.

Chu M.X., Liu Z.H., Jiao C.L., He Y.Q., Fang L., Ye S.C., Chen G.H. and Wang J.Y. 2007 Mutations in BMPR-1B and BMP-15 genes are associated with litter size in small

tailed Han sheep (*Ovis aries*). Journal of Animal Science 85, 598–603.

Davis G.H. 2004. Fecundity genes in sheep. Animal Reproduction Science 82–83, 247–253.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway S.M., Lumsden B.M., Hanrahan J.P., Mullen M., Mao X.Z., Wang G.L., Zhao Z.S., Zeng Y.Q., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranj J.J. and Notter D.R. 2006. Investigation of the Booroola (*FecB*) and Inverdale (*FecXI*) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92, 87–96.

Davis G.H., Dodds K.G. and Bruce G.D. 1999. Combined effect of the Inverdale and Booroola prolificacy genes on ovulation rate in sheep. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 13, 74–77.

Davis G.H., Elsen J.M., Bodin L., Fahmy M.H., Castonguay F., Gootwine E., Bor A., Braw-Tal R., Lengyel A., Paszthy G. and Cummins L. 1991. A comparison of the production from Booroola and local breed sheep in different countries. Pp. 315–323 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB*) mutation. Biology of Reproduction 66, 1869– 1874.

Davis G.H. and Hinch G.N. 1985. Introduction and management of the Booroola gene in sheep flocks in New Zealand. Pp. 139–148 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Davis G.H., Montgomery G.H. Allison A.J. Kelly R.W. and Bray A.R. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Demirhan O., Türkmen S., Schwabe G.C., Soyupak S., Akgül E., Tatemir D., Karahan D., Mundlos S. and Lehmann K. 2005. A homozygous BMPR1B mutation causes a new subtype of acromesomelic chondrodysplacia with genital anomalies. Journal of Medical Genetics 42, 314–317.

Devendra C. 1992. Goats and rural prosperity. Pp. 6–25 in 'Pre-conference Proceedings of the International Conference on Goats', 2–8 March, New Delhi, India.

Devendra C. 2000. Research on goats: opportunities and challenges. Proceedings of 7th International Conference on Goats 2, 200–201.

Dodds K.G., Davis G.H., Elsen J.M., Isaacs K.L. and Owens J.L. 1991. The effect of Booroola genotype on some reproductive traits in a Booroola Merino flock. Pp. 359 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'institute National de la Recherche Agronomique (INRA): Paris.

Dorji T., Tshering G., Wangchuk T., Rege J.E.O. and Hannote O. 2003. Indigenous sheep genetic resources and management in Bhutan. Animal Genetic Resources Information (FAO/UNEP) 33, 81–91.

Elsen J.M., Bodin L., Francois D., Poivey J.P. and Teyssier J. 1994. Genetic improvement of litter size in sheep. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, 16, 237–244.

Feng W., Ma Y., Zhang Z. and Zhou D. 1996. Prolific breeds of China. Pp. 146–151 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Geng R.Q., Chang H., Yang Z.P., Sun W., Wang L.P., Lu S.X., and Tsunoda K.J. 2002. Study on origin and phylogeny status of Hu sheep. Journal of Northwest Sci-Tech University of Agriculture and Forestry (Natural Science Edition) 3, 21–28.

Ghalsasi P.M. and Nimbkar B.V. 1993. The 'Garole'—microsheep of Bengal, India. Animal Genetics Resources Information 12, 73–79.

Gootwine E. 2005. Variability in the rate of decline in birth weight as litter size increases in sheep. Animal Science 81, 393–398.

Gootwine E., Zenu A., Bor A., Yossafi S., Rosov A. and Pollott G.E. 2001. Genetic and economic analysis of introgression the B allele of the *FecB* (Booroola) gene into the Awassi and Assaf dairy breeds. Livestock Production Science 71, 49–58.

Guan F., Liu S.R., Shi G.Q., Ai J.T., Mao D.G. and Yang L.G. 2006. Polymorphism of *FecB* gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Acta Genetica Sinica 33,117–124.

Inounu I., Thomas N., Sitorus P. and Bell M. 1984. Lambing characteristics of Javanese thin-tail ewes at Cicadas experiment station and under village conditions. Small Ruminant Collaborative Research Support Program. Indonesia, Working Paper 41, 1–7.

Jia C.L., Li N., Zhao X.B., Zhu X.P. and Jia Z.H. 2005. Association of single nucleotide polymorphisms in exon 6 region of BMPR1B gene with litter size traits in sheep. Asian–Australian Journal of Animal Science 18, 1375–1378.

Kumar S., Kolte A.P., Mishra A.K., Arora A.L. and Singh V.K. 2006. Identification of the *FecB* mutation in Garole × Malpura sheep and its effect on litter size. Small Ruminant Research 64, 305–310.

Kumar S., Mishra A.K., Kolte A.P., Arora A.L., Singh D. and Singh V.K. 2007. Effects of the Booroola (*FecB*) genotypes on growth performance, ewe's productivity efficiency and litter size in Garole × Malpura sheep. Animal Reproduction Science 105(3), 319–331.

Lehman K., Seeman P., Sammar S.S.M., Meyer B., Majewski K.S.F., Tinschett S. Grzeschik K.H., Muller D. Nurenberg P.K.P. and Mundlos S. 2003. Mutation in bone morphogenetic protein receptor-IB causes brachydactyly type A2. Proceedings of National Academy of Sciences 100(21), 12277–12282.

Liu S.F., Jiang Y.L. and Du L.X. 2003. Study of BMPR1B and BMP15 as candidate genes for fecundity in little tailed Han sheep. Acta Genetica Sinica 8, 755–760.

Markham C.R. (ed.) 1986. Narratives of the Mission of George Bogle to Tibet, and of the Journey of Thomas Manning to Lhasa, edited, with notes, and introduction and lives of Mr Bogle and Mr Manning. London 1876. Reprinted (1971). Manjusri Publishing House: New Delhi.

Mason I.L. 1980a. Prolific sheep in Java. Pp. 65–76 in 'Prolific tropical sheep', ed. by I.L. Mason. FAO Animal Production and Health Paper 17. FAO: Rome.

Mason I.L. 1980b. Prolific sheep in Java. Pp. 90–91 in 'Prolific Tropical Sheep', ed. by I.L. Mason FAO Animal Production and Health Paper 17. FAO: Rome.

Mazumder B., Seshadri V. and Fox P.L. 2003. Translational control by the 3'-UTR: the ends specify the means. Trends in Biochemical Sciences 28, 91–98.

Meyer H.H. 1985. Breed differences in ovulation rate and uterine efficiency and their contribution to fecundity. Pp. 185–191 in 'Genetics of reproduction in sheep' ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Meyer H.H., Baker R.L., Harvey T.G. and Hickey S.M. 1994. Effects of Booroola Merino breeding and the *FecB* gene on performance of crosses with longwool breeds. 2. Effects on reproductive performance and weight of lamb weaned by young ewes. Livestock Production Science 39, 191–200.

Meyer H.H. and Piper L.R. 1992. Embryo survival relative to ovulation rate in Booroola and control Merinos and crosses with medium wool breeds. Proceedings, Western Section, American Society of Animal Science 43, 103–106.

Montgomery G.W., Crawford A.M., Penty J.M., Dodds K.G., Ede A.J., Henry H.M., Pierson C.A., Lord E.A., Galloway S.M., Schmack A.E., Sise J.A., Swarbrick P.A., Hanrahan V., Buchanan F.C. and Hill D.F. 1993. The ovine Booroola fecundity gene (*FecB*) is linked to markers from a region of human chromosome 4q. Nature Genetics 4, 410–414.

Montgomery G.W., Lord E.A., Penty J.M., Dodds K.G., Broad T.E., Cambridge L., Sunden S.L., Stone R.T. and Crawford A.M. 1994. The Booroola fecundity (*FecB*) gene maps to sheep chromosome 6. Genomics 22, 148–153.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y., Chitour N. and Elsen J.M. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of National Academy of Sciences of the United States of America 98, 5104–5109.

Nimbkar C., Ghalsasi P.M., Ghatge R.R. and Gray G.D. 1998. Establishment of prolific Garole sheep from West Bengal in the semi-arid Deccan Plateau of Maharashtra. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production 25, 257–260.

Nimbkar C., Ghalsasi P.M., Maddox J.F., Pardeshi V.C., Sainani M.N., Gupta V. and Walkden-Brown S.W. 2003. Expression of FecB gene in Garole and crossbred ewes in Maharashtra, India. Proceedings of the 15th Conference of Association for Advancement of Animal Breeding and Genetics (AAABG), Melbourne, Australia, 15, 111–114.

Nimbkar C., Ghalsasi P.M., Walkden-Brown S.W. and Kahn L.P. 2002. Breeding program for the genetic improvement of Deccani sheep of Maharashtra, India. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France. CD-ROM Communication 25-11, 4.

Piper L.R. and Bindon B.M. 1996. The Booroola Merino. Pp. 152–160 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Willingford, UK.

Piper L.R., Bindon B.M. and Davis G.H. 1985. Single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Piper L.R., Bindon B.M., Davis G.H. and Elsen J.M. 1988. Control of litter size: major genes and industry utilization of the Booroola F gene. Proceedings of the 3rd World Congress on Sheep Breeding Beef Cattle Breeding 2, 589–609.

Roberts J.A. 2000. Frequency of the prolificacy gene in flocks of Indonesian thin tail sheep: a review. Small Ruminant Research 36, 215–226.

Schulze K.S., Waldron D.F., Willingham T.D., Shelby D.R., Engdahl G.R., Gootwine E., Yoshefi S., Montgomery G.W., Tate M.L. and Lord E.A. 2003. Effect of the FecB gene in half-sib families of Rambouillet-cross ewes. Sheep and Goat Research Journal 18, 83–88.

Sharma R.C., Arora A.L. and Khan B.U. 2001. Garole: a prolific sheep of India. Research Bulletin 5–11. Central Sheep and Wool Research Institute: Avikanagar, Rajasthan, India.

Sharma R.C., Arora A.L., Narula H.K. and Singh R.N. 1999. Characteristics of Garole sheep in India. Animal Genetics Resources Information 26, 57–64.

Shimasaki S., Zachow R.J., Li D., Kim H., Imemura S.-I., Ueno N., Sampath K., Chang R.J. and Erickson G.F. 1999. A functional bone morphogenetic protein receptor system in the ovary. Proceedings of the National Academy of Sciences USA 96, 7282–7287.

Smith P.O., O W.S., Hudson N.L., Shaw L., Heath D.A., Condell L., Phillips D.J. and McNatty K.P. 1993. Effects of the Booroola gene (*FecB*) on body weight, ovarian development and hormone concentrations during fetal life. Journal of Reproduction and Fertility 98, 41–54.

Southey B.R., Thomas D.L., Gottfredson R.G., Zelinksy R.D. 2002. Ewe productivity of Booroola Merino–Rambouillet crossbred sheep during early stages of the introgression of the *FecB* allele into a Rambouillet population. Livestock Production Science 75, 33-44.

Souza C.J.H. and Baird D.T. 2004. The Booroola (*FecB*) mutation is associated with smaller adrenal glands in young adult ewes. Reproductive Biomedicine Online 8, 414–418.

Souza C.J.H., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type IB (BMPR1B) gene. Journal of Endocrinology 169, R1–R6.

Teyssier J., Elsen J.M., Bodin L., Bosc P., Lefevre C. and Thimonier J. 1998. Three-year comparison of productivity of Booroola carrier and non carrier Merino d'Arles. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, New South Wales, 24,117–120.

Thimonier J., Davis G.H., Fahmy M.H., Castonguay F., Fernandez-Abella D., Greef J.C., Hofmeyr J.H., Gootwine E., Bor A., Braw-Tal R., Haley C.S., Klewiec J., Gabryszuka M., Slowak M., Piper L.R., Bindon B.M., Veress L., Lengyel A., Paszthy G., Horn P., Visscher A.H., Wassmuth R. and Young L.D. 1991. The F gene in the world: use and research objectives. Pp. 3–13 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Tu Y.R. 1989. Small tailed Han sheep. Pp. 50–52 in 'The sheep and goat breeds in China'. Shanghai Science and Technology Press: Shanghai, China.

Turner H.N. 1982. Origins of the CSIRO Booroola. Pp. 1–7 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24-25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Visscher A.H., Dijkstra M., Lord E.A., Suss R., Rosler H.J. Heylen K. and Veerkamp R.F. 2000. Maternal and lamb carrier effects of the Booroola gene on food intake, growth and carcass quality of male lambs. Animal Science 71, 209–217.

Wang G.L., Mao X.Z., Davis G.H., Zhao Z.S., Zhang L.J. and Zeng Y.Q. 2003. DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (*FecB*) mutation. Journal of the Nanjing Agricultural University 1, 104–106 (in Chinese with English abstract).

Wang J.Y., Li J.X. and Wei J.C. 1990. Selection and improvement on small tailed Han sheep. China Sheep and Goat Farming 1, 1–3.

Wang Q.G., Zhong F.G., Li H., Wang X. H., Liu S.R. and Chen X.J. 2003. The polymorphism of BMPR1B gene associated with litter size in sheep. Grass-feeding Livestock (2), 20–23 (in Chinese with English abstract).

Wang, Q.G., Zhong F.G., Li H., Wang X.H., Liu S. R., Chen X.J. and Gan S.Q. 2005. Detection on major gene on litter size in sheep. Hereditas (Beijing) 27, 80–84.

Wang Y.X., Wang C., Cheng H., Wang Y.S., Wu J.X. and Sheng B.G. 1998. The characteristics and approach for improvement of Hu sheep in Jiangsu province Wu country Hu sheep conservative region. Domestic Animal Ecology 3, 26–28.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Yan Y.D., Chu M.X., Zeng Y.Q., Fang L., Ye S.C., Wang L.M., Guo Q.K., Han D.Q., Zhang Z.X., Wang X.J. and Zhang X.Z. 2005. Study on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in small tailed Han sheep and Hu sheep. Journal of Agricultural Biotechnology 13, 66–71.

Yi S.E., Daluiski A., Pederson R., Rosen V. and Lyons K.M. 2000. The type I BMP receptor BMPR1B is required for chondrogenesis in the mouse limb. Development 127, 621–630.

Yi S.E., LaPolt P.S., Yoon B.S., Chen J.Y.C., Lu J.K.H. and Lyons K.M. 2001. The type 1 BMP receptor BMPR1B is essential for female reproductive function. Proceedings of National Academy of Sciences USA 98, 7994–7999.

Young L.D. and Dickerson G.E. 1991. Comparison of Booroola Merino and Finnsheep: effects on productivity of mates and performance of crossbred lambs. Journal of Animal Science 69, 1899–1911.

Yue G.H. 1996. Reproductive characteristics of Chinese Hu sheep. Animal Reproduction Science 44, 223–230.

Environmental modulation of *FecB* expression

N.M. Fogarty[1]

Abstract

This paper reviews studies on the effects of the Booroola *FecB* gene on production traits in a range of genetic comparisons, environments and production systems. The comparisons involve Booroola Merino (BM) crosses with various other breeds and *FecB* homozygous (FF), heterozygous (F+) and non-carrier (++) contrasts in comparable background genotypes.[2] A summary of 40 studies showed the effect of F+ for ovulation rate to range from +1.1 to +2.0, with the effect for FF generally being additive. The effect of F+ for litter size was reduced, in the range +0.5 to +1.3, with little or no increase for FF among BM crosses. Poorer lamb survival and lamb growth further reduced the effect of *FecB* for lambs weaned and weight of lamb weaned. The effects of *FecB* on a range of other traits, including fertility, embryo and lamb survival, lamb growth, carcass and meat quality, and wool production are reviewed. In addition, the role of management and opportunities for nutritional modulation of the *FecB* effects are examined.

Introduction

The use of sheep carrying the Booroola *FecB* gene offers the opportunity for a quantum increase in fecundity and reproductive rate in one generation. Turner and Young (1969) first showed an advantage of about 30% in lambs born for the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Booroola flock compared to their twinning selection flock. This stimulated several research experiments in Australia and New Zealand to use the Booroola genotype to increase reproduction in Merino flocks and other breeds. Many of these experiments were set up in the 1970s before it was known that the improved reproduction of the Booroola Merino (BM) was due to a major gene (*FecB*) that was first postulated in 1980 by Piper and Bindon (1982). The source of the Booroola sheep was described by Turner (1982) and expanded upon by Piper and Bindon (1996), including an account of the events leading to the hypothesis of a major gene. A series of experiments in Australia and New Zealand confirmed the existence of a major gene (Davis et al. 1982; Piper et al. 1985). Molecular markers were later found on sheep chromosome 6 (Montgomery et al. 1993; Montgomery et al. 1994) and the effect of *FecB* was subsequently shown to be due to a mutation (BMPR1B) (Wilson et al. 2001). A DNA test for carriers was developed in New Zealand and is now available commercially (Davis 2004). The origin of the Booroola mutation is most likely from the Garole breed in India, and the mutation is also found in the prolific Javanese breed (Davis 2005) and the Hu and Han breeds in China (Davis et al. 2006).

This paper reviews the effects of *FecB* on ovulation rate (OR), lambing performance and other production traits, and the opportunities for management and nutritional modulation of *FecB* expression.

FecB effect on reproduction

Booroola rams have been used for crossing with other breeds in several countries and the effects of *FecB* on the performance of ewes in these studies are

[1] New South Wales Department of Primary Industries, Orange, New South Wales 2800, Australia; neal.fogarty@dpi.nsw.gov.au

[2] FF, F+ and ++ are an alternative notation for *FecB* genotypes, equivalent to $FecB^{BB}$, $FecB^{B+}$ and $FecB^{++}$, respectively, used in other papers in these proceedings.

summarised in the appendix to this paper. The results are from 40 studies that cover a considerable range of crossing breeds and locations in widely varying environments and production systems. Appendix 1 also includes reports for Indian and Chinese breeds in which *FecB* has recently been found.

The early experiments and introductions to commercial flocks involved crossing Booroola rams with ewes of various strains of Merino or other breeds to improve lambing rates in both the wool and lamb industries. These experiments were set up prior to knowledge of a major gene being involved, and the Booroola rams and their progeny were of unknown *FecB* genetic status, which hampered improvements. However, there was generally a quantum increase in litter size (LS) and lambing rate among Booroola cross ewes, although lamb survival was reduced. Piper and Bindon (1987) summarised the average effects of one copy of the *FecB* gene as increasing OR by +1.0 to +1.5 ova and LS by +0.8 to +1.2 lambs across a range of genetic and environmental backgrounds. For two copies the effect on OR appeared to be additive, and for LS it varied from additive to dominant depending on the background genotype.

The contrasts in the early reports in Appendix 1 are likely to be underestimates of the F+ effect. There would have been varying proportions of non-carrier ewes (++) included in the Booroola crosses because of the unknown genetic status of the Booroola sires. The *FecB* status of ewes and sires in the reports up to the late 1990s was generally assigned using OR and LS performance data and progeny test information, as suggested by Davis et al. (1982), while most of the more recent reports have used DNA testing.

The effect of one copy of *FecB* (F+) in the ewes generally ranged from +1.1 to +2.0 for OR, which is close to the earlier summary by Piper and Bindon (1987). The greatest effect on OR (+2.0) was reported in a Romney background genotype that was highly selected for reproduction (Farquhar et al. 2006). The authors suggested there may be a second major gene for reproduction present in this flock. The effect of two copies of *FecB* (FF) generally appears to be additive for OR. The effect of the *FecB* gene on OR was reduced in young ewes in their first autumn (Montgomery et al. 1985; Fogarty et al. 1995). The response in OR to exogenous hormone stimulation also appears to be greater in F+ than ++ ewes (Quirke et al. 1987; Boulton et al. 1995).

The effect of *FecB* (F+) on LS was reduced, ranging from +0.5 to +1.3 lambs born, with little or no increase for FF ewes among the BM crosses. Recent reports in Indian (Kumar et al. 2008) and Chinese (Guan et al. 2007) breeds, in which the *FecB* mutation also occurs, have shown a further increase in LS for FF of about half the effect of one copy (F+). While there was an enhanced response in OR among F+ ewes to exogenous follicle stimulation, the response in LS was negligible or negative (Quirke et al. 1987). Poorer lamb survival and lamb growth further reduced the effect of *FecB*. However, in most reports there was still an advantage of *FecB*, with the effect ranging from –0.4 to +0.6 for lambs weaned and –9.8 kg to +8.4 kg for total weight of lamb weaned per ewe joined. Fogarty and Hall (1995) suggested there may be additional genes with small effect for reproduction in the Booroola as BM × Dorset ++ ewes had similar OR and LS to selected Merino (Trangie Fertility) × Dorset ewes. This was supported by results from an unrelated study, in which Trangie Fertility × SA Merino ewes were superior to SA Merino ewes for OR (+0.3), LS (0.1) and lambs weaned (0.1) (Ponzoni et al. 1985b).

FecB effect on components of ewe productivity

Fertility and embryo survival

The reduced advantage in LS relative to OR for *FecB*-carrier ewes in all the reports indicates that *FecB* may reduce fertilisation and/or embryo survival. Fertilisation rates vary and it may not be an 'all or none' process (Michels et al. 1998). However, fertilisation failure was regarded as a minor source of ova wastage by Kleemann et al. (1990) (F+ ewes, 9.4%; ++ ewes, 6.7%; not significant), with most wastage of ova occurring in the first 21 days after insemination. Embryo survival is reduced at higher ORs (Hanrahan 1980) and any comparisons need to be made after accounting for differences in OR. Southey et al. (2002) reported the *FecB* gene resulted in 15–19% higher embryo mortality and *FecB* was still significant when OR was included as a covariate. At the same OR, Farquhar et al. (2006) found FF ewes were 0.17 lower than F+ ewes ($P < 0.05$) for LS. In contrast, Meyer et al. (1994c) showed that Booroola ewes had a higher uterine efficiency (marginal response in LS with an increase of one ova in OR) than Merino ewes at an OR of 2.0,

and Booroola crosses were superior to non-Booroola cross ewes (and Booroola ewes) at higher ORs. They suggested the latter effect may be due to heterosis for embryo survival. Interpretation of embryo wastage in a study by Kleemann et al. (1990) was obscured by significant interactions of genotype and OR with groups of ewes assessed at different ages post insemination. However, calculations from these data by Meyer et al. (1994c) showed uterine efficiency for two ovulations to be 0.75 for F+ compared to 0.46 for ++ ewes. In addition, my calculations from these data show the uterine efficiency to be 0.5 for both three and four ovulations among the F+ ewes. In a study involving Booroola crosses and Finnsheep and Suffolk ewes, Castonguay et al. (1990) found no difference in prenatal mortality between genetic groups at the equivalent OR. In an extensive review, Michels et al. (1998) emphasised the need to distinguish between uterine efficiency and uterine capacity (the maximum number of foetuses a dam is able to support at birth). They also concluded that there were clear indications of genetic differences in prenatal survival, with the BM (and Finnsheep) contributing to superiority in uterine efficiency among twin ovulating crossbred ewes at least. In contrast, Young and Dickerson (1991) reported higher embryo mortality among BM-sired compared to Finnsheep-sired ewes.

Higher levels of non-pregnant ewes have been reported as OR increases to very high levels (Farquhar et al. 2006). These authors also reported significant increases in non-pregnant ewes associated with *FecB* (16.4%, 7.6% and 4.1% for FF, F+ and ++, respectively), and when compared at the same OR the FF ewes still had significantly more non-pregnant ewes than ++ (9.4%, $P < 0.01$) and F+ (7.4%, $P < 0.01$) ewes. The authors noted that this flock has continued selection for OR after introgression of *FecB* and the FF ewes have a very high OR, which may have exceeded the optimum. Lower fertility of FF ewes has also been reported by Walkden-Brown et al. (2007).

Lamb survival

All of the reports with weaning data in Appendix 1 showed a decline in the advantage of *FecB*-carrier ewes between LS and lambs weaned, indicating lower lamb survival. Industry exploitation of the advantages of the *FecB* ewes is dependent on reducing lamb losses, especially among higher order births (Piper and Bindon 1987; Davis et al. 1991; Meyer et al. 1994a). Poorer lamb survival from BM compared to control Merino ewes was attributed to low survival of triplet and higher order births, as survival rates were similar for singles and twins (Piper and Bindon 1982), although the authors conceded that the survival of the Booroola lambs may have been somewhat enhanced by the extra care they received at birth. Davis et al. (1991) reported in their review that lamb survival was an average of 9.8% lower among Booroola cross than local breed ewes. Subsequent experiments have endeavoured to improve lamb survival by introgressing *FecB* into breeds with superior maternal merit in several countries. However, they have generally had limited success and resulted in only minor or negative improvement in overall ewe productivity (e.g. Castonguay et al. 1990; Fogarty et al. 1992b; Meyer et al. 1994a; Bunge et al. 1995; Southey et al. 2002).

Birth weight

Birth weight is the most important factor affecting survival in Booroola-infused flocks, with other factors largely explained by differences in birth weight (Hinch et al. 1985). Lambs from higher order births have low birth weights and this contributes to their lower survival, especially under extensive production systems (Owens et al. 1985) and where adverse weather conditions with high chill factors occur during lambing (Fogarty and Hall 1995). While lower birth weight among lambs of the same birth type from FF or F+ compared to ++ ewes have been reported (Gootwine et al. 1993), others have shown no significant effect (Abella et al. 2005). In a recent study in the dairy Assaf breed, Gootwine et al. (2006) reported significantly lower birth weight for FF compared to F+ and ++ ewe lambs ($P = 0.01$). They also detected a significant maternal effect, with lambs born to FF ewes being lighter than those born to F+ and ++ ewes (3.9 kg and 4.3 kg respectively).

Lamb growth

Poor growth of Booroola cross lambs, along with low lamb survival, is a major barrier to widespread industry adoption (Davis et al. 1991; Meyer et al. 1994a). Low birth weight and reduced pre-weaning growth due to higher order births and reduced milk availability for individual lambs contribute to lower lamb growth. The BM is also derived from a Merino fine-wool breed that has smaller mature size and

lower growth relative to many of the other breeds (often maternal and meat) that it has been crossed with to improve productivity.

Several studies have reported lower growth from BM crosses, ranging from –4% to –16% for weaning weight and –7% to –12% for later weights (Beetson and Lewer 1985; Davis et al. 1991), although others have reported no effect (Meyer et al. 1994b; Abella et al. 2005). Booroola × Dorset F+ ewes were about 2 kg heavier than ++ ewes during their first year (Fogarty et al. 1995). There was a similar difference as adults, although there was a significant strain × cohort interaction (Fogarty and Hall 1995), and in an earlier study with the same crosses there was no difference (Fogarty et al. 1992b). In Assaf sheep there were no differences in pre-weaning growth rate, although FF lambs had lower post-weaning growth than F+ or ++ lambs, which also resulted in the mature ewes being approximately 3 kg lighter ($P < 0.001$) (Gootwine et al. 2006). Southey et al. (2002) reported that growth of lambs from crossbred F+ ewes was significantly lower ($P < 0.001$) than from ++ ewes, although differences due to birth type were not taken into account in the analysis. Several reports have shown no effect of Booroola *FecB* status (FF, F+, ++) on live weight (Ponzoni et al. 1985a; Bodin et al. 1991; Walkden-Brown et al. 2007). In the Garole × Malpura cross Kumar et al. (2008) reported lower birth weight, lamb growth and 12-month weight for FF and F+ compared to ++ sheep. In contrast, in Chinese meat Merinos the FF and F+ lambs were 17% heavier at 90 days than were ++ lambs (Guan et al. 2007).

Visscher et al. (2000) reported that lambs from dams that were carriers of *FecB* grew slower than those from non-carrier dams, and the lambs needed significantly more intake of energy and protein per kilogram of average daily gain. Fogarty et al. (1995) also reported a trend for lower growth of lambs from Booroola Merino × Dorset F+ compared to ++ ewes.

FecB effect on other production traits

Carcass composition and quality

BM × Merino lambs had greater fat (13% carcass chemical fat and 15% subcutaneous fat) and 6% less bone than other Merino strains at the same lean tissue content, but there were no differences between progeny of Booroola FF, F+ and ++ sires (Kleemann et al. 1988). Several other studies have reported higher fat levels among BM progeny in various crossbreed comparisons (Young and Dickerson 1991; Fogarty et al. 1992a; Janiuk et al. 1998; Visscher et al. 2000). The significantly greater ($P < 0.05$) subcutaneous carcass fat levels among Booroola Leicester (BM × Border Leicester backcross for 1–3 generations) sired crossbred wether lambs than the first-cross lambs from several other maternal sire breeds (Fogarty et al. 2005b) persisted to a lesser extent ($P < 0.05$) in the second-cross progeny of these first-cross ewes (Afolayan et al. 2007). There were no differences between the breed crosses for carcass eye muscle area or muscle colour in either study. While the Booroola Leicester cross lambs have higher subcutaneous fat levels in these studies, it may be partly due to the higher estimated breeding values for fat among the Booroola-sired progeny tested (Fogarty et al. 2005b).

In a Texel backcross population, lambs carrying the *FecB* had higher dressing percentage and eye muscle depth and area, as well as an effect on meat colour, compared with non-carriers, while carrier dams had lambs with smaller eye muscle and slower growth, which reduced feed efficiency (Visscher et al. 2000). Male Booroola cross lambs had less intramuscular fat and marbling than Bulgarian fine-wool controls (Dimitrov and Nedelchev 1999). Booroola cross lambs had more tender and larger eye muscle than Romney and Finnsheep crosses (Fahmy et al. 1992).

Mezoszentgyorgyi et al. (2001) reported small differences ($P < 0.05$) in fatty acid composition between BM and Suffolk breed lambs, with the BM having a higher proportion of saturated fats and a lower proportion of unsaturated fats. This was somewhat contrary to Suess et al. (2000), who found that intramuscular and kidney fat had a lower melting point in BM backcross compared to German Mutton Merino lambs, although they concluded that the small differences were unlikely to affect consumer preferences regarding carcass quality. They also found that the ratio between linoleic (18:2, n-6) and linolenic (18:3, n-3) was higher in progeny of *FecB* carriers, although this was contrary to the results of Mezoszentgyorgyi et al. (2001).

Wool production

Several reports have shown no significant difference between F+ and ++ Booroola cross sheep for wool production or wool quality traits (Ponzoni et al. 1985a; Ponzoni et al. 1985b; Meyer et al. 1994b;

Fogarty et al. 1995; Walkden-Brown et al. 2007). The difference in greasy fleece weight for BM × Dorset F+ and ++ ewes was not significant when the number of lambs born was included in the model (Fogarty and Hall 1995). Differences reported in some early studies were no doubt due to differences in the wool production of the BM and Merino strains used for crossing. For example, the BM × WA Merino had 15% lower clean fleece weight than the WA Merino sheep, which was compensated to some extent by a 0.7 µm lower fibre diameter (Beetson and Lewer 1985).

Other traits

Among BM cross ewes, 9% more F+ than ++ ewes reached puberty ($P < 0.001$) and had 0.18 more cycles per ewe ($P < 0.01$) in their first autumn (Meyer et al. 1994b), although Montgomery et al. (1985) found no evidence of a longer breeding season in hoggets carrying the F gene and there was no effect on puberty in Mérinos d'Arles (Abella et al. 2005). Booroola cross ewe lambs reached puberty 3 weeks later than Finnsheep ewe lambs (Castonguay et al. 1990).

There was little difference in the pattern of oestrous activity throughout the year between Border Leicester × Merino ewes with and without Booroola breeding (Dunstan and Phillips 1984), although there was a differential response to melatonin (Moore et al. 1988). Among mature Booroola × Dorset ewes joined three times at 8-monthly intervals the F+ ewes had lower fertility in all seasons and lambed less frequently than the ++ ewes, and also had higher ewe losses (Fogarty et al. 1992b). The difference in OR between F+ and ++ young ewes in the normal autumn breeding season was reduced during the winter and spring (Fogarty et al. 1995). There was no effect of the *FecB* on the length of the post-lambing anoestrus (Fogarty and Hall 1995).

The gestation length for Merino ewes mated to Booroola Leicester rams was approximately 1 day longer ($P < 0.05$) than for those mated to Border Leicester rams, which was still apparent after accounting for slight differences in the average birth weight of lambs (Fogarty et al. 2005a).

Booroola × Awassi ewes had 50% less milk production than Awassi ewes, although there was no difference between F+ and ++ ewes (Gootwine et al. 1995). There was also no difference in suckling behaviour between BM × Romney and Romney ewes (Hinch 1989).

BM (and Polypay) rams had greater sperm output than Rambouillet and Columbia rams (Fitzgerald and Stellflug 1990), although Oldham and Gray (1984) found no evidence of any increase in testicular growth in young Booroola cross rams.

Nutrition and management

Prolific Booroola crossbreds and local breed controls have very different nutritional and management requirements, and need to be evaluated at stocking rates that reflect these differences in order to determine the full effect on productivity (Davis et al. 1991). OR in ewes responds to improved nutrition prior to mating, and Montgomery et al. (1983) showed that both F+ and ++ ewes responded to improved feeding levels with no significant genotype × treatment interaction. Reduced nutrition prior to mating has been suggested as a way of modifying the very high OR among *FecB*-carrier ewes. Low nutrition for 10 weeks before joining reduced OR in both Booroola-infused and non-infused crossbred ewes, resulting in a subsequent reduction in fertility, LS and lamb marking rate with no genotype × treatment interaction (King 1987). In another study low nutrition of Booroola × Merino (F+) ewes for 11 weeks before mating reduced body weight at mating and OR by 0.4, but resulted in no difference in fertility, LS or lamb weaning rate from the subsequent lambing (Kleemann et al. 1991).

Supplementation of Booroola ewes with lupins for 9 days before parturition increased birth weight by 0.2 kg and lamb survival to weaning by 12%, which resulted in an increase of LS at weaning from 1.26 to 1.52 (Hall et al. 1992). They suggested that the lupin supplement may have affected production and intake of colostrum. Hinch et al. (1996) also reported an increase in lamb survival among prolific crossbred ewes (40% F+) from 58% to 73% following supplementation with cottonseed meal, and the improvement was independent of birth weight.

Conclusions

The effect of the Booroola *FecB* gene on reproduction traits has been remarkably consistent following its introgression into many different breeds and its evaluation in a range of environments and production systems. The comparisons have involved BM crosses with various other breeds and *FecB* homozygous (FF), heterozygous (F+) and non-

carrier (++) contrasts in comparable background genotypes. A summary of 40 studies showed the effect of F+ for OR to range from +1.1 to +2.0, with the effect for FF generally being additive. The effect of F+ for LS was reduced, ranging from +0.5 to +1.3, with little or no increase for FF among BM crosses. However, in Indian and Chinese breeds, in which the *FecB* mutation also occurs, a further increase in LS for FF of about half the effect of one copy (F+) seems to occur. Poorer lamb survival and lamb growth further reduced the effect of *FecB* for lambs weaned and weight of lamb weaned. Most studies still showed a small advantage of *FecB*, although several reported negative effects. There is also some evidence of the Booroola having additional genes with small effect for OR and LS, as well as an additional major gene becoming apparent in a population under intense selection.

Embryo survival declines at higher ORs and the effects of *FecB* per se on embryo survival are equivocal. While there is evidence that F+ may enhance uterine efficiency at moderate ORs (e.g. 2), there may be a decline in embryo survival at higher ORs. In selected high-ovulating flocks with FF ewes, the OR may be beyond optimum uterine capacity. There is also evidence of a higher rate of non-pregnancy among FF ewes in highly selected flocks.

Most studies reported lower birth weight and growth rate from BM cross lambs and lambs from Booroola-introgressed crossbred ewes. However, it is difficult to separate the effects of *FecB*, the BM low background genetic merit for growth, and the lower birth weight and growth rate of lambs from larger litters. Most studies have reported little or no difference in lamb growth rate between FF, F+ and ++ genotypes. In those studies that have shown a small advantage of F+ over ++ genotypes, there may be some bias, as the genetic status was generally derived from reproduction records and there is a positive genetic correlation between growth and reproduction (Safari et al. 2005). For a range of other traits reviewed, including seasonal oestrous activity, carcass and meat quality, and wool production, there was no evidence of major effects of *FecB*.

Poor embryo and postnatal lamb survival, and associated low birth weight and growth rate of lambs, are major barriers to the more widespread exploitation of *FecB* in extensive sheep production systems. However, the evidence suggests that these undesirable characteristics are associated more with high OR and LS than with *FecB* per se. Nutritional restriction of ewes before joining reduces OR and could be used to reduce the incidence of high-order multiple births. Improved nutrition and management at lambing, especially the use of protein supplements to enhance colostrum production, should improve lamb survival.

The *FecB* mutation produces a quantum increase in OR and LS. There are opportunities to exploit *FecB* to improve productivity with F+ ewes that have a high background genetic merit for maternal performance and are run in more intensive production systems with benign lambing environments. However, FF ewes, especially in flocks selected for higher OR, are likely to lead to lower ewe productivity. There is considerable genetic variance for all the component traits of reproduction (Safari et al. 2005), and use of a selection index of overall ewe productivity may result in a more balanced biological outcome (Snowder and Fogarty 2009).

References

Abella D.F., Cognie Y., Thimonier J., Seck M. and Blanc M.R. 2005. Effects of the Fec(B) gene on birth weight, postnatal growth rate and puberty in Booroola × Merinos d'Arles ewe lambs. Animal Research 54, 283–288.

Afolayan R.A., Fogarty N.M., Gilmour A.R., Ingham V.M., Gaunt G.M. and Cummins L.J. 2008. Reproductive performance and genetic parameters in first cross ewes from different maternal genotypes. Journal of Animal Science 86, 804–814.

Afolayan R.A., Fogarty N.M., Ingham V.M., Gilmour A.R., Gaunt G.M., Cummins L.J. and Pollard T. 2007. Genetic evaluation of crossbred lamb production. 3: Growth and carcass performance of second-cross lambs. Australian Journal of Agricultural Research 58, 457–466.

Allison A.J., Stevenson J.R. and Kelly R.W. 1977. Reproductive performance and wool production of Merino and high fertility strain (Booroola) × Merino ewes. Proceedings of the New Zealand Society of Animal Production 37, 230–234.

Beetson B.R. and Lewer R.P. 1985. Productivity of Booroola cross Merinos in Western Australia. Pp. 391–398 in 'Genetics of reproduction in sheep' ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Bindon B.M., Piper L.R. and Ch'ang T.S. 1984. Reproductive performance of crossbred ewes derived from Booroola and control Merinos and joined to rams of two terminal sire breeds. Pp. 243–246 in 'Reproduction in sheep', ed. by D.R. Lindsay and D.T. Pearce. Australian Academy of Science: Canberra, Australia.

Bodin L., Cornu C., Elsen J.M., Molenat G. and Thimonier J. 1991. The effect of Booroola genotype on some traits

in a Merinos d'Arles flock. Pp. 371–379 in 'Proceedings of the 2nd International Workshop on Major Genes for Reproduction in Sheep', Toulouse, 16–18 July 1990. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Boulton M., Haley C., Springbett A. and Webb R. 1995. The effect of the Booroola (*FecB*) gene on peripheral FSH concentrations and ovulation rates during oestrus, seasonal anoestrus and on FSH concentrations following ovariectomy in Scottish Blackface ewes. Journal of Reproduction and Fertility 103, 199–207.

Bunge R., Thomas D.L. and Nash T.G. 1995. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: lamb production of F1 adult ewes. Journal of Animal Science 73, 1602–1608.

Castonguay F., Minvielle F. and Dufour J.J. 1990. Reproductive performance of Booroola × Finnish Landrace and Booroola × Suffolk ewe lambs, heterozygous for the *F* gene, and growth traits of their three-way cross lambs. Canadian Journal of Animal Science 70, 55–65.

Davis G.H. 2004. Fecundity genes in sheep. Animal Reproduction Science 82–83, 247–253.

Davis G.H. 2005. Major genes affecting ovulation rate in sheep. Genetics Selection Evolution 37, S11–S23.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway S.M., Lumsden B.M., Hanrahan J.P., Mullen M., Mao X.Z., Wang G.L., Zhao Z.S., Zeng Y.Q., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranz J.J. and Notter D.R. 2006. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92, 87–96.

Davis G.H., Elsen J.M., Bodin L., Fahmy M.H., Castonguay F., Gootwine E., Bor A., Braw-Tal R., Greeff J.C., Lengyel A., Paszthy G. and Cummins L. 1991. A comparison of the production from Booroola and local breed sheep in different countries. Pp. 315–323 in 'Proceedings of the 2nd International Workshop on Major Genes for Reproduction in Sheep', Toulouse, 16–18 July 1990. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Davis G.H. and Hinch G.N. 1985. Introduction and management of the Booroola gene in sheep flocks in New Zealand. Pp. 139–148 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Davis G.H., Montgomery G.W., Allison A.J., Kelly R.W. and Bray A.R. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Dimitrov D. and Nedelchev D. 1999. Study on growth intensity and meat quantity of lambs crosses between Booroola and north-east Bulgarian finewool sheep. Zhivotnovdni Nauki 36, 11–15.

Dodds K.G., Davis G.H., Elsen J.M., Isaacs K.L. and Owens J.L. 1991. The effect of Booroola genotype on some reproductive traits in a Booroola Merino flock. Pp. 359–366 in 'Proceedings of the 2nd International Workshop on Major Genes for Reproduction in Sheep', Toulouse, 16–18 July 1990. L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Dunstan E.A. and Phillips D. 1984. Seasonal oestrous and ovulation patterns of Border Leicester crossbred ewes from three strains of Merino ewes and their Booroola crosses. Pp. 99–101 in 'Reproduction in sheep' ed. by D.R. Lindsay and D.T. Pearce. Australian Academy of Science: Canberra.

Eppleston J. and Robards G.E. 1995. An evaluation of maiden Finn × Merino and Booroola × Merino ewes for lamb production: reproduction and wool parameters. Proceedings of the Australian Association of Animal Breeding and Genetics 11, 662–665.

Fahmy M.H., Boucher J.M., Poste L.M., Gregoire R., Butler G. and Comeau J.E. 1992. Feed efficiency, carcass characteristics, and sensory quality of lambs, with or without prolific ancestry, fed diets with different protein supplements. Journal of Animal Science 70, 1365–1374.

Farquhar P.A., Dodds K.G. and Davis G.H. 2006. Introgression of the Booroola mutation (FecB) leads to hyper-prolificacy in a Romney sheep flock. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Minas Gerais, Brazil, 13–18 August, 2006.

Fitzgerald J.A. and Stellflug J.N. 1990. Comparison of scrotal circumferences, sperm output and libodo of Booroola Merino, Polypay, Rambouillet and Columbia rams in a controlled photoperiod. Sheep Research Journal 6, 11–14.

Fogarty N.M. and Hall D.G. 1995. Performance of crossbred progeny of Trangie Fertility Merino and Booroola Merino rams and Poll Dorset ewes. 3: Reproduction, liveweight and wool production of adult ewes. Australian Journal of Experimental Agriculture 35, 1083–1091.

Fogarty N.M., Hall D.G. and Atkinson W.R. 1992a. Management of highly fecund ewe types and their lambs for 8-monthly lambing. 2: Effect of weaning age and sex on lamb growth and carcass traits. Australian Journal of Experimental Agriculture 32, 1031–1036.

Fogarty N.M., Hall D.G. and Atkinson W.R. 1992b. Productivity of three crossbred ewe types mated naturally at 8-monthly intervals over two years. Australian Journal of Agricultural Research 43, 1819–1832.

Fogarty N.M., Hall D.G. and Gilmour A.R. 1995. Performance of crossbred progeny of Trangie Fertility Merino and Booroola Merino rams and Poll Dorset ewes. 2: Reproductive activity, liveweight and wool production of ewe lambs. Australian Journal of Experimental Agriculture 35, 1075–1082.

Fogarty N.M., Ingham V.M., Gilmour A.R., Cummins L.J., Gaunt G.M., Stafford J., Hocking Edwards J.E. and Banks R.G. 2005a. Genetic evaluation of crossbred lamb production. 1: Breed and fixed effects for birth and weaning weight of first-cross lambs, gestation length, and reproduction of base ewes. Australian Journal of Agricultural Research 56, 443–453.

Fogarty N.M., Ingham V.M., Gilmour A.R., Cummins L.J., Gaunt G.M., Stafford J., Hocking Edwards J.E. and Banks R.G. 2005b. Genetic evaluation of crossbred lamb production. 2: Breed and fixed effects for post-weaning growth, carcass, and wool of first-cross lambs. Australian Journal of Agricultural Research 56, 455–463.

Gootwine E., Bor A., Braw-Tal R. and Zenou A. 1993. Inheritance of birthweight and growth traits in crosses between the Booroola Merino and Assaf sheep breeds. Livestock Production Science 33, 119–126.

Gootwine E., Bor A., Braw-Tal R. and Zenou A. 1995. Reproductive performance and milk production of the improved Awassi breed as compared with its crosses with the Booroola Merino. Animal Science 60, 109–115.

Gootwine E., Rozov A., Bor A. and Reicher S. 2006. Carrying the FecB (Booroola) mutation is associated with lower birth weight and slower post-weaning growth rate for lambs, as well as a lighter mature bodyweight for ewes. Reproduction Fertility and Development 18, 433–437.

Guan F., Liu S.R., Shi G.Q. and Yang L.G. 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Animal Reproduction Science 99, 44–52.

Hall D.G., Piper L.R., Egan A.R. and Bindon B.M. 1992. Lamb and milk production from Booroola ewes supplemented in late pregnancy. Australian Journal of Experimental Agriculture 32, 587–593.

Hanrahan J.P. 1980. Ovulation rate as the selection criterion for litter size in sheep. Proceedings of the Australian Society of Animal Production 13, 405–408.

Hinch G. 1989. The sucking behaviour of triplet, twin and single lambs at pasture. Applied Animal Behaviour Science 22, 39–48.

Hinch G., Crosbie S., Kelly R., Owens J. and Davis G. 1985. Influence of birth weight and litter size on lamb survival in high fecundity Booroola-Merino crossbred flocks. New Zealand Journal of Agricultural Research 28, 31–38.

Hinch G.N., Lynch J.J., Nolan J.V., Leng R.A., Bindon B.M. and Piper L.R. 1996. Supplementation of high fecundity Border Leicester × Merino ewes with a high protein feed: its effect on lamb survival. Australian Journal of Experimental Agriculture 36, 129–136.

Isaacs K.L., Owens J.L., Littlejohn R.P., Johnstone P.D. and Fennessy P.F. 1991. Influence of maternal liveweight on reproductive performance and wool production of heterozygous Booroola Merino × Coopworth (Fec^B Fec^+) and Merino × Coopworth ewes. New Zealand Journal of Agricultural Research 34, 55–67.

Janiuk W., Baranowski A. and Klewiec J. 1998. Performance and slaughter value of Polish Merino, Booroola and Suffolk crossbred lambs. Journal of Animal and Feed Sciences 7, 161–170.

King C.F. 1987. Regulation of the Booroola effect by pre-joining nutrition. Pp. 293–296 in 'Merino improvement programs in Australia', ed. by B.J. McGuirk. Australian Wool Corporation: Melbourne, Australia.

Kleemann D.O., Ponzoni R.W., Stafford J.E. and Grimson R.J. 1988. Carcass composition of the South Australian Merino and its crosses with the Booroola and Trangie Fertility Merino. Australian Journal of Experimental Agriculture 28, 167–171.

Kleemann D., Walker S., Grimson R., Smith D., Grosser T. and Seamark R. 1991. Exogenous progesterone and embryo survival in Booroola-cross ewes. Reproduction, Fertility and Development 3, 71–77.

Kleemann D.O., Walker S.K., Walkley J.R.W., Smith D.H., Grimson R.J. and Seamark R.F. 1990. Fertilization and embryo loss in Booroola Merino × South Australian Merino ewes: effect of the *F* gene. Theriogenology 33, 487–498.

Kumar S., Mishra A., Kolte A., Arora A., Singh D. and Singh V. 2008. Effects of the Booroola (*FecB*) genotypes on growth performance, ewe's productivity efficiency and litter size in Garole × Malpura sheep. Animal Reproduction Science 105, 319–331.

McGuirk B.J., Killeen I.D., Piper L.R., Bindon B.M., Wilson R., Caffery G. and Langford C. 1984. Lamb production from Booroola × Collinsville ewes. Proceedings of the Australian Society of Animal Production 15, 464–467.

Meyer H.H., Baker R.L., Harvey T.G. and Hickey S.M. 1994a. Effects of Booroola Merino breeding and the Fec(B) gene on performance of crosses with longwool breeds. 2: Effects on reproductive performance and weight of lamb weaned by young ewes. Livestock Production Science 39, 191–200.

Meyer H.H., Bigham M.L., Baker R.L., Harvey T.G. and Hickey S.M. 1994b. Effects of Booroola Merino breeding and the Fec^B gene on performance of crosses with longwool breeds. 1: Effects on growth, onset of puberty, wool production and wool traits. Livestock Production Science 39, 183–190.

Meyer H.H., Piper L.R., Bindon B.M. and Woolaston R.R. 1994c. Litter size and uterine efficiency of Booroola Merinos, control Merinos and their crosses with Border

Leicester and Dorset. Livestock Production Science 38, 217–223.

Mezoszentgyorgyi D., Husveth F., Lengyel A., Szegleti C. and Komlosi I. 2001. Genotype-related variations in subcutaneous fat composition in sheep. Animal Science 72, 607–612.

Michels H., Vanmontfort D., Dewil E. and Decuypere E. 1998. Genetic variation of prenatal survival in relation to ovulation rate in sheep: a review. Small Ruminant Research 29, 129–142.

Mishra A.K., Arora A.L., Kumar S. and Prince L.L.L. 2009. Studies on effect of Booroola (*FecB)* genotype on lifetime ewe's productivity efficiency, litter size and number of weaned lambs in Garole × Malpura sheep. Animal Reproduction Science 113, 203–298.

Montgomery G.W., Bray A.R. and Kelly R.W. 1983. Ovulation rates of first cross Booroola compared with local breed ewes following differential feeding. Animal Reproduction Science 6, 209–215.

Montgomery G.W., Crawford A.M., Penty J.M., Dodds K.G., Ede A.J., Henry H.M., Pierson C.A., Lord E.A., Galloway S.M., Schmack A.E., Sise J.A., Swarbrick P.A., Hanrahan V., Buchanan F.C. and Hill D.F. 1993. The ovine Booroola fecundity gene (*FecB*) is linked to markers from a region of human chromosome 4q. Nature Genetics 4, 410–414.

Montgomery G.W., Kelly R.W., Davis G.H. and Allison A.J. 1985. Ovulation rate and oestrus in Booroola genotypes: some effects of age, season and nutrition. Pp. 237–243 in 'Genetics of reproduction in sheep' ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Montgomery G.W., Penty J.M., Lord E.A., Henry H.M., Cambridge L.M. and Broad T.E. 1994. Mapping production traits in farm animals: the Booroola (FecB) locus. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada 7–12 August, 21, 17–20, .

Moore R., Miller C., Dow B. and Staples L. 1988. Effects of melatonin on early breeding of *F*+ and ++ Booroola × Perendale and Romney ewes. Proceedings of the New Zealand Society of Animal Production 48, 109–111.

Nieuwhof G.J., Visscher A.H., Engel B., van der Werf J.H.J., Jong F.H. and Dijkstra M. 1998. Identification of early predictors of carriers of the Booroola gene in sheep using a mixed inheritance model. Animal Science 67, 317–325.

Nimbkar C., Ghalsasi P.M., Chavan K.M., Nalawade M.H., Walkden-Brown S.W., Gupta V.S., Pardeshi V.C. and van der Werf J.H.J. 2006. Lamb production by FecB heterozygous carrier and non-carrier ewes in smallholder flocks in Maharashtra state of India. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Minas Gerais, Brazil, 13–18 August. CD-ROM 04-03.

Nimbkar C., Ghalsasi P.M., Maddox J.F., Pardeshi V.C., Sainani M.N., Gupta V. and Walkden-Brown S.W. 2003. Expression of the *FecB* gene in Garole and crossbred ewes in Maharashtra, India. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 15, 111–114.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Walkden-Brown S.W., Maddox J.F., Gupta V.S., Pardeshi V.C., Ghalsasi P. and van der Werf J.H.J. 2007. Reproductive performance of Indian crossbred Deccani ewes carrying the *FecB* mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 430–433.

Oldham C.M. and Gray S.J. 1984. Testicular growth and the expression of the 'F' gene in young Merino rams. Pp. 257–259 in 'Reproduction in sheep', ed. by D.R. Lindsay and D.T. Pearce. Australian Academy of Science: Canberra, Australia.

Owens J.L., Bindon B.M., Edey T.N. and Piper L.R. 1985. Behaviour at parturition and lamb survival of Booroola Merino sheep. Livestock Production Science 13, 359–372.

Piper L.R. and Bindon B.M. 1982. The Booroola Merino and the performance of medium non-Peppin crosses at Armidale. Pp. 9–19 in 'The Booroola Merino: proceedings of a workshop held at Armidale, New South Wales, 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO: Melbourne, Australia (reprinted in Wool Technology and Sheep Breeding 1983, 31, 14–19, 33).

Piper L.R. and Bindon B.M. 1987. Industry utilisation of the Booroola Merino in Australia. Pp. 279–282 in 'Merino improvement programs in Australia', ed. by B. McGuirk. Australian Wool Corporation: Melbourne, Australia.

Piper L.R. and Bindon B.M. 1996. The Booroola Merino. Pp. 152–160 in 'Prolific sheep' ed. by M.H. Fahmy. CAB International: Wallingford, Oxford, UK.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the prolificacy of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Ponzoni R.W., Fleet M.R., Walkley J.R.W. and Walker S.K. 1985a. A note on the effect of the *F* gene on wool production and live weight of Booroola × South Australian Merino rams. Animal Production 40, 367–369.

Ponzoni R.W., Walker S.K., Walkley J.R.W. and Fleet M.R. 1985b. The productivity of Bungaree, Booroola × Bungaree and Trangie Fertility × Bungaree Merino ewes in South Australia. Pp. 127–137 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Quirke J.F., Meyer H.H., Lahlou-Kassi A., Hanrahan J.P., Bradford G.E. and Stabenfeldt G.H. 1987. Natural and induced ovulation rate in prolific and non-prolific breeds of sheep in Ireland, Morocco and New Zealand. Journal of Reproduction and Fertility 81, 309–316.

Safari E., Fogarty N.M. and Gilmour A.R. 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 92, 271–289.

Schulze K.S., Waldron D.F., Willingham T.D., Shelby D.R., Engdahl G.R., Gootwine E., Yoshefi S., Montgomery G.W., Tate M.L. and Lord E.A. 2003. Effects of the FecB gene in half-sib families of Rambouillet-cross ewes. Sheep and Goat Research Journal 18, 83–88.

Snowder G.D. and Fogarty N.M. 2009. Composite trait selection to improve reproduction and ewe productivity: a review. Animal Production Science 49, 9–16.

Southey B.R., Thomas D.L., Gottfredson R.G. and Zelinsky R.D. 2002. Ewe productivity of Booroola Merino–Rambouillet crossbred sheep during early stages of the introgression of the Fec(B) allele into a Rambouillet population. Livestock Production Science 75, 33–44.

Suess R., Heylen K. and von Lengerken G. 2000. The effect of Booroola-Merinos on the fat content and fat quality of carcasses in crosses with German Mutton Merinos. Archiv fur Tierzucht 43, 45–56.

Teyssier J., Elsen J.M., Bodin L., Bosc P., Lefevre C. and Thimonier J. 1998. Three-year comparison of productivity of Booroola carriers and non-carrier Merino d'Arles ewes. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, 24, 117–120, Armidale, Australia.

Turner H.N. 1982. Origins of the CSIRO Booroola. Pp. 1–7 in 'The Booroola Merino: Proceedings of a workshop held at Armidale, New South Wales 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO: Melbourne, Australia.

Turner H.N. and Young S.S.Y. 1969. Quantitative genetics in sheep breeding. MacMillan: Melbourne, Australia.

Visscher A.H., Dijkstra M., Lord E.A., Suss R., Rosler H.J., Heylen K. and Veerkamp R.E. 2000. Maternal and lamb carrier effects of the Booroola gene on food intake, growth and carcass quality of male lambs. Animal Science 71, 209–217.

Walkden-Brown S.W., Wolfenden D.H., Charles R.J. and Maddox J.F. 2007. Expression of reproductive and production traits in commercial Merino ewes having 0, 1 or 2 copies of the *FecB* mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 426–429.

Williams G.A. and Nicoll G.B. 1990. Management and selection in a Booroola × Romney breeding scheme. Proceedings of the Australian Association of Animal Breeding and Genetics 8, 261–264.

Wilson T., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Young L.D. and Dickerson G.E. 1991. Comparison of Booroola Merino and Finnsheep: effects on productivity of mates and performance of crossbred lambs. Journal of Animal Science 69, 1899–1911.

Appendix

Studies showing the Booroola *FecB* (F) gene advantage for ovulation rate, litter size, number of lambs weaned and total weight of lamb weaned, and the approximate numbers of ewes or records

Ovulation rate	Litter size	Lambs weaned	Weight weaned (kg)	Records (n)	F gene contrast and ewe breeds[a]	Country	Reference
	+0.56	–0.01[b]	–1.9[b]	2,000	B°L × M *v.* BL × M	Australia	Afolayan et al. (2008)
	+1.14 +0.70		+2.3 +3.4	252	FF *v.* ++, G × Mal intercross F+ *v.* ++	India	Kumar et al. (2008)
	+0.70	+0.60	+2.3	208	F+ *v.* ++, G × Mal	India	Mishra et al. (2009)
	+1.61 +1.11			53	FF *v.* ++, Chinese meat M F+ *v.* ++	China	Guan et al. (2007)
	+0.54 +0.72	+0.41 +0.45	+1.9 +0.7	2,161	FF *v.* ++, G × Dec backcross F+ *v.* ++	India	Nimbkar et al. (2007)
+2.80 +1.01	+0.81 +0.96	–0.09[bc] +0.26[bc]	–2.6[b] +3.3[b]	599	FF *v.* ++, BM × M backcross F+ *v.*++	Australia	Walkden-Brown et al. (2007)
+4.21 +2.02	+0.67 +0.77			2,200	FF *v.* ++, BM × RomS backcross F+ *v.* ++	New Zealand	Farquhar et al. (2006)
	+0.49	+0.09		2,815	F+ *v.* ++, G × Dec	India	Nimbkar et al. (2006)
+0.82	+0.61			353	F+ *v.* ++, G crosses	India	Nimbkar et al. (2003)
+1.18	+0.51	+0.18		93	F+ *v.* ++, BM × Ram	USA	Schulze et al. (2003)
+1.62	+0.68	+0.20	–2.7[b]	278	F+ *v.* ++, BM × Ram & backcross	USA	Southey et al. (2002)
+1.48	+0.73			590	F+ *v.* ++, BM × Tex backcross	Netherlands	Nieuwhof et al. (1998)
+1.19	+0.89	+0.58	+8.4[b]	686	F+ *v.* ++, BM × Arles M backcross	France	Teyssier et al. (1998)
+1.8 +1.3					FF *v.* ++, BM × SB, F2 & backcross F+ *v.* ++	UK	Boulton et al. (1995)
+0.76 +1.07	+0.45 +0.56	+0.04[b] –0.37[b]	–0.7[b] –9.8[b]	155 137	(BL × BM) × M *v.* BL × M B°L (FF) × M *v.* BL × M	Australia	Eppleston and Robards (1995)
+0.67				260	F+ *v.* ++, BM × D (9–19 months)	Australia	Fogarty et al. (1995)
+1.15	+0.79	+0.02[b]	–1.3[b]	965	F+ *v.* ++, BM × D	Australia	Fogarty and Hall (1995)

Appendix (cont'd)

Studies showing the Booroola *FecB* (F) gene advantage for ovulation rate, litter size, number of lambs weaned and total weight of lamb weaned, and the approximate numbers of ewes or records

Ovulation rate	Litter size	Lambs weaned	Weight weaned (kg)	Records (n)	F gene contrast and ewe breeds[a]	Country	Reference
+1.30	+0.74			269	F+ *v.* ++, BM × Aw backcross	Israel	Gootwine et al. (1995)
	+1.16	+0.36	+0.[b]	925	F+ *v.* ++, BM × Rom, BM × Per	New Zealand	Meyer et al. (1994a)
+1.15	+0.48	–0.32[b]	–6.5[b]	443	F+ *v.* ++, BM × D	Australia	Fogarty et al. (1992b)
+1.13	+0.68	+0.39		580	F+ *v.* ++, BM × M d'Arles backcross	France	Bodin et al. (1991)
+0.99	+0.61	+0.33[b]	+1.8[b]	3,782	BM × *v.* local ×, 11 experiments	9 countries	Davis et al. (1991)
+2.69 +1.27	+1.05 +0.88			2,200	FF *v.* ++; combined flock data F+ *v.* ++	New Zealand	Dodds et al. (1991)
+1.51	+1.00	+0.54	+4.0[b]	246	BM × Cp *v.* M × Cp	New Zealand	Isaacs et al. (1991)
+1.60	+0.85	+0.45		77	BM × Fi & BM × Suf *v.* Fi & Suf	Canada	Castonguay et al. (1990)
+1.64				277	F+ *v.* ++, BM × M	Australia	Kleemann et al. (1990)
	+0.90	+0.22[b]	+7.0[b]	6,000	BM × Rom backcross nucleus *v.* Rom control	New Zealand	Williams and Nicoll (1990)
+1.93 +0.62	+1.20 +0.68	+0.15 +0.49		n.a.	BM × P *v.* P BL (BM × P) *v.* BL × P	Australia	King (1987)
+1.93	+1.33			116	F+ *v.* ++, BM × Rom	New Zealand	Quirke et al. (1987)
	+0.49	+0.27[b]	+2.5[b]	614	BM × M *v.* M	Australia	Beetson and Lewer (1985)
+0.70 +1.14	+0.57 +0.81	+0.27[b] +0.54[b]		n.a.	BM(F+) × Var *v.* Var, 6 comm. flocks BM(FF) × Var *v.* Var, 3 comm. flocks	New Zealand	Davis and Hinch (1985)
+0.55 +1.29 +0.34 +1.48				209 240 118 146	F+ *v.* ++, BM × Rom (7–9 months) F+ *v.* ++, BM × Rom (1.5–2.5 years) FF *v.* F+, BM × Rom (7–9 months) FF*v.* F+, BM × Rom (1.5–2.5 years)	New Zealand	Montgomery et al. (1985)
+0.95	+0.49	+0.15[b]		617	BM × M *v.* M	Australia	Ponzoni et al. (1985b)
+1.08	+0.73	+0.32	+6.9[b]	525	BL & D × BM *v.* BL & D × M	Australia	Bindon et al. (1984)
+0.42				283	BL × (BM × M) *v.* BL × M	Australia	Dunstan and Phillips (1984)

Appendix (cont'd)

Studies showing the Booroola *FecB* (F) gene advantage for ovulation rate, litter size, number of lambs weaned and total weight of lamb weaned, and the approximate numbers of ewes or records

Ovulation rate	Litter size	Lambs weaned	Weight weaned (kg)	Records (n)	F gene contrast and ewe breeds[a]	Country	Reference
+0.64	+0.46[b]	+0.29[b]		n.a.	BM × M *v.* M	Australia	McGuirk et al. (1984)
+1.54				600	F+ *v.* ++, BM × M, BM × Rom	New Zealand	Montgomery et al. (1983)
+1.24	+0.69			283	F+ *v.* ++, BM × M backcross	New Zealand	Davis et al. (1982)
[3.66[d]] +0.92	+1.00 +0.57	+0.27[b] +0.14[b]		1,500 448	BM *v.* M BM × M *v.* M	Australia	Piper and Bindon (1982)
+0.83	+0.56			1,008	BM × M *v.* M	New Zealand	Allison et al. (1977)

[a] Aw = Awassi; BM = Booroola Merino; BºL = Booroola Leicester (BM × BL 87.5% backcross); BL = Border Leicester; Cp = Coopworth; D = Dorset; Dec = Deccani; Fi = Finnsheep; G = Garole; M = Merino; Mal = Malpura; P = Polwarth; Per = Perendale; Ram = Rambouillet; Rom = Romney; RomS = Romney (selected); Suf = Suffolk; Tex = Texel; Var = various breeds

[b] Per ewe joined

[c] Based on ewes scanned

[d] Ovulation rate of 210 mixed-age BM ewes

n.a. = not available

Effects of multiple ovulation and litter size on maternal and foetal physiology: prenatal and postnatal consequences

G.N. Hinch[1]

Abstract

This review examines the consequences of large litter size on the physiology of the ewe and lamb and subsequent reproductive efficiency, and highlights that there are major difficulties in managing litters of more than two lambs. The paper addresses the impact of high ovulation rates on subsequent embryo loss, and on placental and foetal development. It identifies that increases in eggs shed are linked to increases in embryo and foetal mortality and also with reduced placental capacity per lamb. The review reports that increased litter size also impacts on ewe energy reserves, particularly in late pregnancy when there is also a reduction in alimentary tract weight in ewes carrying three or more lambs. The postpartum outcomes of high litter size are also seen in reduced neonatal survival and lower colostrum and milk availability per lamb.

Introduction

Examination of the patterns of development in mammalian species show that high ovulation rate (OR) and large litter size (LS) are most often associated with species with immature young at birth, highly efficient placenta transfer of nutrients (Perry 1981) and a relatively short lactation that reduces the impact of the litter on the energy reserves of the mother. It can be reasonably argued that ruminants are not well equipped to care for litters of more than two, both in terms of efficient nutrient transfer across the placenta to ensure adequate birth weights but also in terms of their capacity to maintain the body fat reserves needed for foetal growth and, more particularly, adequate milk supply to the newborn. Multiple ovulation in ruminant species in reality is a major physiological challenge to the ewe, and can put both mother and offspring at risk (Echternkamp 1992), particularly if the nutritional needs of the mother are not carefully monitored.

Small ruminants such as sheep are responsive to good nutrition at mating time (predominantly autumn), with multiple ovulations usually resulting from high body energy stores. These increase the likelihood that placental development will be adequate to ensure optimal birth weights of lambs, and sufficient colostrum and milk supply in the postnatal period. If nutritional status is poor at mating, the outcome is usually a lower OR, thus reducing the risk to both lamb and ewe for that gestation.

Multiple ovulation unrelated to good energy stores in the prolific ewe could be argued to be counterintuitive to the needs of the ewe, and have profound effects both during gestation and in early lactation. This paper will examine the effect of multiple ovulations (3+) in terms of embryo and foetal loss, placental development and interactions with foetal growth and birth weight, and subsequent impact on lamb survival and ewe lactation.

[1] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia; ghinch@une.edu.au

Embryo and foetal loss

Embryo loss (loss until day 15 of pregnancy) in high fecundity sheep has not been widely documented, although it is generally accepted that embryo loss increases with increase in OR. Rhind et al. (1980) suggested that an increase in OR of up to 4 or 5 was associated with an increase in mean LS, but that higher ORs were counterproductive. More recently, embryo transfer studies suggest that the probability of survival declines once the number of embryos transferred increases above 3, although the probability of conception may continue to increase (Fahmy et al. 1994).

In the last decade there have been a number of reviews (although not many focused on high fecundity ewes) examining the impact of increasing OR on embryo loss; possibly the most extensive of these is by Michels et al. (1998a). Their review examined in detail the relationship between survival and OR, and reported a linear relationship (around 0.65 lambs born per egg shed) in an OR range of 1.4–2.5. Thereafter, there was a greater decrease in prenatal survival as OR increased. Michels et al. (1998a) suggested that there is genetic variation in prenatal survival, possibly mediated through uterine efficiency, with a marginal increase in LS for an extra egg shed. Meyer et al. (1994) concluded that, independent of OR, the Romanov breed appeared to have the greatest variation in uterine efficiency.

Most of the high fecundity breeds examined, for example D'Man (Bradford et al. 1989) and Cambridge (Owen 1988), appear to have some variation in embryo survival. However, studies comparing the control, heterozyous and homozygous Booroola and crossbred ewes (Kleemann et al. 1990; Dodds et al. 1991) show minimal differences in embryo loss after adjustment for OR differences.

It has been suggested that breeds with evidence of distributive embryo migration have lower embryo loss (Michels et al. 1998b). While this may be true for ovulations of 1 or 2, there appears very limited data for higher OR scenarios. In general, it seems that losses at this stage of gestation are associated largely with asynchrony of embryo development and ewe uterine environment (e.g. Wilmut et al. 1985). Various manipulations in early pregnancy to facilitate high progesterone levels could be advantageous in this context, with improvements in both survival and foetal growth. For example, Kleemann et al. (1991, 1994), Manalu and Sumaryadi (1998) and Michels et al. (1998b) reviewed some of the factors that influence prenatal survival in ewes, arguing that the endocrine environment prior to ovulation as well as subsequently has a significant influence on prenatal survival via effects on luteal function.

The negative effects of high nutrition on progesterone levels during the first 30 days of pregnancy have been reported by Parr (1992), and interactions between the genotype of the ewe and her nutritional environment may be important in determining embryo survival and uterine efficiency. The study of West et al. (1991) showed that uterine efficiency in high fecundity ewes (mean OR 2.6) was greatest in those in good condition at mating, irrespective of the subsequent nutritional level of ewes with both twin or triplet ovulations. It seems that optimisation of embryo survival in prolific ewes is possibly best achieved by maintaining high nutritional levels pre mating with maintenance levels post mating.

Foetal loss is not well documented in most species (Jonker 2004). However, the recent report by Dixon et al. (2002) has shown that losses, as determined by ultrasound measurements in Dorset and Suffolk ewes (mean foetal number of 1.79), is relatively large, with 19.9% of ewes in the study experiencing embryo and/or foetal loss and 21.2% of these losses occurring after day 25 of pregnancy. The latter foetal losses occurred throughout pregnancy, with around 3-4% of losses for each 20-day period post 25 days. Most of these losses were partial losses and did not impact significantly on conception rates of ewes. A very similar overall level of loss (19.7%) was found in high fecundity Booroola Merinos (BMs) in a slaughter trial conducted by Owens and Hinch (1983, unpublished), although in this study, when ewes with no conceptus and not recycling by day 25 were included, the average losses were estimated to be 33.5%. This suggests that losses may be higher in ewes with ORs of more than 4, such as those in this study. The overall ova loss (adjusted to a mean OR of 2.9) and foetal losses in this study are shown in Table 1. There were no significant differences between years or stage of gestation for the proportion of embryo/foetal loss, nor in the proportion of foetuses found to be dead (mean 2.1%).

An evaluation of the reasons for embryo and foetal losses suggest that low maternal progesterone (< 2 ng/mL) post 25 days was significantly correlated to subsequent loss (Dixon et al. 2007). In the

BM (ORs of 4–6) foetal losses between 40 and 60 days have been estimated to be 5–9% and from 60 days to term around 1–9% compared to negligible losses in the low fecundity control Merinos (OR 1.3–1.8; Wilkins et al. 1984). A similar pattern has also been reported for twin-bearing Merinos by Kelly et al. (1989). High foetal mortality between 30 and 70 days of pregnancy has also been reported in super-ovulated ewes (Fahmy et al. 1994), with losses of around 30%.

Losses during the post-implantation stage are most likely associated with placental insufficiency issues, as originally proposed by Robinson et al. (1977) and Rhind et al. (1980) from their studies of Finnish Landrace × Dorset Horn ewes. It is possible that reduced placental efficiency in high LSs will impact on foetal growth and survival, reducing foetal numbers at birth significantly.

Placental development

There have been a limited number of studies evaluating the impact of large LS on placental development and subsequent foetal size and survival. The most significant of these is possibly the study of Finnish Landrace × Dorset ewes by Robinson et al. (1977), who reported on conceptus development in single, twin, triplet and quadruplet lambs. This study described the impact of LS on the development of the placenta, and clearly illustrated a reduction in cotyledonary attachments per lamb as LS increased. The associated decrease in foetal and placental size for each foetus was evident as early as at 50 days of pregnancy. The same authors comment that interspecies relationships between total foetal weight and maternal weight would predict that a 70 kg ewe (approximate size of Finn × Dorset ewes) would carry 5.8 kg of foetus to term. Given that twins were 1.5-fold, triplets 2-fold and quads 2.4-fold higher than this estimate, it would seem that these prolific ewes were under considerable physical (as in space available for abdomen expansion) and metabolic stress in late pregnancy. Such stressors may increase the likelihood of reduced feed intake in late pregnancy and the incidence of metabolic diseases such as pregnancy toxaemia, although documentation of these effects is lacking for prolific sheep.

In an experiment reported by Owens et al. (1986), the effects of undernutrition to 100 days of gestation in fat and lean Coopworth lines carrying singles, twins and triplets were examined. The weight of the placenta and placentome number declined with increases in LS, as previously reported by Robinson et al. (1977), although submaintenance nutrition until mid pregnancy resulted in an increase in average placental weight per foetus for the litter bearers. According to this study, placental size reached a maximum at around 100 days of gestation, and LS and ewe nutrition interacted until mid pregnancy to determine placental size and efficiency and, consequently, the capacity of the foetuses to respond to changes in nutrient availability in late pregnancy.

While there have been numerous studies examining the effects of nutrition on placental development of ewes with single or twin lambs (e.g. Kelly 1992), there appears little information on ewes carrying large litters. The unpublished study of Owens and Hinch (1983) reported below examined the impact of LS on placental development and the interactions between LS and nutrition in high fecundity BM ewes. The study was replicated over 2 years and conducted on Tara Hills Research Station, New Zealand, using multiparous Merino ewes all heterozygous for the Booroola (*FecB*) gene.

The ewes used in this study were allocated to feed treatment groups on the basis of OR and age, and were

Table 1. Reproductive measures of embryo and foetal losses at different stages of gestation and for different nutrition treatments (SE in brackets)

Stage of gestation/nutrition	Day 32	Day 97	Day 146	Maintenance 32–97 days	Submaintenance 32–97 days
Number of ewes	35	57	49	50	55
Ovulation rate	3.03	3.0	2.71	3.03	2.76
Proportion of ova lost	0.267 (0.05)	0.243 (0.04)	0.168 (0.04)	0.2 (0.04)	0.21 (0.04)
Total number of foetuses	2.36 (0.16)	2.29 (0.13)	2.46 (0.14)	2.36 (0.12)	2.29 (0.11)
Proportion of foetuses dead	0.053 (0.01)	0.016 (0.01)	0.034 (0.01)	0.028 (0.01)	0.014 (0.01)

Source: adapted from Owens and Hinch (1983, unpublished)

'blocked' according to day of conception so that three slaughter groups included animals at 31–33, 96–98 and 145–147 days of gestation. Ewes were fed ad-libitum pasture from conception to 30 days (> 1 kg dry matter (DM)/day) and from 97 days until slaughter (> 2 kg DM/day). The differential feeding treatments occurred between 31 and 97 days of gestation and consisted of a submaintenance (10% loss in bodyweight over the 66 days) and a maintenance treatment (slight increase in body weight through the mid-pregnancy period).

The ewes were euthanased at a mean 32, 97 or 146 days of gestation, and alimentary tract, liver, kidney fat, omental fat and mammary gland weights were recorded, as were carcass fat measurements at GR and C sites (standard sites used in composition assessment).

The gravid uterus was weighed immediately after being removed from the ewe and the number of corpora lutea recorded. The uterus was then cut along the dorsal surface to expose the foetal membranes, and foetal position was recorded relative to the left and right tips of the uterine horns. Foetal status (live/dead) was assessed subjectively, and membranes were then removed from the uterus, with placentomes being excised from the uterine wall and remaining attached to foetal membranes. After separation of the membranes for each foetus, placentome number, membrane, placentome and foetal weights were recorded. The number of abnormal placentomes was also identified and classified as degenerating, everted or fused.

The impact of LS on foetal weight and placental development in twins and triplets to day 146 is illustrated in Table 2 (Owens and Hinch 1983, unpublished), showing a dramatic decline in number of placentomes and weight of placentome between twins and triplets, and confirming the earlier patterns reported by Robinson et al. (1977).

Position in utero was shown to have a significant effect on foetal weight, with foetuses in the uterine tip having lower placental weights and body weights and a greater likelihood of mortality. This was most likely to occur in triplet litters (Table 3) as twins were normally evenly dispersed, with one twin located in each horn. It is apparent that sharing a uterine horn in the ewe is detrimental to foetal growth, with a reduction of around 17%. This is clearly correlated to placental weight (12% reduction) and placentome number (28% reduction), and reflects the fact that cotyledonary placentation is not well suited to optimal litter growth, with only a limited ability to compensate for greater demand.

Ewe energy stores

As LS increases, the capacity of the ewe to consume adequate energy to maintain foetal growth potential in late pregnancy is increasingly limited. Consequently, the availability of ewe fat reserves is of some consequence to foetal growth in the latter third of pregnancy (Cowan et al. 1980). There are very few studies that have examined the impact of foetal number of ewe energy stores, but the serial slaughter study of Owens and Hinch (1983, unpublished) using Booroola ewes provides an insight into the impact of LS. Table 4 shows the differences in carcass parameters for the various treatment groups, indicating similar lowered energy reserves in the ewes carrying litters of 2+ when compared with the effect of submaintenance nutrition in mid pregnancy (live weight difference of 6.42 kg at 97 days). Omental and kidney fat levels at 146 days remained relatively constant (between 5–6% and 2–2.5% of body weight respectively) for both LSs and nutrition groups, but subcutaneous fat depths were significantly lower for litters of 3+ on the submaintenance nutritional treatment ($P < 0.01$), indicating the need for nutrition levels well above maintenance in the

Table 2. Least squares means (SE in brackets) for placental parameters at 146 days of pregnancy for twins and triplets

Litter size	2	3
Foetal weight (g)	3,913.3 (708.0)	3,481.1 (781.0)
Number of placentomes per foetus	40.6 (9.2)	28.7 (7.9)
Weight per placentome (g)	8.8 (2.8)	9.4 (2.6)
Total weight of placentomes per foetus (g)	343.6 (77.0)	258.4 (63.0)
Foetal membrane weight (g)	215.0 (34.0)	213.0 (29.0)

Source: adapted from Owens and Hinch (1983, unpublished)

last trimester for multiple-bearing ewes. It also seems that subcutaneous fat depth may be a good indicator of the capacity of ewes to maintain litter foetal growth in the last trimester of pregnancy. LS increases also reduced alimentary tract weight at day 146 (3.37 kg vs. 3.14 kg for twins and triplets, $P < 0.01$), and this probably reflects the lower food intake of litter-bearing ewes at this time.

Lamb birth weight and survival

The consequences of a small placenta are likely to be equivalent to undernutrition in late pregnancy (Robinson 1990), namely hypoglycaemia and hypoxaemia, the latter leading to depressed thermogenic responses. Reduced thermogenic responses in 'growth retarded' low birth weight lambs are also associated with a higher surface to weight ratio and greater susceptibility to hypothermia. Consequently, the risk of mortality increases with each additional lamb in a litter.

The impact of birth weight on survival probability was demonstrated for litters in the early paper of Maund et al. (1980) and subsequently in many other studies, such as Hinch et al. (1985a) and Hinch et al. (1996). The report of Hinch et al. (1985a) included data collected over 7 years for grazing BM flocks in New Zealand, and is possibly the most extensive examination of LS effects on survival and its relationship to birth weight in high fecundity flocks. Mean birth weight declined (i.e. growth was restricted) with increasing LS, with a decline of around 23% between singles and twins, 22% between twins and triplets, and 14.5% between triplets and quadruplets. This translates to a ratio for single:twin:triplet:quad that is consistently around 1:0.75:0.6:0.5, and recent studies in a range of breeds have confirmed this pattern for Dorset, Rambouillet, Suffolk, Finnsheep, Romanov and Composite III ewes (Freetly et al. 2004). A detailed examination of the mechanisms contributing to LS-dependent growth restriction can be found in the recent review of Gootwine et al. (2007).

Birth weight is a significant factor influencing survival of lambs in flocks of various fecundity levels but is most apparent in prolific flocks. In the study of Hinch et al. (1985a) birth weight was also shown to explain the positive effect of ewe age and

Table 3. Least squares means (SE in brackets) for foetal and placental weights and placentome number for lambs from twin and triplet litters sharing or not sharing a uterine horn at 146 days of gestation

Measure	Litter size	Number in uterine horn 1	Number in uterine horn 2
Foetal weight (gm)	2	3,913.3 (567.0)	
	3	3,749.2 (593.0)	3,346.9 (840.0)
Placentome weight (gm)	2	343.7 (76.8)	
	3	280.4 (69.1)	247.4 (57.9)
Number of placentomes	2	40.6 (9.2)	
	3	35.4 (7.2)	25.3 (5.9)

Source: adapted from Owens and Hinch (1983, unpublished)

Table 4. Least squares means for ewe hot carcass, subcutaneous fat depth, and kidney and omental fat weights at 146 days of gestation

Litter size	Carcass weight (kg)	Subcutaneous fat at C-site (mm)	Internal fat kidney + omental fat (kg)
Maintenance[a]			
2	23.70	11.00	2.55
3+	20.20	11.50	2.35
Submaintenance[a]			
2	20.40	5.86	2.15
3	19.80	5.22	1.68

[a] Nutrition treatment at days 30–97 of gestation
Source: adapted from Owens and Hinch (1983, unpublished)

the negative effect of being male on survival. The traditional quadratic relationship between birth weight and survival reported for single lambs was not appropriate for multiples in this study, and hyperbolic relationships showing lower birth weight lambs having lower survival were defined for various LSs. At a given birth weight an increase in LS also reduced the probability of survival, suggesting that maternal as well as lamb size may be important to survival of litters. Age at death data for these flocks showed that, for all LSs, the highest proportion of lambs died within the first day of birth, with the next most significant period of loss in multiples (including twins) being 24–48 hours after birth (Hinch et al. 1986). These data suggest that losses are not simply associated with mismothering, and detailed autopsy studies demonstrated that lambs from large litters were most likely to die from causes associated with prenatal or prolonged birth (hypoxia symptoms—often categorised as 'weak' lambs in earlier studies such as that of Maund et al. (1980)). Starvation as a primary cause of death was of lower incidence than previous reports, although prolonged birth symptoms were often linked to starvation at 24–48 hours postpartum.

A later study using larger mature ewes (Hinch et al. 1996) confirmed that differences in LS survival to weaning were significantly related to birth weight, and that increases in LS reduce birth weight in a similar ratio to that reported above. This particular study demonstrated that protein supplementation during the last half of pregnancy, and according to LS, had only a small effect on birth weight but significantly increased survival in all LSs (overall 58% vs. 73%), most particularly for triplets (42% vs. 61%). Hall et al. (1992) reported similar differences in survival of triplets supplemented with lupins in late pregnancy (48% vs. 56%). If such effects are not mediated by birth weight changes, it is possible that they are linked to nutritional impact on lactation, which will be discussed later. However, overall these autopsy data suggest that lambs from litters of 3+ are physiologically not well adapted for survival postnatally.

ln the study of Tiddy et al. (1991, unpublished) using BM × Border Leicester ewes, differences in total lipid content of term foetal lambs (mean weights of 3.5–4.5 kg) were significantly lower in triplets compared to twins (1.06 vs. 1.16 g/100 g) and these lambs also had higher PCV (50.2 vs. 49.1 vs. 43.4: triplet vs. twin vs. single) and higher blood lactate levels, both indicating chronic hypoxia. The greater levels of hypoxia and hypoglycaemia, which are linked to growth retardation, are clearly likely contributors to lower survival probabilities of litters of 3+.

A retrospective examination of the factors that influence birth weight in large litters was conducted by Hinch et al. (1985b) for high fecundity Booroola cross ewe flocks (average OR of 2.2). These authors found that year, ewe weight at joining, LS and pregnancy wastage (difference between OR and lambs born) all contributed significantly to variation in birth weight. The largest proportion of variation was attributable to LS (71–83%), 6.7–11.8% was due to year (possibly nutritional effects), 0.9–4.4% to ewe joining weights and a small but significant contribution of 1.1–3.5% was from pregnancy wastage. The effect of wastage during pregnancy suggested that high OR and potential embryo or foetal losses have a negative effect on birth weights. This is presumably associated with allocation of placental attachment sites in early pregnancy, meaning that fewer are available per lamb as wastage increases. In this particular study, with an average OR of 2.2, it was estimated that around 8% of lambs had birth weights reduced by more than 0.3 kg. Such differences can have profound effects on survival probabilities, particularly in higher LSs, as illustrated in Table 5. In this table the probability of survival of a triplet born from an OR of 3 is some 15% higher than one surviving from an OR of 6.

Lactation

It is well documented that undernutrition in late pregnancy reduces mammary development and colostrum availability at birth (e.g. Mellor 1987; Mellor and Murray 1985), and in fact can also delay the onset of lactogenesis, with major survival implications for lambs. As indicated earlier, the impact of increases in LS can equate to lowered nutrition. Consequently, there is the potential for lambs born in litters to have low levels of colostrum available per lamb or even delayed availability. While not well documented, it seems that, unless litter-bearing ewes have access to energy-dense diets in late pregnancy, colostrum availability is likely to be restricted. A study evaluating BM × Border Leicester ewes (Tiddy et al. 1991, unpublished) found that mammary weights at 145 days of

pregnancy were not significantly different between twin- and triplet-bearing ewes (1.21 kg vs. 1.06 kg) but were greater than for single bearers (0.65 kg). Colostrum scores at this time indicated significantly more colostrum present in twins compared to singles and triplets, suggesting potential lower transfer of immunoglobulin for the triplets postpartum. The poorer passive immunity transfer of larger litters was demonstrated by Hall et al. (1992) in BM ewes with single, twin and triplet lambs at birth The correlation between immunoglobulin levels and subsequent survival was not recorded in this study, but the lower levels of maternally derived immunity are likely to be associated with greater susceptibility to infection in early life.

There have been many studies on ewes that have shown interactions between milk production and condition score, for example Peart et al. (1972) and Gibb and Treacher (1980). However, a limited number of such studies have evaluated in detail the impact of large LSs on ewe milk production. Generally, this group has shown that a one-lamb increase in LS results in an increase in milk yield of 20–30% (see series of papers in Boyazogly and Treacher (1978)). Studies by Hinch and Kyle (1982, unpublished), summarised in Davis and Hinch (1985), confirmed similar percentage increases in milk production for each increase in LS for twin- and triplet-rearing Booroola × Romney ewes in good condition. However, triplet-rearing ewes had significantly lower condition scores at 20 days of lactation, which had disappeared by day 50, suggesting that milk production dropped quickly after the lactation peak. Milk production between birth and 70 days for triplet-rearing ewes reached a peak earlier and declined more rapidly than for twin-rearing ewes, with peak yields for triplets reaching just over 2 L/day. The consequence of this lactation pattern is considerably lower growth rates and potentially earlier natural weaning for triplet-reared lambs (Hinch 1989).

Conclusions

Increases in OR of greater than 2 are likely to create a number of challenges to the physiology of ewe and lamb that require careful management. On average, an increase in OR above 2 increases the probability of embryo loss and later foetal mortality. These effects are difficult to manage as they appear to be at least partially associated with the placental physiology of ruminants, which have a limited number of uterine attachment sites. Where conception and implantation do occur, the consequence of large litters is a reduction in placental size per foetus, and this is associated with reductions in foetal weight and the probability of foetal survival. There is little that can be done to compensate for these effects except possibly ensuring maximal placental size at 100 days of gestation facilitated through nutritional monitoring in mid pregnancy.

The impact of large litters can also be seen in terms of the fat reserves of the pregnant ewe, with ewes carrying three or more lambs using large amounts of fat reserves in late pregnancy to maintain foetal growth. Even if high fat reserves are available, birth weights of multiple-birth lambs are lower, and the impact of intra-uterine competition for placental sites can result in hypoxia and hypoglycaemia and lowered survival.

Increases in birth weight through optimal late pregnancy nutrition may be possible but survival levels of multiples will be lower than singles, even at equivalent birth weights.

The impact of being born as a multiple continues into the postnatal phase, with high LS usually associated with reduced colostrum availability at birth and, subsequently, with reduced milk availability per lamb. Overall, it seems that the ewe is not

Table 5. Predicted birth weights and survival probabilities of litter-bearing ewes with various levels of pregnancy wastage (PW)

Litter size	Birth weight (kg)				Predicted survival (%)			
	PW-0	PW-1	PW-2	PW-3	PW-0	PW-1	PW-2	PW-3
1	4.67	4.51	4.41	4.23	98	96	94	92
2	3.74	3.58	3.48	3.30	82	80	77	75
3	2.98	2.82	2.72	2.54	65	63	60	50
4	2.45	2.29	2.19	2.01	41	37	35	27

Source: adapted from Hinch et al. (1985b)

well adapted for carrying litters of more than two lambs through pregnancy, and early lactation and specific and targeted nutritional strategies are necessary where ewes are carrying more than two lambs.

References

Boyazogly J.G. and Treacher T.T. (eds) 1978. Milk production in the ewe. European Association for Animal Production (EAAP) Publication 23, 115.

Bradford G.E., Lahlou-Kassi M., Berger Y.M., Boujenane I. and Derquoui L. 1989. Performance of D'Man and Sardi sheep on accelerated lambing: II. Ovulation rate and embryo survival. Small Ruminant Research 2, 242–252.

Cowan R.T., Robinson J.J., McDonald I. and Smart R. 1980. Effects of body fatness at lambing and diet in lactation on body tissue loss, feed intake and milk yield of ewes in early lactation. Journal Agricultural Science Cambridge 95, 497–514.

Davis G.H. and Hinch G.N. 1985. Introduction and management of the Booroola gene in sheep flocks in New Zealand. Pp 139–148 in 'Genetics of reproduction in sheep', ed. by R.B. Land and R.W. Robinson. Butterworths: London.

Dixon A.B., Knights M., Winkler J.L., Marsh D.J., Pate J.L, Wilson M.E., Daily R.A., Seidel G. and Inskeep E.K. 2007. Patterns of late embryonic and fetal mortality and association with several factors in sheep. Journal of Animal Science 85(5), 1274–1284.

Dodds K.G., Davis G.H., Elsen J.M., Isaacs K.L. and Owens J.L. 1991. The effect of Booroola genotype on some reproductive traits in a Booroola Merino flock. Pp. 359–366 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Boon and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Versailles, France,

Echternkamp S.E. 1992. Fetal development in cattle with multiple ovulations. Journal of Animal Science 70, 2309–2321.

Fahmy M.H., Castonguay F. and Laforest J.P. 1994. Uterine morphology and reproductive phenomena in relation to number of embryos at different stages of gestation in prolific sheep. Small Ruminant Research 13, 159–168.

Freetly H.C. and Leymaster K.A. 2004. Relationship between litter birth weight and litter size in six breeds of sheep. Journal Animal Science 82(2), 612–618.

Gibb M.J. and Treacher T.T. 1980. The effect of ewe body condition at lambing on performance of ewes and their lambs at pasture. Journal of Agricultural Science Cambridge 95, 631–640.

Gootwine E., Spencer T.E. and Bazer F.W. 2007. Litter-size independent intrauterine growth restriction in sheep. Animal 1, 547–564.

Hall D.G., Piper L.R., Egan A.R. and Bindon B.M. 1992. Lamb and milk production from Booroola ewes supplemented in late pregnancy. Australian Journal of Experimental Agriculture 32, 587–593.

Hinch G.N. 1989. The sucking behaviour of triplet, twin and single lambs at pasture. Applied Animal Behaviour Science 22, 39–48.

Hinch G.N., Crosbie S.F., Kelly R.W., Owens J.L. and Davis G.H. 1985a. The influence of birth weight and litter size on lamb survival in high fecundity Booroola Merino crossbred flocks. New Zealand Journal Agricultural Research 28, 31–38.

Hinch G.N., Davis G.H., Crosbie S.F., Kelly R.W. and Trotter R.W. 1986. Causes of lamb mortality in two highly prolific crossbred flocks and a Romney flock. Animal Reproduction Science 12, 47–61.

Hinch G.N., Kelly R.W., Davis G.H., Owens J.L. and Crosbie S.F. 1985b. Factors affecting lamb birthweights from high fecundity Booroola ewes. Animal Reproduction Science 8, 53–60.

Hinch G.N. and Kyle B. 1982 Effects of differential nutrition during lactation on the milk production of high fecundity ewes. Unpublished annual progress report, New Zealand Ministry of Agriculture and Fisheries.

Hinch G.N., Lynch J.J., Nolan J.V., Leng R.A., Bindon B.M. and Piper L.R. 1996 Supplementation of high fecundity Border Leicester × Merino ewes with a high protein feed: its effects on lamb survival. Australian Journal of Experimental Agriculture 36, 129–136.

Jonker F.H. 2004. Fetal death: comparative aspects in large domestic animals. Animal Reproduction Science 82–83, 415–430.

Kelly R.W. 1992. Nutrition and placental development. Proceedings of the Nutrition Society of Australia 17, 203–211.

Kelly, R.W., Wilkins, J.F. and Newnham, J.P. 1989. Fetal mortality from day 30 of pregnancy in Merino ewes offered different levels of nutrition. Australian Journal of Experimental Agriculture 29, 339–342.

Kleemann D.O., Walker S.K., Grimson R.J., Smith D.H., Grosser T.L. and Seamark R.F. 1991. Exogenous progesterone and embryo survival in Booroola-cross ewes. Reproduction Fertility Development 3, 71–77

Kleemann D.O, Walker S.K. and Seamark R.F. 1994. Enhanced foetal growth in sheep administered progesterone during the first 3 days of pregnancy. Journal Reproduction and Fertility 102, 411–417.

Kleemann D.O., Walker S.K., Walkley J.R.W., Smith D.H., Grimson R.J. and Seamark R.F. 1990. Fertilization and embryo loss in Booroola Merino × South Australian Merino ewes: effect of the F gene. Theriogenology 33, 487–498.

Manalu W. and Sumaryadi M.Y. 1998. Maternal progesterone concentration during pregnancy and lamb birth weight at parturition in Javanese thin-tail ewes with different litter sizes. Small Ruminant Research 30, 163–169.

Maund B.A., Duffell S.I. and Winkler C.E. 1980. Lamb mortality in relation to prolificacy. Experimental Husbandry 36, 99–112.

Mellor D.J. 1987. Nutritional effects on the fetus and mammary gland during pregnancy. Proceedings of the Nutrition Society 46, 249–257.

Mellor D.J. and Murray. L. 1985. Effect maternal nutrition on availability of energy in the body reserves of foetuses at term and colostrum from Scottish Blackface ewes with twin lambs. Research in Veterinary Science 39, 235–240.

Meyer H.H., Piper L.R., Bindon B.M. and Woolaston R.R. 1994. Litter size and uterine efficiency of Booroola Merinos, control Merinos and their crosses with Border Leicester and Dorset. Livestock Production Science 38, 217–223.

Michels H., Vanmontfort D., Dewil E. and Decuypere E. 1998a. Genetic variation of prenatal survival in relation to ovulation rate in sheep: a review. Small Ruminant Research 29, 129-–142.

Michels H., Vanmontfort D., Dewil E. and Decuypere E. 1998b. Prenantal survival in relation to pre-ovulatory phenomena and the site of ovulation in sheep: a review. Small Ruminant Research 29, 157–166.

Owen J.B. 1988. Breeding for fecundity in sheep. Veterinary Record 123, 308–310.

Owens J.L. and Hinch G.N. 1983. The influence of ovulation rate, litter size, and mid-pregnancy feeding on placental and foetal development in Booroola ewes. Unpublished annual progress report – experiment I616, New Zealand Ministry of Agriculture and Fisheries.

Owens J.L., Kyle B. and Fennessy P.F. 1986. Observations on the effects of litter size, pregnancy nutrition and fat genotype on ewe and foetal parameters. Proceedings of New Zealand Society of Animal Production 46, 41–44.

Parr R.A. 1992. Nutrition progesterone interactions during early pregnancy in sheep. Reproduction, Fertility and Development 4, 297–300.

Peart J.N., Edwards R.A. and Donaldson E. 1972. The yield and composition of the milk of Finnish Landrace × Blackface ewes. 1: Ewes and lambs maintained indoors. Journal of Agricultural Science Cambridge 85, 315–324.

Perry J.S. 1981. The mammalian fetal membranes. Journal of Reproduction and Fertility 62, 321–335.

Rhind S.M., Robinson J.J., Fraser C., McHattie I. 1980. Ovulation and embryo survival rates and plasma progesterone concentrations of prolific ewes treated with PMSG. Journal of Reproduction and Fertility 58(1), 139–144.

Robinson J.J. 1990. Nutrition in reproduction of farm animals. Nutrition Research Reviews 3, 253–276.

Robinson J.J., McDonald I., Fraser C. and Crofts R.M.J. 1977. Studies on reproduction in prolific ewes. 1: Growth of the products of conception. Journal of Agricultural Science Cambridge 88, 539–552.

Tiddy R.M., Nolan J.V., Lynch J.J. and Hinch G.N. 1991. By-pass protein supplement modifies pregnant ewe and foetal lamb metabolism in litter bearing ewes. Unpublished report, University of New England, Australia.

West K.S., Meyer H.H. and Nawaz M. 1991. Effects of differential ewe condition at mating and early postmating nutrition on embryo survival. Journal of Animal Science 69, 3931–3938.

Wilkins J.F., Fowler D.G., Bindon B.M., Piper L.R., Hall D.G. and Fogarty N.M. 1984. Measuring foetal loss with real time scanning. Proceedings of the Australian Society of Animal Production 15, 768.

Wilmut I., Sales D.I. and Asworth C.J. 1985. Physiological criteria for embryo mortality: is asynchrony between embryo and ewe a significant factor? Pp. 275–289 in 'Genetics of reproduction in sheep', ed. by R.B. Land and R.W. Robinson. Butterworths: London.

Session 3:
Case studies on introgression of *FecB* in local breeds

Biological and economic consequences of introgression of the *FecB* (Booroola) gene into Deccani sheep

C. Nimbkar[1,2], P.M. Ghalsasi[1], B.V. Nimbkar[1], P.P. Ghalsasi[1], V.S. Gupta[3], V.C. Pardeshi[3], J.F. Maddox[4], J.H.J van der Werf[5], G.D. Gray[6] and S.W. Walkden-Brown[5]

Abstract

Deccani sheep are reared on the Deccan plateau by smallholders under a system of supervised grazing on fallow and harvested fields and public lands. Lamb production is the main source of income. Deccani ewes usually give birth to only one lamb. The Nimbkar Agricultural Research Institute (NARI) introduced the *FecB* mutation, which increases prolificacy, into the Deccani breed from the Garole breed of West Bengal to increase lamb production and incomes of smallholder shepherds. Two fecund strains were developed—the NARI Suwarna, with contributions from only Garole and Deccani breeds, and the NARI Composite, with additional contributions from Awassi and/or Bannur breeds. One copy of *FecB* led to an increase in ovulation rate from 1.0 to 2.0 eggs, and an increase in live litter size (LS) at birth from 1.0 to 1.6 in the NARI flock and from 1.0 to 1.4 in smallholder flocks. Less than 5% of the litters of B+ ewes were triplets. The increased LS was found to be manageable under the existing production system of smallholders by keeping young lambs behind in the pens when ewes went grazing, and by providing lambs with a small amount of supplementary feed. The LS at 3 months of B+ ewes was 1.3 and 1.2 in NARI and smallholder flocks, respectively, compared to 0.9 of ++ ewes. In a smallholder flock with 8, 41 and 50 B+ ewes in 2005 to 2008, respectively, the gross margin per B+ ewe over the 3-year period was found to be 37–50% higher than the gross margin per ++ ewe. It is likely that the significantly larger LS of B+ ewes in the NARI flock, compared to B+ ewes in smallholder flocks, was due to the 100 g/day supplementary feed given to all ewes at NARI for 2 months at breeding and to the better body condition of ewes owing to longer lambing intervals. It could be termed a *FecB* genotype by environment interaction because the supplementary feeding did not influence the LS of non-carrier ewes. In conclusion, the introduction of the *FecB* mutation in Deccani sheep proved to be successful in increasing both lamb production and incomes.

[1] Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
[2] Corresponding author; chanda.nimbkar@gmail.com
[3] Division of Biochemical Sciences, National Chemical Laboratory, Pune, Maharashtra, 411 008, India
[4] School of Veterinary Science, University of Melbourne, Melbourne, Victoria 3010, Australia
[5] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia
[6] PO Box 290, Kingscote, South Australia 5223, Australia

Introduction

Deccani sheep are reared on the Deccan plateau in Maharashtra, Karnataka and Andhra Pradesh states of India by smallholders. They are traditionally reared by communities such as the Dhangar in Maharashtra, the Kuruba in Karnataka and the Kuruma in Andhra Pradesh. The traditional system of rearing is still followed although there is some use of vaccination and modern veterinary medicines. Sheep are usually reared in flocks of 25 to 200 breeding ewes along with their progeny and one to four breeding rams. Rams are always with the flock and lambs are not artificially weaned by separating them from their mothers. For about 8 months of the year—from November to June—80% of the flocks migrate to areas of higher rainfall or irrigated areas, over distances of 100–500 km.

More than 90% of the income from rearing Deccani sheep is earned from the sale of 3–4-month-old unweaned lambs weighing about 15 kg. Lambs are sold young because there is market demand for them and sheep owners consider it beneficial to have the dams return to oestrus and conceive again once the lamb is sold. Sheep are herded for grazing by flock owners (referred to as 'smallholders' in this paper) all day. They are grazed mainly on public, often degraded, grazing lands including hilly areas, road and canal sides, and farm bunds. They also graze on crop residues of grain sorghum, sunflower, groundnut, pearl millet, maize, chickpea, cotton, okra, vegetable and other crops. Sheep are penned at night either on a field for manuring or near the owner's house. The sale of manure collected from the pen and the manuring of fields are sources of cash income. Deccani ewes usually have only a single lamb (average litter size (LS) 1.02), with an average lambing interval of about 10 months. Lambs are reared with careful personal attention, and lambs that do not get sufficient milk from their mothers are cross-fostered to ewes that have surplus milk. Usually there are a few goats in the flock and lambs may also be cross-fostered to goat does. If there are no surplus lambs in the flock for cross-fostering to ewes whose lambs die, orphan lambs are purchased and suckled to such ewes. Lambs are given a small amount of supplement such as groundnut cake and wheat flour dough. Given the system of management and the high and increasing demand and market price of lambs, a moderate increase in the LS of Deccani ewes would lead to an increase in the number of saleable lambs and in the incomes of shepherds. An increase in sheep productivity and meat production also conforms to Indian national agricultural priorities and would help to tackle the serious problem of protein malnutrition, especially among women and children.

A breeding program was initiated in 1998 at the Nimbkar Agricultural Research Institute (NARI), Phaltan, in Maharashtra State of India to introduce prolificacy in the Deccani breed from the prolific Garole breed of Sundarbans in West Bengal state (Nimbkar et al. 2002). Garole ewes are small, weighing only 15 kg compared with 28 kg for an adult Deccani ewe. They are also prolific, with an average LS of 1.74 in the management system at NARI (Nimbkar et al. 1998). Garole sheep are likely to be the 'diminutive' Bengal sheep that were taken to Australia from Calcutta, India, in 1792–93 and were postulated as the source of the *FecB* (*Fec* for fecundity and *B* for Booroola) gene discovered in a highly prolific strain of Merino sheep in the 1980s (Turner 1982; Ghalsasi and Nimbkar 1993). It was confirmed later that the Garole indeed possessed the same *FecB* mutation as the highly prolific Booroola Merino (BM) (Davis et al. 2002). *FecB* is an autosomal dominant gene having a large effect on ovulation rate (OR) in sheep. $FecB^{B}$ is the allele promoting higher fecundity at this locus, while $FecB^{+}$ is the wild-type allele (COGNOSAG ad hoc committee 1995). The genotype carrying two $FecB^{B}$ alleles is called the genotype homozygous for $FecB^{B}$ (or BB), the heterozygous B+ and the non-carrier ++.

The *FecB* mutation has now been introduced into a flock of the Lonand strain, which is one of four strains within the Deccani breed based on geographic separation: Lonand, Sangamneri, Sangola and Kolhapuri (Gokhale 2003). This paper reports and discusses the performance results of two newly developed twinning strains of the Lonand Deccani—the NARI Suwarna and the NARI Composite—in the nucleus flock maintained under experimental field conditions at NARI and in flocks belonging to local shepherds around Phaltan in Maharashtra state.

Introgression of the *FecB* gene into Deccani sheep

The beginning

The Garole is the only reported prolific sheep breed in India (Fahmy and Mason 1996). A Garole flock in Phaltan (18° N 74° E) in Maharashtra state was established by importing two rams and two ewes in April 1993 and 10 rams and 30 ewes in May 1994 from the Sundarbans, West Bengal state, over a distance of about 1,500 km (Nimbkar et al. 1998). The Bannur is a non-prolific meat sheep breed from Karnataka state adjacent to Maharashtra and is known for its blocky conformation and superior meat quality. Lamb production and gastrointestinal parasite resistance of the three breeds—the Deccani, Garole and Bannur—were evaluated at NARI from 1996 to 1999 with the aim of developing recommendations for the appropriate breed combination for a likely composite (Nimbkar et al. 2003a). It was only speculation at that time that the prolificacy of the Garole was due to the *FecB* gene. An 'incomplete diallel' mating design was used in which both ewes and rams of Deccani and Bannur breeds were used with only rams of the Garole breed included. Over 4 years 290 Deccani ewes and 265 Bannur ewes were inseminated with the semen of 8 Deccani, 9 Bannur and 15 Garole rams, and the resulting progeny were evaluated. Artificial insemination was used because the small size of Garole rams precluded natural mating. It was found that crossing the Deccani and Bannur with Garole reduced live weight at birth, 3 and 6 months, and thus reduced lamb growth rates significantly compared with Deccani. However, lambs sired by Garole rams (i.e. carrying 50% Garole genes) were more resistant to naturally acquired gastrointestinal nematode infections and to artificial challenge with *Haemonchus contortus* than those sired by Deccani or Bannur rams (i.e. not carrying any Garole genes). Despite the enhanced resistance to *H. contortus*, lambs sired by Garole rams had reduced rates of survival (Nimbkar et al. 2003a).

Early investigation of the genetic basis of Garole prolificacy

Matings were also carried out to demonstrate segregation of a postulated single major gene for prolificacy in the Garole breed (Nimbkar et al. 2002). B+ F1 Garole cross rams were mated to Deccani ewes and the OR of the resulting 25% Garole ewes was measured. If the Garole prolificacy was due to a single gene, half the ewes would be expected to inherit one prolificacy allele of the gene from the sire and the other half would be expected to inherit the wild-type allele. After the first lambing about half of the daughters of each F1 sire had a mean OR of 1.0, while the other half had a mean OR of 2.0, indicating single gene inheritance of prolificacy in the Garole (Nimbkar et al. 2003b). After confirmation of the existence of the *FecB* mutation in the Garole, the PCR–RFLP DNA test to identify the mutation (Wilson et al. 2001) was established in 2002 at the National Chemical Laboratory (NCL) in Pune, India. All crossbred progeny produced in the breeding program thereafter were genotyped for the *FecB* locus using this test. Deccani and Bannur animals and their crosses were not routinely genotyped for *FecB* because they are not prolific and are unlikely to carry the *FecB* mutation. This was confirmed by genotyping a number of sheep of these two breeds (Pardeshi et al. 2005).

Development of the breed improvement program using the *FecB* gene

In 2000, 86 F1 ewes generated from the diallel breeding program were added to the main breeding ewe flock of Deccani and Bannur ewes. Inseminations with Deccani, Bannur, Garole and Garole × Deccani ram semen were carried out to compare the maternal performance of the crosses with that of pure Deccani and Bannur ewes and to produce further crosses for determination of appropriate contributions of each breed to a possible composite. It was also planned that no lamb should have more than 25% Garole genes, the remaining being made up by Deccani and Bannur breeds. A reduction in Garole proportion was deemed necessary because of their small size, poor lamb-rearing ability, poor milk production, existence of horns in males, wide foreheads and other features of appearance of Garole crosses considered undesirable by local shepherds. The benefit from the better worm resistance of the Garole therefore had to be sidelined. The Improved Awassi Dairy Sheep strain from Israel, which was available at NARI (Nimbkar and Ghalsasi 1992), was used for crossbreeding from 2001 to improve milk yield, lamb weight and growth rate. Awassi rams and crossbred rams with > 50%

Awassi genes were used for breeding. In each year Deccani × Deccani and Bannur × Bannur matings were also carried out to produce contemporary animals for comparison with crosses. In 2002 four 25% Garole–75% Deccani rams genotyped to be B+ were used widely to cross with Deccani and Bannur ewes. Additionally, Awassi–Garole B+ crossbred rams were mated to Deccani–Bannur crossbred ewes.

From 2003 onwards the focus was on developing two prolific strains: the fecund Deccani (later named the NARI Suwarna) and the fecund Composite (later named the NARI Composite) with the attributes of high fertility and prolificacy, good lamb-rearing ability and fast growth. The NARI Suwarna was to have up to 25% Garole proportion, with the remaining being Deccani. The NARI Composite was to have up to 25% Awassi and/or Bannur genes, < 25% Garole genes and the remaining Deccani. The objective was to generate BB, B+ and ++ ewes of both the NARI Suwarna and Composite breeds for testing in shepherds' flocks. The mate selection approach (Kinghorn et al. 2002) was used for allotting rams to ewes using the Total Genetic Resource Management Program (TGRM™) (X'Prime Pty Ltd 2005). An index of estimated breeding values (EBVs) for reproductive and weight traits was used. The ratio of index weights on the different traits was 1:4:12 for fertility, 3-month weight and live LS at birth, respectively. This was based on economic values for a breeding objective calculated by Nimbkar (2006) for the animals in this introgression program. There was a trade-off between increasing the frequency of the $FecB^B$ allele and controlling inbreeding because of the relationships among *FecB*-carrier sires and between *FecB*-carrier sires and dams. There was also a trade-off between increasing frequency of the $FecB^B$ allele and increasing the progeny EBV for 3-month weight (Nimbkar 2006). An attempt was therefore made to maximise genetic merit, increase frequency of the $FecB^B$ allele up to a target of 0.5–0.6, and control inbreeding by keeping the predicted progeny mean inbreeding coefficient around 0.02 and the mean co-ancestry of selected parents around 0.02.

Forty-eight B+ NARI Suwarna (fecund Deccani) ewes, 40 ++ ewes having Deccani and Garole genes, 12 B+ NARI Composite ewes and 20 ++ Composite ewes were introduced into 14 smallholder flocks in 2003 and 2004. Two of these flocks were seasonally migratory. In addition, B+ and BB rams were introduced into 25 smallholder flocks every year from 2003 to 2007. Rams were kept in smallholder flocks for 35–90 days and then brought back to NARI as the same rams were used for breeding in NARI's flock.

Animals with lambing performance records

The number, breed proportions and *FecB* genotypes of ewes inseminated at NARI during 2002–07 are shown in Table 1. There were 808 non-carrier (++) ewes including 306 Deccani, 111 Bannur and 391 crossbred ewes (104 with only Deccani and Garole breed proportions and 287 Composite). There were 532 heterozygous (B+) ewes (200 fecund Deccani (NARI Suwarna) and 332 NARI Composite) and 47 homozygous (BB) ewes (9 NARI Suwarna and 38 NARI Composite).

In smallholder flocks 2,465 and 325 lambing records were available from ++ and B+ ewes respectively. These records were of 959 ++ and 115 B+ ewes born in those flocks and 51 ++ ewes and 52 B+ ewes introduced by NARI. These records were from February 2004 to January 2008.

Influence of the *FecB* mutation on ewe reproductive performance

Ovulation rate

The primary action of the *FecB* mutation is on OR (eggs shed per ewe ovulating) and the effect is additive (Piper et al. 1985). Nimbkar et al. (2003b) reported that one copy of $FecB^B$ increased the mean OR from 1.03 (in non-carrier Deccani, Bannur and 25% Garole ewes) to 2.02 (in F1 and 25% Garole B+ ewes) after the first parity. This was the first report of the difference in OR between ++ and B+ ewes where *FecB* genotypes were assigned by the PCR–RFLP direct DNA test. They also reported that 25% Garole B+ maiden ewes had a least squares mean OR of 1.76 compared to 1.03 in 25% Garole ++ maiden ewes. The number and breed of B+ and ++ ewes with OR measurements in this study are shown in Table 2. This study reported the mean OR of BB Garole ewes as 3.37. If the effect of *FecB* is assumed to be the same in a breed background of 25% Garole and Garole, the effect of the first copy is 0.99 and the second copy 3.37–2.02 = 1.35, which indicates an

additive effect. This is, however, a much smaller effect than the literature reports of an increase in OR of 1.5 and 3 for one and two copies of *FecB*^{B} respectively (Davis 2004). A similar effect on OR of an increase of 1.01 with one copy of *FecB*^{B} in commercial Merino ewes in Australia, genotyped using the DNA test, was reported by Walkden-Brown et al. (2007). Their estimate of the effect of the second copy on OR was, however, 1.79, which was much higher than 1.35 in the BB Garole.

All the reports before 2003, of OR in *FecB*-carrier Booroola Merino (Davis et al. 1982; Piper et al. 1985), Awassi (Gootwine et al. 1995) and Javanese Thin Tail (Bradford et al. 1986) ewes indicated that one copy of *FecB* increased OR by about 1.5, the range being 1.24 to 1.65. However, only the Gootwine et al. (1995) study used the OarAE101 marker to assign genotypes in addition to the OR criterion. The other studies used either the criterion of there being at least one LS record or one OR record greater than or equal to 3 to classify the ewe as a heterozygous *FecB* carrier. This criterion is likely to bias the genotype effect upward. In the Nimbkar et al. (2003b) study only 11 of 113 (9.7%) maiden B+ ewes had at least one OR record of 3 and none had any record greater than 3, while 15 of 69 second parity B+ ewes (21.7%) had at least one OR record of 3 and one had a record of 4, which was the maximum (unpublished data).

Live litter size at birth

NARI flock

The live LSs at birth of Deccani and Deccani crossbred ++, B+ and BB ewes were 1.03, 1.58 and 1.65, respectively, indicating an almost dominant effect of the mutation (Table 3). However, there were only 45 records of BB ewes and most of these were for the first parity. As more records of later parities become available, the mean LS of BB ewes

Table 1. Number, breed / breed composition and *FecB* genotype of ewes inseminated at NARI during 2002–07 (A = Awassi; B = Bannur; D = Deccani; G = Garole)

Breed / cross / breed composition	Ewe *FecB* genotype		
	FecB^{++}	*FecB*$^{B+}$	*FecB*^{BB}
D	306	–	–
B	111	–	–
0.5 D, 0.5 G	4	43	–
0.75 D, 0.25 G	72	106[a]	7[a]
0.81–0.88 D, 0.12–0.19 G	28	51[a]	2[a]
0.5 D, 0.5 B	66	–	–
GB crosses (F1 and inter se)	12	26	–
Crosses consisting of D, B and G	60	72[b]	4[b]
F1 crosses A×D, A×B, A×G	30	6[b]	–
Crosses consisting of D, G and A	55	84[b]	12[b]
Crosses consisting of D, G, B and A	64	144[b]	22[b]
Total ewes inseminated/mated	808	532	47

[a] NARI Suwarna; [b] NARI Composite
Source: unpublished data

Table 2. Number, breed and *FecB* genotype of ewes whose ovulation rate (OR) was measured

	Breed of ewes						
	G	F1 (GD, GB)	25% G		D	B	Total
FecB genotype of ewes	BB	B+	B+	++	++	++	
1–3 OR records before first lambing	5	25	113	114	2	1	260
1–3 OR records after first parity or at later parities	53	18	69	57	48	30	275

D = Deccani; B = Bannur; G = Garole, GD = F1 Garole × Deccani; GB = F1 Garole × Bannur
Source: modified from Nimbkar et al. (2003b)

may be found to increase. However, 1.58 can be regarded as a reasonably accurate estimate of the mean LS of B+ ewes because it is based on 806 lambing records of 447 ewes from first to sixth parity. The proportion of triplet litters of B+ ewes increased from 1% in the first parity to a maximum of 12% in the fourth parity. There were no litters of more than three lambs. Breed composition did not have a significant effect on LS. NARI Suwarna and NARI Composite ewes had similar mean LSs.

Compared to the literature estimates of one and two copies of *FecB* resulting in about 1.0 and 1.5 extra lambs per ewe lambing, respectively (Davis 2004), this increase of about 0.5 and 0.6 is very low. Arora and Mishra (2009) reported slightly higher increases in prolificacy of 0.7 and 0.8, respectively, with one and two copies of *FecB* in Garole × Malpura ewes in Rajasthan. However, Gootwine et al. (2003) reported increases of only 0.45 and 0.49 live lambs born per lambing with one and two copies of *FecB*, respectively, in Booroola–Assaf ewes housed indoors in Israel and fed to meet their nutritional requirements.

Live litter size at birth: smallholder flocks

The mean live LS of Deccani crossbred $FecB^{B+}$ ewes in 22 local smallholders' flocks around Phaltan was found to be 1.42, which was lower than in NARI's flock (Table 3; unpublished data). There were 325 lambing records over 4 years from February 2004 from a total of 115 B+ ewes born in smallholder flocks and 52 B+ ewes introduced by NARI into those flocks. The 187 records of B+ ewes born in smallholder flocks were from parities 1 to 4 (96, 60, 22 and 9 records respectively).

Of the lambings of B+ ewes in NARI and shepherds' flocks, 47% and 58%, respectively, were singles, 49% and 40% were 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 average mortality among single- and twin-born lambs from birth to 3 months was 6% and 9%, respectively, in smallholder flocks.

Live litter size at 3 months

The live LSs at 3 months of Deccani and Deccani crossbred ++, B+ and BB ewes were 0.95, 1.35 and 1.34, respectively, in the NARI flock. The LSs at 3 months of ++ and B+ Deccani and crossbred ewes in smallholders' flocks were 0.95 and 1.21, respectively. In NARI's flock B+ ewes weaned 0.4 more lambs of 3 months of age than ++ ewes, while in smallholders' flocks B+ ewes weaned 0.3 more lambs per ewe lambing. Twin-bearing ewes in both NARI and smallholder flocks weaned 0.8 more lambs than single-bearing ewes.

A small quantity of supplementary feed was given to lambs and occasionally to ewes in some of the larger smallholder flocks. In five of the smallholder flocks with better management, B+ ewes weaned 0.4 more lambs of 3 months of age than ++ ewes. The numbers of B+ ewes in these five flocks were 46, 26, 22, 3 and 3. In some of these flocks, young lambs

Table 3. Least squares means and standard errors for ewe's *FecB* genotype for Deccani and crossbred ewes in NARI and smallholders' flocks. Traits are for ewes lambing with at least one live lamb.

Trait	Flock	$FecB^{++}$	$FecB^{B+}$	$FecB^{BB}$
Live litter size at birth	NARI	1.03 ± 0.01^{a} (1,632)	1.58 ± 0.02^{b} (806)	1.65 ± 0.09^{b} (45)
	Smallholder	1.03 ± 0.01^{a} (2,465)	1.42 ± 0.02^{b} (325)	Few records available
Live litter size at 3 months	NARI	0.95 ± 0.01^{a} (1,632)	1.35 ± 0.02^{b} (806)	1.34 ± 0.10^{b} (45)
	Smallholder	0.95 ± 0.01^{a} (2,406)	1.21 ± 0.02^{b} (321)	Few records available
Total weight of 3-month-old lamb	NARI	10.63 ± 0.24^{a} (1,326)	13.52 ± 0.26^{b} (739)	12.93 ± 1.08^{b} (41)
	Smallholder	13.91 ± 0.10^{a} (1,385)	14.96 ± 0.27^{b} (186)	Few records available

Figures in brackets are the number of records.
[a,b,c] Least squares means with different superscripts within a row are significantly different ($P < 0.05$).
Source: analysis of unpublished data

were kept behind in the pen when the ewes went grazing every day and given nutritious fodder such as lucerne. There was average rainfall (500 mm) in the 4 years during which the data were recorded in smallholder flocks. There were, however, dry periods of 4–6 months with severe feed shortage during each of these years since the rain usually falls between June and October. It was thus evident that smallholders were able to control lamb mortality with better management. The extra feeding to lambs was needed only when there was scarcity of nutritious grazing.

Lamb weight produced per ewe lambing

The total weight of 3-month-old lamb produced by Deccani crossbred B+ ewes in the NARI flock was 13.5 kg, which was 2.9 kg higher than that produced by non-carrier ewes (Table 3). Deccani crossbred BB ewes produced a lamb weight of 12.9 kg, which was 2.3 kg higher than non-carrier ewes. Kumar et al. (2008) reported similar figures of total litter weight of lamb at weaning produced by Garole–Malpura crossbred ewes (10.6 kg for 33 ++ ewes, 14.0 kg for 110 B+ ewes and 12.9 kg for 5 BB ewes) although the mean LS of 1.73 and 2.17 for B+ and BB ewes, respectively, reported by them was higher than in Deccani crosses. It needs to be remembered here that only slightly more than half the lambings of B+ ewes (345 of 739) produced twins or triplets and the rest of them produced singles. *FecB*-carrier and non-carrier ewes giving birth to twin lambs weaned 15.1 kg weight of 3-month-old lamb compared to 10.6 kg weaned by ewes that had singles. The weight of 3-month-old lamb produced in 29 lambings with triplets was 20.3 kg. In shepherds' flocks the total weight of 3-month-old lamb produced by B+ ewes was 15 kg, 1.1 kg higher than that produced by ++ ewes, while the weight of 3-month-old lamb produced by twin-bearing ewes was 18.3 kg, 4.7 kg higher than that produced by single-bearing ewes.

B+ ewes thus produced 27% more weight of 3-month-old lamb than ++ ewes, while ewes with twins produced 42% more weight of 3-month-old lamb than ewes bearing singles, in the NARI flock. In shepherds' flocks B+ ewes produced 8% more weight of 3-month-old lamb than ++ ewes, and ewes with twins produced 35% more weight of 3-month-old lamb than ewes bearing singles.

Lambing interval

It was sensible to analyse lambing interval only from smallholders' flocks, because in NARI's flock lambing intervals typically exceeded 12–14 months and they were the result of human decisions rather than an expression of the ewe's natural reproductive ability. The mean lambing interval in smallholder flocks was 10 ± 0.2 months.

Gross margin per breeding ewe

The income and expenditure from 16 smallholder flocks from 1 February 2004 to 31 March 2008 were compiled and analysed. Only 3 years' data were available from four of these flocks. 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 ++ breeding ewe was found to be Rs450–800/year. Only one of the flocks had data from a reasonable number of B+ breeding ewes, namely 8, 41 and 50 ewes in the 3 years in the interval 2005–08 respectively. The numbers of ++ breeding ewes in that flock were 98, 89 and 74, respectively, in the 3 years. In this flock the gross margin per B+ breeding ewe was Rs150–300 higher than for ++ ewes. This amounted to an increase in gross margin of between 37% and 50%. Lamb mortality was 31%, 24% and 19% among lambs of B+ ewes, and 14%, 18% and 24% among lambs of ++ ewes, in the 3 years, respectively. Despite the higher mortality, B+ ewes weaned a higher number of lambs per ewe than ++ ewes, which led to the higher gross margin. Twin-born lambs were sold at about Rs100 less on average than single-born lambs and they were 2–4 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, in conformity with the prediction of an economic model of the Deccani sheep production system (Nimbkar 2006). 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.

Discussion

When the genetic basis of the prolificacy of the Garole breed was confirmed to be the *FecB* mutation, there was apprehension that carrier ewes may end up having too large a LS, as indicated by the experience in other breeds such as the Merino in Australia. Deccani ewes have excellent lamb-rearing ability but they are not known for a particularly high milk yield. There was therefore doubt whether Deccani ewes would be able to rear twin lambs and whether smallholder shepherds would be able to manage twins in their extensive production system. The results presented in this paper show that the LS of Deccani crossbred B+ ewes was 1.4 and 1.6 in smallholders' and NARI's flocks, respectively. This was manageable in the existing production system with some additional feed to pregnant and lambed ewes and young lambs. In fact, the small proportion of multiple births was a constraint on the production by B+ ewes under the smallholders' system of management. The lower effect of the *FecB* mutation on OR and LS in Deccani crossbred ewes, compared to the Merino and some other breeds, could be partially attributed to the effect of poorer nutrition available to them, leading to poorer body condition of the ewes and extensive (at least 5 km every day) walking done by them in search of grazing. Piper et al. (1985) found highly significant between-year differences in the mean difference in OR between putative B+ and ++ Merino ewes due to plane of nutrition effects.

The significantly higher proportion of twin and triplet births among B+ ewes in NARI's flock compared to smallholder flocks could be attributed to the extra feed given to NARI's ewes for about 2 months during breeding, starting 3 weeks before the commencement of breeding. A supplement of 50 g of maize grain and 50 g of mixed, pelleted concentrate with 16–18% of 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 1 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 LS in only B+ ewes, indicating a FecB genotype × feeding interaction.

During the periods when there was no supplementary feed given to NARI ewes, they probably got worse nutrition than shepherds' ewes because they were grazed in large flocks of 200–300, while the smallholder flocks had 40–100 ewes. It can also be reasonably expected that flocks grazed by their owners would be better fed than the NARI flock, which was grazed by paid employees working for a certain number of hours rather than until the ewes' stomachs were full.

It is thus likely that OR and LS of *FecB*-carrier ewes (but not of non-carrier ewes) increased with an increase in available nutrition at mating and consequent improvement in body condition. This finding indicates the suitability of the *FecB* gene to the smallholder production system because it means that they would be able to take advantage of good seasons by feeding the ewes at breeding. This feature might induce entrepreneurs to start commercial sheep enterprises rearing *FecB*-carrier ewes for meat production. The return on the expenditure made on feeding ewes for 2 months is likely to be 300% at current prices in Maharashtra. The returns on supplementary feeding to lambs directly might be even higher.

More lambing records of BB ewes are needed before we can get an accurate estimate of their average LS. It is likely, however, that the OR and LS of BB ewes are also influenced by the nutrition available to the ewes at breeding and their body condition.

Conclusion

The OR of B+ Deccani crossbred ewes was around 2.0 compared to 1.0 in ++ ewes. This is lower than the increase of 1.5 with one copy in most other breeds. The lower OR led to a lower and more manageable LS of 1.58 in B+ ewes in the NARI flock and 1.42 in local smallholder flocks. The lower expression of the *FecB* mutation meant that B+ ewes weaned 0.4 more lambs of 3 months of age than ++ ewes in the NARI flock and in well-managed smallholder flocks. This translated into a 37–50% higher gross margin per B+ breeding ewe compared to that per ++ breeding ewe in a smallholder flock which had an adequate number of B+ ewes for comparison. The gross margin per twin-bearing ewe was 30% higher than that per single-bearing ewe. It is likely that the significantly higher mean LS of B+ ewes in the NARI flock was due to the supplementary feed

given to ewes for 2 months at breeding and the better body condition of ewes. The better nutrition did not lead to an increased LS in non-carrier ewes in the NARI flock, indicating an interaction between *FecB* genotype and nutrition at breeding.

With the currently available small number of records of BB ewes in the NARI flock, the effect of the *FecB* mutation on LS at birth and 3 months appears to be almost dominant. This indicates that homozygosity for *FecB* is unlikely to induce any unmanageable or undesirable increase in LS. More records are, however, needed before this result can be confirmed.

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- the office staff at NARI who helped with the correspondence and bookkeeping for the project, and the administrative staff at the University of New England who handled the finances
- the smallholder shepherds who accepted introduction of *FecB*-carrier rams and ewes into their flocks; allowed ear tagging, measurement and recording of their animals; and cooperated with NARI in many other ways.

References

Arora A.L. and Mishra A.K. 2009. Consequences of introgression of FecB gene into Malpura sheep in Rajasthan. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 111–118. Australian Centre for International Agricultural Research: Canberra. [These proceedings].

Bradford G.E., Quirke J.F., Sitorus P., Inounu I., Tiesnamurti B., Bell F.L., Fletcher I.C. and Torell D.T. 1986. Reproduction in Javanese sheep: evidence for a gene with large effect on ovulation rate and litter size. Journal of Animal Science 63, 418–431.

COGNOSAG ad hoc committee 1995. Revised guidelines for gene nomenclature in ruminants 1993. Genetics Selection Evolution 27, 89–93.

Davis G.H. 2004. Fecundity genes in sheep. Animal Reproduction Science 82–83, 247–253.

Davis G.H., Galloway S.M., Ross I.K., Gregan S., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB)* mutation. Biology of Reproduction 66, 1869–1874.

Davis G.H., Montgomery G.W., Allison A.J., Kelly R.W. and Bray A.R. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Fahmy M.H. and Mason I.L. 1996. Less known and rare breeds. Pp. 178–186 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Ghalsasi P.M. and Nimbkar B.V. 1993. The 'Garole' – microsheep of Bengal, India. Animal Genetic Resources Information 12, 73–79. FAO.

Gokhale S.B. (ed.) 2003. Final report of the Network Project on Survey, Evaluation and Characterization of Deccani Sheep Breed. National Bureau of Animal Genetic Resources and BAIF Development Research Foundation: Pune.

Gootwine E., Bor A., Braw-Tal R. and Zenou A. 1995. Reproductive performance and milk production of the improved Awassi breed as compared with its crosses with the Booroola Merino. Animal Science 60, 109–115.

Gootwine E., Rozov A., Bor A. and Richer S. 2003. Effects of the *FecB* (Booroola) gene on reproductive and productive traits in the Assaf breed. Proceedings of the international workshop on major genes and QTL in sheep and goat, Toulouse, France. CD-ROM communication no. 2-12.

Kinghorn B.P., Meszaros S.A. and Vagg R.D. 2002. Dynamic tactical decision systems for animal breeding. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France. CD-ROM communication no. 23-07.

Kumar S., Mishra A.K., Kolte A.P., Arora A.L., Singh D. and Singh V.K. 2008. Effects of the Booroola (FecB) genotypes on growth performance, ewe's productivity efficiency and litter size in Garole × Malpura sheep. Animal Reproduction Science 105, 319–331.

Nimbkar C. 2006. Genetic improvement of lamb production efficiency in Indian Deccani sheep. PhD thesis, University of New England, Armidale, New South Wales, Australia.

Nimbkar C. and Ghalsasi P.M. 1992. Observations on the performance of the first flock of improved Awassi sheep in India. In 'Recent advances in animal production: Proceedings of the Sixth AAAP Animal Science Congress', III, 250, Bangkok, Thailand.

Nimbkar C., Ghalsasi P.M., Ghatge R.R. and Gray G.D. 1998. Establishment of prolific Garole sheep from West Bengal in the semi-arid Deccan plateau of Maharashtra. In 'Proceedings of the 6th World Congress of Genetics Applied to Livestock Production', 25, 257–260, Armidale, Australia, 7–13 January 1998.

Nimbkar C., Ghalsasi P.M., Maddox J.F., Pardeshi V.C., Sainani M.N., Gupta V. and Walkden-Brown S.W. 2003b. Expression of the *FecB* gene in Garole and crossbred ewes in Maharashtra, India. In '50 Years of DNA: Proceedings of the 15th Conference of the Association for the Advancement of Animal Breeding and Genetics', 15, 111–114, Melbourne, Australia.

Nimbkar C., Ghalsasi P.M., Swan A.A., Walkden-Brown S.W. and Kahn L.P. 2003a. Evaluation of growth rates and resistance to nematodes of Deccani and Bannur lambs and their crosses with Garole. Animal Science 76, 503–515.

Nimbkar C., Ghalsasi P.M., Walkden-Brown S.W. and Kahn L.P. 2002. Breeding program for the genetic improvement of Deccani sheep of Maharashtra, India. Proceedings of the 7th World Congress on Genetics Applied to Livestock Production. Montpellier, France. 19–23 August 2002. CD-ROM communication no. 25-11.

Pardeshi V.C., Sainani M.N., Maddox J.F., Ghalsasi P.M., Nimbkar C. and Gupta V.S. 2005. Assessing the role of FecB mutation in productivity of Indian sheep. Current Science. 89, 887–890.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Turner H.N. 1982. Origins of the CSIRO Booroola. Pp. 1–7 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24-25 August 1980' ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Walkden-Brown S.W., Wolfenden D.H., Charles R.J. and Maddox J.F. 2007. Expression of reproductive and production traits in commercial Merino ewes having 0, 1 or 2 copies of the *FecB* mutation. In 'Proceedings of the 17th Conference of the Association for the Advancement of Animal Breeding and Genetics', 17, 426–429, Armidale, Australia.

Wilson T., Wu X., Juengel J., Ross I., Lumsden J., Lord E., Dodds K., Walling G., McEwan J., O'Connell A., McNatty K. and Montgomery G. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

X'Prime Pty Ltd 2005. Total Genetic Resource Management™. At: <http://www.xprime.com.au/products/tgrm/>

Biological and economic consequences of introgression of the *FecB* mutation into Merino sheep in Australia

S.W. Walkden-Brown[1], D.H. Wolfenden[2] and L.R. Piper[3]

Abstract

The *FecB* mutation has probably been present in Australia since 1792 but to date has had little impact on the local commercial sheep industry. This is despite considerable research efforts, which commenced in the late 1950s. In Merino sheep the combination of poor lamb survival of twins and triplets under extensive Australian management systems and the comparatively lower economic incentives to boost reproductive rate have been the major impediments to commercial success. The gene has also been introgressed into meat breeds, but competition from traditional or novel alternative breeds or crosses that also exhibit high fecundity has limited the impact of *FecB*. The development of the direct DNA test for *FecB* genotyping in 2001 was a major advance that provided a modest rekindling of research activity in Australia. Recent work with DNA genotyped sheep in a commercial Merino flock has confirmed many of the early findings in the Booroola Merino, but has also demonstrated a previously unreported 'homozygote penalty'. On the New South Wales property 'Allandale', homozygote carriers of *FecB* exhibit significantly lower conception rate and lamb survival rates than non-carriers, something not observed in the heterozygote. It is postulated that this is due to uterine effects associated with excessive ovulation rate in homozygotes. Attempts to modulate litter size and lamb survival by restricting nutrition at mating time have proved unsuccessful in Merinos, although there are unpublished reports of successful modulation of these traits in a new meat composite breed carrying *FecB*. The comparatively lower ovulation rates and litter sizes of *FecB*-carrier ewes observed in Deccani crosses in India is encouraging as it suggests that there are environmental and/or genetic mechanisms which modulate *FecB* expression. If these can be identified and effectively exploited, the future may yet be bright for the *FecB* in Australian Merino sheep. However, on the basis of long experience, cautious scepticism is warranted.

Background

The *FecB* mutation has probably been present in Australia since 1792 or 1793 when the first shipments of the diminutive but highly fecund 'Bengal sheep' arrived from Calcutta (Turner 1982). These sheep were likely the same as or related to the diminutive but highly fecund Garole sheep from West Bengal, in which the *FecB* mutation occurs at a very high frequency (Davis et al. 2002; Nimbkar et al. 2003). The detailed history of the gene is presented elsewhere in these proceedings (Davis 2009). Suffice it to say here that, at some point in the mid 1940s, the Seears brothers established a multiple birth flock at their property 'Booroola' near Cooma, New South Wales. Historical links between this flock and the early introduction of Bengal sheep

[1] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia; swalkden@une.edu.au
[2] 'Allandale', Rand, New South Wales 2642, Australia
[3] F.D. McMaster Laboratory, CSIRO Livestock Industries, Armidale, New South Wales 2350, Australia

have been documented (Turner 1982). Interestingly, selection for prolificacy in this flock was on the dam side only, with the rams used being introduced from a different stud. In 1958 the Commonwealth Scientific and Industrial Research Organisation (CSIRO) purchased 12 ewes and 2 rams from the Booroola multiple birth flock. Several more rams and ewes were subsequently donated or purchased, including 91 ewes when the multiple birth flock at Booroola was dispersed (Piper and Bindon 1982b, 1991; Turner 1982). On the basis of the unique method of selection at Booroola, and analysis of the lifetime lambing records of the original purchased ewes and their daughters, it was postulated, at the First World Congress on Sheep and Beef Cattle breeding in New Zealand in 1980, that the increased prolificacy of the Booroola Merino (BM) was due to the segregation of a single major gene affecting litter size (LS) (Piper and Bindon 1982a). This conclusion was subsequently supported by analysis of lambing records of Booroola cross ewes in New Zealand (Davis et al. 1982). The combined evidence in favour of this hypothesis was presented by Piper et al. (1985). In the decade 1980–90 there was intensive research, mostly at CSIRO, into the physiology of the BM and the production consequences of carrying the gene under Australian conditions.

BM rams carrying the *FecB* gene were made widely available to industry by CSIRO from the late 1980s. In 1987 only 20 flocks were actively incorporating the gene into their sheep and by 1990 the number of flocks had fallen to 14 (Piper and Bindon 1991). Between 1980 and 1995 CSIRO was also funded by industry to examine the potential of the *FecB* mutation in the Australian sheep meat industry, and the impact of introgressing the gene into the Border Leicester breed to produce the 'Booroola Leicester' (BL). This new breed was proposed to be used in place of traditional Border Leicester rams to generate BL × Merino prime lamb dams carrying the FecB gene. Following modest industry uptake of these animals, both of the CSIRO Booroola flocks (BM and BL) were dispersed in 1995. Part of the CSIRO BM flock was transferred to the University of New England, where research continues under the supervision of Dr Jim McFarlane. Some of the BL flock was taken to Struan Agricultural Research Station, near Naracoorte, South Australia, and work there has subsequently led to the development of a composite meat breed homozygous for *FecB*, the Multimeat (Colin Earl, pers. comm.). The aim of the Multimeat is to produce first-cross Merino prime lamb dams, all heterozygous for *FecB*.

The difficulty and/or delay in accurately determining the *FecB* genotype of individual sheep, particularly rams, which has limited the use of *FecB* in industry, was alleviated by the discovery of the *FecB* mutation on chromosome 6 (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001) and the advent of direct DNA tests for genotyping. However, there is little evidence of a marked increase in interest or uptake of *FecB* within industry as a result of the availability of direct DNA tests for the gene. It has enabled some investigation into the effects of carrying the mutation in a commercial flock, into which it was introduced over 20 years ago (Walkden-Brown et al. 2007), and greatly accelerated the development of the Multimeat composite breed in South Australia. Despite the long history of the gene in Australia, no systematic investigation of its frequency in the wider sheep population has been undertaken.

The objectives of this paper are to a) review more recent data from DNA genotyped animals in commercial flocks and compare this with earlier studies in research flocks genotyped by using LS or ovulation rate (OR) records; b) summarise our current understanding of the reproductive and production consequences of carrying the *FecB* gene under Australian conditions; and c) draw some broad conclusions about the major challenges and opportunities relating to the use of *FecB* by the industry.

Impact of *FecB* on reproduction and lamb survival

In the early studies at CSIRO, Armidale, homozygous and heterozygous ewes in the Booroola flock were not differentiated. Table 1 summarises the reproductive findings for lambings of this flock and the control flock from 1977 to 1979 (Piper and Bindon 1982b).

The basis for the high fecundity was clearly elevated OR, with combined data from 1970–80 revealing mean ORs of 2.49, 3.0 and 2.88 for BMs at ages 2, 3–5 and 6–10 years, respectively, compared with 1.03, 1.26 and 1.40 in control Merino ewes of the same ages (Bindon et al. 1982). The data in Table 1 demonstrate an overall superiority of the Booroola ewes in net reproductive rate. However,

the survival rate of Booroola lambs was comparatively low relative to that of controls. Unpublished analyses of lamb survival by Piper and Bindon suggested that most of the mortality occurred in the first week of life, and it was decided in 1980 to trial a system of intensive care at lambing (Cornu et al. 1982). This generally involved lambing indoors under close supervision for the first 12–24-hours after birth and physical assistance to multiple-birth lambs when necessary. Further analysis revealed that Booroola lambs had similar survival to control lambs for singles and twins, and the overall lower survival for the Booroola lambs in this study was due to the lower survival of higher order births (Figure 1a).

During the 1970s BM rams from CSIRO were crossed with ewes of many Merino bloodlines in divergent environments, and the effects on reproduction and productivity were assessed. Major studies included those in Collinsville and Murray ewes in Western Australia (WA) (Beetson and Lewer 1985); Bungaree in South Australia (SA) (Ponzoni et al. 1985); and Collinsville in New South Wales (NSW) (McGuirk et al. 1982). Reproductive performance was broadly similar in all three studies, with the first cross Booroola conferring an increase of approximately 0.5 in lambs born per ewe lambing, but much smaller increases in lambs weaned per ewe lambing: 12–19% in WA, 28.6% in NSW and 27% in SA. The higher lamb losses between birth and weaning in the Booroola were due primarily to LSs of 3 and above (Figure 1b). Indeed, in the NSW study the survival of single- and twin-born lambs was higher in the Booroola cross than the Collinsville ewes (McGuirk et al. 1982). The increase in LS of 0.5 in these studies is lower than the 0.9 conferred by one copy of the *FecB* in Merinos (see below) because some of the Booroola rams used to generate the Booroola crosses were undoubtedly heterozygous for *FecB*.

Researchers in New Zealand developed criteria for identifying homozygous ($FecB^{BB}$), heterozygous ($FecB^{B+}$) and non-carriers ($FecB^{++}$) of the gene in Merino ewes based on at least two reproductive records of OR or LS (Davis et al. 1982), with maximum values of > 5, 3–4 or 1–2 resulting in classification as $FecB^{BB}$, $FecB^{B+}$ or $FecB^{++}$, respectively. Based on these criteria, Piper et al. (1985) used an analysis of published and new data to confirm the single gene inheritance of the *FecB* and to provide strong evidence in support of their earlier hypothesis that the action of the gene was primarily on OR, with an effect that was additive to the underlying OR for the breed. However, the size of the additive effect of a copy of the gene varied considerably, with most values falling within the range 0.5–1.5. On the other hand, the effect on LS showed considerable dominance in the CSIRO Booroola flock, with the first and second copies of the gene conferring increases of 0.9 and 0.4 in LS respectively.

A key question arising from these findings was whether or not all the effects of the *FecB* gene on reproductive performance could be explained solely by the action of the gene on OR. This issue was comprehensively examined in BMs in New Zealand by Dodds et al. (1991), who showed that the effects of *FecB* genotype on embryonic mortality, LS, uterine efficiency and lamb birth weight were all removed after adjustment for OR. There were approximately linear negative effects of OR on embryonic mortality, uterine efficiency and lamb birth weight; and a curvilinear positive relationship with LS. Between ORs of 1 and 5, LS increased (from 1 to 2.6) at a declining rate. LS was maintained at around 2.6 for ORs of 6 or 7 before

Table 1. Least squares means (± SE[a]) for reproduction rate and its components in mixed age Booroola (2–7 years) and control (2–6 years) Merino ewes between 1977 and 1979

Flock	Number joined	Fertility (EL/EJ)	Fecundity (LB/EL)	Survival[b] (LW/LB)	Reproduction rate (LW/EJ)
Booroola	596	0.88 ± 0.01	2.30 ± 0.03	0.62 ± 0.02	1.25 ± 0.03
Control	904	0.92 ± 0.01	1.30 ± 0.03	0.84 ± 0.02	0.98 ± 0.03

[a] The model fitted included effects due to year of measurement, age of ewe and all first order interactions; [b]For this analysis age of ewe refers to age of the lambs' dam.

Note: EL/EJ = ewes lambed / ewes joined; LB/EL = lambs born / ewes lambed; LW/LB = lambs weaned / lambs born; LW/EJ = lambs weaned / ewes joined

Source: reproduced from Piper and Bindon (1982b)

declining with higher ORs. This study also provided the first set of contemporaneous OR and LS measurements in BMs of all three *FecB* genotypes (Table 2).

Table 2. Least squares means for the genotype effect (adjusted for year and age) on ovulation rate and litter size in New Zealand Booroola Merino ewes between 1975 and 1985, based on 847 ewes with 2,514 records

Trait	$FecB^{++}$	$FecB^{B+}$	$FecB^{BB}$	Max SE
Ovulation rate	1.91	3.18	4.60	0.07
Litter size	1.51	2.39	2.56	0.06

Source: reproduced from Dodds et al. (1991)

In the 1990s Australian studies focused on the management of Merino flocks carrying the *FecB* mutation, particularly nutritional management to improve lamb survival by either restricting OR or improving milk production and mothering ability of ewes, as discussed later. Other studies, which are beyond the scope of this review, investigated the consequences of using *FecB* in crossbreeding systems for meat production by introgressing it into breeds such as the Border Leicester and Poll Dorset.

Since the development of a direct DNA test to detect the number of copies of the *FecB* mutation carried (Wilson et al. 2001), there have been few further investigations into *FecB* in Australian Merinos. Under an extension of ACIAR project AS1/1994/022, some Australian investigations were permitted to complement the work in India, and a study commenced on David Wolfenden's property 'Allandale' near Rand, between Wagga Wagga and Albury in southern New South Wales. This is a commercial fine-wool (19.5 µm) Merino operation with approximately 3,500 breeding ewes into which BM rams had been introduced in 1982, 1985 and 1991. In 2002 an experimental flock was established to enable valid comparisons of the three *FecB* genotypes in a common commercial environment by genotyping maiden ewes from the commercial flock. This is detailed by Walkden-Brown et al. (2007), who reported on some reproductive and production variables for the 2004–06 lambings. As with earlier studies, the effect of *FecB* on OR was approximately additive but, unlike other studies, there was no increase in LS with the second copy of the gene (Figure 2).

Furthermore, there was a clear homozygote penalty as $FecB^{BB}$ ewes had a combination of lower fertility and higher reproductive wastage between scanning and lamb marking, leading to significantly lower weaning rates than $FecB^{B+}$ ewes. Fitting LS as a covariate in the analysis of reproductive wastage

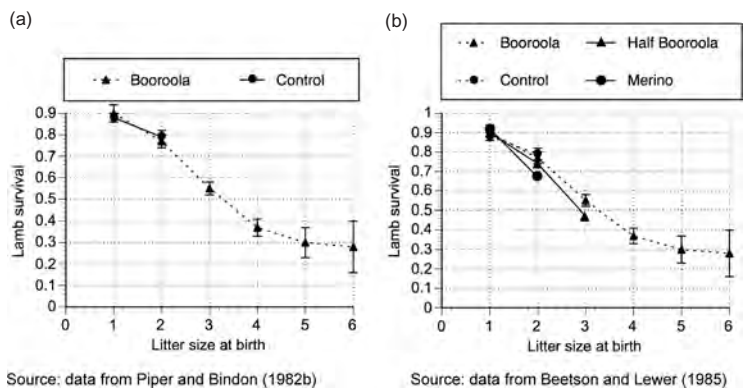


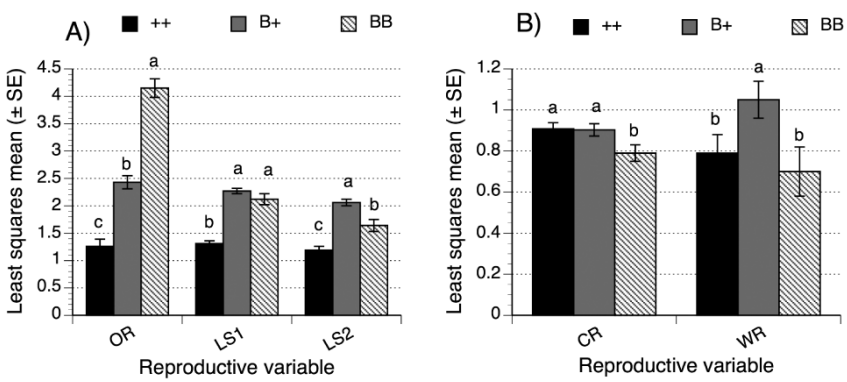
Figure 1. (a) Least squares means (± SE) for lamb survival according to litter size at birth for Booroola (*n*=1195) and control (*n*=1016) Merino lambs born 1977–79. The model fitted included the effects due to year of measurement, age of the dam, flock, litter size at birth, all the first order interactions involving flock, and the interaction of year and age. (b) Data from Figure 1a with lamb survival data from lambs born to Western Australian Merinos (*n*=285) and first-cross Booroola ewes (*n*=897) superimposed

did not remove the effect of genotype, whereas fitting the effect of OR did, suggesting that the penalty was due mostly to effects on embryo and foetal viability prior to scanning for LS. These may relate to effects of high OR on oocyte quality, fertilisation rate, embryo quality and/or uterine crowding.

In 2006 and 2007 the ewes in the experimental flock were managed similarly and had the same measurements made on them. In both years attempts were made to reduce OR by restricting nutrition in the period leading up to and including mating. In 2006 we were unsuccessful in inducing major changes in body weight by varying grazing but in 2007 we were successful, and the effects of this in that year are reported separately at this workshop (Wolfenden and Walkden-Brown 2009). The results of analysis of reproductive data from these lambings are summarised in Table 3. Data were analysed in appropriate REML mixed models in JMP 6.0 (SAS Institute, NC), with ewe fitted as a random variable and the listed effects fitted as fixed effects. Significant interactions or those approaching significance were retained in the models. Genotype was a highly significant effect on all traits. The homozygote penalty is again clear in this dataset, with $FecB^{BB}$ ewes weaning significantly fewer lambs per ewe scanned than $FecB^{B+}$ despite similar LS per ewe lambing. This effect is due to significantly reduced conception rate (Table 3) and poorer lamb survival (Table 4; Figure 3).

In the earlier analysis of Walkden-Brown et al. (2007) the adverse effects of the Fec^{BB} genotype were rendered non-significant if the effect of OR at conception, but not scanned LS, was included in the model. In the present analysis both covariates rendered the effect non-significant. Nevertheless, given that $FecB^{BB}$ and $FecB^{B+}$ ewes do not differ significantly in LS even though the former have a significantly higher OR (Table 3), it is tempting to ascribe the homozygote penalty to excessive OR in line with our current understanding of the consequences of multiple ovulation on embryo, foetal and lamb wellbeing (Hinch 2009).

The effect of scanned LS on lamb survival is shown in Figure 3. Lamb survival under extensive commercial conditions is lower than under research conditions at CSIRO for all LSs other than 3. At every LS, survival of $FecB^{BB}$ lambs was lower than that of $FecB^{B+}$ or $FecB^{++}$, a statistically significant effect (Table 4). When the BM has been crossed with other breeds of sheep, ewe progeny often go on to have lower lamb survival than that of straightbred progeny of the non-Booroola parent, even after adjustment for LS (e.g. Fogarty and Hall 1995).



Source: data from Walkden-Brown et al. (2007)

Figure 2. Least squares means (± SE) for the *FecB* genotype effect (adjusted for year and age) on ovulation rate on (A) the cycle of conception (OR), ultrasound scanned litter size per pregnant ewe (LS1), ultrasound scanned litter size per ewe scanned (LS2); and (B) conception rate (CR: ewes pregnant / ewes mated) and weaning rate (WR: lambs weaned / ewes scanned). Data are for commercial Merino ewes for lambings in 2004–06, with 599 records in total. OR was only measured in 2006.

Table 3. Least squares means (± SE) and significance (*P* value) for the effect of *FecB* genotype on selected reproductive traits of fixed effects in lambing ewes at 'Allandale' in 2006 and 2007. Other fixed effects fitted in the model included peri-mating nutritional treatment, parity and year.

Genotype	*n*	Trait[A]							
		OR	CR	LS1	MR1	WR1	LS2	MR2	WR2
		P < 0.001	*P* = 0.029	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* = 0.006
FecB^{++}	152	1.27^{c} ± 0.09	0.85^{a} ± 0.04	1.22^{b} ± 0.07	0.94^{b} ± 0.06	0.94^{b} ± 0.07	1.00^{b} ± 0.09	0.79^{b} ± 0.07	0.79^{b} ± 0.07
FecB^{B+}	136	2.48^{b} ± 0.10	0.75^{ab} ± 0.04	2.19^{a} ± 0.08	1.46^{a} ± 0.07	1.36^{a} ± 0.07	1.71^{a} ± 0.09	1.12^{a} ± 0.07	1.05^{a} ± 0.07
FecB^{BB}	94	3.86^{a} ± 0.12	0.69^{b} ± 0.05	2.06^{a} ± 0.10	1.13^{b} ± 0.09	1.09^{ab} ± 0.09	1.51^{a} ± 0.11	0.79^{b} ± 0.08	0.77^{b} ± 0.08

[A] OR = ovulation rate on cycle of conception, CR = conception rate (ewes pregnant / ewes scanned), LS = scanned litter size, MR = lamb marking rate; WR = lamb weaning rate; 1 means the divisor is ewes pregnant at scanning, 2 means the divisor is ewes present at scanning.

[a,b,c] means not sharing a common letter in the superscript within traits differ significantly (*P* < 0.05).

Source: Walkden-Brown and Wolfenden (unpublished)

Table 4. Lamb/foetal survival and individual lamb weaning weight at 'Allandale' during lambing seasons 2006 and 2007. Each record is a scanned foetus. Least squares means (± SE) and *P* values for the effect of dam *FecB* genotype (dam G) on survival to weaning and weaning weight are presented. Other fixed effects fitted in the model included peri-mating nutritional treatment, parity and year (data not shown). To investigate the role of scanned litter size (LS) and ovulation rate on the cycle of conception (OR) on these variables, models were fitted with and without them as a covariate, and *P* values for these are shown in the bottom two rows.

Effect	Level	*n*	Trait[A]					
			Surv	Surv-LS	Surv-OR	WWt	WWt-LS	WWt-OR
Dam G			*P* = 0.001	*P* = 0.070	*P* = 0.536	*P* < 0.001	*P* = 0.024	*P* = 0.069
	FecB^{++}	150	0.76^{a} ± 0.04	0.63^{a} ± 0.05	0.70^{a} ± 0.06	24.4^{a} ± 0.46	22.6^{a} ± 0.46	23.2^{a} ± 0.61
	FecB^{B+}	215	0.67^{a} ± 0.04	0.71^{a} ± 0.04	0.67^{a} ± 0.04	21.3^{b} ± 0.44	22.3^{a} ± 0.41	21.5^{a} ± 0.45
	FecB^{BB}	129	0.53^{b} ± 0.05	0.57^{a} ± 0.05	0.60^{a} ± 0.06	20.3^{b} ± 0.61	20.7^{b} ± 0.55	21.0^{a} ± 0.76
LS			n.f.	*P* < 0.001	n.f.	n.f.	*P* < 0.001	n.f.
OR			n.f.	n.f.	0.044	n.f.	n.f.	*P* = 0.024

[A] Surv = probability of a scanned foetus surviving to weaning; WWt = weaning weight of individual lambs surviving to weaning; -LS and -OR indicates including these variables as covariates (n.f. = not fitted).

[a,b,c] means not sharing a common letter in the superscript within traits differ significantly (P < 0.05).

Source: Walkden-Brown and Wolfenden (unpublished)

Impact of *FecB* on other production traits

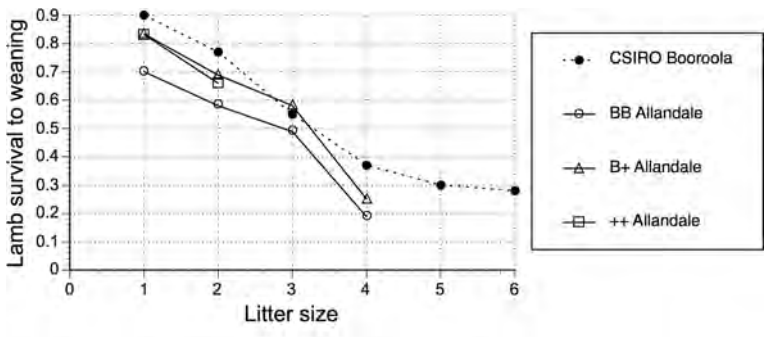
In the original Booroola flock the Seears brothers continually outbred the ewes to purchased sires, and so could be expected to maintain some parity with other commercial sheep in terms of wool traits. However, during the period at CSIRO there was no selection emphasis on meat or wool traits, and so it could be expected that the BM fell below the genetic improvement in these traits occurring in the commercial industry. BMs have often been thought to be inferior for wool and meat traits, but it has rarely been possible to tease out the effects of multiple births and poorer performance in foundation stock from any truly detrimental effect on productivity.

Early studies at CSIRO suggested that there was little difference in wool traits between BMs and control medium-wool non-Peppin Merinos apart from a slightly lower clean scoured yield (Piper and Bindon 1982b, 1996). When crossed or compared with Merinos of different genetic background, BM crosses tended to have lower body weights and carry lighter fleeces than the base breed under comparison (Ponzoni et al. 1985; Fogarty and Hall 1995). However, these results were as expected given the normal fleece weights and body weights of the Merino strains involved in these studies. Working with a single commercial flock (Allandale) into which the *FecB* gene had been introduced for more than two decades, Rachel Charles (nee Flanigan) was able to show that there was no significant effect of the *FecB* mutation, per se, on body weight or wool production traits when birth type was included in the model (Flanigan 2004). Her analysis was based on 472 *FecB* genotypings (67 $FecB^{BB}$, 204 $FecB^{B+}$ and 188 $FecB^{++}$) on the basis of the PCR–RFLP DNA test, although only 206 had birth type records (25 $FecB^{BB}$, 80 $FecB^{B+}$ and 90 $FecB^{++}$). A follow-up study with two further years of data confirmed this finding (Walkden-Brown et al. 2007) (Table 5).

Flock management

Sheep production in Australia is characterised by a strong emphasis on wool production from Merinos under relatively extensive grazing systems with no housing and limited stock supervision. The high lamb mortality associated with the prolificacy of the BM under these conditions, and the reduced importance of reproduction as a driver of profit in wool-producing enterprises (as evidenced by the keeping of non-reproductive wethers purely for wool production), have restricted the interest of industry in the *FecB* gene. Nevertheless, there has been considerable research into modulating its expression and/or improving lamb survival.

Researchers in New Zealand had previously shown that OR in $FecB^{B+}$ Merinos was as responsive to differential nutrition prior to mating as that in non-carrier ewes (Montgomery et al. 1983). In a large experiment Kleemann et al. (1991) again



Source: Walkden-Brown and Wolfenden (unpublished)

Figure 3. Unadjusted mean lamb survival to weaning by litter size and dam genotype. CSIRO data are from Piper and Bindon (1982b) and relate to litter size at birth (n=1195). Allandale data relate to scanned litter size in the years 2006 and 2007 (n=481).

showed that OR in five groups of $FecB^{B+}$ ewes fed at varying levels prior to and during mating varied broadly in proportion to pre-mating live weight (LWT) (mean OR range 2.62–3.01; mean LWT range 44.0–55.2 kg). No other measures of reproductive performance, lamb survival or lamb productivity were influenced by the ewe nutritional treatments. However, ewe fleece weights were significantly reduced and wool tenderness increased in ewes on the more restricted diets. A more recent investigation into nutritional restriction at joining in $FecB^{BB}$, $FecB^{B+}$ or $FecB^{++}$ Merinos confirmed the responsiveness of all three genotypes to differential nutrition in terms of OR (Wolfenden and Walkden-Brown 2009). In this study lamb wastage between scanning and weaning was reduced by the nutritional restriction, but the adverse effects of this treatment on conception rate were too large for it to be considered a practical proposition.

With regard to nutritional manipulation during pregnancy, Australian results are somewhat equivocal. Low-level (80 g/head/day) supplementation of prolific Border Leicester × Merino ewes (40% $FecB^{B+}$) with a protein supplement during mid pregnancy (days 50–100) followed by higher levels of supplementation until parturition (80, 160 and 220 g/head/day for ewes bearing 1, 2, or 3 or more lambs respectively) increased lamb birth weight and survival to weaning from 58% to 73% (Hinch et al. 1996). In South Australia Kleemann and his colleagues attempted to separate out the effects of nutrition during mid and late pregnancy. They found that nutritional variation during mid gestation (days 50–100) had no effect on foetal survival or growth (Kleemann et al. 1993), although improved nutrition tended to improve lamb survival (Kleemann et al. 1998). Improved nutrition during late pregnancy (> day 100) appeared to be more beneficial, with significant positive effects on the survival of twins and a similar trend in triplets (Kleemann et al. 1998). However, there was a strong trend towards reduced survival in single lambs. A shorter period (4 weeks) of differential nutrition in mid pregnancy (from day 75) had no effect on lamb birth weight or survival in BM × Dorset ewes (Fogarty et al. 1992). The optimum use of the Multimeat composite × Merino prime lamb dam requires some careful manipulation of ewe condition and live weight at both joining and prior to lambing (Colin Earl, pers. comm.).

Economics

The economic importance of improved prolificacy in both wool and meat sheep can be demonstrated (Dickerson 1996), including with prolificacy genes (Amer et al. 1999). However, the low uptake of the *FecB* by the Australian sheep industry is testament to the difficulties associated with this route to improved reproduction rate. In fact, uptake of non-genetic methods of increasing LS (e.g. vaccination) is also comparatively low, suggesting that demand for increased LS is low, particularly in the wool industry. In the meat industry the traditional Border Leicester × Merino cross remains popular as a maternal type as it couples high reproductive rate with superior lamb-rearing ability and meat characteristics.

Few formal economic comparisons have been conducted following the introduction of *FecB*. Ponzoni et al. (1985) carried out an economic analysis of the performance of the Bungaree Merino and its crosses with the Trangie fertility Merino

Table 5. Least squares means (± SE) and significance (*P* value) for the effect of *FecB* genotype on selected production traits in ewes entering the experimental flock at Allandale

Effect	*n*	Trait			
		Weaning weight (kg)	15-month weight (kg)	Greasy fleece weight 15 months of age (kg)	Mean fibre diameter 15 months of age (μm)
Genotype		*P* = 0.809	*P* = 0.383	*P* = 0.949	*P* = 0.066
$FecB^{++}$	138	20.7 ± 1.00	40.2 ± 0.6	2.37 ± 0.05	18.34 ± 0.17
$FecB^{B+}$	177	20.7 ± 0.63	39.9 ± 0.6	2.38 ± 0.05	18.58 ± 0.15
$FecB^{BB}$	65	21.2 ± 0.70	39.4 ± 0.7	2.39 ± 0.06	18.80 ± 0.19

Source: adapted from Walkden-Brown et al. (2007)

(selected for increased LS) and the BM. Under no scenario was the BM superior—improved wool prices favoured the Bungaree and higher lamb prices favoured the Trangie fertility Merino. Our own work at Allandale suggests that the significant increase (~ 25%) in weaning rate observed in $FecB^{B+}$ ewes may be economically useful, but even these ewes have lamb survival rates 10% lower than non-carriers and require ultrasound scanning, separation of multiple-bearing ewes, and allocation of additional nutrition prior to lambing. In addition, twin- and triplet-born sheep pay a permanent penalty in wool production, primarily by having broader wool, something we have observed at Allandale. However, the most disturbing finding at Allandale to date has been the 'homozygote penalty' observed in $FecB^{BB}$ ewes, primarily with regards to conception rate and lamb survival. This would require the implementation of strategies to maintain heterozygosity in the population, many of which are available but all of which add additional cost and complexity into the management of the flock.

Conclusions and future challenges

Although the *FecB* mutation has been present in Australia for more than 200 years and has been introgressed into various strains of Merino sheep and into the Border Leicester breed, it has had little industry impact and has a poor reputation in the country in which it was discovered. With the advent of a direct DNA test for the gene, it would be an interesting exercise to measure its frequency in different Australian flocks, particularly the numerous high-fertility Merino flocks and the Border Leicester breed. The gene may be contributing more to improved reproduction rate in industry than it is given credit for, particularly in flocks where OR in *FecB* carriers is restricted by nutrition.

The most promising developments with Australian implications in the last decade or so are:

- the development of the direct DNA test for the gene. Although expensive as an individual sheep test, it has enabled: flexibility in flock structures and decision-making; genotype comparisons in commercial flocks where the gene has been present for some time; surveys of gene frequency; and the development of the homozygous Multimeat composite breed
- the finding that the gene is virtually fixed in some breeds of sheep, which infers an adaptive advantage in these breeds. Furthermore, in some Indian breeds into which it has been introgressed, *FecB* does not produce the extremes in LS (numerous triplets and above) that are seen in Merinos and other breeds. Discovery of the underlying mechanism (possibly nutritional) may assist in the use of the gene in Australia.

On the other hand, some other developments have negative implications for use of the *FecB* gene:

- the finding in an Australian commercial flock of a 'homozygote penalty' in ewes carrying two copies of the gene. These ewes have lower fertility and reduced lamb survival relative to heterozygote carriers and non-carriers. This finding needs to be confirmed in other studies and the underlying basis for it determined
- the failure to develop effective management practices that will reduce LS and improve lamb survival in sheep carrying the *FecB* mutation. Although OR in carriers is very responsive to nutrition, we have yet to find a practical means of exploiting this
- growing public interest in animal welfare means that practices resulting in excessive mortality of lambs will increasingly be called into question.

It is to be hoped that the positive developments provide avenues for understanding and ameliorating the negative ones.

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References

Amer P.R., McEwan J.C., Dodds K.G. and Davis G.H. 1999. Economic values for ewe prolificacy and lamb survival in New Zealand sheep. Livestock Production Science 58, 75–90.

Beetson B.R. and Lewer R.P. 1985. Productivity of Booroola cross Merinos in Western Australia. Pp. 391–398 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London UK.

Bindon B.M., Piper L.R. and Evans R. 1982. Reproductive biology of the Booroola Merino. Pp. 21–33 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Cornu C., Bindon B.M. and Piper L.R. 1982. Artificial rearing of lambs: evaluation of French equipment and techniques in N.S.W. Wool Technology and Sheep Breeding 30, 171–174.

Davis G.H. 2009. Origin, spread, use and management of the *FecB* gene. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 22–32. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Davis G.H., Galloway S.M. et al. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB*) mutation. Biology of Reproduction 66, 1869–1874.

Davis G.H., Montgomery G.W., Allison A.J., Kelly R.W. and Bray A.R. 1982. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research 25, 525–529.

Dickerson G.E. 1996. Economic importance of prolificacy in sheep. Pp. 205–213 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Oxon, UK.

Dodds K.G., Davis G.H., Elsen J.M., Isaacs K.L. and Owens J.L. 1991. The effect of Booroola genotype on some reproductive traits in a Booroola Merino flock. Pp. 359–366 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Flanigan R.J. 2004. Reproductive performance and production characteristics of Merino ewes carrying the FecB mutation in a commercial flock. BRurSci (Hons) thesis, University of New England, Australia.

Fogarty N.M. and Hall D.G. 1995. Performance of crossbred progeny of Trangie Fertility Merino and Booroola Merino rams and Poll Dorset ewes. 3: Reproduction, liveweight and wool production of adult ewes. Australian Journal of Experimental Agriculture 35, 1083–1091.

Fogarty N.M., Hall D.G., Holst P.J. 1992. The effect of nutrition in mid pregnancy and ewe liveweight change on birth-weight and management for lamb survival in highly fecund ewes. Australian Journal of Experimental Agriculture 32, 1–10.

Hinch G.N. 2009. Effects of multiple ovulation and litter size on maternal and foetal physiology: prenatal and postnatal consequences. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 79–87. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Hinch G.N., Lynch J.J., Nolan J.V., Leng R.A., Bindon B.M. and Piper L.R. 1996. Supplementation of high fecundity Border Leicester × Merino ewes with a high protein feed: its effect on lamb survival. Australian Journal of Experimental Agriculture 36, 129–136.

Kleemann D.O., Walker S.K., Walkley J.R.W., Ponzoni R.W., Smith D.H., Grimson R.J. and Seamark R.F. 1993. Effect of nutrition during pregnancy on fetal growth and survival in FecB Booroola × South Australian Merino ewes. Theriogenology 39, 623–630.

Kleemann D.O., Walker S.K., Walkley J.R.W., Smith D.H., Grimson R.J., Stafford J.E. and Seamark R.F. 1998. The effect of nutrition during mid and late pregnancy on lamb birthweight and survival in F+ Booroola × SA Merino ewes. Proceedings of the Australian Society for Animal Production 17, 428.

Kleemann D.O., Walkley J.R.W., Ponzoni R.W., Smith D.H., Grimson R.J. and Seamark R.F. 1991. Effect of premating nutrition on reproductive-performance of Booroola merino × South Australian merino ewes. Animal Reproduction Science 26, 269–279.

McGuirk B.J., Killeen I.D., Piper L.R., Bindon B.M., Caffery G. and Langford C. 1982. Evaluation of the Collinsville Merino and its crosses with the Booroola and the Border Leicester in southern N.S.W. Pp. 69–75 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Montgomery G.W., Bray A.R. and Kelly R.W. 1983. Ovulation rates of first cross Booroola compared with local breed ewes following differential feeding. Animal Reproduction Science 6, 209–215.

Mulsant P., Lecerf F. et al. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences of the United States of America 98, 5104–5109.

Nimbkar C., Ghalsasi P.M., Maddox J.F., Pardeshi V.C., Sainani M.N., Gupta V. and Walkden-Brown S.W. 2003. Expression of the FecB gene in Garole and crossbred ewes in Maharashtra, India. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 15, 111–114.

Piper L.R., Bindon B.M. 1982a. Genetic segregation for fecundity in Booroola Merino sheep. Pp. 394–400 in 'Proceedings of the World Congress on Sheep and Beef Cattle Breeding, vol. I'. The Dunsmore Press Limited: Palmerston North, New Zealand.

Piper L.R., Bindon B.M. 1982b. The Booroola Merino and the performance of medium non-peppin crosses at Armidale. Pp. 9–19 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South

Wales, 24-25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Piper L.R. and Bindon B.M. 1991. The Booroola gene, FecB, in Australia. Pp. 43–45 in 'Major genes for reproduction in sheep. 2nd International Workshop, Toulouse, 16–18 July, 1990.' L'Institut Scientifique de Recherche Agronomique (INRA): Paris, France.

Piper L.R. and Bindon B.M. 1996. The Booroola merino. P. 542 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Oxon, UK.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Ponzoni R.W., Walker S.K., Walkley J.R.W. and Fleet M.R. 1985. The productivity of Bungaree, Booroola × Bungaree and Trangie Fertility × Bungaree Merino ewes in South Australia. Pp. 127–137 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Souza C.J.H., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology 169, R1–R6.

Turner H.N. 1982. Origins of the CSIRO Booroola. Pp. 1–7 in 'The Booroola Merino: proceedings of a workshop held in Armidale, New South Wales, 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO, Melbourne, Australia.

Walkden-Brown S.W., Wolfenden D.H., Charles R.J. and Maddox J.F. 2007. Expression of reproductive and production traits in commercial merino ewes having 0, 1 or 2 copies of the FecB mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 426–429.

Wilson T., Wu X.Y. et al. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Wolfenden D.H. and Walkden-Brown S.W. 2009. Use of nutritional restriction at mating to dampen reproductive performance of FecB carrier merino ewes. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 227–228. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Consequences of introgression of the *FecB* gene into Malpura sheep in Rajasthan

A.L. Arora[1,2], A.K. Mishra[1] and L.L.L. Prince[1]

Abstract

The performance of Garole × Malpura (GM) half-bred sheep was evaluated in this study. The presence of the *FecB* gene in GM sheep ($FecB^{BB}$, $FecB^{B}$+ or *FecB*++) was determined using DNA typing by forced PCR–RFLP. In Garole carriers 82% and 14% of animals were homozygous and heterozygous, respectively, for *FecB*. In GM crosses the *FecB* gene was detected in 13% of animals in the homozygous (*BB*) state and in 59% animals in the heterozygous (*B*+) state. The *FecB* genotype significantly increased prolificacy, weaning rate and litter size at 6 months of age, giving GM ewes advantage over Malpura ewes. Lambing rate averaged 91.8% and 148.2% in Malpura and GM ewes respectively. The twin lambing percentage was 4.71% in the Malpura flock and 51.10% in the GM flock within 5 years of inter se mating among GM crosses. Of the GM ewes, 5.72% produced triplets. *B*+ ewes weaned more litter weight than *BB* and ++ ewes. The ewe productivity of GM ewes bearing twin and triplet lambs in terms of lamb weight per kilogram of body weight of ewes at weaning, 6 months of age and 12 months of age was much higher than for Malpura ewes. The average body weights at birth, weaning and 6 months indicate significantly higher growth of lambs produced after backcrossing of Malpura ewes with GM rams compared to the reciprocal backcross. Introgression of the *FecB* allele into the Malpura breed via backcrossing has potential, with the ability to track the presence of *FecB* alleles in such crosses.

Introduction

India is endowed with a wide diversity of sheep genetic resources, which form the backbone of its rural livelihood security systems. Sheep rearing now faces a dilemma to produce more mutton and wool for the growing human population against the reality of shrinking grazing resources, which are creating a major constraint to the further growth of the sheep population. In the present scenario the demand for meat in India has increased rapidly, and the emphasis has shifted from wool towards mutton as the main produce from sheep rearing (Mishra 2008). There is an acute shortage of meat for domestic needs, apart from the huge demand in the international market. The gap between demand and production of mutton could be bridged by augmenting the reproductive rate of low-producing Indian sheep breeds. In order to improve the fecundity of sheep, incorporation of genetic material of prolific sheep is an ideal approach to evolve a large-sized breed capable of multiple births for economic and remunerative mutton production.

The Booroola gene (*FecB*), a dominant autosomal gene located on the 6th autosomal chromosome, is responsible for increasing ovulation rate (OR) and, in turn, prolificacy. The effect of *FecB* is additive for OR, each copy of the allele increasing OR by about 1.6, and litter size (LS) by approximately one to two extra lambs in the Booroola Merino (BM) (Piper et al. 1985; Piper and Bindon 1996). Turner (1969)

[1] Division of Animal Genetics and Breeding, Central Sheep and Wool Research Institute, Avikanagar–304 501, Rajasthan, India
[2] Corresponding author; alarora_avk@yahoo.co.in

concluded that LS seemed to be the most useful selection criterion for genetic improvement of reproduction. Davis et al. (2002) identified the *FecB* mutation in Garole and Indonesian Javanese Thin Tail sheep and proved that it was the same as that reported in the Australian BM. There has been an increasing interest worldwide in the identification and use of major genes affecting LS in sheep in the last two decades. So far, the *FecB* gene mutation has been discovered in only five breeds in the world, namely Garole, Hu, Han, Javanese and BM (Davis et al. 2002, 2006), excluding the breeds into which the gene has been introgressed from the BM. Recent research has confirmed that the *FecB* mutation is fixed in populations of Garole and Hu sheep, but is segregating in the Javanese, BM and Han breeds. Ewe productivity is dependent on the component traits of fertility, LS, lamb survival and growth (Fogarty et al. 1984) and is a major concern for the sheep industry. Improving female reproductive performance is an important objective for increasing the prolificacy of sheep (Abdulkhaliq et al. 1989). There is greater potential for increasing both biological and economic efficiency of lamb production through genetic improvement in reproductive rate than through improvement in growth rate or body composition (Dickerson 1978).

Considering the importance of prolificacy genes, a crossbreeding scheme was initiated in 1997 at the Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan. Its purpose was to introgress the *FecB* gene from India's most valuable germplasm Garole sheep, from a hot and humid environment, into the non-prolific and large-sized mutton sheep breed Malpura, which is best adapted to a semi-arid tropical environment, to produce the Garole × Malpura (GM) crossbreed carrying the *FecB* gene.

Garole is an exceptionally prolific breed among other non-prolific sheep breeds in India. It is a rare micro-sheep known for its high prolificacy (Ghalsasi and Nimbkar 1993; Davis et al. 2002; Mishra et al. 2005a) and the ability to thrive well under an adverse hot and humid climate. The average adult body weight of this breed ranges between 10 kg and 14 kg with a mean LS of 2.27 in its native habitat (Ghalsasi and Nimbkar 1993; Bose et al. 1999; Sharma et al. 2001; Mishra et al. 2006). On the other hand, the Malpura is a popular mutton breed of Rajasthan and is known for its hardiness and adaptability to the local environment, but its prolificacy is low and it usually produces single lambs. The average adult body weight of the Malpura ranges from 30 kg to 40 kg with a mean LS of 1.05 and a 4.71% twinning rate (Arora et al. 2004; Sharma et al. 2004; Mishra et al. 2005b, 2007b).

The purpose of this paper is to describe this introgression program and to present results on the crossbred animals that have arisen from the program.

Location and methods

The study was conducted at the CSWRI, Avikanagar, Rajasthan, India, located at 75° 22' E and 27° 17' N and an altitude of 320 m above mean sea level. The climate of the location is typically hot and semi-arid, with yearly minimum and maximum temperatures of 4 °C and 46 °C, respectively. The Garole (G) sheep was purchased from the native tract, i.e. the hot, humid region of West Bengal, in 1997. Malpura (M), a native mutton-type sheep breed usually having single births, was used as the dam breed, and the Garole was used as the sire breed, for developing GM crossbreds in which the *FecB* gene would segregate. The GM sheep included in the study were obtained by either crossing Malpura ewes with Garole rams or from interbreeding among GM half-breds. Ewes were bred in two breeding seasons, namely autumn (August–September) and spring (March–April). Most of the ewes were bred in autumn and only those not exposed in autumn were bred in spring. Contemporary Malpura lambs were produced through selective breeding within that breed (mating of selected males and females). Reproductive traits on GM ewes started to become available from 2000 onward. All the sheep were raised under a semi-intensive management system and were provided with similar grazing/feeding conditions, comprising 8–10 hours of grazing in the field interspersed with seasonal shrubs and forbs. An extra allowance of concentrate at 250 g/ewe/day was provided under group feeding for 3 months every year from the last month of pregnancy to completion of the 2nd month of lactation. The grazing diet was supplemented with minerals and vitamins. Lambs were allowed to suckle their dams from birth until weaning at 3 months of age. Prophylactic measures against various sheep diseases were carried out as prescribed in the health calendar of the CSWRI in addition to curative treatment of sick animals when required.

The presence of the *FecB* gene ($FecB^{BB}$, $FecB^{B+}$ or $FecB^{++}$) in GM sheep was determined using

DNA typing. The DNA was isolated by standard proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. The *FecB* mutation assay was carried out by the forced RFLP-PCR technique (Wilson et al. 2001).

The reproductive traits studied were prolificacy (number of live lambs born per ewe lambing during a year), weaning rate (WR; the number of lambs weaned / number of ewes lambed) and LS/ewe lambing at 6 months of age (LS6). Reproductive data were available from 2000 to 2006. Weights of the lambs at birth, 3, 6 and 12 months of age were recorded from 1998 to 2006. The ewe productivity efficiency (EPE) was calculated as the sum of total lamb weight harvested at birth, weaning, 6 or 12 months of age / number of ewes lambed, and ewe efficiency (EE) was calculated as the total litter weight of lambs harvested at birth, weaning, 6 or 12 months of age / body weight of ewes at lambing.

Least squares procedures (Harvey 1990) were used to analyse the growth data. Significant differences between means were detected using Duncan's multiple range tests (Kramer 1957). The data on ewe productivity were classified according to genotype, parity and generation.

Frequency of *FecB*

FecB was detected in 96% of Garole sheep and 72% of GM crosses (Table 1). The presence of the *FecB* mutant allele could not be detected in Malpura animals. In Garole carriers 74 (82%) and 13 (14%) animals were homozygous and heterozygous, respectively, for *FecB*. Davis et al. (2002) also reported that *FecB* is present at a high frequency in Garole sheep. In GM crosses the *FecB* gene was detected in 13% of animals in the homozygous (BB) state and 59% of animals in the heterozygous (B+) state. The results indicated that most GM individuals (72%) carried the *FecB* mutation and genotypic frequency of $FecB^{B+}$ was 0.59. As the *FecB* allele has been nearly fixed in the Garole population and is absent in Malpura sheep, the observed allele frequency in GM sheep is close to the expected frequency of 50%. In GM the *FecB* gene is present in the heterozygous state ($FecB^{B+}$) in the F1 generation and then segregates in F2, F3 and F4 after interbreeding among half-breds (Table 1).

Effect of *FecB* genotype on prolificacy and weaning rate

The *FecB* genotype significantly ($P > 0.01$) affects prolificacy, WR and LS6. The GM ewes had 45.7% (1.53 vs. 1.05), 35.1% (1.31 vs. 0.97) and 29.5% (1.23 vs. 0.95) advantage over Malpura for these traits respectively. One or two copies of the *FecB* allele resulted in prolificacy of 1.71 and 1.83, and WR of 1.46 and 1.42 in GM ewes, respectively. The second copy of the mutation increased prolificacy by about 0.12 lambs. The result clearly indicates that a second copy of the mutation has a partially dominant effect on prolificacy. The reason for comparatively lower overall prolificacy in GM ewes (Table 2) is due to the cross being a mix of carrier and non-carriers individuals with a close to 50% frequency of the *FecB* allele in the flock. Prolificacy and WR decreased with generation, probably due to the segregation of non-carriers (++) after inter se mating (Table 1). Prolificacy, WR and LS6 increased with parity.

Table 1. *FecB* genotypes (in numbers) and genotypic frequencies in Garole (G), Malpura (M) and GM sheep

Genetic group	Genotypes / genotypic frequencies			Total
	$FecB^{BB}$	$FecB^{B+}$	$FecB^{++}$	
Garole	74 (0.82)	13 (0.14)	3 (0.03)	90
Malpura	–	–	62 (100)	62
GM				
F1	00 (0.00)	59 (0.84)	11 (0.16)	70
F2	21 (0.14)	80 (0.52)	52 (0.34)	153
F3	17 (0.24)	35 (0.49)	19 (0.27)	71
F4	2 (0.29)	3 (0.43)	2 (0.28)	7
Total (GM)	40 (0.13)	177 (0.59)	84 (0.28)	301

Figures in parentheses are genotypic frequencies.

Reproductive performance

Reproduction is a good indicator of adaptation and contributes to the overall economics of the flock. The GM ewes had a non-significantly higher lambing rate (90.4%) on the basis of ewes available (Mishra et al. 2007a) than Malpura ewes (87.7%). Lambing rate on a ewes-available basis determines the flock reproductive efficiency as a whole and is measured as the number of lambs born in a year / number of ewes available for exposure in a year × 100 in terms of live lambs born out of available ewes in the flock in a specified period. Lambing rate averaged 91.8% and 148.2% in Malpura and GM ewes respectively (Mishra et al. 2007a). The twin lambing percentage was only 4.71% in the Malpura flock. It reached 51.1% in the GM flock within 5 years of inter-mating between GM crosses, with 5.7% of GM ewes producing triplets (Mishra et al. 2007a). There was a tremendous increase in multiple births and in other reproductive efficiency traits in GM ewes compared to contemporary Malpura ewes. These results provided the first evidence for increased lambing rate and LS in GM, with the possibility of further exploitation using appropriate breeding and selection strategies. Other studies (Nimbkar et al. 1998; 2003b) have also shown Garole crosses to have high reproductive efficiency. The overall distribution of single, twin, triplet and quadruplet lambs was 33.5%, 47.9%, 17.0% and 1.6%, respectively, in Garole sheep (Mishra et al 2005a), showing that twinning is more common than single births in Garoles. Sharma et al. (2004) reported prolificacy of GM as 1.19.

Growth performance

It was obvious that, on crossing the small-sized Garole rams with large-sized Malpura ewes, the half-bred progenies would weigh less than the dam parent and be higher than the sire parent. The main purpose was to attain multiple births from the *FecB*-introgressed GM sheep. Malpura lambs were always heavier than GM lambs at all stages of growth up to 1 year (Arora et al. 1999; Mishra et al. 2005b). Two reasons contributed to these differences: the fact that the cross contained genes from a significantly lighter breed; and many of the crossbred lambs were born twins or triplets, which are mostly lighter than single-born lambs. Significantly lower body weights and growth rates of lambs sired by Garole rams with Deccani/Bannur as the dam breed compared to the lambs sired by Deccani and Bannur rams with Deccani/Bannur as the dam breed were also reported by Nimbkar et al. (2000) and Nimbkar et al. (2003a).

Table 2. Prolificacy, weaning rate and litter size at 6 months age among *FecB*-carrier and non-carrier GM ewes

	n	Prolificacy	Weaning rate	Litter size at 6 months
Overall	268	1.53 ± 0.04	1.31 ± 0.04	1.23 ± 0.04
FecB genotype		**	**	**
$FecB^{BB}$	12	1.83^{a} ± 0.21	1.42^{a} ± 0.23	1.33^{a} ± 0.19
$FecB^{B+}$	187	1.71^{a} ± 0.04	1.46^{a} ± 0.05	1.36^{a} ± 0.05
$FecB^{++}$	69	1.01^{b} ± 0.01	0.88^{b} ± 0.04	0.84^{b} ± 0.05
Generation		**	**	**
F1	119	1.61^{a} ± 0.05	1.46^{a} ± 0.06	1.40^{a} ± 006
F2	70	1.63^{a} ± 0.08	1.39^{b} ± 0.09	1.23^{b} ± 0.09
F3	69	1.33^{b} ± 0.06	1.04^{c} ± 0.08	0.99^{c} ± 0.08
F4	10	1.40^{b} ± 0.22	0.80^{a} ± 0.04	0.80^{d} ± 0.04
Parity		*	**	*
1	120	1.39^{a} ± 0.05	1.11^{a} ± 0.06	1.06^{a} ± 0.06
2	72	1.65^{b} ± 0.07	1.47^{b} ± 0.08	1.36^{b} ± 0.08
3	38	1.68^{bc} ± 0.11	1.58^{b} ± 0.12	1.42^{b} ± 0.18
4	23	1.52^{c} ± 0.14	1.26^{c} ± 0.13	1.26^{c} ± 0.13
5	10	1.70^{d} ± 0.15	1.50^{b} ± 0.22	1.40^{bd} ± 0.27
6	5	1.80^{d} ± 0.34	1.60^{d} ± 0.51	1.40^{bd} ± 0.51

n = number of ewes lambed; * $P < 0.05$; ** $P < 0.01$; $^{a\ b\ c\ d}$ same superscript within column indicates that results did not differ significantly

Survivability

Survivability is a very crucial factor in deciding the economic viability of any breed improvement program. It is the indicator of adaptability of a genetic group under the prevailing climatic and management conditions of a particular region. Lamb survival percentage during pre-weaning, weaning to 6 months and weaning to 6–12 months, was 97.3%, 94.1% and 96.8% for Malpura and 94.2%, 92.2% and 95.9% for GM, respectively. The survivability of single, twin and triplet lambs of GM up to 12 months of age was 91.3%, 82.2% and 62.2% respectively. The lower survival in GM may be due to higher twinning (Mishra et al. 2007a).

Ewe productivity efficiency (EPE)

The *FecB* genotype significantly affected EPE and EE traits. Regarding EPE, B+ ewes weaned more litter weight (12.8 kg) compared to BB (11.6 kg) and ++ (10.4 kg) ewes (Table 3). Nimbkar et al. (2003a) also reported that the body weight of lambs weaned was higher in ewes bearing twin lambs (*FecB* carriers) compared to bearing single lambs (non-carriers). It is well established that the survivability decreases as the number of lambs born increases. The EPE of Malpura ewes was 13.3 kg and 20.3 kg at weaning and 6 of months age respectively (Mishra et al 2007a). The results indicate that the production of B+ ewes is more beneficial than BB or ++ ewes for achieving higher EPE and EE, but that EPE remains similar or lower than that observed in the parent Malpura breed. The total lamb production from *FecB*-carrying GM ewes bearing twin and triplet lambs was more than non-carriers at birth, weaning, 6 months and 12 months of age. Generation and parity significantly affected the EPE from birth to 6 months. In the present study it was found that the EPE and EE increased up to the third parity and after that an erratic trend was noticed.

Significance of multiple births in Garole crosses

Increasing multiple births is vital for augmenting mutton production and economic returns from the sheep rearing profession. The benefits of multiple births in GM ewes compared to single births in Malpura ewes are reflected in terms of high kg of lamb production per kg of ewe body weight (EE); and high litter size at birth, weaning and 6 months of age. Comparative evaluation of twin- and triplet-bearing ewes of GM with the monotocous Malpura ewes reveals the significant potential of prolific GM germplasm for enhancing mutton production. The ewe productivity of GM ewes bearing twin and triplet lambs in terms of per kg body weight of ewes was 0.63 kg and 0.65 kg at weaning, 1.04 kg and 1.11 kg at 6 months of age and 1.41 kg and 1.35 kg at 12 months of age, respectively. The corresponding values of Malpura ewes were very much lower, at 0.44 kg, 0.67 kg and 0.86 kg at weaning, 6 months and 12 months of age, respectively (Mishra et al. 2007b). The LS of GM ewes bearing twin and triplet lambs was 1.98 and 2.71 at birth, 1.72 and 2.14 at weaning, and 1.64 and 2.00 at 6 months of age, respectively (Mishra et al. 2007b). The EPE from GM ewes bearing twins and triplets was greater than Malpura ewes at birth, weaning, 6 months and 12 months of age (Mishra et al. 2007b). Nimbkar et al. (2005) reported that in Garole × Deccani cross twin-bearing ewes weaned 0.7 more lambs and produced 2.4 kg more weight of lambs at 105 days than single-bearing ewes, while triplet-bearing ewes weaned 0.5 lambs and 4.7 kg more weight of lamb than twin-bearing ewes.

Backcrossing of Garole × Malpura half-bred with Malpura for enhanced growth

Having developed prolific GM half-bred sheep, there is now scope to exploit this for relatively better growth by backcrossing with native Malpura. In the backcrossing program the *FecB*-gene carrier GM rams were used as sires and Malpura ewes as the dam breed to produce the GM × Malpura (GM (M)); 75% Malpura and 25% Garole), and reciprocal crosses were also attempted to produce M (GM). The average body weight of GM (M) (numbers in parentheses) at birth, weaning and 6 months of age were 2.85 ± 0.05 kg (161), 13.7 ± 0.2 kg (146) and 19.6 ± 0.3 kg (145), respectively, and the corresponding figures for M (GM) were 2.14 ± 0.04 kg (201), 11.0 ± 0.2 kg (157) and 17.1 ± 0.3 kg (150). There were 49.2%, 35.3% and 27.9% increases in body weight of GM (M) lambs over GM half-bred at birth, weaning and 6 months of age, respectively. The results indicate that there is pronounced significantly higher gain in body weight and growth rate of lambs produced after backcrossing of Malpura ewes with GM rams compared to the reciprocal backcrossing.

Table 3. Ewe productivity efficiency (EPE) and kg of lamb produced per kg of ewe body weight (EE) of *FecB*-carrier and non-carrier GM ewes

	n	EPE at:				EE at:			
		Birth	Weaning	6 months	12 months	Birth	Weaning	6 months	12months
Overall	268	2.83 ± 0.06	12.10 ± 1.10	18.28 ± 0.64	21.81 ± 0.91	0.12 ± 0.00	0.51 ± 0.02	0.78 ± 0.03	0.93 ± 0.04
FecB genotype		**	*	**	**	**	**	**	**
$FecB^{BB}$	12	3.03^{a} ± 0.37	11.55^{a} ± 1.89	17.83^{a} ± 3.42	22.53^{a} ± 4.02	0.13^{a} ± 0.01	0.49^{a} ± 0.08	0.78^{a} ± 0.14	0.99^{a} ± 0.17
$FecB^{B+}$	187	2.97^{a} ± 0.72	12.77^{b} ± 0.45	19.61^{a} ± 0.79	24.19^{a} ± 1.12	0.13^{a} ± 0.00	0.56^{b} ± 0.02	0.86^{a} ± 0.04	1.06^{a} ± 0.05
$FecB^{++}$	69	2.42^{c} ± 0.06	10.39^{c} ± 0.61	14.74^{b} ± 0.96	15.22^{c} ± 1.43	0.09^{c} ± 0.00	0.39^{c} ± 0.02	0.56^{c} ± 0.04	0.57^{c} ± 0.06
Generation		*	**	**	**	NS	*	**	**
F1	119	2.91^{a} ± 0.07	13.29^{a} ± 0.46	21.09^{a} ± 0.81	27.42^{a} ± 1.13	0.12 ± 0.00	0.56^{a} ± 0.02	0.89^{a} ± 0.04	1.16^{a} ± 0.05
F2	70	3.01^{a} ± 0.13	12.52^{a} ± 0.75	17.95^{b} ± 1.38	19.25^{b} ± 1.90	0.13 ± 001	0.53^{a} ± 0.03	0.74^{b} ± 0.06	0.81^{b} ± 0.08
F3	69	2.52^{b} ± 0.11	10.06^{b} ± 0.79	14.51^{c} ± 1.2	16.12^{c} ± 1.80	0.11 ± 0.05	0.45^{b} ± 0.03	0.64^{c} ± 0.05	0.71^{c} ± 0.08
F4	10	2.69^{c} ± 0.32	9.06^{b} ± 2.29	13.15^{c} ± 3.50	12.34^{d} ± 4.34	0.12 ± 0.02	0.38^{c} ± 0.09	0.55^{d} ± 0.14	0.53^{d} ± 0.19
Parity		**	**	**	NS	*	**	NS	NS
1	120	2.46 ± 0.07	9.85 ± 0.50	15.53 ± 0.91	19.20 ± 1.34	0.11 ± 0.00	0.44 ± 0.02	0.69 ± 0.04	0.86 ± 0.06
2	72	3.07 ± 0.10	13.97 ± 0.66	20.29 ± 1.21	23.86 ± 1.85	0.13 ± 0.01	0.58 ± 0.03	0.84 ± 0.05	1.00 ± 0.08
3	38	3.29 ± 0.15	14.84 ± 0.87	21.85 ± 1.63	25.98 ± 2.33	0.13 ± 0.01	0.59 ± 0.04	0.88 ± 0.07	1.03 ± 0.09
4	23	2.98 ± 0.23	12.67 ± 1.29	19.79 ± 1.98	21.94 ± 2.62	0.12 ± 0.01	0.51 ± 0.06	0.80 ± 0.91	0.88 ± 0.12
5	10	3.37 ± 0.31	14.19 ± 2.31	20.50 ± 1.31	22.52 ± 5.50	0.13 ± 0.12	0.54 ± 0.09	0.79 ± 0.17	0.87 ± 0.22
6	5	3.16 ± 0.69	11.80 ± 3.69	16.74 ± 5.89	21.18 ± 7.55	0.14 ± 0.03	0.51 ± 0.02	0.74 ± 0.26	0.92 ± 0.22

n = number of ewes lambed; * $P < 0.05$; ** $P < 0.01$; NS = non-significant; $^{a\ b\ c\ d}$ same superscript indicates that results did not differ significantly

The significant gains achieved in body weight of GM(M) and M(GM) *FecB*-carrier lambs (having 25% inheritance of Garole and 75% of Malpura) over GM lambs at birth and during the growth stage has a wider implication for meeting the current and future shortfall of mutton production in India. Quarter-bred rams (viz. GM × M) carrying the *FecB* gene can also be distributed to farmers to improve the prolificacy of their sheep flocks by natural mating or artificial insemination.

Conclusion

There was a large increase in the prolificacy, WR and other ewe productive efficiency traits in GM half-breds carrying the *FecB* gene compared to non-carriers. The study showed that B+ ewes increased WR and lamb weight produced compared to BB and ++ ewes. Hence, there is a need to increase the number of *FecB*-carrying GM ewes that produce twins or triplets by inter se mating among carriers, and to discard non-carriers using marker assisted selection. Introgression of the *Fec*B allele via backcrossing crossbreds to Malpura also has potential, with the ability to track the *FecB* alleles in such crosses. The DNA test should be applied in routine breeding programs as a marker for identifying *FecB*-gene carriers at an early stage. This will accelerate breeding strategies for improving the prolificacy and genetic improvement of non-prolific sheep breeds.

References

Abdulkhaliq A.M., Harwey W.R. and Parker C.F. 1989. Genetic parameters for ewe productivity traits in the Columbia, Suffolk and Targhee breeds. Journal Animal Sciences 67, 3250–3257.

Arora A.L., Sharma R.C. and Narula H.K. 2004. Evaluation of Awassi × Malpura half-bred sheep in semi-arid region of Rajasthan. Indian Journal Animal Science 74(12), 1219–1222.

Arora A.L., Sharma R.C., Narula H.K. and Ravindra K. 1999. Comparative performance of Avikalin and Malpura sheep. Indian Journal Small Ruminants 5, 4–8.

Bose S., Dutta Gupta R. and Moitra D.N. 1999. Reproductive performance of Bengal sheep in Sunderbans. Indian Journal of Animal Production and Management 15(4), 157–160.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway S.M., Lumsden B.M., Hanrahan J.P., Mullen M., Mao X.Z., Wang G.L., Zhao Z.S., Zeng Y.Q., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranz J.J. and Notter D.R. 2006. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92(1–2), 87–96.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray, G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.C and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Dickerson G.E. 1978. Animal size and efficiency: basic concepts. Animal Productive 27, 367–379.

Fogarty N.M., Dickerson G.E and Young L.B. 1984. Lamb production and its components in purebreds and composite lines II. Breed effects and heterosis. Journal of Animal Science 58, 301–311.

Ghalsasi P.M. and Nimbkar B.V. 1993. The 'Garole' micro sheep of Bengal, India. Animal Genetics Resource Information 12, 73–79.

Harvey W.R. 1990. User's Guide for LSMLMW MIXMDL PC-2 Version. Columbus, Ohio, USA.

Kramer C.Y. 1957. Extension of multiple range tests to group correlated adjusted means. Biometrics 13, 13–18.

Mishra A.K. 2008. Significance of prolificacy trait in intensive mutton production. Pp. 267–280 in 'Small ruminant production in India: strategies for enhancing', ed. by S.A. Karim et al. Satish Serial Publishing House: New Delhi.

Mishra A.K., Arora A.L., Kumar S., Gupta D.C. and Singh V.K. 2007b. Studies on ewes' efficiency of single and multiple bearing lambs of non-prolific Malpura, prolific Garole and Garole × Malpura sheep. The Indian Journal of Animal Sciences 77(8), 759–762.

Mishra A.K., Arora A.L., Kumar S., Sharma R.C. and Singh V.K. 2005b. Malpura: a mutton type sheep breed. Research Bulletin, Central Sheep Wool Research Institute (CSWRI), Avikanagar via Jaipur (Rajasthan).

Mishra A.K, Arora A.L., Kumar S. and Singh V.K. 2005a. Prolificacy of Garole in semi-arid tropics of Rajasthan. The Indian Journal of Small Ruminants 11(1), 1–5.

Mishra A.K., Arora A.L., Kumar S. and Singh V.K. 2006. Performance evaluation of Garole sheep in semi-arid region of Rajasthan. Indian Journal of Animal Science 76, 393–397.

Mishra A.K., Arora A.L., Kumar S. and Singh V.K. 2007a. Improving productivity of Malpura breed by crossbreeding with prolific Garole sheep in India. Small Ruminant Research 70,159–164.

Nimbkar C., Ghalsasi P.M., Ghatge R.R. and Gray G.D. 1998. Establishment of prolific Garole sheep from West

Bengal in the semi-arid Deccan plateau of Maharastra. Pp. 257–260 in 'Proceedings of the 6th World Congress on Genetics Applied to Livestock Production', Armidale, New South Wales.

Nimbkar C., Ghalsasi P.M., Maddox J.F., Pardeshi V.C., Sainani M.N., Gupta V. and Walkden-Brown S.W. 2003b. Expression of FecB gene in Garole and Crossbred ewes in Maharastra, India. Pp. 111–114 in 'Proceedings of the 15th conference of AAABG', Melbourne, Australia.

Nimbkar C., Ghalsasi P.M., Swan A., Walkden-Brown S.W. and Kahn L.P. 2003a. Evaluation of growth rates and worm resistance of Deccani and Bannur lambs and their crosses with Garole. Animal Science 76, 503–515.

Nimbkar C., Ghalsasi P.M., Walkden-Brown S. W., Kahn L.P. and Gray G.D. 2000. A comparison of the growth performance and worm resistance of lambs produced by diallel crossing of three Indian sheep breeds. Asian–Australasian Journal of Animal Science 13, 72–75.

Nimbkar C., Pardeshi V.C. and Ghalsasi P.M. 2005. Evaluation of the utility of the FecB gene to improve the productivity of Deccani sheep in Maharashtra, India. In 'Applications of gene-based technologies for improving animal production and health in developing countries', ed. by H.P.S. Makkar and G.J. Vilijoen. FAO–IAEA, Springer: the Netherlands.

Piper L.R. and Bindon B.M. 1996. The Booroola Merino. In 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, U.K.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep' ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Sharma R.C., Arora A.L., Khan B.U. 2001. Garole: a prolific sheep of India. P. 17 in Research Bulletin, Central Sheep and Wool Research Institute, Avikanagar.

Sharma R.C., Arora A.L., Mishra A.K., Kumar S. and Singh V.K. 2004. Breeding prolific Garole with Malpura sheep for increased reproductive efficiency in semi-arid tropics of India. Asian–Australasian Journal of Animal Science 17(6), 737–742.

Turner H.N. 1969. Genetic improvement of reproduction in sheep. Animal Breeding Abstracts 37, 545 (abstract).

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEvan J.C., O'Connel A.R., McNatty K.P. and Montgomery, G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Biological and economic consequences of introgressing the *B* allele of the *FecB* (Booroola) gene into Awassi and Assaf sheep

E. Gootwine[1]

Abstract

Lamb production in Israel is the main source of income from non-dairy flocks and contributes about half of the gross income of dairy flocks. The prolificacy of the local breeds, the Awassi and the Assaf, is low to intermediate, being about 1.3 and 1.7 lambs born per lambing (LB/L) respectively. Breeding work aimed at improving the prolificacy of the Awassi and the Assaf through introgression of the *B* allele (Booroola mutation) of the *FecB* locus started in 1986 with the import of homozygous BB Booroola Merino rams. Identification of B carriers among the crossbreds was initially carried out using physiological indicators, later by using genetic markers linked to the Booroola gene, and finally by direct genotyping for the *FecB* locus. The breeding work resulted in the development of sheep with > 94% Improved Awassi or Assaf inheritance that carried the Booroola mutation in either a homozygous or heterozygous state. These sheep were designated Afec–Awassi and Afec–Assaf respectively. Prolificacy of B+ and BB ewes was 1.90 and 1.92 LB/L respectively, in the Afec–Awassi; and 2.40 and 2.55 LB/L, respectively, in the Afec–Assaf. Lamb survival at birth in the ++ Awassi and the ++ Assaf averaged 0.98 and 0.94 respectively. It declined to 0.93 and 0.86, and 0.85 and 0.78, in the B+ and BB Awassi, and B+ and BB Assaf, respectively. Carrying the B allele adversely affected lambs' birth weight and survival rate at lambing, as well as ewes' body weight and milk production. Despite those limitations, introduction of the Afec–Awassi and Afec–Assaf into flocks under intensive management improved flock profitability. The results of introducing the Booroola mutation into flocks under semi-extensive management are still under investigation.

Sheep production in Israel

The sheep population in Israel (about 420,000 head) is maintained under a wide range of production systems that coexist in close geographical proximity: from traditional transhumance and extensive lamb production to the most intensive dual-purpose dairy and meat production. The native unimproved Awassi, a fat-tail breed most common in the Middle East, comprises more than half of the national flock, and is managed mainly by Bedouin producers under extensive to semi-extensive conditions in the dry southern region of the country. The native Awassi is a hardy, lowly prolific breed (about 1.1 lambs born per lambing (LB/L)) and is raised under extensive conditions where feed availability and other environmental constraints are the rate-limiting factors for production.

Within-breed selection for high milk production in the native Awassi started in the 1930s, resulting in the formation of the Improved Awassi dairy strain (Epstein 1985). Under intensive conditions this large-framed strain manifests high milk production ability, about 550 L/lactation (Gootwine and Pollott

[1] Institute of Animal Science, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel; gootwine@volcani.agri.gov.il

2000). However, prolificacy of the Improved Awassi is still rather low—about 1.3 LB/L.

Under intensive conditions the ewe's genetic makeup is the rate-limiting factor for production. To improve milk and lamb production in the intensive dairy flocks, the Improved Awassi was crossed with the East Friesian milk sheep, which was imported from Germany in the 1950s (Gootwine and Goot 1966). The crossbreeding work resulted in the development of the Assaf breed, which has a prolificacy of about 1.65 LB/L and average milk production of about 450 L/lactation (Pollott and Gootwine 2004). Today, most of the dairy ewes in the country (some 70,000 head) belong to the Assaf breed. Due to their high milk production capacity and their adaptability to the Mediterranean climate, both the Improved Awassi and the Assaf have gained worldwide recognition and distribution (Rummel et al. 2006).

Intensive non-dairy sheep production was initiated in Israel in the 1950s by massive importation of the German Mutton Merino (GMM) breed, which has a prolificacy of about 1.4 LB/L. Improvements in lamb production in the non-dairy sector were achieved in the 1970s by crossing the GMM with the Finn and Romanov breeds, resulting in a prolificacy of about 1.9 LB/L in those crosses (Eyal et al. 1984). Further breeding steps in the non-dairy flocks included integration of the Assaf breed due to its high growth-rate potential, along with some heavy mutton breeds such as the Dorper, the Charollais and the American Suffolk. Today, most of the non-dairy flocks (some 100,000 head) are made up of crossbreds, with an average prolificacy of about 1.8 LB/L.

As wool is not economically important in the Israeli market, lamb production is the main source of income in non-dairy flocks and contributes about half of the gross income of the dairy flocks. To maximise lamb production, reproductive management in most of the intensive flocks includes an accelerated lambing program with several lambing periods per year. To achieve this, hormonal oestrus synchronisation of the ewes at about 70 days after lambing is commonly applied. In most intensively managed dairy and non-dairy flocks, lambs are removed from their mothers on the day of lambing and are raised in artificial rearing units until weaning at about 35 days of age. They are then fattened on a concentrated diet and some hay until about 5 months of age, and sold at a weight of about 60 kg for US$5–6/kg live weight.

As intensive management for lamb production demands a high level of financial input for housing, feeding, hormonal treatments, labour and veterinary treatments, high prolificacy has become a major breeding goal in both dairy and non-dairy intensive flocks. Higher prolificacy has also become crucial for the extensive and semi-extensive flocks as pasture availability is seasonal and, from midsummer to winter, flocks are fed indoors.

Introgressing the Booroola mutation into the Awassi and Assaf breeds

Breeding for high prolificacy through introgression of the Booroola mutation (Piper et al. 1985) into the Improved Awassi and Assaf breeds started in 1986 with the import of five homozygous BB Booroola Merino rams from The Invermay Agricultural Centre, New Zealand. F_1 B+ heterozygous rams produced by artificial insemination of Improved Awassi and Assaf ewes were backcrossed to the respective breeds to produce the first backcross generation. Advanced backcross generations in the two breeding trials were produced by mating non-carrier purebred rams with B+ crossbred females. Initially, identification of B+ females among the backcross populations was based on monitoring lambing rate and natural ovulation rate (OR) (Gootwine et al. 1995), and on recording induced ovulation rate (IOR) at 5 months of age (Gootwine et al. 1993), as suggested by Davis and Johnstone (1985). Selection based on IOR was implemented mainly in the Booroola Assaf crosses, where ewe lambs that exhibited three or more induced ovulations were kept as replacements.

The use of molecular tools rather than physiological indicators in the Booroola crosses became common practice once genetic markers linked to the Booroola gene had been identified (Montgomery et al. 1993). Those markers were used mainly for the Booroola–Awassi crosses (Gootwine et al. 1998), as they were not informative in the Booroola–Assaf crossbreeding trial. However, since the identification of the Booroola gene in 2001 (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001), B-carrier identification has been based on genotyping directly for the *FecB* locus (Gootwine et al. 2008). Selection for the presence of the Booroola mutation based on IOR records was found, in hindsight, to be relatively efficient, as about 90% of the ewe lambs with records of three or more induced ovulations indeed carried

the Booroola mutation, as confirmed by molecular means (Table 1). However, considerable numbers of B carriers with two or fewer ovulations were missed. Today, sheep with > 94% of Improved Awassi or Assaf inheritance that carry the Booroola mutation in either a homozygous or heterozygous state are designated Afec Awassi and Afec Assaf respectively.

Distribution of Afec–Awassi and Afec–Assaf sheep to commercial flocks

An Afec–Awassi breeding nucleus of about 500 ewes is maintained in the Ein Harod flock alongside about 700 Improved Awassi ewes. Afec–Awassi rams and ewes have been distributed from the Ein Harod flock to the extensive local Awassi flocks in the south of the country, to Jordan and to the Palestinian Authority. An Afec–Assaf breeding nucleus of about 400 ewes is maintained at the Volcani Center. Since 2002 more than 300 BB-homozygous Afec–Assaf rams have been distributed from the Volcani Center, mainly to non-dairy commercial flocks.

Lamb production in the Afec–Awassi and the Afec–Assaf strains

Prolificacy up to the fifth parity and lamb survival at birth were investigated in ≥ 96.875% Awassi and ≥ 96.875% Assaf sheep belonging to the ++, B+ and BB genotypes (Gootwine et al. 2008). It was found that carrying one copy of the B allele increases prolificacy by 0.62 and 0.72 LB/L in the Awassi and the Assaf respectively (Table 2). BB ewes manifest somewhat higher prolificacy than the respective B+ genotypes, indicating an overall partial dominance mode of action for the B allele on prolificacy.

Table 1. Frequency and prolificacy of B+ and ++ Booroola Assaf crossbred ewes grouped according to their induced ovulation rate (IOR) record at 5 months of age. Ewe genotype was verified by genotyping for the *FecB* locus. Prolificacy estimates (lambs born/lambing) were based on at least three lambing records/ewe.

		Frequency of:		Prolificacy of:	
IOR	*n*	++	B+	++	B+
0	21	0.52	0.48	1.81	2.56
1	14	0.47	0.43	1.77	2.78
2	49	0.43	0.57	1.71	2.39
3	74	0.12	0.88	1.80	2.42
4	29	0.03	0.97	1.89	2.50
5	13	0.07	0.93	2.00	2.53
6–7	7	0.00	1.00	–	2.64
Average				1.83	2.53

Table 2. Lambs born/lambing, lambs born alive/lambing and lamb survival rate of Awassi, Assaf, Afec–Awassi and Afec–Assaf ewes (from Gootwine et al. 2007b)

Genotype	Genotype at the *FecB* locus	Litter size (LSM ± SEM)	Litter size born alive (LSM ± SEM)	Lamb survival rate
Awassi	++	1.31 ± 0.02^{b}	1.28 ± 0.02^{b}	0.98
≥ 96.875% Awassi	++	1.28 ± 0.02^{b}	1.24 ± 0.02^{b}	0.97
Afec–Awassi	B+	1.90 ± 0.02^{a}	1.76 ± 0.02^{a}	0.93
Afec–Awassi	BB	1.92 ± 0.07^{a}	1.65 ± 0.07^{a}	0.86
≥ 96.875% Assaf	++	1.68 ± 0.06^{c}	1.58 ± 0.06^{b}	0.94
Afec–Assaf	B+	2.40 ± 0.05^{b}	2.05 ± 0.04^{a}	0.85
Afec–Assaf	BB	2.55 ± 0.07^{a}	1.98 ± 0.07^{a}	0.78

Within a column and breed group, means with different superscript letters differ significantly ($P < 0.05$).

Lamb survival rate at birth, which averaged 0.98 and 0.94 in the Awassi and the Assaf, respectively, declined significantly ($P < 0.05$) to 0.93 and 0.86 in the B+ and to 0.85 and 0.78 in the BB Afec–Awassi and Afec–Assaf respectively (Gootwine et al. 2008). Thus, on average, B+ ewes produced an additional 0.5 live-born lambs per lambing than Awassi or Assaf ewes. Production of lambs born live was slightly lower in BB ewes than in B+ ewes, despite the relatively higher prolificacy of the former, because of the lower lamb survival rate.

Effect of the *FecB* gene on lamb survival at birth

Uterine capacity is defined as the maximal number of foetuses the uterine environment can support to birth (Bazer et al. 1969; Leymaster and Johnson 1994). Thus, low uterine capacity may prevent full economic exploitation of high prolificacy (Wu et al. 2006; Gootwine et al. 2007b). Indeed, the relatively lower survival rate of lambs born in large litters to Afec ewes was related mainly to insufficient uterine capacity, as most of the lamb losses recorded at birth resulted from prenatal foetal death rather than dystocia (data not shown). However, variability in uterine capacity among Afec–Assaf has been noted, as some ewes can successfully carry large litters to term (Figure 1) while other fail to do so.

Analysis of variance of records of average lamb survival rates at birth from 652 Afec–Assaf ewes with two or more lambing records revealed that ewe's sire and ewe's number of parities do not significantly ($P > 0.05$) affect lamb survival rate. On the other hand, lamb survival rate was affected by ewe's average prolificacy ($P < 0.001$) and, interestingly, by her genotype at the *FecB* locus ($P < 0.003$). Lamb survival rate was relatively low for BB ewes, high for ++ ewes and intermediate for B+ ewes (Table 3). Whether carrying the Booroola mutation affects uterine capacity directly, or indirectly via reduction in ewe body size (see below), is under investigation.

Figure 1. Afec–Assaf ewe with a litter of six lambs born with average birth weight of 3.7 kg (photograph taken by Prof. Anne Valle Zárate)

Lamb and ewe losses due to pregnancy toxaemias

Despite the fact that the recommended management for Afec ewes includes feeding to meet their metabolic needs, some animals are affected by pregnancy toxaemia (PT). PT is characterised by hypoglycaemia and hyperketonaemia as a result of the animal's inability to maintain an adequate energy balance. A study of PT frequency among Afec–Assaf ewes (2,776 lambing records) showed

Table 3. Least squares means ± SEM for lambs' survival rate index according to ewe genotype at the *FecB* locus and her prolificacy

Effect	Level	*n*	Lamb survival index (LSM ± SEM)
Maternal *FecB* genotype	++	107	0.93 ± 0.02^{a}
	B+	444	0.88 ± 0.01^{b}
	BB	101	0.83 ± 0.02^{c}
Average prolificacy (lambs born/ lambing)	1.0–1.4	32	0.96 ± 0.03^{a}
	1.5–1.9	138	0.91 ± 0.02^{ab}
	2.0–2.4	194	0.89 ± 0.02^{bc}
	2.5–2.9	134	0.84 ± 0.02^{cd}
	3.0–3.4	105	0.85 ± 0.02^{d}
	≥ 3.5	40	0.73 ± 0.03^{e}

[a b c d e] Within effects, means with different superscript letters differ significantly ($P < 0.05$).

PT frequencies of 0%, 0.9%, 4.0%, 9.0%, 18% and 37% for ewes carrying 1, 2, 3, 4, 5 and 6 foetuses respectively.

The most common treatment for PT is to drench the affected ewe with propylene glycol. However, it is seldom possible to save the mother and lead her to normal lambing. Aiming at developing appropriate management for handling highly prolific ewes, we found (Zamir et al. 2009) that combining the propylene glycol treatment with flunixin meglumine, a potent analgaesic and antipyretic non-steroidal anti-inflammatory drug (Kopcha and Ahl 1989), dramatically improves both ewe and lamb survival rates (Table 4).

Production traits of Afec strains

Body weight

Carrying the B allele was found to have an adverse effect on mature body weight of Afec–Assaf ewes (Gootwine et al. 2006). A similar association between ewe body weight and genotype at the *FecB* locus was obtained with Afec–Awassi ewes after their parity number and body score were included in the statistical analysis (Table 5). The association between low body weight and presence of the B allele might be due to a pleiotropic effect of the B allele on body weight or to the effect of a gene closely linked to the *FecB* locus, which controls growth ability (Walling et al. 2000).

Birth weight

Birth weight is an important economic trait as it is associated with postpartum lamb survival (Fogarty et al. 2000; Kleemann and Walker 2005). Presence of the B allele in the lamb or its mother was found to have direct adverse effects on the birth weight of Afec–Assaf ewe lambs, independent from the effect of litter size (LS) on birth weight (Gootwine et al. 2006).

An attenuation in foetal development has been shown to occur in foetuses carrying the Booroola mutation at as early a time as mid gestation (Smith et al. 1993, 1996). Support for this observation was recently obtained in our study with Afec–Assaf ewes whose heart rate was monitored during pregnancy. From mid pregnancy onwards, maternal

Table 4. Results of treating ewes affected with pregnancy toxaemia (PT) with propylene glycol alone or in combination with flunixin meglumine

	Treatment	
	Propylene glycol	Propylene glycol + flunixin meglumine
No. of ewes experiencing PT	60	67
Ewes that died before lambing (%)	20	3
Ewes that died at or right after lambing (%)	58	7
Ewe survival rate	0.22	0.90
Prolificacy (lambs born/lambing)	3.66	3.73
Lambs born alive/lambing	1.85	2.25
Lamb survival rate	0.49	0.70

Table 5. Least squares means ± SEM for body weight of Awassi and Assaf ewes belonging to different genotypes at the *FecB* locus

Genotype	Body condition score [A]		*FecB* genotype	Body weight (kg)	Reference
	n	range (units)			
Assaf	294	1.0–3.5	BB B+ ++	67.3 ± 1.4^{a} 70.8 ± 1.1^{b} 70.1 ± 1.7^{b}	Gootwine et al. (2006)
Awassi	86	2.0–3.5	BB B+ ++	62.5 ± 2.7^{a} 70.4 ± 1.4^{b} 76.1 ± 1.4^{c}	Unpublished results

[a b c] Within rows, means with different superscript letters differ significantly ($P < 0.05$).
[A] on a scale of 1–5 where 1 is very thin and 5 is very fat

heart rate was monitored once a week using a Polar Sports Tester^{TM} (Polar Electro Oy, Finland) adjusted to record the heart rate every 5 seconds. It was found that maternal heart rate increases as pregnancy progresses and as number of foetuses increases (Figure 2).

Least squares analysis of heart rate records, where the effect of LS and maternal genotype at the *FecB* locus were included in the model, showed that both LS and maternal *FecB* genotype significantly ($P < 0.05$) affected heart rates, with BB ewes manifesting a lower heart rate than B+ and ++ ewes (Table 6). Whether the lower heart rate of BB ewes during pregnancy is associated with lower foetal demand for nutrients, due to their intrinsically slower growth rate, or whether it is the maternal genotype that attenuates heart function leading to attenuation in foetal growth, remains to be determined.

Milk production

Information regarding milk production was obtained mainly from the Booroola–Awassi cross-breeding trial. Milk production/lactation of B+ ewes increased as upgrading to the Awassi progressed; at the BC4 level it was about 94% that of the Improved Awassi (Gootwine et al. 2001). Further analysis (Gootwine 2006) showed that BB Afec–Awassi ewes produce less milk than B+ ewes. Analysis of variance of records up to the fifth lambing of Awassi ewes (n = 1,136), Afec–Awassi (BC4–BC6) B+ ewes (n = 653), Booroola–Awassi ((BC4–BC6) non-carrier ++ ewes (n = 261) and (BC4–BC5) BB ewes (n = 28), all lambed between the years 2000 and 2008, showed that average milk production/lactation was significantly different ($P < 0.05$) among the different genotypes, being (LSM ± SEM) 503 ± 14 L, 467 ± 14 L, 485 ± 15 L and 399 ± 25 L

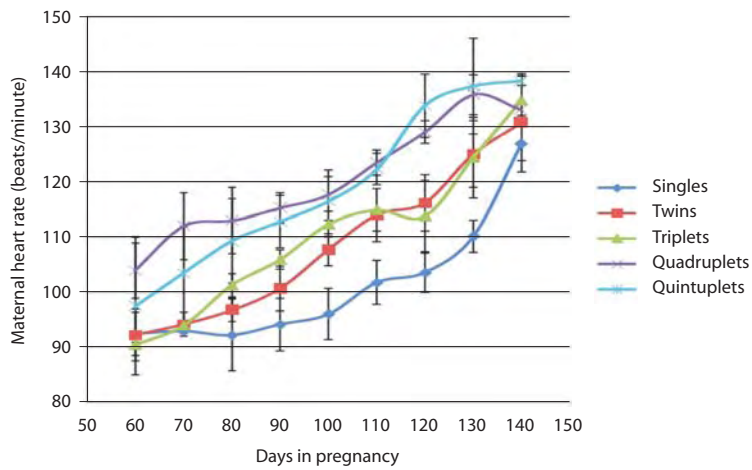

Figure 2. Maternal heart rate during pregnancy in Afec–Assaf ewes carrying singles (n = 18), twins (n = 13) triplets (n = 10), quadruplets (n = 5) and quintuplets (n = 4)

Table 6. Least squares means ± SEM for heart rate in Assaf and Afec–Assaf ewes according to their genotype at the *FecB* locus

Days of pregnancy		80	90	100	110	120	130
FecB genotype	*n*	Heart rate (beats/minute)					
BB	7	97 ± 5	95 ± 4^{a}	98 ± 5^{a}	102 ± 5^{a}	107 ± 5^{a}	116 ± 5^{a}
B+	27	104 ± 2	106 ± 2^{b}	110 ± 2^{b}	116 ± 2^{b}	121 ± 2^{b}	129 ± 3^{b}
++	16	101 ± 4	111 ± 4^{b}	117 ± 4^{b}	121 ± 4^{b}	122 ± 4^{b}	125 ± 5^{ab}

$^{a\,b}$ Within columns, means with different superscript letters differ significantly ($P < 0.05$).

respectively. A comparison of average milk production of B+ and ++ BC4–BC6 ewes in that study suggested that carrying the B allele negatively affects milk production, either directly or indirectly.

Economic assessment of the breeding work

Economic assessment of a breeding program aimed at increasing prolificacy by using the Booroola mutation should consider both the economics of the R&D process up until the appropriate breeding material becomes available for distribution and, once the breeding material is ready, the economics of using the new breeding material by commercial flocks.

From the performance point of view, the recommended genotype for the commercial flocks is B+. Changing an Awassi or Assaf flock to Afec–Awassi or Afec–Assaf, respectively, usually starts by introducing BB rams or by inseminating the ewes with BB semen. The next step is to raise B+ daughters as replacements and, further on, mate B+ ewes with non-carrier rams and select their B+ ewe lambs as replacements following genotyping for the *FecB* gene.

In Israel, as in most Middle Eastern countries, selling lambs is the main source of income for non-dairy flocks. The flock's revenue should cover fixed costs, for example for ewe maintenance, labour and housing, as well as non-fixed costs such as that of feeding the growing lambs. An economic assessment of using Afec strains should take into consideration, on the one hand, the additional revenue from selling more lambs and, on the other, the additional fixed costs for genotyping the mothers and the non-fixed costs associated with raising more progeny of B+ ewes.

The economic aspects of introducing the Booroola mutation into non-dairy flocks managed under semi-intensive to intensive conditions was addressed by Spharim and Gootwine (1997). It was concluded that, in the range of the economic parameters tested, the genetic change is profitable in most scenarios, and the higher the ratio between lamb price and feed costs, the greater the benefit of using the Booroola mutation. The dramatic increase in grain prices in the years 2006–08 severely affected the economics of intensive flocks. Under the new economic constraints, use of the Afec strains has become, in many cases, a prerequisite for sustainable production rather than simply a means of increasing flock profitability.

The economics of introgression of the Booroola mutation into Awassi and Assaf dairy flocks was addressed by Gootwine et al. (2001). Afec ewes produce less milk than their contemporary Awassi or Assaf ewes. Thus, the increase in revenue per ewe from selling more lambs from dairy flocks is followed by a decrease in the income from selling milk. However, under a milk quota policy, which is customary in Israel, the use of Afec sheep takes on a new dimension. As flock milk production cannot be increased due to the quota policy, producing more lambs is the only way to increase revenue. Using Afec ewes may lead to higher revenue, not only because of the higher prolificacy of Afec ewes, but also because the number of animals in the flock can be increased without deviating from the flock's milk quota.

Introgression of the Booroola mutation into semi-extensive local Awassi flocks

As indicated, about half of the national flock in Israel belongs to the hardy but low-prolific local Awassi breed. Most of the local Awassi sheep are kept by Bedouin farmers in the Negev, the southern arid part of the country, under traditional semi-extensive management where animals rely for about half the year on pasture. Decreases in recent years in the availability of grazing land have forced Bedouin growers to spend more on feeding their animals by purchasing costly grains and fodder, rendering their sheep production nearly unprofitable.

Traditionally, Bedouin farmers prefer the Awassi phenotype, and introducing the Afec–Awassi into the semi-extensive flocks may therefore be the best way of increasing their profitability. Ewes of more highly prolific genotypes need proper feeding year-round, and intensive care must be given to both the pregnant ewes and the lambs born in large litters. The extent to which traditional Bedouin farmers are willing and able to change their management methods is currently being studied.

A study, launched in January 2007, is aimed at following the incorporation of the Afec–Awassi into Bedouin flocks. This is being done by monitoring changes in both flock productivity and socioeconomic parameters associated with sheep production (Gootwine et al. 2009). As the transition from

extensive to intensive sheep production becomes a necessity, not only in Israel but also in neighbouring countries, similar projects, in which the Afec–Awassi is tested along with the local Awassi, are being conducted in other locations in the Middle East (Herold et al. 2009).

Conclusions

Introgression of the Booroola mutation offers, under specific economic conditions, a fast way of increasing the prolificacy and hence profitability of sheep flocks, as has been experienced with the Afec–Awassi and the Afec–Assaf strains in Israel. This is regardless of the direct and indirect negative effects that carrying the Booroola mutation has on some economic traits. To exploit the full benefits of carrying the Booroola mutation, further R&D is needed, aimed at increasing ewes' uterine capacity as well as reducing the adverse effects of carrying the Booroola mutation on productive traits like growth rate and milk production. Working with the Booroola mutation offers an opportunity to intensify sheep production, a move that may facilitate the adaptation to socioeconomic changes in traditional sheep-producing communities.

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References

Bazer F.W., Clawson A.J., Robison O.W. and Ulberg L.C. 1969. Uterine capacity in gilts. Journal of Reproduction and Fertility 18, 121–124.

Davis G.H. and Johnstone P.D. 1985. Ovulation response to pregnant mares' serum gonadotrophin in prepubertal ewe lambs of different Booroola genotypes. Animal Reproduction Science 9, 145–151.

Epstein H. 1985. The Awassi sheep with special reference to the improved dairy type. Animal Production and Health Paper no. 57. FAO: Rome.

Eyal E., Goot H., Kali J., Amir D., Rosenberg M., Schindler H., Davidson M., Tamarin R., Foote W.C., Matthews D.H. and Hogue D.E. 1984. The promotion of prolific strains of sheep by nutritional and managerial means. Report submitted to the United States–Israel Binational Agricultural Research and Development Fund. The Volcani Center: Bet Dagan, Israel.

Fogarty N., Hopkins D. and van der Ven R. 2000. Lamb production from diverse genotypes. 1. Lamb growth and survival and ewe performances. Animal Science 70, 135–145.

Gootwine E. 2006. Increasing prolificacy of the Awassi and the Assaf breeds using the FecB (Booroola) gene. Proceedings of the 57th Annual Meeting of the European Association for Animal Production, Antalya, Turkey.

Gootwine E., Al Baqain A., Abu Siam M., Leibovich H., Herold P., Reicher S. and Valle Zárate A. 2009. Impact of introducing new technologies on Bedouin sheep production in the Negev in Israel. Options Méditerranéenes (in press).

Gootwine E., Bor A., Braw-Tal R. and Zenou A. 1995. Reproductive performance and milk production of the improved Awassi breed as compared with its crosses with the Booroola Merino. Animal Science 60, 109–115.

Gootwine E., Braw-Tal R., Shalhevet D., Bor A. and Zenou A. 1993. Reproductive performance of Assaf and Booroola–Assaf crossbred ewes and its association with plasma FSH levels and induced ovulation rate measured at prepuberty. Animal Reproduction Science 31, 69–81.

Gootwine E. and Goot H. 1996. Lamb and milk production of Awassi and East-Friesian sheep and their crosses under Mediterranean environment. Small Ruminant Research 20, 255–260.

Gootwine E. and Pollott G.E. 2000. Factors affecting milk production in improved Awassi dairy ewes. Animal Science 71, 607–615.

Gootwine E., Reicher S. and Rozov A. 2008. Prolificacy and lamb survival at birth in Awassi and Assaf sheep carrying the FecB (Booroola) mutation. Animal Reproduction Science 108, 402–411.

Gootwine E., Rozov A., Bor A. and Reicher S. 2006. Carrying the FecB (Booroola) mutation is associated with lower birth weight and slower post-weaning growth rate for lambs, as well as a lighter mature bodyweight for ewes. Reproduction, Fertility, Development 18, 433–437.

Gootwine E., Spencer T.E. and Bazer F.W. 2007b. Litter-size-dependent intrauterine growth restriction in sheep. Animal 1, 547–564.

Gootwine E., Yossefi S., Zenou A. and Bor A. 1998. Marker assisted selection for FecB carriers in Booroola Awassi crosses. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production.

University of England, Armidale, New South Wales, Australia.

Gootwine E., Zenou A., Bor A., Yossefi S., Rozov A. and Pollott G.E. 2001. Genetic and economic analysis of introgression the B allele of the FecB (Booroola) gene into the Awassi and the Assaf dairy breeds. Livestock Production Science 71, 49–58.

Herold P., Gootwine E., Abulkahliq A., Jawasreh K. and Valle Zárate A. 2009. Evaluation of the performance of improved Awassi strains under a range of sheep farming systems in the Middle East. Options Méditerranéenes (in press).

Kleemann D.O. and Walker S.K. 2005. Fertility in South Australian commercial Merino flocks: source of reproductive wastage. Theriogenology 63, 2075–2088.

Kopcha M. and Ahl A.S. 1989. Experimental uses of flunixin meglumine and phenylbutazone in food-producing animals. Journal of the American Veterinary Medical Association 194, 45–49.

Leymaster K.A. and Johnson R.K. 1994. Second thoughts on selection for components of reproduction. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada.

Montgomery G.W., Crawford A.M., Penty J.M., Dodds K.G., Ede A.J., Henry H.M., Pierson C.A., Lord E.A., Galloway S.M., Schmack A.E., Sise J.A., Swabrick P.A., Hanrahan V., Buchanan F.C. and Hill D. 1993. The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. Nature Genetics 4, 410–414.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y., Chitour N. and Elsen J.M. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences USA 98, 5104–5109.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the prolificacy of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworth: London, UK.

Pollott G.E. and Gootwine E. 2004. Reproductive performance and milk production of Assaf sheep in an intensive management system. Journal of Dairy Science 87, 3690–3703.

Smith P., Hudson N.L., Corrigan K.A., Shaw L., Smith T., Phillips D.J. and McNatty K.P. 1996. Effects of the Booroola gene (FecB) on bodymass, testis development and hormone concentrations during fetal life. Journal of Reproduction and Fertility 108, 253–261.

Smith P., O W.S., Hudson N.L., Shaw L., Heath D.A., Condell L., Phillips D.J. and McNatty K.P. 1993. Effects of the Booroola gene (FecB) on body weight, ovarian development and hormone concentrations during fetal life. Journal of Reproduction and Fertility 98, 41–54.

Souza C.J., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology 169, R1–R6.

Spharim I. and Gootwine E. 1997. Economic evaluation of breeding for higher prolificacy in Awassi flocks. Options Méditerranéenes 33, 157–61.

Rummel T., Valle Zárate A. and Gootwine E. 2006. The world wide gene flow of the Improved Awassi and Assaf sheep breeds from Israel. Pp. 305–358 in 'Gene flow in animal genetic resources: a study on status, impact and trends', ed. by A. Valle Zárate, K. Musavaya and C. Schafer. GTZ, German Federal Ministry for Economic Cooperation and Development (BMZ).

Walling G.A., Dodds K.G., Galloway S.M., Beattie A.E., Lord E.A., Lumsden J.M., Montgomery G.W. and McEwan J.C. 2000. The consequences of carrying the Booroola fecundity (FecB) gene on sheep live weight. Proceedings of the British Society of Animal Science, Midlothian, UK.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Wu G., Bazer F.W., Wallace J.M. and Spencer T.E. 2006. Intrauterine growth retardation: implication for animal science. Journal of Animal Science 84, 2316–2337.

Zamir S., Rozov A. and Gootwine E. 2009. Treatment of pregnancy toxaemia in sheep with flunixin meglumine. The Veterinary Records 165, 265–266.

Biological and economic consequences of introgression of the *FecB* gene into the French Mérinos d'Arles sheep

J. Teyssier[1], L. Bodin[2], C. Maton[1], P.M. Bouquet[3] and J.M. Elsen[2,4]

Abstract

The information analysed comes from three datasets collected on an experimental farm ('Le Merle') where the Booroola gene was first introgressed into the Mérinos d'Arles breed in 1983, and a private farm where Booroola-carrier ewes were bred for production. Reproduction (ovulation rate, litter size, survival rate) and production (body weight, carcass weight, dressing percentage) traits were compared between B+ and ++ animals as well as controls from the Mérinos d'Arles breed. The increase in ovulation rate caused by the Booroola gene had a dramatic effect on litter size at birth, and consequently changed the survival and growth of the lambs. Globally, B+ ewes were able to produce about 50–65% extra lambs, the result being consistent across comparisons. Efforts are now under way by the breeders to exploit the gene at a higher level.

Historical introduction

Copies of the Booroola gene were imported three times by the French National Institute for Agricultural Research (INRA) for experimental purposes. The first importation occurred in 1982, when five Booroola rams from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) flock, Armidale, Australia, were given to the institute. The second resource, imported in 1986, consisted of frozen semen of six BB and three ++ rams that came from New Zealand. The third introduction of Booroola genetic material originated from the United Kingdom, where semen was collected from two BB rams and sent to France in 1989.

Two introgression processes of the *FecB* allele were organised by INRA to non-carrier breeds differing in their prolificacy: the Mérinos d'Arles (MA), a lowly prolific breed, and the highly prolific Romanov breed. The processes started in 1983 and 1986, respectively. Different objectives were assigned to the experiments: inferring the Booroola genotype of the five rams from the CSIRO (from the ovulation rate (OR) of daughters born to MA dams); exploring the physiological effects of the *FecB* allele in two polygenic environments; evaluating the economical impact of the gene in the MA background; and trying to localise and identify the Booroola locus.

As described in Boomarov (1991), the introgression into the MA breed was performed in a single flock belonging to the École Nationale Supérieure Agronomique de Montpellier ('Le Merle' farm). In a first period (1983–90) a succession of backcrosses, up to the 87.5% MA level, was realised. At each gener-

[1] INRA/CIRAD/Agro-M, UMR868 Élevage des Ruminants en Régions Chaudes, F-34060 Montpellier, France
[2] INRA, UR631 Station d'Amélioration Génétique des Animaux, F-31320 Castanet-Tolosan, France
[3] INRA/Agro-M, Domaine du Merle, Route d'Arles, 13300 Salon de Provence, France
[4] Corresponding author: Jean-Michel.Elsen@toulouse.inra.fr

ation the males (pure Booroola, F1, 75%, 87.5%) were progeny tested, measuring ORs of a set of daughters during three consecutive cycles at the end of their first year of age. The genotypes were assigned according to the method of Elsen and Le Roy (1991). At the end of this first period, intercrosses between 87.5% males and females provided BB animals.

A second period (1991–98) then started with three major objectives: (a) creation, selection and maintenance of the nucleus of homozygous Booroola carriers possessing at least 87.5% MA blood; (b) production of experimental animals both for physiological and molecular genetics studies; and (c) implementation of a detailed biological and economical comparison of prolific (B+ or F1 Romanov–MA) vs. non prolific (87.5% ++ or pure MA) ewes bred in the context of a terminal cross with Ile de France rams. All the objectives of the second period were accomplished: a small flock of about 100 BB ewes was created; the Booroola mutation was identified in 2001 by INRA (Mulsant et al. 2001) and others (Souza et al. 2001; Wilson et al. 2001); and the B allele proved to be of economical value in the MA farming conditions (Teyssier et al. 1998).

During a third period (1999–2008) our aims were mostly the production of experimental animals for research aimed at understanding the physiological role of the BMPR1B gene in ovulation control, the maintenance of the BB nucleus flock, the dissemination of the B gene to a few private farms, and the use of B+ ewes for lamb production on Le Merle experimental farm. Enough information about the productivity of B+ vs. ++ MA females mated to Ile de France rams was collected in 2005 and 2006 to allow a second sensible comparison of these genotypes. This paper gives a synthesis of results from datasets of these three periods.

Material and methods

Close to 30,000 ORs were measured during the introgression process of the Booroola gene into the MA breed, in order to sample animals for various experiments and for the maintenance of the BB nucleus. Results of part of those measurements were reported by Bodin et al. (1991) and Teyssier et al. (2003). In addition to that information, results from additional measurements obtained during 2000–08 are also reported here.

Three datasets were analysed to evaluate the differences between B+ and ++ ewes. The first (Le Merle 1), already presented in Teyssier et al. (1998) and Teyssier et al. (2003), was collected at Le Merle farm during the second period, as described above, and more precisely over 3 years from 1993 to 1995. A total of 417 B+ and 269 ++ ewes from the introgression program were compared. The genotypes were inferred from OR measurements and pedigree information. To check the quality of the backcrossing process, a subset of 299 pure MA animals was also included in the comparison. All the ewes were mated to Ile de France rams. The second dataset (Le Merle 2) comes also from Le Merle farm and corresponds to data recorded in 2005–06. Results from 329 pure MA and 86 B+ ewes mated with Ile de France rams were included in the analysis. The genotypes of the females were known from molecular information (markers of the Booroola locus or genotype at the BMPR1B). The third dataset (Raymond) was organised from performance recorded at a private farm (Raymond farm) from 2000, in which the Booroola gene was used for demonstration purposes. The studied population included 71 B+ and 21 ++ females born on the farm after insemination of pure MA ewes with semen of BB, B+ and ++ sires.

Under Le Merle conditions, all females were run as one group under the extensive management of the Mediterranean Crau plain of southern France: natural matings were conducted in spring (from end of April to mid June); transhumance to alpine pasture was done in summer (June to September); and lambing took place in autumn (October and November). Ewe lambs were first mated in spring at approximately 18 months of age. In this system there is only one mating period per year.

Natural matings were made following the 'ram effect'. About 14 days after the introduction of vasectomised rams, all females were exposed to Ile de France rams. At lambing the ewes were identified and their lambs were weighed and tagged. In the first dataset (Le Merle 1) rams were harnessed and ORs of ewes marked within a week were assessed by laparoscopy during the following week. Dates of oestrus, laparoscopy and lambing were used to check that the ovulation record corresponded to the fertilisation cycle. When the litter size (LS) exceeded the OR, the latter was adjusted to the LS. From multiple births, one or two lambs were left with their dam according to the decision of the experienced staff. The remaining lambs were artificially reared. Weaning was at 2.5–3 months of age

for lambs suckled by their dam and at 12–15 kg for artificially reared lambs. In the other datasets (Le Merle 2 and Raymond) the use of artificial rearing was strongly limited or abandoned, and compensated by lamb fostering.

All lambs were weighted three or four times at 3-weekly intervals according to the French national performance recording scheme. Body weight and carcass weight were recorded at slaughter for male lambs born in 1994 and 1995 (Le Merle 1), and all lambs born in 2005 (Le Merle 2), in order to determine the dressing percentage.

At Raymond farm the young females were mated for the first time at 1 year of age in autumn for lambing in spring, which is the most favourable period. They were mated for the second time in May at the main mating period of the flock. For adult ewes the reproductive rhythm was generally one mating/year, in spring. However, ewe lambs and adult ewes that were not pregnant in spring were given a chance to be mated in autumn.

Results

Ovulation rate

Table 1 reports the mean OR corresponding to fertile matings, as observed in 1993–95 during the comparison of pure MA ++ and B+. The data show that the Booroola gene doubles the OR in the MA breed, with an increase of about 1.2 ovulations. As described in Teyssier et al. (1998), the variability in OR was much higher in Booroola carriers than in non-carriers.

Figure 1 presents data regarding this variability in OR in BB and B+ young (1-year-old) and adult MA ewes.

Reproduction data

The reproductive performances of the different genotypes at the *FecB* locus are presented in Table 2. The general picture is rather consistent across the different datasets, with generally lower performances in the private farm conditions. The Booroola heterozygous females were more fertile (3–10 points) and prolific (0.7–1 extra lambs at birth) than the non-carriers. As expected with this genotype, the proportion of LSs exceeding 2 (triplets or more) was high—over 29% in Le Merle experiments, which means that in this case more that 50% of lambs were born as triplets or more. However, a large variability in OR distribution among sire families has been found. As an example, the distribution difference between two particular sire families having similar mean ORs was significant ($P = 0.037$) (Table 3). Understanding the genetic basis of these phenotypes would be useful.

Table 1. Mean ovulation rate (OR) of lambing ewes (Le Merle 1, 1993–95)

	MA	++	B+
No. of ewes lambing	269	237	380
Mean OR at mating	1.27	1.37	2.46
Corrected OR[a]	1.30	1.38	2.57

[a] When litter size was higher than the corresponding OR, the OR was corrected to the observed litte size.

It must be emphasised that the birth weights were lighter for lambs born to B+ dams compared to ++ dams. This difference was higher in the second set (Le Merle 2). Consequently, more lambs were artificially reared or fostered, and the mortality was higher for the progeny of B+ ewes.

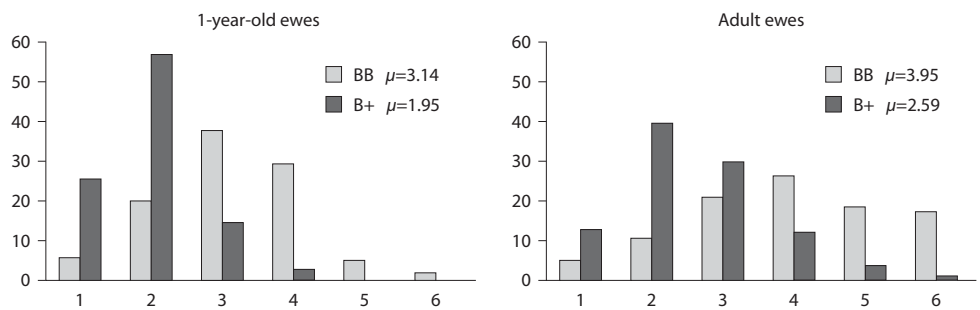

Figure 1. Distribution of ovulations in BB and B+ Mérinos d'Arles ewes (Le Merle 2, 2000–08). Note: μ = estimated population mean.

Globally, heterozygous carrier females were able to produce 0.5–0.7 more lambs than non-carrier females (about 50–65% extra lambs).

Production data

Information on growth ability and meat yield production of the animals from the two experiments carried out at Le Merle farm is reported in Table 4. The results were consistent between experiments. The lambs were sold at similar weight, with a very limited difference between sons of carrier and non-carrier rams (less than 1 kg). Average carcass weights did not differ between genotypes but were a little higher for the most recent comparison. On average, male lambs from B+ dams needed 8–15 days more than those from pure MA to reach the fixed slaughter live weight.

The comparison between pure MA and ++ females demonstrated that the 87.5% and more animals can be considered as pure bred, at least in terms of reproductive and productive abilities.

Discussion and conclusions

Although some bias could be suspected due to selection for hypo- or hyper-ovulating ewes during the introgression process of the Booroola gene, the results of genotype comparisons did not change with the discovery of the causal mutation, which allows an accurate identification of the different genotypes. At the farm level the mutation of the BMPR1B gene has a direct effect on OR but indirectly changes other traits as a consequence of this increase. Therefore, the increase in prolificacy induced by the

Table 2. Reproductive performance of Pure Mérinos d'Arles (MA), ++ and B+ introgressed Booroola to Mérinos d'Arles ewes for three datasets (LM1, LM2, R)

	MA		++		B+		
	LM1	LM2	LM1	R	LM1	LM2	R
No. of ewes joined	299	329	269	21	417	86	71
% of lambing ewes	90.0	86.3	88.1	71.4	91.1	95.3	81.7
Litter size at birth	1.19	1.26	1.21	1.22	2.10	2.23	1.95
% of litter size:							
1	80.7	75.0	79.8	78.5	22.4	22.0	28.4
2	19.3	24.3	19.8	21.5	48.1	40.2	50.0
3 or 4		0.7	0.4		29.5	37.8	21.6
Lamb birth weight (kg):							
single	4.4	5.0	4.4	n.a.	4.1	4.3	n.a.
twins	3.8	4.1	3.7	n.a.	3.3	3.6	n.a.
triplets		2.6		n.a.	2.8	3.0	n.a.
% of artificial rearing	6.9		8.2	0.8	34.1	<1.0	2.2
% of fostering	n.a.	1.3	n.a.		n.a.	6.2	3.9
Lamb mortality (%):							
within 2 days	6.2	5.1	3.1	1.9	11.8	7.3	11.3
within 70 days	8.4	13.2	5.6	4.4	18.4	19.3	19.0
Litter size alive at 70-days	1.09	1.10	1.14	1.16	1.72	1.81	1.58

LM1 = data collected at Le Merle INRA farm, 1993–95
LM2 = data collected at Le Merle INRA farm, 2005–06
R = Raymond private farm, 2000–07

Table 3. Distribution of ovulation records in two sire families of BB sires having similar mean ovulation rates (OR)

Sire	No. of records	OR = 1	OR = 2	OR = 3	OR mean
904369	44	6.8%	84.1%	9.1%	2.02
904392	36	16.7%	58.3%	25.0%	2.08

Booroola gene was associated with the same negative output as those which come from a classical polygenic selection:

- Higher LS obliges artificial rearing of some multiple-born lambs. However, good ewe management using lamb fostering allows the need for artificial rearing to be minimised, as can be observed in the Le Merle 2 dataset where more than 50% of lambs were born as triplets and the percentage of lambs artificially reared was less than 1%.
- Higher prolificacy was associated with a decrease in the average lamb's birth weight, since lambs born as multiples had lower weight at birth than single lambs.
- Lamb mortality increased with increase in prolificacy.
- The growth rate of lambs born in large litters, up to 30 days of age and even later, was slightly lower than the growth weight of singletons. Thus, these lambs needed a few days more to be ready for slaughter at a fixed live body weight.

These negative points increase production costs directly and indirectly. However, this was widely counterbalanced by the higher prolificacy (giving more kilograms of lamb meat produced per ewe) and the fact that these ewes had a genetic background similar to ++ pure MA, which ensured their ability to cope with harsh environments.

In spite of the large benefit it could bring to breeders, the Booroola gene was not taken up in commercial populations in France, even after the discovery of the causal mutation that allows a fast and accurate genotyping of carrier animals. A similar situation was observed elsewhere in the world with this gene, as well as with other known major genes for OR (Inverdale, Hanna, Galway etc.). This may be due to the fact that, during the period 1984–2002, the importance of high productivity was almost banished in agricultural and livestock European policies, as breeders were obtaining subsidies from the European Community of up to 21 euros per head, whatever their production.

However, economic studies showed that the variability in numeric productivity between flocks explained more the variability in breeder profit than any other traits (lamb growth rate, carcass quality etc.). Although there has been a recent revival of interest in livestock productivity, the demand for the Booroola gene in France remains limited.

For the Booroola gene, as well as for the other major genes controlling OR, homozygous carrier genotypes are unfavourable. They are either too prolific (Booroola, Lacaune) and far beyond the threshold of the economic optimum, or sterile (all BMP15 mutations—Inverdale, Hanna, Belclare, Galway, Lacaune, Rasa Aragonesa—and the GDF9 mutation). The optimum management of such genes

Table 4. Productive performances for two datasets of pure Mérinos d'Arles (MA), ++ and B+ introgressed Booroola to Mérinos d'Arles lambs sired by Ile de France rams

	MA		++	B+	
	LM1	LM2	LM1	LM1	LM2
Male:					
no. of lambs followed	80	71	65	174	28
sale weight (kg)	37.9	37.7	37.0	36.9	36.9
age at sale (days)	124	113	127	132	128
cold carcass weight (kg)	16.8	17.1	16.1	16.2	17.1
dressing percentage	44.3	45.1	43.7	44.2	45.8
Female:					
no. of lambs followed		76			32
sale weight (kg)		31.7			30.1
age at sale (days)		115			132
cold carcass weight (kg)		14.7			14.0
dressing percentage		46.4			45.9

LM1 = Data collected at Le Merle INRA farm, 1993–95
LM2 = Data collected at Le Merle INRA farm, 2005–06

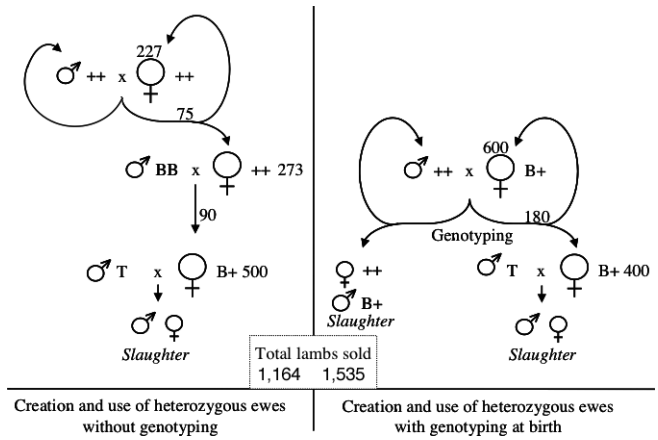

Figure 2. Number of each type of ewe and replacement ewe lambs, matings and lambs at slaughter in two flocks of 1,000 adult ewes with and without genotyping at birth

within a flock or within a population is not easy. There are two ways to avoid the procreation of homozygous carrier ewes, depending on the possibility or otherwise of genotyping. Without genotyping, heterozygous ewes might come from mating homozygous carrier sires with homozygous non-carrier ewes which have to be replaced from a group of homozygous non-carrier ewes (Figure 2). With genotyping, all adult ewes of the flock can be heterozygous. They are mated either with non-carrier sires to produce heterozygous replacements after genotyping or with terminal sires. The total number of lambs produced is then much higher and offsets the genotyping cost.

The use of the Booroola gene in MA on private farms should increase in the near future thanks to the efforts of breeder organisations. Some breeders will act as multipliers to produce heterozygous females from pure MA ewes mated to homozygous BB sires from Le Merle experimental farm. These B+ ewe lambs will be sold at weaning to users, who will cross them with terminal sires to produce lambs to slaughter.

In order to keep the Le Merle BB nucleus connected to the MA population, at each generation a few homozygous BB females are inseminated with the semen of Elite Mérinos d'Arles sires. B+ sons are bred and mated to BB females to produce replacement BB sires.

References

Bodin L., Cornu C., Elsen J.M., Molenat G. and Thimonier J. 1991. The effect of Booroola genotype on some traits in a Merinos d'Arles flock. Pp. 371–379 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. 2nd International Workshop, Toulouse, 16–18 July 1990.

Boomarov 1991. The Booroola gene in France. In 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. 2nd International Workshop, Toulouse, 16–18 July 1990. Les Colloques de l'INRA vol. 57.

Elsen J.M. and Le Roy P. 1991. Genotype determination at major locus in a progeny test design. In 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. 2nd International Workshop, Toulouse, 16–18 July 1990. Les Colloques de l'INRA vol. 57.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y., Chitour N. and Elsen J.M. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences of the United States of America 98(9), 5104–5109.

Souza C.J.H., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. Journal of Endocrinology 169(2), R1–R6.

Teyssier J., Elsen J.M., Bodin L., Bosc P., Lefevre C. and Thimonier J. 1998. Three-year comparison of productivity of Booroola carriers and non-carrier

Merino d'Arles ewes. Pp. 117–120 in 'Sheep and goats (fibre); sheep and goats (meat and milk); poultry; horses; buffaloes'. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production', vol. 24, Armidale, Australia, 11–16 January 1998.

Teyssier J., Elsen J.M., Bodin L., Bouquet P.M., Mulsant P. and Thimonier J. 2003. The Booroola gene in Merinos d'Arles sheep: introduction of the gene (FECB), productivity of FECB heterozygous carrier ewes under farm conditions. International Workshop on Major Genes and QTL in Sheep and Goat, Toulouse, France, 8–11 December 2003.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64(4), 1225–1235.

Biological and economical consequences of the *FecB* mutation in Indonesian Thin Tail sheep

I. Inounu[1] and A. Priyanti[1]

Abstract

The Javanese Thin Tail (JTT) breed, the predominant sheep breed in West Java, is one of the Indonesian thin-tailed sheep breeds. The JTT is also known as the Indonesian prolific sheep. It was found that its high prolificacy is due to carrying the *FecB* Booroola mutation. The origin of the mutation in the JTT is not known—JTT sheep could have acquired the Booroola FecB gene directly from Garole sheep from India or via Merinos from Australia. Performance studies of JTT sheep showed that ewes with the $FecB^B/FecB^+$ genotype had the highest gross margin when treated with a high level of feeding management, followed by $FecB^B/FecB^B$ genotype and $FecB^+/FecB^+$. In situations where a low level of feeding management was practised, ewes carrying the *FecB* gene did not show their superiority. Crosses between the JTT and St Croix rams or Mouton Charollais rams were evaluated. Results from those studies showed that crossbred sheep were more profitable as they had a higher mature body weight, were faster in reaching a standard slaughter body weight of 35 kg (440 days), and were more efficient in both biological and economic terms.

Introduction

The sheep population in Indonesia is about 9.8 million, with 92% found on the island of Java (Directorate General of Livestock 2008). Sheep in Indonesia can be classified according to their tail type as thin- or fat-tailed sheep. Most of the Indonesian Thin Tail (ITT) sheep found in West Java are known as Garut or Priangan sheep. However, some scientists have named them Javanese Thin Tail (JTT) sheep. ITT sheep are also raised on the island of Sumatra, where they are called Sumatran Thin Tail (STT) sheep.

ITT sheep are not seasonal and ewes can express reproductive activity throughout the year. It was found that, during the dry season (April–September), the conception rate of JTT sheep, but not of STT sheep, is a little lower compared to the conception rate during the rainy season (Iniguez et al. 1991; Setiadi et al. 1995). Under intensive conditions the mean lambing interval of JTT sheep is around 203 days (Fletcher et al. 1985), while Iniguez et al. (1991) reported a mean lambing interval of 201 days in STT sheep. Bradford et al. (1986) reported on the evidence for segregation in JTT sheep of a gene with a large effect on ovulation rate (OR) and litter size (LS), with heritability for LS of 0.5 and repeatability for OR of 0.6. Segregation analysis of OR from ewes and their daughters indicated that prolificacy in JTT is indeed affected by a major gene named $FecJ^F$ (Bradford et al. 1991). The genotype classification was based on Bradford et al. (1991) and Inounu et al. (1993) criteria, as follows:

- $FecJ^+/FecJ^+$: ewes which never have more than 2 corpora lutea (CL) or lambs born, and with ≥ 3 records with a mean of ≤ 1.7

[1] Indonesian Center for Animal Research and Development, Jl Raya Pajajaran Kav E 59, Bogor 16151, Indonesia; i_inounu@yahoo.com; atienpriyanti@ yahoo.com

- $FecJ^F/FecJ^+$: ewes with at least one record (CL or LS) of 3, but never > 3 or with a high frequency of 2s (a mean of 3 or more independent records > 1.7)
- $FecJ^F/FecJ^F$: ewes with one or more records ≥ 4.

This genotype classification system requires time for multiple recordings on ewes and a sound recording system, but the results have been found to be in good agreement with blood DNA tests. Davis et al. (2002) found that JTT sheep carry the Booroola gene previously found also in Garole sheep in India. It is unknown whether JTT sheep acquired the Booroola gene from Garole sheep imported into Indonesia by Arabian traders about two centuries ago, or via Merino sheep imported from Australia by Dutch colonials at the beginning of the 18th century. JTT sheep are well adapted to the local Indonesian conditions, especially in West Java, as this breed is resistant to internal parasites (Fletcher et al. 1985; Roberts et al. 1997).

Females reach puberty at 6–8 months of age (Sutama et al. 1988; Sutama 1992), and can express oestrus all year long so that lambing can happen at any time during the year. Postpartum oestrus occurs 1 month after lambing (Setiadi et al. 1995). Under rural conditions JTT sheep are generally raised in small-scale units and provide additional sources of income for farmers (Bradford and Inounu 1996). Consequently, farmers pay less attention to their animals, especially in providing feed. Under these conditions prolificacy is not advantageous to farmers, since the pre-weaning lamb mortality rate is high and the lamb growth rate is relatively low (Inounu et al. 1999). Therefore, to reach the desirable slaughter weight of 35 kg, the progeny of prolific ewes need a longer time and a higher level of input than lambs of non-prolific sheep. To overcome this limitation, cross-breeding studies between JTT and heavier breeds were conducted. These involved mating St Croix hair sheep (H) rams with JTT ewes (J), resulting in the HJ cross; and insemination of JTT ewes with frozen semen of Mouton Charollais (M), resulting in the MJ cross and making MHJ (MJ rams × HJ ewes) sheep and HMJ (HJ rams × MJ ewes) sheep.

FecJ/FecB mutations and their effects

Davis et al. (2002) suggested that the *FecJ* locus in JTT sheep should be designated as *FecB* and, similarly, that the $FecJ^F$ allele should be designated $FecB^B$ in recognition of the discovery that the Javanese and Booroola sheep carry the same mutation. Table 1 shows the reproductive traits of JTT ewes carrying the *FecB* gene. Homozygous carriers of the mutation are designated $FecB^B/FecB^B$, non-carriers as $FecB^+/FecB^+$ and heterozygous carriers as $FecB^B/FecB^+$. The effect of one copy of the $FecB^B$ allele on OR in the JTT is about 0.93, which is less than the 1.65 reported by Piper et al. (1985) in other breeds. The difference could be due to a lower prolificacy potential from the background genotype, to environmental factors such as the relatively low nutritional value of the tropical forages available to these ewes, or to a combination of these factors.

LSs of ewes that were non-carrier, heterozygous or homozygous for $FecB^B$ have been measured at 1.22, 2.02 and 2.50 respectively (Inounu et al. 1999). In this study one copy of the $FecB^B$ gene increases LS by 0.81, and the second copy by 0.53. $FecB^B/FecB^B$ ewes produced 84% of multiple births (2–5 lambs), while $FecB^+/FecB^+$ ewes produced only 17% of twins. Unfortunately, the increase in LS was not accompanied by an equivalent increase in litter weight. As a result, the survival rate of lambs decreases from non-carrier, through heterozygous, to homozygous. Based on total litter weaning weight, there is no advantage in having homozygous ewes in either low or medium nutritional conditions, as reported by Inounu et al. (1999).

Table 1. Effect of carrying the Booroola mutation on reproductive traits of Javanese Thin Tail ewes

Genotype	OR	LS	LWT (kg)	SURV (%)	WWT (kg)
$FecB^+/FecB^+$	1.19	1.22	3.10	84.3	12.6
$FecB^B/FecB^+$	2.12	2.02	3.88	71.9	14.4
$FecB^B/FecB^B$	2.96	2.50	4.06	59.2	14.7

OR = ovulation rate (Inounu et al. 1997); LS = litter size at birth; LWT = litter weight at birth; SURV = lamb survival at 90 days of age; WWT = litter weaning weight at 90 days of age
Source: Inounu et al. (1999)

Table 2 indicates that ewes of the three genotypes ($FecB^{+}/FecB^{+}$, $FecB^{B}/FecB^{+}$ and $FecB^{B}/FecB^{B}$) are very sensitive to level of feeding. Homozygous ewes have too many lambs for Indonesian farming conditions, heterozygous ewes would be best under good feed conditions, and non-carrier ewes would be best under poorer farming conditions. Inounu and Soedjana (1998) reported an economic analysis of performance of JTT sheep, showing that ewes with the $FecB^{B}/FecB^{+}$ genotype had the highest gross margin (6% higher than JTT ewes) when treated with a high level of feeding management, followed by $FecB^{B}/FecB^{B}$ genotype (5% higher than JTT ewes). Control of the genetic make-up of the flock at this locus is therefore important in achieving an efficient match between the genetic potential and its management level (Bradford and Inounu 1996). In Indonesia it is suggested to use ewes with $FecB^{B}/FecB^{+}$ genotype and to breed them with non-prolific rams with high growth rate (as the terminal sire breed) to produce commercial sheep or a composite breed.

Table 2. Litter weaning weight (WWT (kg)) of Javanese Thin Tail sheep under different feeding conditions

Genotype	Feeding condition		
	Low	Medium	High
$FecB^{+}/FecB^{+}$	9.9	11.5	16.3
$FecB^{B}/FecB^{+}$	10.1	13.5	19.4
$FecB^{B}/FecB^{B}$	10.2	14.3	19.4

Source: Inounu et al. (1999)

Results of crossbreeding experiments

In general, breeds of livestock developed in temperate climates do not perform well in high-rainfall tropical climates like Indonesia. Where temperate breeds are used, they should probably be as contributors to a composite population involving substantial local breed inheritance. This should follow evaluation of the life-cycle performance of the crosses under local environment and management conditions (Bradford and Inounu 1996), as the Indonesian Research Institute for Animal Production (IRIAP) has done since 1991. In Sei Putih Medan crossing of STT sheep with St Croix and Barbados Black Belly rams has been evaluated, while in Bogor crossing JTT sheep with St Croix and Mouton Charollais rams has been evaluated.

The goal of these crossbreeding experiments is to improve feed efficiency and growth rate to 35 kg body weight. Growth curves of the crossbred animals using the Von Bertalanffy model are reported by Inounu et al (2008) and reproduced in Table 3. The study shows that crossbreeding indeed improved mature weight and weight at puberty, but not growth rate toward mature weight and age at puberty. JTT reach puberty sooner than MHJ. In general, the results of this study show a younger age of maturity (around 4 months) than in the study by Gatenby (1991), which reported maturity for sheep in the tropics at about 5–6 months, as well as that of Farid and Fahmy (1996), which reported an age of puberty for Mouton Charollais at its origin of 12–13 months.

Based on the growth curve model of Von Bertalanffy (Figure 1), if we assume that 35 kg is a market standard for live weight, crossbreeding will accelerate the time to market from 752 days (25.1 months) in JTT to 467 days (15.7 months) in MJ, 567 days (18.9 months) in HJ, 440 days (14.7 months) in MHJ and 445 days (14.8 months) in HMJ.

The ewe productivity index (EPI) was calculated using total weaning weight of two consecutive lambings as:

$$EPI = \{(LWT_1 + LWT_2)/LI\} \times 365 \text{ kg/year} \quad (1)$$

where LWT_1 and LWT_2 are litter weaning weights for the two consecutive lambings and LI is the ewe lambing interval.

The results are summarised in Table 4. Based on the growth characteristics and ewe productivity index in Table 4, the crossbreeding results show that these sheep are biologically and economically efficient. MHJ and HMJ, in particular, show potential to be developed further in Indonesia (Inounu et al. 2008).

Economic evaluation of the JTT and its crossbred

Several approaches are available to quantify the economic benefits of new animal technology, in this case a genetic improvement of the JTT sheep with its crossbred. Moav (1973) in Weller (1994) described how animal genetic differences could be economically

Table 3. Growth curve parameters for Javanese Thin Tail and crossbred sheep using the Von Bertalanffy model

Sheep genotype	Growth curve parameter				
	A ± SE (kg)	k ± SE (10^{-3}%/day)	b ± SE (unit)	Ui*A ± SE (kg)	Ti ± SE (days)
JTT	$37.013^{a} \pm 0.71$	$4.400^{a} \pm 0.24$	$0.50^{a} \pm 0.0061$	$10.97^{a} \pm 0.22$	$117.13^{a} \pm 5.5$
MJ	$44.143^{c} \pm 1.16$	$4.184^{a} \pm 0.37$	$0.52^{ab} \pm 0.0094$	$13.08^{c} \pm 0.34$	$133.45^{ab} \pm 8.5$
HJ	$40.003^{b} \pm 0.93$	$4.341^{a} \pm 0.30$	$0.51^{a} \pm 0.0076$	$11.85^{b} \pm 0.28$	$117.21^{ab} \pm 6.9$
MHJ	$43.284^{c} \pm 1.04$	$4.716^{a} \pm 0.33$	$0.54^{b} \pm 0.0084$	$12.83^{c} \pm 0.31$	$135.56^{b} \pm 7.6$
HMJ	$44.423^{c} \pm 1.28$	$4.326^{a} \pm 0.41$	$0.51^{a} \pm 0.0103$	13.11^{c} 6± 0.38	$125.56^{ab} \pm 9.4$

A = mature weight; k = mean growth toward mature weight; b = integral constant; Ui*A = weight at inflection point (puberty); Ti = age at inflection (age of puberty); SE = standard error; JTT = Javanese Thin Tail; MJ = Mouton Charollais–JTT; HJ = St Croix hair–JTT; MHJ = MJ rams x HJ ewes; HMJ = HJ rams x MJ ewes

[a b c] Means within column with different superscript letters differ significantly ($P < 0.05$)

Source: Inounu et al. (2008)

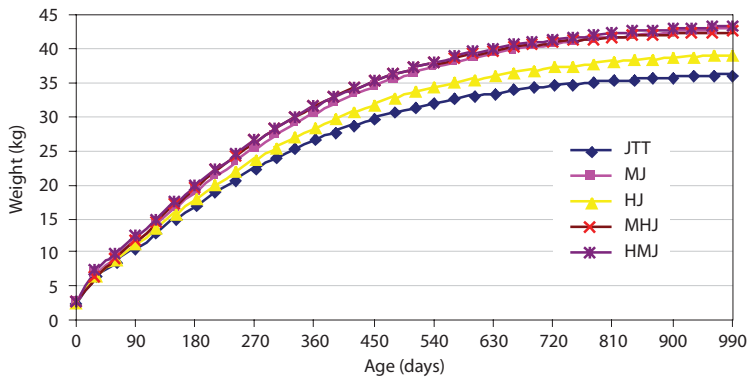

Figure 1. Growth curve of Javanese Thin Tail sheep and its crosses with other breeds using the Von Bertalanffy model
JTT = Javanese Thin Tail; MJ = Mouton Charollais–JTT; HJ = St Croix hair–JTT; MHJ = MJ rams × HJ ewes; HMJ = HJ rams × MJ ewes
Source: Inounu et al. (2008)

evaluated based on profit, which is defined as returns from a unit of production minus its production costs.

For this economic evaluation the body weights of JTT and crossbred sheep up to 450 days of age were used. Growth rate is usually highly correlated with feed efficiency, and rearing animals to various ages illustrates this. The growth rate is also used in the relative economic evaluation of breeds used as terminal sires of crossbred market lambs (Dickerson 1996). There was a rough increase in average growth rates until the age of 270 days and then a decline. Table 5 shows that sheep genotype did not affect the growth rate over any of the 90-day periods from birth to 450 days ($P > 0.05$). Based on the growth rate over the different periods, the profit above feed cost was estimated using the following equation (Moav 1973 in Weller 1994):

$$I = \Delta Q \times P_Q - k \times P_k \quad (2)$$

where:

I = profit (Rp/period)
ΔQ = growth rate of the sheep (kg/day)
k = total amount of feed (kg/day)
P_k = price of feed cost (Rp/day)
P_Q = price of sheep (Rp/kg live weight)

All of the production cost was related to feed cost, namely costs for grass (elephant grass) and concentrates (commercially available) that were estimated using the current market price for each component.

Table 4. Ewe productivity index (EPI) and percentage change of Javanese Thin Tail sheep and its crosses with other breeds

Breed	EPI[A] (kg/year)	Percentage change relative to JTT (%)
JTT	29.4[a]	100
MJ	32.7[a]	111
HJ	42.3[b]	144
MHJ	40.6[b]	138
HMJ	38.9[b]	132

[A] EPI = {(LWT_1 + LWT_2)/LI} × 365 kg/year (where LWT_1 and LWT_2 are litter weaning weights for the two consecutive lambings and LI is the ewe lambing interval); JTT = Javanese Thin Tail; MJ = Mouton Charollais–JTT; HJ = St Croix hair–JTT; MHJ = MJ rams × HJ ewes; HMJ = HJ rams × MJ ewes
[a b] Means within column with different superscript letters are significantly different ($P < 0.05$)
Source: Inounu et al. (2008)

The labour cost to manage and feed the animals was not taken into account as it was considered an opportunity cost, since the study took place on a research station rather than a farm. The gains in growth rate multiplied by the live weight market sheep price will generate revenue and yield a profit above the feed cost. The estimated profit shows a similarity with the growth rate curve that increases until the age of 270 days and then declines substantially (Figure 2). The MHJ showed the highest profit among all breeds, followed by the HMJ. Compared to the JTT, the advantage in profit was 34% and 17% for MHJ and HMJ, respectively, and 8% for both MJ and HJ. This result indicates that crossbred JTT sheep produced higher profits, a finding supported by a previous study showing that crossing JTT with St Croix (HJ) increased income by 26% (Priyanti et al. 1996). Priyanti et al. (2001) reported an evaluation of gross margins of the second generation of these crossbred sheep over different production periods. These included yield of offspring to weaning age followed by production of lambs to a market age of 8 months. The evaluation showed that the gross margin for MHJ was highest among the genotypes, being 30% higher than for JTT. Gross margin estimation is derived from a partial budget analysis that is simple to use and provides information about changes in production cost and benefits caused by application of the new technology (Amir and Knipscheer 1989).

Profit analysis may be based on the assumption that sheep are slaughtered at a certain constant body weight, e.g. 35 kg, as an optimum marketable live weight. Clearly, the number of days from birth to slaughter varies across breed genotypes. with JTT taking the longest and MHJ the shortest time to reach this target (Figure 1). This results in an increase in profit due to the increase in growth rate and the shortened raising time. According to this analysis, the highest profit is generated by MHJ (Rp732,758/sheep/year), followed by HMJ (Rp726,148/sheep/year) and JTT (Rp427,635/sheep/year). Thus, the MHJ and HMJ are 71% and 69% more economically efficient, respectively, than JTT (Priyanti and Inounu 2009).

Under alternative management and marketing systems, developing crossbred sheep is prospective to further development of the animals commercially after a certain time period. This could be managed in a system of fattening for a private enterprise.

Table 5. Average growth rates of Javanese Thin Tail sheep and its crosses with other breeds (kg/head/day)

Sheep genotype	Age (days)				
	0–90	90–180	180–270	270–360	360–450
	n.s.	n.s.	n.s.	n.s.	n.s.
JTT	0.0650	0.0711	0.0813	0.0712	0.0468
MJ	0.0717	0.0796	0.0921	0.0826	0.0571
HJ	0.0684	0.0753	0.0863	0.0763	0.0513
MHJ	0.0768	0.0876	0.0997	0.0867	0.0578
HMJ	0.0755	0.0833	0.0946	0.0833	0.0570

JTT = Javanese Thin Tail; MJ = Mouton Charollais–JTT; HJ = St Croix hair–JTT; MHJ = MJ rams × HJ ewes; HMJ = HJ rams × MJ ewes
n.s. = not significantly different ($P > 0.05$)
Source: Priyanti and Inounu (2009)

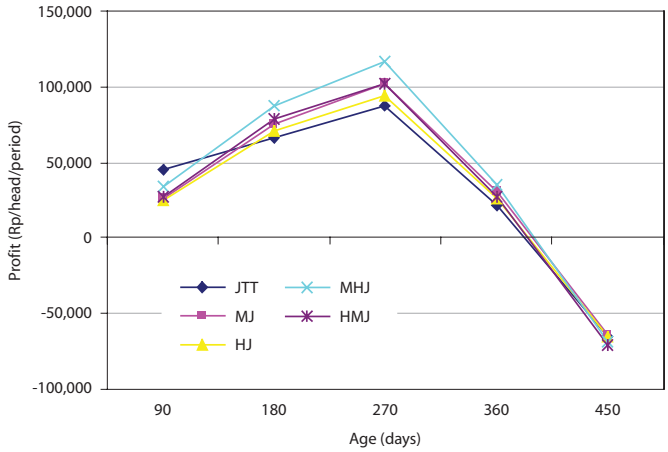

Figure 2. Profit over feed cost of Javanese Thin Tail sheep and its crosses with other breeds. The calculations are shown in the body of the paper
JTT = Javanese Thin Tail; MJ = Mouton Charollais–JTT; HJ = St Croix hair–JTT; MHJ = MJ rams × HJ ewes; HMJ = HJ rams × MJ ewes
Source: Priyanti and Inounu (2009)

Conclusions

- The origin of the Indonesian prolific sheep breed is not known, but the prolificacy gene of the Booroola Merino mutation *(FecB)* has been found in Garole and JTT sheep. JTT sheep could have acquired the gene directly from Garole sheep from India or via Merinos from Australia.
- $FecB^{B}/FecB^{+}$ sheep had the highest gross margin when treated with a high level of feeding management, followed by the $FecB^{B}/FecB^{B}$ genotype.
- In situations where a low level of feeding management was practised, ewes carrying the *FecB* gene did not show their superiority.
- Results from crossbreeding studies showed that MHJ sheep (50% JTT, 25% St Croix hair sheep, 25% Mouton Charollais) had the best prospects for further development based on performance in terms of high mature body weight, faster growth rate to standard slaughter body weight (35 kg at 440 days), and greater efficiency, both biological and economic.
- The MHJ shows the highest profit among all breeds in crossbreeding studies, followed by the HMJ (HJ × MJ). Profits from crossbred sheep were higher than from JTT— 34% for MHJ, 17% for HMJ and 8% for both MJ and HJ, respectively.

References

Amir P. and Knipscheer H.C. 1989. Conducting on-farm animal research: procedures and economic analysis. Winrock International Institute for Agricultural Development, USA; and International Development Research Centre, Canada.

Bradford G.E. and Inounu I. 1996. Prolific breeds of Indonesia. Pp. 137–145 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Bradford G.E., Inounu I., Iniguez L.C., Tiesnamurti B., Thomas D.L. 1991. The prolificacy gene of Javanese sheep. Pp. 67–73 in 'Major genes for reproduction in sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier (eds.) 2nd International Workshop, Toulouse, France. Les Colloques de l'INRA, vol. 57.

Bradford G.E., Quirke J.F., Sitorus P., Inounu I., Tiesnamurti B., Bell F.L., Fletcher I.C. and Torell D.T. 1986. Reproduction in Javanese sheep: evidence for a gene with large effect on ovulation rate and litter size. Journal of Animal Science 63,418-431.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA test in prolific sheep from eight countries provides new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Dickerson G.E. 1996. Economic importance of prolificacy in sheep. In 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, Oxon, UK.

Directorate General of Livestock. 2008. Sheep population by province 2003–2007. http://www.deptan.go.id/infoeksekutif/nak/NAK07/Populasi Domba.htm.

Farid A.H. and Fahmy M.H. 1996. The East Friesien and other European breeds. Pp. 146–151 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, UK.

Fletcher I.C., Gunawan B., Hetzel D.J.S., Bakrie B., Yates N.G. and Chaniago T.P. 1985. Comparison of lamb production from indigenous ewes in Indonesia. Tropical Animal Health 25,161–167.

Gatenby R.M. 1991. Sheep. In 'The tropical agriculturalist'. McMillan Education Ltd.: London, UK, in cooperation with CTA Wageningen: Netherlands.

Iniguez L., Sanchez M. and S. Ginting S. 1991. Productivity of Sumatran sheep in a system integrated with rubber plantation. Small Ruminant Research 5, 303–317.

Inounu I., Iniguez L.C., Bradford G.E., Subandriyo and Tiesnamurti B. 1993. Production performance of prolific Javanese ewes. Small Ruminant Research 12, 243–257.

Inounu I., Mauluddin D. and Subandriyo 2008. Growth characteristics of Garut sheep and its crossbreeds. Jurnal Ilmu Ternak dan Veteriner 13(1), 13–22.

Inounu I. and Soedjana T.D. 1998. Productivity of prolific sheep: economic analysis. Jurnal Ilmu Ternak dan Veteriner 3(4), 215–224.

Inounu I., Tiesnamurti B., Subandriyo and Martojo H. 1999. Lamb production of prolific sheep. Jurnal Ilmu Ternak dan Veteriner 4(3), 148–160.

Moav R. 1973. Economic evaluation of genetic differences. Pp. 319–352 in 'Agricultural genetics, selected topics'. John Wiley and Sons: New York.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Priyanti A. and Inounu I. 2009. Economic liability of JTT sheep and its crosses with other breeds. Jurnal Ilmu Ternak dan Veteriner (in press).

Priyanti A., Inounu I., Priyanto D. and Soedjana T.D. 2001. Pertambahan nilai ekonomi usaha ternak domba persilangan generasi kedua. Laporan Penelitian. Balai Penelitian Ternak. Ciawi-Bogor.

Priyanti A., Inounu I. and Tiesnamurti B. 1996. Utilization of $FecJ^F$ gene in developing commercial sheep farming: economic analysis. Jurnal Ilmu Ternak dan Veteriner 2(1).

Roberts J.A., Estuningsih E., Widjayanti S., Partoutomo S. and Spithill T.W. 1997. Resistance of Indonesian thin tail sheep against *Fasciola gigantica* and *F. hepatica*. Veterinary Parasitology 68, 308–314.

Setiadi B., Subandriyo and Iniguez L.C. 1995. Reproductive performance of small ruminant in outreach pilot project in West Java. Jurnal Ilmu Ternak dan Veteriner 1(2), 73–80.

Sutama I.K. 1992. Reproductive development and performance of small ruminant in Indonesia. Pp. 7–14 in 'New program for small ruminant production in Indonesia', ed. by P. Ludgate and S. Scholz. Winrock International Institute for Agricultural Development.

Sutama I.K., Edey T.N. and Fletcher I.C. 1988. Study on reproduction of Javanese thin tailed ewes. Australian Agricultural Research 39, 703–711.

Weller J.I. 1994. Evaluation of genetic differences from profit equations. In 'Economic aspects of animal breeding'. Chapman & Hall: London.

Biological and economic consequences of the *FecB* mutation in Chinese breeds of sheep

G.H. Hua[1] and L.G. Yang[1,2]

Abstract

The *FecB* gene, first identified in Booroola Merino sheep, is a major gene responsible for high prolificacy. Many other aspects of the *FecB*, including endocrinology, foetal and postnatal growth, have subsequently been studied. The forced PCR–RFLP method of DNA testing was used by researchers to screen some Chinese breeds or strains of sheep to determine whether the mutation is responsible for their high prolificacies. These results showed that *FecB* gene distribution was most imbalanced in the different Chinese breeds and strains of sheep. The *FecB* mutation was present in some Chinese prolific breeds, such as Hu, Small Tail Han, Cele, Duolang and Chinese Merino prolific strains, but absent in low-prolificacy breeds such as Mongolian, Chinese Merino, Tan, Xinjiang, Hulunbeier, Inner Mongolian fine-wool and North-eastern Half-fuzz. The *FecB* gene was indeed associated with high prolificacy in some breeds or strains; however, it cannot be precluded that there are other major genes responsible for the fecundity mechanism. Notwithstanding, introducing the *FecB* mutation to some low prolificacy breeds by crossbreeding can improve the reproductive traits economically.

Background

Sheep (*Ovis aries*) is a highly diverse species with more than 900 different breeds. China is home to more than 15 different domestic breeds that vary substantially in their physiological characteristics, including ovulation rate (OR) and fecundity. In some instances the differences in OR have been attributed to the action of a single gene or a closely linked group of genes (Davis 2005). Recent discoveries have revealed that high prolificacy in Booroola sheep is the result of a mutation (*FecB*) in the bone morphogenetic protein receptor 1B (BMPR1B) gene (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001). In $FecB^B$ animals a single A to G substitution at nucleotide position 830 results in an arginine replacing a glutamine amino acid in a highly conserved region of this receptor. Knowledge of the mutation has prompted researchers to screen other prolific sheep breeds to determine whether either of the mutations is responsible for their high prolificacy.

Biological consequences of the *FecB* mutation in sheep

The Booroola gene (*FecB*) is a dominant autosomal gene mapped to sheep chromosome 6 (Piper et al. 1985). In recent years many aspects of the *FecB* gene, including reproductive endocrinology (Smith et al. 1993), ovary development (Cognie et al. 1998), litter size (LS), organ development and body mass (Smith et al. 1996), have been studied. The major effect of the *FecB* gene mutation in Chinese sheep is to increase OR (eggs shed per ewe ovulating) and, as

[1] Key Laboratory under Education Ministry of China for Agricultural Animal Genetics, Breeding and Reproduction, Huazhong Agricultural University, Wuhan, 430070, China

[2] Corresponding author: yangliguo2006@yahoo.com.cn

a consequence, LS (lambs born per ewe lambing). One copy of the *FecB* gene increases OR by about 1.5 and two copies by about 3.0. These extra ovulations typically increase LS by about 1.0 and 1.5 respectively (Davis 2004). The *FecB* mutation in the BMPR1B leads to differences in foetal development, pituitary function and particularly in ovarian cell function (McNatty et al. 1995; Montgomery et al. 2001). Essentially, these *FecB* effects in the various tissues relate to the timing of events. For example, the *FecB*-specific effects on organ development are first observed during early foetal life, but during mid to late foetal life, these effects appear to be restricted to ovarian development (McNatty et al. 2001). Meanwhile, the mutation also has negative effects on foetal growth and development and body mass during gestation. Carrying the *FecB* (Booroola) mutation is associated with lower birth weight and slower post-weaning growth rate for lambs, as well as lighter mature body weight for ewes (Gootwine et al. 2006). However, studies in the Chinese Merino prolific meat strain showed that the *FecB* gene had a positive effect on early postnatal body growth (Guan et al. 2007).

In different ages and physiological states, some evidence indicates that BB animals have a greater follicle stimulating hormone (FSH) output per cell relative to non-carrier (++) animals (McNatty et al. 1991; Heath et al. 1996). FSH receptor (FSHR) mRNA levels (relative to reference gene GAPDH) from the right ovary of BB (1.14 ± 0.11) ewes were higher than those of ++ (0.44 ± 0.11) and B+ (0.36 ± 0.08) ewes ($P < 0.01$) (Jia et al. 2005a). In addition, luteinising hormone receptor (LHR) mRNA levels from the right ovary of BB (0.42 ± 0.02) ewes were significantly higher than those of ++ (0.23 ± 0.02) and B+ (0.25 ± 0.04) ewes ($P < 0.01$). Oestrogen receptor α(ERα) mRNA levels from the right ovary of BB (0.48) ewes were also higher than those of ++ (0.27) and B+ (0.24) ewes (Jia et al. 2005b). Similarly, progestin receptor (PR) mRNA levels of BB (0.82) ewes were higher than those of ++ (0.37) and B+ (0.20) ewes (Jia et al. 2005b). Besides, as the recipients of frozen embryo transfer, the pregnancy rates of BB animals (66.67%) were higher than both B+ (45.71%) and ++ animals (38.78%) (Yao et al. 2006).

Frequency distributions of *FecB* gene in different sheep breeds or strains

Based on the methods described by Wilson et al. (2001) and Davis et al. (2002), the forced PCR–RFLP DNA test was used to detect the mutations of *FecB* in different breeds or strains of sheep in China. The tested population included both the high prolificacy breeds or strains such as Hu, Small Tail Han, Cele, Duolang sheep and Chinese Merino prolific strain, and the low prolificacy breeds such as Mongolian, Chinese Merino, Tan, Xinjiang, Hulunbeier, Inner Mongolian fine-wool and North-eastern Half-fuzz sheep. In addition, the test was also performed in some crossbreeds or strains.

These results showed that *FecB* gene distribution was most imbalanced in the different Chinese breeds and strains of sheep (Table 1). *FecB* is almost fixed in the population of Hu sheep, as the allele B frequency (0.92–1) is extremely high in different flocks from different areas in China (Wang G.L. et al. 2003; Wang Q.G. et al. 2003; Yan et al. 2005; Davis et al. 2006; Zhu et al. 2006; Guan et al. 2007; Wang et al. 2007). Segregations are found in some other prolific breeds or stains such as Small Tail Han, Cele, Duolang and Chinese Merino prolific. The presence of three different *FecB* genotypes in the Small Tail Han samples shows that the gene is not fixed in the breed. However, the BB and B+ genotypes are dominant in this breed, and the allele B frequency is higher than 0.6 (Liu et al. 2003; Wang G.L. et al. 2003; Yan et al. 2005; Zhang 2005; Zhong et al. 2005a; Davis et al. 2006; Liu 2006; Yin et al. 2006; Zhu et al. 2006; Chu et al. 2007; Wang et al. 2007; Chen et al. 2008). There are three different Booroola genotypes (BB, B+ and ++) detected in Chinese Merino prolific strains. The frequency distributions varied in different populations (Wang G.L. et al. 2003; Wang Q.C. et al. 2003; Wang et al. 2004; Zhong et al. 2004, 2005a, 2006; Zhu et al. 2006; Liu et al. 2007; Guan et al. 2007; Chen et al. 2008). Genotype segregation is also found in Cele sheep. The B+ and ++ genotypes are dominant (Zhu et al. 2006). Two Booroola genotypes are found in the Duolang. The ++ genotype is dominant and the frequency of allele + is higher than 0.8 (Chen et al. 2004; Zhong et al. 2005a, b; Shi et al. 2006). In Mongolian sheep, from which high prolificacy breeds such as Hu and Small Tail Han are descended,

Table 1. Booroola gene distributions in Chinese breeds and strains of sheep

Breed	*n*	Genotypic frequency			Allele frequency		Source
		BB	BB	++	B	+	
Hu	12	1	0	0	1	0	Wang G.L. et al. (2003)
	38	0.84	0	0.16	0.92	0.08	Wang Q.G. et al. (2003)
	34	0.882	0.118	0	0.941	0.058	Yan et al. (2005)
	32	0.97	0	0.03	0.97	0.03	Zhu (2006)
	12	1	0	0	1	0	Davis et al. (2006)
	77	0.909	0.091	0	0.955	0.045	Wang et al. (2007)
	305	1	0	0	1	0	Guan et al. (2007)
Small Tail Han	164	0.537	0.396	0.067	0.735	0.265	Liu et al. (2003)
	12	0.33	0.58	0.08	0.62	0.37	Wang G.L. et al. (2003)
	32	0.303	0.6061	0.0909	0.6061	0.3939	Zhang et al. (2005)
	34	0.882	0.118	0	0.941	0.058	Yan et al. (2005)
	93	0.548	0.397	0.054	0.747	0.253	Zhong et al. (2005a, b)
	12	0.33	0.58	0.08	0.62	0.37	Davis et al. (2006
	188	0.52	0.42	0.06	0.73	0.27	Liu F.L. (2006); Liu Z.H. (2006)
	299	0.5418	0.3645	0.0937	0.7241	0.2759	Yin et al. (2006)
	37	0.43	0.46	0.11	0.66	0.34	Zhu et al. (2006)
	16	0.563	0.437	0	0.7815	0.2185	Wang et al. (2007)
	188	0.52	0.42	0.06	0.73	0.27	Chu et al. (2007)
	40	0.475	0.350	0.175	0.650	0.350	Chen et al. (2008)
Chinese Merino prolific strain	49	0.12	0.51	0.27	0.48	0.52	Wang Q.G. et al. (2003)
	38	0	0.76	0.24	0.38	0.62	Zhong et al. (2004)
	47	0.2553	0.4894	0.2553	0.5	0.5	Wang et al. (2004)
	40	0.05	0.7	0.25	0.4	0.6	Zhu et al. (2006)
	47	0.2553	0.4894	0.2553	0.5	0.5	Zhong et al. (2005a, b)
	60	0.1	0.12	0.78	0.16	0.84	Zhong et al. (2006)
	53	0.32	0.49	0.19	0.565	0.435	Liu et al. (2007)
	53	0.51	0.30	0.19	0.66	0.34	Guan et al. (2007)
	31	0.0330	0.2258	0.7419	0.1459	0.8541	Chen et al. (2008)
Cele	44	0.16	0.48	0.36	0.4	0.6	Zhu et al. (2006)
Duolang	68	0	0.05	0.95	0.025	0.975	Chen et al. (2004)
	77	0	0.039	0.961	0.021	0.979	Zhong et al. (2005a, b)
	49	0	0.35	0.65	0.17	0.83	Shi et al. (2006)

Table 1. (cont'd) Booroola gene distributions in Chinese breeds and strains of sheep

Breed	*n*	Genotypic frequency			Allele frequency		Source
		BB	BB	++	B	+	
Mongolian	14	0	0.0714	0.9286	0.0357	0.9642	Zhang (2005)
Chinese Merino monotocous	47	0	0	1	0	1	Wang Q.G. et al. (2003)
	47	0	0	1	0	1	Zhong et al. (2004)
	47	0	0	1	0	1	Zhong et al. (2005a, b)
	47	0	0	1	0	1	Zhu et al. (2006)
	24	0	0	1	0	1	Guan et al. (2007)
	51	0	0	1	0	1	Liu et al. (2007)
	33	0	0	1	0	1	Chen et al. (2008)
Tan	152	0	0	1	0	1	Liu et al. (2003)
Xinjiang	12	0	0	1	0	1	Wang G.L. et al. (2003); Wang Q.G. et al. 2003)
Hulunbeier	49	0	0	1	0	1	Liu et al. (2007)
Inner Mongolian fine-wool	53	0	0	1	0	1	Liu et al. (2007)
Northneastern Half-fuzz	185	0	0.03	0.97	0.02	0.98	Yao et al. (2006)
Hu cross	16	0	1	0	0.5	0.5	Guan et al. (2005)
Dorper × Hu	16	0	0.875	0.125	0.4375	0.5625	Wang et al. (2007)
Small Tail Han cross	112	0.11	0.56	0.33	0.39	0.61	Yao et al. (2006)
Dorper × Han	28	0	0.786	0.214	0.393	0.607	Wang et al. (2007)
(Tan × Han) × German Merino	32	0	0.47	0.53	0.23	0.77	Yu et al. (2008)
(Tan × Han) × Poll Dorset	31	0	0.42	0.58	0.21	0.79	Yu et al. (2008)

no BB genotype is found, while the ++ genotype was dominant (Zhang 2005). *FecB* mutation was absent in the other low prolificacy breeds such as Chinese Merino, Tan, Xinjiang, Hulunbeier, Inner Mongolian fine-wool and North-eastern Half-fuzz (Liu et al. 2003; Wang Q.G. et al. 2003; Zhong et al. 2004, 2005a, b; Zhu et al. 2006; Liu et al. 2007; Guan et al. 2007; Chen et al. 2008). Meanwhile, the B+ genotype was found in the crossbred progenies of Hu and Small Tail Han sheep, which exhibited a simple Mendelian pattern of segregation (Guan et al. 2005; Yao et al. 2006; Wang et al. 2007; Yu et al. 2008).

The different frequency distributions among breeds or strains indicated that the *FecB* mutation is possibly related to the differences in prolificacy among flocks.

FecB mutation associated with prolificacy of sheep

The DNA tests in Chinese sheep breeds showed that some of the breeds carried the *FecB* mutation, namely Hu, Small Tail Han, Chinese prolific meat strains and some crossbreeds or strains. The fecundity of these breeds is similar to that reported in Booroola sheep. In general, the results in the Chinese sheep breeds or strains support the view that the *FecB* gene increases OR and LS (co-dominant for OR and partially dominant for LS).

The LSs of some breeds or strains with different *FecB* genotypes are summarised in Table 2. This shows that LS with the same genotype varied among different flocks of Small Tail Han (Liu et al. 2003; Yan et al. 2005; Zhang 2005; Davis et al. 2006; Liu 2006; Yin et al. 2006; Zhu et al. 2006; Chu et al. 2007; Chen et al. 2008). This may be caused by differences in the husbandry systems in which they have evolved, and may also be affected by whether a spontaneous mutation leading to large litters is retained or lost. However, all the studies of Small Tail Han showed that BB ewes had consistently greater LS, followed by B+ and ++. This was also true within the Chinese Merino prolific strains (Liu et al. 2003; Yan et al. 2005; Zhang 2005; Davis et al. 2006; Liu et al. 2007; Yin et al. 2006; Zhu et al. 2006; Chu et al. 2007; Guan et al. 2007; Chen et al. 2008). In the crossed strains (Tan × Han) × German Merino and (Tan × Han) × Dorset, the LS of the heterozygote was higher compared to non-carriers of ++ ewes (Yu et al. 2008). These studies indicated that one copy of the *FecB* mutation could increase LS. In addition, the average lambing rate of BB (209.1%) and B+ (208.7%) was significantly higher than ++ Chinese Merino prolific wool ewes (Wang Q.G. et al. 2003). Furthermore, the Chinese Merino prolific ewes with BB and B+ had 4.0 and 3.5 ovulations, significantly more than ++ ewes at only 1.56 (Zhong et al. 2006).

These findings indicate that the *FecB* gene is significantly related to the high prolificacy of some Chinese breeds of sheep. The *FecB* gene is therefore an important molecular marker for fecundity and could be used in the marker-assisted selection (MAS) system in Chinese breeds of sheep. Introgression of the *FecB* gene assisted by molecular techniques has the potential to improve the fecundity of sheep breeds with poor prolificacy.

Other major genes for prolificacy may be present in some Chinese breeds of sheep

Although the *FecB* gene is associated with high prolificacy in some Chinese sheep breeds or strains, some other findings indicate that *FecB* is possibly not the only reason for high fecundity (Hua et al. 2008). It cannot be precluded that there are other major genes with effects on prolificacy. For example, as one of the most famous prolific sheep breeds in China, Hu sheep were almost all homozygous BB carriers (Table 1). The *FecB* mutation is therefore almost fixed in the Hu sheep. However, the LS was significantly different among flocks (Guan et al. 2007). Furthermore, the prolificacy can be promoted after selection within the breed (Guan et al. 2005). Although the *FecB* genotypes were segregating in the Small Tail Han, and the genotypes were significantly associated with LS, consistently large litters were found successively from three non-carrier ewes (the LSs of the first and second parities were 2.33 and 3.33) (Zhang 2005) and one non-carrier Han ewe (four sets of triplets, one set of quadruplets and one set of quintuplets) (Davis et al. 2006). When the *FecB* genotypes were used to predict LS (BB and B+ > 2.0, ++ = 1), the accordance rates were only 78.8% 80.4% and 80.0% for BB, B+ and ++, respectively, compared to the factual lambing number (Wang Q.G. et al. 2003). Therefore, the prolificacy of the ewes could not be totally accurately predicted by the *FecB* genotypes alone.

All this evidence indicates that other major genes related to high prolificacy may also be present. There have been several recent research findings in relation to inheritance patterns and DNA testing of major genes for prolificacy that have the potential to significantly increase the reproductive performance of sheep flocks throughout the world. These findings will also enhance knowledge of the control of reproduction regulation mechanisms, for example studies on the inhibin α gene (INHA) (Hiendleder et al. 2002), the melatonin receptor 1A gene (MTNR1A) (Chu et al. 2003) and the prolactin gene (PRL) (Dai et al. 2007). More extensive screening is required to fully reveal the mechanisms underlying the prolificacy of Chinese sheep.

Application of *FecB* gene in the sheep breeding system and the economic consequences

Improving prolificacy of sheep through within-breed selection is a slow process. It is more efficient to improve the flock prolificacy by crossbreeding with known prolific breeds such as Hu and Small Tail Han sheep. The incorporation of a major gene for prolificacy into a flock using MAS allows increased selection pressure on the traits leading to increased genetic gain (Davis 2005). The major gene has the advantage that it can be introduced into any new breed while retaining the new breed's characteristics (Davis 2005). Now that the criterion to identify the ovine fecundity gene *FecB* has been established in China, MAS using *FecB* is warranted to increase LS in sheep, which will be of considerable economic value to mutton producers. The actual economic benefits realised will depend on the different economic environments, such as the price of lamb and mutton, and the feed costs.

Application to development of high fecundity strains

To increase profitability through improved prolificacy, a breeding program was initiated from the 1990s by introducing the blood of prolific Hu sheep, which is almost homozygous for *FecB*, into Chinese Merino (Xinjiang type) sheep via reciprocal crossing by phenotype selection. After several generations of selection, a Chinese Merino prolific strain was successfully developed. Subsequently, some other specific lines such as prolific meat and wool lines were bred using the Chinese Merino prolific strain as the foundation. These crossbred strains increased income substantially by improving productivity. For example, the Chinese Merino prolific strain carrying the *FecB* gene was used as the dams to cross with the South African Mutton Merino to breed a new prolific strain that is used for meat and fine-wool production (Figure 1).

Table 2. Comparison of litter size in some Chinese breeds and strains of sheep by *FecB* genotype

Breed/strain	*n*	Genotype			Source
		BB	B+	++	
Small Tail Han	164	2.47 ± 0.79	2.05 ± 0.73	1.5 ± 0.71	Liu et al. (2003)
	12	2.99 ± 0.24	2.73 ± 0.13		Davis et al. (2006)
	32	3.67 ± 1.03	3.53 ± 0.99	3.33 ± 1.53	Zhang (2005)
	227	2.27 ± 0.08	2.18 ± 0.10	1.35 ± 0.12	Yan et al. (2005)
	299	2.87 ± 0.12	2.47 ± 0.19	1.85 ± 0.35	Yin et al. (2006)
	37	2.81 ± 0.14	2.76 ± 0.14	2.25 ± 0.29	Zhu et al. (2006)
	188	2.65 ± 0.17	2.36 ± 0.15	1.25 ± 0.10	Liu F.L (2006); Liu Z.H. (2006)
	188	2.65 ± 0.17	2.36 ± 0.15	1.25 ± 0.10	Chu et al. (2007)
	38	2.89 ± 0.76	1.92 ± 0.95	1.00 ± 0.00	Chen et al. (2008)
Chinese Merino prolific	40	3.00 ± 0.40	2.11 ± 0.11	1.60 ± 0.18	Zhu et al. (2006)
	53	2.84 ± 0.74	2.34 ± 0.63	1.23 ± 0.68	Guan et al. (2007)
(Tan × Han) × German Merino	32		2.21 ± 0.17	2.10 ± 0.10	Yu et al. (2008)
(Tan × Han) × Dorset	31		2.33 ± 0.27	1.50 ± 0.10	Yu et al. (2008)

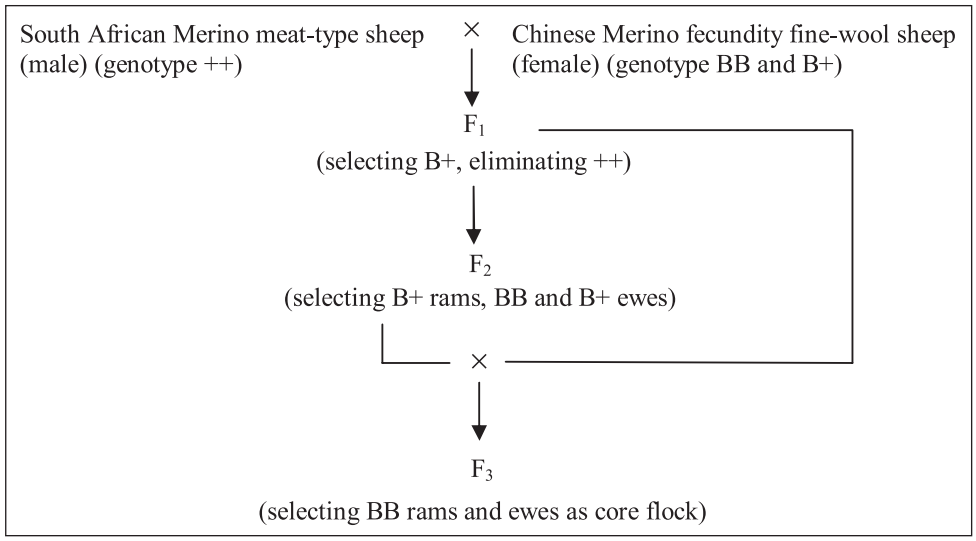

Figure 1. Breeding system to produce a South African Merino prolific strain for meat purposes

An MAS approach and routine selection were incorporated into the breeding programs, resulting in efficient improvement in prolificacy and meat traits (Yang et al. 2006).

Application to commercial crossbreeding

Economic traits such as prolificacy and growth traits can be improved significantly and efficiently using crossbreeding systems. *FecB*-mutation carriers such as the Hu and Small Tail Han are used widely to improve the fecundities of different breeds, with increases in profit as a consequence. The frequencies of *FecB* mutation can be increased significantly by crossbreeding. For example, two genotypes (B+ and ++) were found in Dorper × Hu (DH) sheep and Dorper × Small Tail Han (DS) sheep. B+ and ++ frequencies were 0.875 and 0.125, respectively, in DH sheep and 0.786 and 0.214, respectively, in DS sheep. However, only one genotype (++) was detected in Dorper sheep (Wang et al. 2007). Introducing the *FecB* gene into Romilly Hills sheep by crossing with the Chinese Merino prolific strain increased the lambing rate of the F1 generation by 37.14% (Zhang et al. 2004). The reproduction traits of the crossbred Charollais × Small Tail Han were improved by 27% relative to the Charollais, while growth traits were improved relative to the Small Tail Han (Lu et al. 2008). It was also reported that the F2 generation of the Charollais × Small Tail Han improved the lambing rate by 193.7% (Wang et al. 2005). Yang and Zhang (2002) investigated the reproductive traits of the Tan × Small Tail Han in the F1 generation and found that the lambing rate increased by 121.1% compared to the Tan ewe.

Conclusions

The *FecB* mutation was ubiquitous in the highly prolific sheep breeds and strains in China but absent in some Chinese non-prolific breeds. In general, the results in Chinese sheep breeds or strains support the view that the *FecB* gene increases OR and LS. However, there is some evidence that *FecB* may not be the only factor responsible for high fecundity. Nevertheless, introgression of the *FecB* gene can improve the fecundity of non-prolific sheep flocks and this has already been widely demonstrated in China.

References

Chen X.J., Zhong F.G., Luo S.P. and Wang X.H. 2004. The primary studies on polymorphism of BMPR1B gene of Duolang sheep. Xinjiang Agricultural Science 41(1), 6–9.

Chen Y., Luo Q.J., Li D.Z., Zhang Y.J., Yang F.Y., Yang J.Q. and Zhu W.Y. 2008. Relationship between BMPR1B polymorphism and litter size in six breeds or strains of sheep. Journal of Xinjiang Agricultural University 31(2), 12–16.

Chu M.X., Ji C.L. and Chen G.H. 2003. Association between PCR–RFLP of melatonin receptor 1a gene and high prolificacy in small tail Han sheep. Asian–Australasian Journal of Animal Science 16, 1701–1704.

Chu M.X., Liu Z.H., Jiao C.L., He Q.Y., Fang L., Ye S.C., Chen G.H. and Wang J.Y. 2007. Mutations in BMPR1B and BMP15 genes are associated with litter size in Small tailed Han sheep (*Ovis aries*). Journal of Animal Science 85, 598–603.

Coqnie Y., Benoit F., Poulin N., Khatir H. and Driancourt M.A. 1998. Effect of follicle size and of the FecB Booroola gene on oocyte function in sheep. Journal of Reproduction and Fertility 112(2), 379–386.

Dai O., Chu M.X., Bai H.Q., Bai L.H., Li X.W., Fang L., Ye S.C., Yi J.H. and Gao F.C. 2007. Polymorphism of prolactin gene and its relationship with prolificacy of small tail Han sheep. Journal of Agricultural Biotechnology 15(2), 222–227.

Davis GH. 2004. Fecundity genes in sheep. Animal Reproduction Science 82–83, 247–253.

Davis G.H. 2005. Major genes affecting ovulation rate in sheep. Genetics Selection Evolution 37(suppl. 1), S11–S23.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway S.M., Lumsden B.M., Hanrahan J.P., Mullen M., Mao X.Z., Wang G.L., Zhao Z.S., Zeng Y.Q., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranz J.J. and Notter D.R. 2006. Investigation of the Booroola (FecB) and Inverdale (FecX(I)) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92(1–2), 87–96.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar C., Gray G.D., Subandriyo Inounu. I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66(6), 1869–1874.

Gootwine E., Rozov A., Bor A. and Reicher S. 2006. Carrying the FecB (Booroola) mutation is associated with lower birth weight and slower post-weaning growth rate for lambs, as well as a lighter mature bodyweight for ewes. Reproduction, Fertility and Development 18(4), 433–437.

Guan F., Ai J.T., Liu S.R. and Shi G.Q. 2005. Study of BMPR1B and BMP15 as candidate genes for prolificacy in Hu sheep. Acta Ecologiae Animal Domastici 2005, 26(3), 9–12.

Guan F., Liu S.R., Shi G.Q. and Yang L.G. 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Animal Reproduction Science 99(1–2), 44–52.

Heath D.A., Caldani M. and McNatty K.P. 1996. Relationships between the number of immunostaining gonadotropes and the plasma concentrations of gonadotrophins in ewes with and without the FecBB gene. Journal of Reproduction and Fertility 106, 73–78.

Hua G.H., Chen S.L., Ai J.T. and Yang L.G. 2008. None of polymorphism of ovine fecundity major genes FecB and FecX was tested in goat. Animal Reproduction Science 108(3–4), 279–286.

Hiendleder S., Lewalshi H., Jaeger C. and Pracht P. 2002. Nucleotide sequence of ovine α- inhibin (INHA) genes and evaluation of RFLP marker effects on reproductive performance. Animal Genetics 33(3), 247–248.

Jia C.L., Li N., Wei Z.H., Zhu X.P., Liu H.Y. and Jia Z.H. 2005a. Study on FSHR and LHR mRNA levels of different BMPR1B genotypes from small tail Han sheep during the oestrum. Scientia Agricultura Sinica 39(1), 170–175.

Jia C.L., Li N., Wei Z.H., Zhu X.P., Liu H.Y. and Jia Z.H. 2005b. Study on ER- and PR-mRNA levels of different BMPR1B genotypes from small tail Han sheep during the oestrum. Chinese Journal of Animal Science 41(11), 27–30.

Liu F.L. 2006. Polymorphism analysis of sheep BMP15 gene and BMPR1B gene. Masters thesis, Inner Mongolia Agricultural University.

Liu F.L., Liu Y.B., Wang F., Wang R., Tina C.Y., Liu M.X., Wei J.M. and Rong W.H. 2007. Study on the polymorphism of bone morphogenetic protein receptor IB in China paitial sheep. Acta Agriculturae Boreali-Sinica 22(4), 151–154.

Liu S.F., Jiang Y.P. and Du L.X. 2003. Studies of BMPR1B and BMP15 as candidate genes for fecundity in little tailed Han sheep. Acta Gentica Sinica 30(8), 755–760.

Liu Z.H. 2006. Study on the gonadotropin releasing hormone receptor (GnRHR), bone morphogenetic protein 15 (BMP15) and bone morphogenetic protein receptor-IB (BMPR1B) as candidate genes of prolificacy in Small tail Han sheep. Masters thesis, Yang Zhou University.

Lu J.Y., Ding F.C. and Zhang L.G. 2008. The reproductive traits test of Charolais × small tail Han sheep. Chinese Livestock and Poultry Breeding 2, 74.

McNatty K.P., Hudson N.L., Shaw L., Condell L.A., Ball K., Seah S.L. and Clarke I.J. 1991. GnRH-induced gonadotrophin secretion in ovariectomized Booroola ewes with hypothalamic–pituitary disconnection. Journal of Reproduction and Fertility 91(2), 583–592.

McNatty K.P., Juengel J.L., Wilson T., Galloway S.M. and Davis G.H. 2001. Genetic mutations influencing ovulation rate in sheep. Reproduction, Fertility and Development 13(7–8), 549–555.

McNatty K.P., Smith P., Hudson N.L., Heath D.A., Tisdall D. J., O W.S. and Braw-Tal R. 1995. Development of the sheep ovary during fetal and early neonatal life and the effect of fecundity genes. Journal of Reproduction and Fertility Supplement 49, 123–135.

Montgomery G.W., Galloway S.M., Davis G.H. and McNatty K.P. 2001. Genes controlling ovulation rate in sheep. Reproduction 121(6), 843–852.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Mnniaux D. and Callebaut I. 2001. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences of the United States of America 98(9), 5104–5109.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Shi H.C., Wu J., Zhu E.Y., Zhang Z.F. and Guo Z.Q. 2006. Studies of BMPR1B as candidate gene for fecundity in Duolang sheep. China Herbivores 26(2),12–14.

Smith P., Hudson N.L., Corrigan K.A., Shaw L., Smith T., Phillips D.J. and McNatty K. P. 1996. Effects of the Booroola gene (FecBB) on body weight, testis development and hormone concentrations during fetal life. Journal of Reproduction and Fertility 108(2), 253–261.

Smith P., O W.S., Hudson N.L., Shaw L., Heath D.A., Condell L., Phillips D.J. and McNatty K.P. 1993. Effects of the Booroola gene (FecB) on body weight, ovarian development and hormone concentrations during fetal life. Journal of Reproduction and Fertility 98(1), 41–54.

Souza C.J., MacDougal C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. Journal of Endocrinology 169(2), R1–R6.

Wang G.J., Dou D.Y., Hua W.H., Nie X.W., Chu G.L., Xu X.B., Zhao W. and Liu Y.B. 2007. Analysis of polymorphism of BMPR1B gene in five sheep populations. Jiangsu Journal of Agricultural Science 23(5), 447–450.

Wang G.L., Mao X.Z., Davis G.H., Zhao Z.S., Zhang L.J. and Zeng Y.Q. 2003. DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (FecB) mutation. Journal of Nanjing Agricultural University 26(1), 104–106.

Wang J.H., He Q.R., Zhang Y.S., Wei R.A., Ma C.P., Kumusihan, Wu W.S., Wang H.W., Mao L.S. and Wang H. 2004. Analysis of multiplets character genotype of Chinese Merino fecundity fine-wool sheep as filial-generation. China Herbivores 24(4), 12–13.

Wang Q.G., Zhong F.G., Li H., Wang X.H., Liu S.R., Chen X.J. 2003. The polymorphism of BMPR1B gene and the relationship with litter size in sheep. Grass-Feeding Livestock 2, 20–22.

Wang R., He Y.T. and Zhao F.L. 2005. The analysis of growth and slaughter performance of different generations of Charollais × small tail Han sheep. Henan Journal of Animal Husbandry and Veterinary Medicine 26(4), 5–7.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64(4), 1225–1235.

Yan Y.D., Chu M.X., Zeng Y.Q., Fang L., Ye S.C., Wang L.M., Guo Q.K., Han D.Q., Zhang Z.X. and Zhang X.Z. 2005. Study on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in small tail Han sheep and Hu sheep. Journal of Agricultural Biotechnology 13(1), 66–71.

Yang W.D. and Zhang Z.K. 2002. The growth performance test of Tan Yang by introducing the fecundity gene of Small Tail Han sheep. Gansu Journal of Animal Husbandry and Veterinary 4, 10–12.

Yang Y.L., Liu S.R. and Wang J.H. 2006. Chinese Merino prolific meat and fine wool strain. China Herbivores, 38–40.

Yao Y.C., Tian L., Han H.B., Chen X.H., Lu M.H., Guo P.C., Zhang C.F., Li N., Lian Z.X. and Li W. 2006. Preponderance genotype of BMPR1B improves the pregnant rate of embryo-transfer in sheep. Endocrinology 33(11), 1074–1079.

Yin Z.H., Jiang Y.L., Fan X.Z., Wang Y., Tang H. and Yue Y.S. 2006. Polymorphism, effect and linkage analysis of BMPR1B gene in small tailed Han sheep. Acta Veterinaria et Zootechnica Sinica 37(5), 510–513.

Yu Z.Q., Yang B.H. Guo J., Lang X., Liu J.B., Cheng S.L. and Sun X.P. 2008. Polymorphism of GDF9 and FecB gene in two types of crossbred sheep and their effects on the litter size and body weight. China Herbivores 2, 5–8.

Zhang Y.S., Wang J.H., Wei R.A., He Q.R., Ma C.P., Fan J.J., Kumusihan, Wu W.S., Wang H.W., Wang H. and Mao L.S. 2004. Analysis of crossbreed effects about Romilly Hills mutton type fine-wool sheep and Chinese Merino fecundity fine-wool sheep. China Herbivore 26, 15–17.

Zhang L.P. 2005. Studies on FecB gene as candidate gene for the fecundity of small tail Han sheep. China Herbivores, 108–111.

Zhong F.G., Wang X.H., Li H., Liu S.R., Gan S.Q., Yang Y.L., Wang J.H. and Zhang Y.S. 2006. Study on the polymorohism of BMPR1B gene associated with ovulations in Chinese Merino fecundity sheep. China Herbivore 26(1), 5–6.

Zhong F.G., Wang X.H., Li H., Liu S.R., Yang Y.L., Gan S.Q., Wang J.H., Zhang Y.S., He Q.H. and Wei R.A. 2005b. Sheep BMPR1B gene as fecundity candidate gene application to the breeding breed for the meat type fecundity sheep. China Herbivores 25(4), 6–7.

Zhong F.G., Wang X.H., Liu S.R., Li H., Chen X.J., Yin J.L. and Ni J.H. 2005a. Study on the polymorphism of BMPR1B gene associated with litter size in small-tailed Han sheep and Xinjiang Duolang sheep. China Herbivore 25(6), 15–16.

Zhong F.G., Wang X.H., Liu S.R., Li H., Shen M., Chen X.J., Yang H. and Gan S.Q. 2004. Studies of BMPR1B and BMP15 as candidate genes for fecundity in China Merino fecundity sheep and Hu-Yang. Animal Biotechnology Bulletin 9(1), 139–145.

Zhu E.Y. 2006. Study on bone morphogenetic protein receptor IB as a candidate gene for fecundity in several sheep breeds in Xinjiang. Masters thesis, Xinjiang Agricultural University.

Zhu E.Y., Shi H.C., Wu J., Liu M.J.M., Jian Z.J., Bai J. and Xu X.M. 2006. Study on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in sheep. Acta Agriculturae Boreali-occidentalis Sinica 15(6), 20–23.

Biological and economic consequences of the *FecB* mutation in the USA

D.R. Notter[1,2], D.L. Thomas[3] and D.F. Waldron[4]

Abstract

Commercial use of *FecB*^{B} in the USA is quite limited. Introductions of prolific Finnish Landrace (FL) sheep into North America in the 1970s were followed by assessment of FL germplasm and development of composite lines containing various percentages of FL breeding. The increase in average litter size (LS) of approximately 0.25 associated with 25% FL breeding, and the associated mean LS of 2.10–2.25 lambs in adult ewes, was accepted by a number of US sheep producers. Introductions of Booroola Merino (BM) sheep in the 1980s provided opportunity to increase LS by use of *FecB*^{B} and to introgress this allele into different genetic backgrounds. Initial evaluations of the effects of *FecB*^{B} in the BM genetic background were not favourable. In Illinois BM F_1 ewes had higher ovulation rates and LSs at lambing but were inferior to F_1 ewes sired by rams of FL, St Croix, Barbados Blackbelly and a composite maternal breed (the Combo-6) in ewe productivity and most measures of lamb performance. In Nebraska the effects of *FecB*^{B} and the polygenic effect of the FL were approximately additive for ovulation rate and LS, but weaning rates of BM F_1 ewes did not exceed those of FL F_1 ewes, and growth of lambs produced by BM F_1 ewes remained inferior. Thus, the mean level of prolificacy conferred by a single copy of *FecB*^{B} in crosses with, or through introgression into, typical US sheep breeds appears to exceed the level that is desired by most producers.

Introduction

The first importations into the USA of Booroola Merino (BM) animals carrying the *FecB*^{B} allele occurred in the early 1980s and were summarised by Young (1991). In 1982 a 75%-BM ram homozygous for *FecB*^{B} and two heterozygous BM × Coopworth rams were imported from New Zealand to the Texas A&M Agricultural Research Center at San Angelo. This introduction was followed in 1983 by importation of five BM rams and 21 Coopworth ewes carrying BM embryos from the Tara Hills flock in New Zealand to the US Meat Animal Research Center in Clay Center, Nebraska (MARC). The imported rams were found to include three *FecB*^{B} homozygotes and two heterozygotes, and the Coopworth ewes produced 17 male and 12 female lambs. Of these 33 animals, 16 males and eight females subsequently produced offspring and were foundation animals for the MARC BM flock. No large-scale commercial importation of animals carrying the *FecB*^{B} mutation occurred until the late 1980s, when 187 purportedly homozygous *FecB*^{B} rams were imported by a private company from the Haldon Station in New Zealand and used to provide breeding rams and semen to producers in the USA.

The assessment and subsequent use of the BM in the USA can be properly understood only in light of previous introductions and widespread comparative evaluation of the prolific Finnish Landrace (FL) breed during the 1970s (Dickerson 1977; Notter and Copenhaver 1979). These studies sensitised US

[1] Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA
[2] Corresponding author: drnotter@vt.edu
[3] Department of Animal Sciences, University of Wisconsin, Madison, Wisconsin, USA
[4] Texas AgriLife Research, Texas A&M System, San Angelo, Texas, USA

producers to opportunities associated with increased prolificacy, but also made them wary of the generally inevitable increases in lamb mortality associated with larger litters and the changes in intensity of shepherding required to keep those losses from becoming excessive. Death losses for lambs born in litters of three or more under extensive production conditions commonly ranged from 25% to 50% (Iniguez et al. 1986; Iman and Slyter 1996), sharply reducing the anticipated contribution of the FL to flock productivity and profitability. Small adult size, associated undesirable correlated effects on growth rate and body fatness at typical US lamb market weights of 45–55 kg, a propensity to preferentially deposit fat in the body cavity, and poor fleece quality further limited enthusiasm for the FL. The BM was thus introduced as an alternative prolific type anticipated to allow maintenance or, perhaps, improvement of fleece characteristics in US wool breeds, and with the hope that the BM would be equal or superior to FL in growth and carcass merit.

The opportunities and challenges implicit in adapting the polygenic prolificacy of the FL or the single-gene prolificacy of the BM to US conditions were understood but perhaps not fully appreciated. Research during the 1970s indicated that each 1% increase in FL breeding in the ewe was associated with an increase of approximately 0.01 in flock average litter size (LS) (Dickerson 1977). Because of the polygenic inheritance of LS in the FL, crossbreeding systems or composite lines containing various proportions of FL breeding could be developed to allow titration of the FL effect on LS to effectively any point between 0 and approximately 1.0 lambs/litter.

Most domestic US breeds have mean LSs as adult ewes of between 1.60 and 1.95 lambs/litter, and most large US flocks would view an average lamb drop of 2.00–2.25 lambs/ewe lambing as an acceptable production goal. By the early 1980s, studies with the FL had shown that this increase in prolificacy could be readily achieved by incorporation of 25–50% FL breeding into the commercial ewe, and development of several composite ewe lines had begun. Prominent among those was the Polypay population developed at the US Sheep Experiment Station in Idaho (Hulet et al. 1984), containing 25% FL breeding. The Polypay is the only FL-derivate breed that is currently in active use in the USA, with an average lamb drop of 2.25 for spring-lambing adult ewes in US National Sheep Improvement Program flocks (D.R. Notter, unpublished).

The effect of the *FecB*^{B} mutation on LS approaches +1.0 lamb in heterozygous ewes and +1.5 lambs in homozygous ewes (Davis 2005). Appropriate use of *FecB*^{B} under US conditions would thus most likely involve structured mating systems or screening of segregating populations to produce heterozygous commercial ewes. However, given current prolificacy levels in domestic US breeds, the addition of one copy of *FecB*$^{+}$ would likely result in a mean LS of 2.3 to 2.9 lambs per ewe lambing in adult ewes. For most US flocks such an increase would require large increases in intensity of shepherding or substantial improvements in ewe mothering ability to avoid unacceptable lamb losses.

Review of literature

Comparison of the Booroola merino to other breeds

Initial evaluations of the effects of *FecB*^{B} in the USA were necessarily confounded with effects from the BM genetic background. Much of the research comparing the BM to the FL and a variety of US breeds was summarised by Young et al. (1996), and included studies at Texas A&M University (Willingham et al. 1988), MARC (Young and Dickerson 1988, 1991a, b) and the University of Illinois (Bunge et al. 1993a, b, 1995, 1996). This review will focus mainly on comparisons of the BM with the FL and other prolific or semi-prolific breeds.

In Illinois homozygous *FecB*^{B} BM rams were compared to FL rams; rams of two hair sheep breeds, the St Croix (SC) and Barbados Blackbelly (BB); and rams of a composite breed, the Combo-6 (C6), derived from crosses among the FL, Suffolk, Border Leicester, Dorset, Targhee and Rambouillet breeds. Rams were mated to either Targhee (TA) or Suffolk (SU) ewes over 4 years (1986–89) in a life-cycle comparison of productivity and profitability of the resulting F_1 ewe types across three lamb crops. Reproductive efficiency of the different sire breeds, growth and survival of F_1 lambs, and wool characteristics and lamb production of F_1 ewe lambs and adult ewes were summarised by Bunge et al. (1993a, b, 1995, 1996).

Briefly, the results were as follows:

- The productivity of TA and SU ewes was lowest (18% below the mean) in matings with BM rams, in association with relatively low levels of ewe fertility, lamb survival and preweaning growth.

- The productivity of yearling ewes sired by BM was lower than that of all breeds except the Combo-6 (8% below the mean), again due to a combination of low ewe lamb fertility, lamb survival and preweaning growth, and despite the highest level of prolificacy (18% above the mean).
- The productivity of 2- and 3-year-old ewes was likewise lowest for F_1 BM ewes (23% below the mean), reflecting accentuated inferiority in fertility, lamb survival and lamb preweaning growth relative to that observed in yearling ewes.
- Compared to all other types, F_1 BM ewes had much heavier fleeces (51% above the mean) with lower fibre diameter (10% below the mean) and fewer coloured, medullated or kempy fibres.

Bunge (1992) also compared the postweaning growth and carcass characteristics of ram lambs and the profitability of the various F_1 ewe types. In F_1 ram lambs harvested at approximately 50 kg live weight (Table 1), lambs sired by BM rams had the least desirable weight at 26 weeks, age at 50 kg, dressing percentage, backfat and lower rib fat thickness, and USDA yield grade; and were superior only to lambs sired by BB rams in postweaning gain and percentage of kidney and pelvic fat. In progeny of F_1 ewe lambs sired by Dorset rams, lambs from dams sired by BM rams were superior only to lambs from dams sired by BB rams in postweaning gain, weight at 29 weeks of age, and age at 50 kg (Table 2). Deterministic simulation of profitability of the different F_1 ewe types (Table 2; Bunge 1992) indicated that BM crosses had much lower profit/ewe. Given existing concerns at the time regarding slow growth and poor carcass merit in FL and Caribbean hair breeds, results of the Illinois study did nothing to create enthusiasm for use of the BM. The poor performance of the BM in this environment may have been due, at least partially, to their poor adaptation to a hot, humid environment. The study was conducted in southern Illinois (37º 26' N, 88º 40' W), with average high temperatures of approximately 35 ºC in July and August, high summer humidity, high internal parasite loads on pastures, and pastures composed primarily of tall fescue (*Festuca arundinacea*) infected with the toxic fungal endophyte *Neotyphodium coenophialum*.

At MARC, 18 BM and 31 FL rams were mated to either FL ewes or ewes of a composite line (C3) composed of 50% Columbia, 25% Suffolk and 25% Hampshire breeding over 4 years (Young and Dickerson 1988, 1991b). This study was significant because it provided the opportunity to assess the additivity of the polygenic effect of the FL and the single-gene effect of $FecB^B$ on ovulation rate (OR) and LS. Effects of breed of sire, breed of dam and their interaction were evaluated; the presence of interaction was considered indicative of differential effects of $FecB^B$ when expressed in a prolific versus non-prolific polygenic genetic background.

Differences between F_1 BM or FL lambs out of non-prolific C3 ewes at MARC were smaller than those observed in Illinois. Matings of C3 ewes to BM

Table 1. Growth and carcass characteristics of lambs sired by Booroola Merino (BM), Finnish Landrace (FL), St Croix (SC), Barbados Blackbelly (BB) and Combo-6 (C6) rams

Item	Sire breed					Avg. SE
	BM	FL	SC	BB	C6	
Weaning weight (kg)	17.2	19.1	17.4	16.5	18.6	4.0
Postweaning ADG[a] (g/day)	220	255	232	217	248	6.4
26-week weight (kg)	47.8	57.7	54.1	48.5	57.2	1.1
Age at 50 kg (days)	192	157	165	184	157	4.0
Dressing percentage	50.7	51.2	52.6	54.7	52.0	0.9
Hot carcass weight (kg)	26.7	27.0	27.2	27.9	27.1	0.5
12th rib fat (mm)	6.3	4.2	4.3	4.4	4.2	0.3
Lower rib fat (mm)	14.3	9.7	10.2	11.1	9.9	0.5
KPF fat (%)[b]	2.7	2.3	2.6	2.9	1.9	0.2
Ribeye area (cm^2)	14.9	14.6	15.1	16.8	15.2	0.3
USDA yield grade[c]	3.4	2.8	2.9	3.0	2.6	0.1

[a] ADG = average daily gain
[b] weight of kidney and pelvic fat as a percentage of carcass weight
[c] range from 1 to 5, with higher numbers indicating greater fatness and lower yield of closely trimmed retail cuts

rams resulted in non-significantly lower percentages of ewes lambing (73% vs. 76%), LSs (1.57 vs. 1.61), lamb survival (89% vs. 94%), and numbers of lambs weaned per ewe lambing (1.39 vs. 1.51) compared to matings to FL rams (Young and Dickerson 1988). Lambs by BM sires were 13% lighter than FL-sired lambs at 63 days of age and 10% lighter at 147 days of age (both $P < 0.001$), had lower dressing percentages at comparable harvest weights ($P < 0.001$) and much greater backfat thickness (0.375 vs. 0.310 cm), but comparable percentages of kidney and pelvic fat at equal carcass weights. Fleeces from 2-year-old BM F_1 ewes were 23% heavier than those of FL F_1 ewes. F_1 BM ewes out of C3 dams had much lower conception rates as ewe lambs (39% vs. 81%) and were equal in LS to FL F_1 ewes at 1 year of age (1.53 lambs), but had somewhat higher conception rates (99% vs. 91%) and substantially larger litters at 2 and 3 years of age (2.13 vs. 1.95 and 2.40 vs. 2.05 lambs, respectively; Young and Dickerson 1991b). However, numbers of lambs weaned favoured FL F_1 ewes at 1 (1.26 vs. 1.02) and 2 years of age (1.79 vs. 1.69), but no difference was observed in 3-year-old ewes (1.84). When backcrossed to C3 rams, FL F_1 ewes produced lambs that were 10% lighter at weaning and 8% lighter at 147 days of age than lambs from BM F_1 ewes. These results were more encouraging than those in Illinois regarding performance of BM F_1 ewes, but weaning rates still did not exceed those of F_1 FL ewes, and lamb growth rates remained inferior.

Effects of *FecB*^{B} on OR and LS were approximately additive in crossbred ewes out of C3 and FL dams. ORs of ewe lambs were 0.27 ova higher for BM compared to FL F_1 ewes out of C3 dams and 0.44 ova higher in F_1 ewes out of FL dams (Young and Dickerson 1988). In terms of LS, the effect of the BM versus FL sire at 1, 2 and 3 years of age was 0.00, 0.18 and 0.35 lambs in crosses with C3 ewes and 0.14, 0.21 and 0.49 lambs in offspring of FL dams, respectively (Young and Dickerson 1991b). Slightly larger effects of *FecB* in matings with FL ewes may have been influenced by differential expression of heterosis in matings of BM and FL rams to FL ewes. Effects of the BM versus FL on lamb growth were somewhat smaller in matings with FL ewes, again perhaps because of lower realised heterosis, but effects on fleece weight were larger (44% vs. 23%).

Results from San Angelo comparing BM × Rambouillet and purebred Rambouillet ewes for OR (2.64 vs. 1.63) and LS (2.04 vs. 1.39) at 2 years of age were generally consistent with the international literature, confirming no particular inconsistency in expression of *FecB*^{B} in crosses with non-prolific US breeds. Crosses of BM and FL to Rambouillet ewes and crosses of BM rams to FL ewes at San Angelo (Willingham et al. 1988) provided additional evidence for the approximate additivity of effects of *FecB*^{B} and the polygenic effect of the FL. Relative to the mean ORs of 1.44 at 1 year of age and 1.63 at 2 years of age for the Rambouillet, the average effect of one copy of *FecB*^{B} was 1.0 ova, the average effect of 50% FL breeding was 0.51 ova, and the BM × FL crosses exceeded the Rambouillet by an average of 1.21 ova. The percentage of ewes with four or more ovulations was considerably higher for BM × FL ewes (18.5%) than for either BM × Rambouillet ewes (9.8%) or FL × Rambouillet ewes (1.6%), suggesting less tightly regulated control of ORs in BM × FL ewes.

Introgression of *FecB*^{B} into other breeds

Experimental comparisons of BM and FL crosses showed no advantage to use of *FecB*^{B} in its original BM genetic background other than that observed in

Table 2. Lamb performance and profitability of crossbred ewes sired by Booroola Merino (BM), Finnish Landrace (FL), St Croix (SC), Barbados Blackbelly (BB) and Combo-6 (C6) rams

Item	Sire breed					Avg. SE
	BM	FL	SC	BB	C6	
Lamb weaning weight (kg)	15.1	17.3	16.6	15.6	17.1	2.7
Lamb postweaning ADG[a] (g/day)	217	231	234	207	245	4.7
Lamb 29-week weight (kg)	48.4	52.7	52.3	47.0	54.6	0.8
Lamb age at 50 kg (days)	234	212	211	240	206	4.2
Profit/ewe ($US)	–2.20	8.86	15.12	7.14	8.71	n.a.

[a] ADG = average daily gain

fleece weight and quality. Increasing emphasis on meat production relative to wool production and increases in lamb harvest weights in US production systems further reduced enthusiasm for use of the BM, and it thus became clear that use of *FecB*^{B} in the USA would require introgression into different genetic backgrounds.

Introgressions of *FecB*^{B} into a Rambouillet genetic background occurred in the mid-1980s at the University of Wisconsin (after transfer of the Illinois BM flock to that location) and at San Angelo, and resulting 'Booroola Rambouillet' populations are being maintained at both locations. Results from the first two backcrosses of BM × Rambouillet ewes to Rambouillet rams were presented by Southey et al. (2002), and provided estimates of effects of a single copy of *FecB*^{B} in a 75% or 87.5% Rambouillet genetic background. The effect of a single copy of *FecB*^{B} on OR did not change in a systematic way during upgrading, averaging 1.61 ova. The effect on *FecB*^{B} on LS was relatively consistent in 50% and 75% Rambouillet ewes, averaging 0.90 lambs, but was reduced to only 0.26 lambs in a small group (34 heterozygous and 17 wild-type) of young 87.5% Rambouillet ewes. Ewes with one copy of the *FecB*^{B} allele had lambs with significantly lower survival rates and weaning weights, and produced a similar weight of lamb per ewe exposed when compared to ewes of similar breed composition without the *FecB*^{B} allele. A subsequent analysis (B.R. Southey and D.T. Thomas, unpublished) was conducted to specifically assess the effects of *FecB*^{B} in the dam on lamb survival and body weights when lamb birth type was included in the models. In this analysis there were no effects of *FecB*^{B} on any lamb body weights or survival rates through 120 days of age, indicating that negative effects of *FecB*^{B} on lamb growth and survival are indirect through the positive effect of *FecB*^{B} on LS, and are not direct effects of *FecB*^{B}.

Production of commercial Rambouillet ewes carrying a single copy of *FecB*^{B} is achieved in the Wisconsin flock by continual backcrossing of Rambouillet–BM ewes to Rambouillet rams and differentiation of heterozygous ewes from wild-type ewes on the basis of laparoscopically determined OR or, currently, DNA testing. Comparisons of effects of *FecB*^{B} in a predominantly Rambouillet genetic background (Crooks et al. 1999, 2000) confirm no effect of *FecB*^{B} on fertility or average lamb weaning weight, but a 5-kg (9.2%) reduction in realised lamb weight (i.e. without adjustment for birth type) at 120 days. Effects of *FecB*^{B} were observed for OR (1.54 ova), LS (0.59 lambs), lamb survival (–8.5%) and ewe productivity at weaning (9.7%) at a lamb age of 180 days (8.9%), and at a lamb weight of 54.4 kg (20.8%).

At Texas A&M–San Angelo, offspring from heterozygous *FecB*^{B} rams mated to Rambouillet ewes were used to estimate the effects of *FecB*^{B} on OR (1.18 ova) and LS (0.51 lambs) (Schulze et al. 2003). Introgression of *FecB*^{B} into a Rambouillet genetic background resulted in a flock that is currently > 93.75% Rambouillet ancestry. Determination of *FecB*^{B} was accomplished by recording OR prior to 1993 and by DNA testing in later years. In the Texas A&M flock, the mean LS of mature Rambouillet ewes (4–7 years of age) with a single copy of *FecB*^{B} is 2.4 lambs.

The only major current commercial use of *FecB*^{B} in the USA is the Tamarack *Prolific* line in Minnesota. The Tamarack program (J. McNally, pers. comm.; <www.tamaracksheep.com>) started in 1987 with introgression of *FecB*^{B} into a Poll Dorset genetic background. Subsequent introduction of Ile de France breeding resulted in development of a segregating crossbred flock with relatively high frequency of *FecB*^{B} and producing a combination of homozygous *FecB*^{B}, wild-type and heterozygous offspring. The Tamarack program emphasised introgression of *FecB*^{B} into breeds of high genetic merit for milk production and mothering ability, and has been critical of programs that chose to use the Rambouillet as the basis for *FecB*^{B} introgression. The generally favourable maternal effects of the Poll Dorset and Ile de France have been accentuated by performance recording and use of a maternal selection index, which places emphasis on estimated breeding values for maternal weaning weights and numbers of lambs born and weaned. Tamarack *Prolific* ewes are claimed to be capable of weaning 240–320% lamb crops (McNally 2008). To our knowledge, this is the only private flock in the USA with an organised program to produce and market *FecB*^{B} breeding animals.

Summary and conclusions

The results summarised above suggest little opportunity for future use of *FecB*^{B} in commercial sheep production in the USA. This outcome mainly reflects the relatively high prolificacy levels already present in several US sheep breeds and the desire by

most US producers to avoid high frequencies of triplet births. However, if the mean LS of local breeds is below approximately 1.3 lambs/litter, use of *FecB*^{B} has the potential to rapidly increase the frequency of twinning with only modest increases in numbers of triplet births.

References

Bunge R. 1992. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois, USA. PhD dissertation, University of Illinois, Urbana-Champaign.

Bunge R., Thomas D.L. and Nash T.G. 1993a. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: lamb production of F_1 ewe lambs. Journal of Animal Science 71, 2012–2017.

Bunge R., Thomas D.L. and Nash T.G. 1995. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: lamb production of F1 adult ewes. Journal of Animal Science 73, 1602–1608.

Bunge R., Thomas D.L., Nash T.G. and Fernando R.L. 1993b. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: effect of breed of service sire on lamb production of Suffolk and Targhee ewes. Journal of Animal Science 71, 321–325.

Bunge R., Thomas D.L., Nash T.G. and Lupton C.J. 1996. Performance of hair breeds and prolific wool breeds of sheep in southern Illinois: wool production and fleece quality. Journal of Animal Science 74, 25–30.

Crooks A.E., Thomas D.L., Zelinsky R.D., Gottfredson R.G. and McKusick B.C. 1999. Introgression of the Fec^{B} allele of the Booroola Merino into a Rambouillet flock—a progress report. Pp. 61–62 in 'Proceedings of the 47th Annual Spooner Sheep Day', University of Wisconsin, Madison.

Crooks A.E., Thomas D.L., Zelinsky R.D., Gottfredson R.G. and McKusick B.C. 2000. Effect of the Fec^{B} allele of the Booroola Merino on weight of lamb marketed per ewe when introgressed into a Rambouillet flock. Pp. 7–9 in 'Proceedings of the 48th Annual Spooner Sheep Day', University of Wisconsin, Madison.

Davis G.H. 2005. Major genes affecting ovulation rate in sheep. Genetics Selection Evolution 37(suppl. 1), 11–23.

Dickerson G.E. 1977. Crossbreeding evaluation of Finnsheep and some U.S. breeds for market lamb production. North Central Regional Publication no. 246, Agricultural Research Service, US Department of Agriculture; and University of Nebraska, Lincoln.

Hulet C.V., Ercanbrack S.K. and Knight A.D. 1984. Development of the Polypay breed of sheep. Journal of Animal Science 58, 15–24.

Iman N.Y. and Slyter A.L. 1996. Lifetime lamb and wool production of Targhee or Finn–Dorset–Targhee ewes managed as farm or range flock. I: Average annual ewe performance. Journal of Animal Science 74, 1757–1764.

Iniguez L.C., Bradford G.E. and Mwai O.A. 1986. Lambing date and lamb production of spring-mated Rambouillet, Dorset and Finnsheep ewes and their F_1 crosses. Journal of Animal Science 63, 715–728.

McNally J. 2008. Origins of Tamarack sheep. At: <www.tamaracksheep.com>. Accessed 27 May 2008.

Notter D.R. and Copenhaver J.S. 1980. Performance of Finnish Landrace crossbred ewes under accelerated lambing. I: Fertility, prolificacy and ewe productivity. Journal of Animal Science 51, 1033–1042.

Schulze K.S., Waldron D.F., Willingham T.D., Shelby D.R., Engdahl G.R., Gootwine E., Yoshefi S., Montgomery G.W., Tate M.L. and Lord E.A. 2003. Effects of the FecB gene in half-sib families of Rambouillet-cross ewes. Sheep & Goat Research Journal 18, 83–88.

Southey B.R., Thomas D.L., Gottfredson R.G. and Zelinsky R.D. 2002. Ewe productivity of Booroola Merino–Rambouillet crossbred sheep during early stages of the introgression of the Fec^{B} allele into a Rambouillet population. Livestock Production Science 75, 33–44.

Willingham T., Shelton M. and Lupton C. 1988. The influence of introducing the Booroola Merino genotype to Rambouillet flocks on reproduction and fleece traits in comparison with other selected breed crosses. SID Research Journal 4(1), 1–5.

Young L.D. 1991. Origin of Booroola Merino genes in the United States. Pp. 57–59 in 'Proceedings of the 2nd International Workshop on Major Genes for Reproduction in Sheep'. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Young L.D. and Dickerson G.E. 1988. Performance of Booroola Merino and Finnsheep crossbred lambs and ewes. Journal of Agricultural Science in Finland 60, 492–499.

Young L.D. and Dickerson G.E. 1991a. Comparison of Booroola Merino and Finnsheep: effects on productivity of mates and performance of crossbred lambs. Journal of Animal Science 69, 1899–1911.

Young L.D. and Dickerson G.E. 1991b. Reproductive performance of ewes produced by mating Finnsheep and Booroola Merino rams to Finnsheep and crossbred ewes in the USA. Pp. 325–328 in 'Proceedings of the 2nd International Workshop on Major Genes for Reproduction in Sheep'. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Young L.D., Fahmy M.H. and Torres-Hernandez G. 1996. The use of prolific sheep in various countries: North America. Pp. 289–349 in 'Prolific sheep', ed. by M.H. Fahmy. CAB International: Wallingford, Oxon, UK.

Session 4:
The way forward—introgression of *FecB* in the wider population

Genetic aspects of Booroola introgression in breeding programs

J.H.J. van der Werf[1]

Abstract

This paper describes the genetic aspects of introgressing a major gene from a donor breed into a commercial recipient breed. 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. This difference can be small for traits that are easy to measure but is larger for reproduction traits, as in the case of Booroola. Various introgression strategies are compared for efficiency, including strategies for efficient dissemination of improved nucleus animals into the wider population.

Introduction

Introgression is the process where a desirable allele of a major gene (or genes) from a donor population is transferred into a commercial population. Usually, the favourable allele is absent in the commercial population. The genetic merit of the donor breed is usually lower than that of the commercial breed; otherwise, breed replacement through upgrading would be a better option. Therefore, introgression commences by producing a first cross (F1) between the two breeds, followed by a backcross (BC) to the commercial recipient population in order to recover the genome of that breed. After several generations of backcrossing, the BC animals will have a sufficiently high content of the original genome, and within-line selection can commence in an intercross (IC) among the final BC animals in order to increase the frequency of the favourable allele and to produce homozygotes for the major gene (Figure 1). Usually, males from the commercial population, which is still undergoing selection, would be mated to BC females, but the reverse is also possible.

The obvious advantage of introgression is to combine the favourable allele at the major gene in one breed with the otherwise favourable genetic background of the commercial breed. However, the gain at the major gene locus is initially offset by a loss in genetic merit at all other loci. Repeated backcrossing allows restoration of that loss to some extent. But there will be a genetic lag due to the time delay, and a permanent loss in genetic gain due to selecting animals for the favourable genotype at the major gene instead of selection for overall merit (Visscher and Haley 1999). If the rates of genetic improvement are low in the commercial population, introgression will usually have a positive outcome after several generations of backcrossing have been completed.

[1] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia; jvanderw@une.edu.au

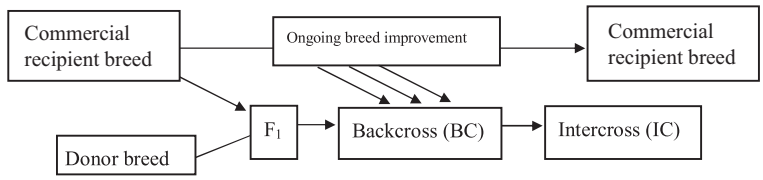

Figure 1. The various phases of an introgression program

It is generally assumed that the efficiency of introgression can be greatly improved when the major genotype can be inferred based on gene markers, a process referred to as marker-assisted introgression (MAI). Without knowledge of genotypes at the major gene, there is a chance of losing the favourable allele in the backcrossing phase. Dominik et al. (2007) found that the additional benefit of using gene marker information is small when the major gene is for a trait affecting wool quality. They found that selection based on best linear unbiased prediction (BLUP) was almost as efficient. However, it should be noted that their example is favourable for phenotypic selection, as the trait involved had a high heritability and can be measured in young animals before they are selected as parents. It is well known that the benefit of marker-assisted selection (MAS) is greatest when conventional selection for a trait is difficult, e.g. in the case of low heritability, sex-limited phenotypes and expression of a trait late in life. It is likely that MAI, like MAS, is most advantageous for such traits. Introgression of the Booroola gene falls into this category because it would result in improvement of reproductive performance, which is a trait that would benefit from information on genes or gene markers.

Various alternative strategies have been described to increase the efficiency of introgression. These include the use of selection, possibly based on additional gene markers, to increase the rate of recovery of the recipient breed background genes during the backcrossing; and mating strategies for crossbred males to commercial females, or vice versa (Hospital et al. 1992; Visscher and Haley 1999; Dominik et al. 2007). Besides the goal of increasing genetic merit, other issues need to be considered during introgression, notably the management of genetic diversity and inbreeding. Typically in an introgression scheme, there will be incentive to select on animals carrying the favourable allele, but the average co-ancestry among those animals will be higher than from average selection candidates (Nimbkar et al. 2006).

This paper will review the various aspects of introgression and the effect of the key parameters that determine the efficiency of the introgression process, including the size of the major gene effect introgressed, the difference between the donor population and the commercial population in overall genetic merit, and the rate of ongoing improvement in the commercial recipient population. The paper is mainly based on the theoretical model described by Visscher and Haley (1999), and examples will be used to illustrate the main points. In addition to discussion of the goal of increasing the merit of the breeding nucleus, dissemination to the population at large will also be outlined.

Allele frequencies in the backcross

Assume the targeted favourable allele is fixed in the donor line and absent in the recipient line. In the first generation, donor line males are crossed with recipient line females and all F1 progeny will be heterozygous. With ongoing backcrossing of BC females with males from the commercial recipient line, the allele frequency differences between BC and recipient lines are expected to be halved at each generation when mating is at random. After five generations of backcrossing, the frequency of the donor breed alleles at each locus would be less than 2%.

Selection would affect this process. If selection is for overall merit, the favourable alleles will increase in frequency. For background loci this would imply that their frequencies will more rapidly return to the commercial line frequencies. However, at the major gene the commercial allele is unfavourable and selection for merit would inhibit the loss of the favourable allele. The amount of selection pressure placed on the major gene will depend on the proportion that its allelic effect contributes to the overall variation in merit. A selection coefficient (SC) can be approximated as the square root of this proportion, basically reflecting the correlation between the

selection criterion and the allelic effect. The SC would be equal to unity when gene markers are used to select carrier BC females. In that case the allele frequency in the BC is kept at a maximum of 50%. The decline in major gene allele frequency is depicted in Figure 2, showing that the allele frequency would decline quickly in the backcrossing stage unless the major gene accounts for most of the variation in merit, or unless gene markers are used.

Selection in the BC generations would speed up the recovery of the recipient genome. It has been proposed to select in the BC using genetic markers scattered across the genome in order to recover the recipient genome more quickly. Hospital et al. (1992) concluded that retrieving the recipient's genome based on marker selection of background genes was approximately two generations faster if selection were used (Table 1). Selection based on markers is only more efficient if the breed difference is very large or if phenotypic selection is based on low accuracy (i.e. low heritability). The rate of retrieval is much slower at the chromosome carrying the major gene. The 'linkage drag' is the chromosomal region surrounding the favourable major gene allele originating from the donor breed, and this region could be 20–50 cM after five generations of backcrossing.

Merit of backcross versus commercial population

The genetic merit in the BC versus the merit of the commercial population depends on three factors: the effect (α) of the favourable major gene allele and its frequency in each line; the initial genetic difference (D) between the donor population and the commercial recipient population, which is due to the cumulative effect of all 'background genes'; and the difference in genetic lag. The latter is due to the

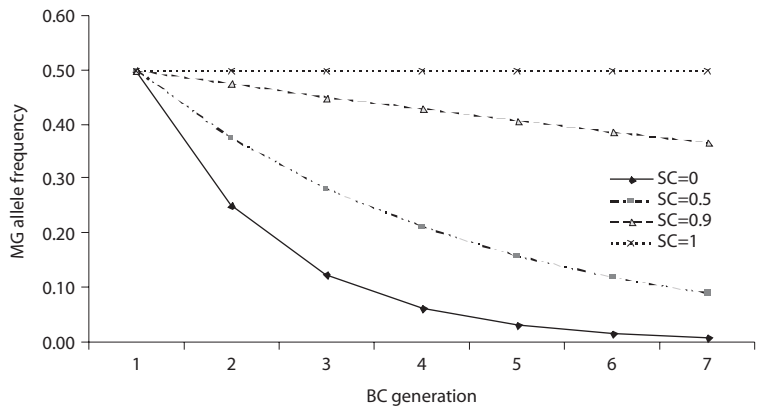

Figure 2. Decline in major gene (MG) allele frequency in the backcross (BC) for different values of the selection coefficient. The selection coefficient can be approximated as the square root of major gene variance as a proportion of total genetic variance (in merit). A selection coefficient (SC) value of 1 can be achieved when using gene markers

Table 1. Proportion of commercial recipient genome in the chromosome carrying the major gene and in non-carrier chromosomes, with and without selection on background markers

Backcross generation	Carrier chromosome		Non-carrier chromosome	
	Selection	No selection	Selection	No selection
1	0.636	0.607	0.817	0.750
2	0.747	0.684	0.943	0.875
3	0.855	0.741	0.983	0.938
4	0.891	0.784	0.993	0.969
5	0.903	0.817	0.996	0.984
6	0.913	0.842	0.998	0.992

Source: Hospital et al. (1992)

difference in rate of genetic gain between the commercial nucleus and the BC and initial IC generations. Usually, the genetic gain that can be achieved in the BC is smaller than in the nucleus because BC animals will need to be preselected based on carrying the favourable allele, i.e. half of the population will not be used as selection candidates. This effect is even stronger in the initial IC generations, where selection candidates will be required to be homozygous for the favourable allele.

There are two further reasons why rate of gain might differ (Visscher and Haley 1999). First, the heritability might be different; it is probably higher in the BC due to additional genetic variance in the crossbred population. Hill (1993) derived the genetic variance in the crossed line as the sum of within-line variance and a variance resulting from the difference between the lines. The variation between lines is proportional to *D* and the variance in genomic proportion, that is the extent to which BC individuals vary in the proportion of genes they carry from each breed compared to the expected proportion. The second reason for a different rate of gain is that the accuracy of selection can be higher in the cross, as genetic markers can be used to speed up the recovery of the recipient line genome. Hill (1993) showed that the variation in genomic proportion reduces very quickly, and Visscher and Haley (1999) showed that the benefit of marker selection on background genes is only efficient in the first generations of backcrossing, and only if the breed difference is large. Phenotypic selection proved to be generally more efficient in quickly reducing the genetic lag between BC and the commercial nucleus, although the differences were small. Therefore, if most traits are easy and cheap to measure, phenotypic selection would be the most cost-effective method to quickly recover the recipient genetic background, whereas genetic markers could help when breeding objective traits are difficult to measure before first matings.

Visscher and Haley (1999) give the following expression for the genetic difference between the commercial line and the BC line after *t* generations, assuming that males from the commercial line are mated to BC females:

$$\Delta_t = \alpha - \left(\frac{1}{2}\right)^t D + \sum_{i=1}^{t=1} \left(\frac{1}{2}\right)^t \delta_{t-i} - \left(1 - \left(\frac{1}{2}\right)^{t-1}\right)\delta_f \quad (1)$$

where the first term is the allele substitution effect of the major gene (α); the second term is the breed difference (D) due to the remainder of the donor genome, which is halved with each generation of backcrossing; and the third term represents the accumulation of the difference in selection response in each generation (δ_i), where δ_f refers to the selection differential in females. The term for male selection differential can be omitted when assuming that both nucleus and introgression lines have used the same sires.

In the absence of any selection other than for the major gene—that is, an introgression program that focuses only on recovering the recipient genome while maintaining the animals carrying the favourable major gene allele—the genetic difference becomes:

$$\Delta_t = \alpha - \left(\frac{1}{2}\right)^t D \quad (2)$$

This expression shows that, in the absence of ongoing selection, the difference between the BC and the commercial lines is simply a function of breed difference (declining over time) and the major gene effect.

From backcrossing to intercrossing

Figure 3 shows two simple examples of merit in recipient and BC populations. The example on the top is a case where the major gene effect is large relative to the breed difference, for example a Booroola case when considering fecundity as the main trait of relevance. In this example the assumed breed mean difference between donor and recipient breeds is 7 phenotypic standard deviations (σP) and the allele substitution effect is 3 σP. In this case the BC is almost immediately as good as the commercial line and it is tempting to immediately start the intercrossing phase. The example on the bottom represents the case where the gene effect is much smaller relative to the breed difference in overall merit. The allele substitution effect is now only 0.5 σP. In this case it is more evident that a few more generations of backcrossing are needed before initiating an intercrossing phase, as in the initial generations the genetic merit of crossbred animals is low compared to that of the commercial animals. Note that the latter example could also refer to the Booroola case, but where fecundity constitutes a much smaller proportion of variation in profit.

Figure 3 may incorrectly suggest that a larger gene effect (relative to the breed difference) justifies a

shorter backcrossing phase. However, this is not true and it should be kept in mind that ongoing backcrossing will increase the proportion of recipient-line background genes in the IC population.

At some generation *T*, backcrossing can cease and further matings can occur among the animals in the BC population. Hence, at generation *T* the introgressed BC animals will be intercrossed and both males and females carrying a copy of the favourable allele will be selected. In generation *T*+1 it would be ideal to use only homozygous carriers of the allele. This may not be possible in lowly fecund species like sheep, and although using additional heterozygous animals will delay the process of fixation by one or two generations, this will not have a large impact on the optimal choice of *T*.

Consider the case where there is no selection in the commercial nucleus population or in the BC. The difference between the introgression population and the commercial nucleus at generation *T*+2 can be determined as:

$$\Delta_{T+2} = 2\alpha - \left(\frac{1}{2}\right)^{T} D \qquad (3)$$

Therefore, choosing a small value for *T* (generations of backcrossing) will limit the potential genetic merit of the introgressed population. The remaining proportion of donor genome will be halved at each generation of backcrossing with random mating, but it will not be affected during the IC stage unless selection takes place. In the case where the gene effect is large relative to the breed difference, such as in the Booroola case, there may be an urge to quickly use BC individuals that carry the favourable allele, for example individuals that carry 75% or 87.5% of the recipient genome. Male carriers could be sold for the purpose of early dissemination of the desirable allele, but it would be more advantageous

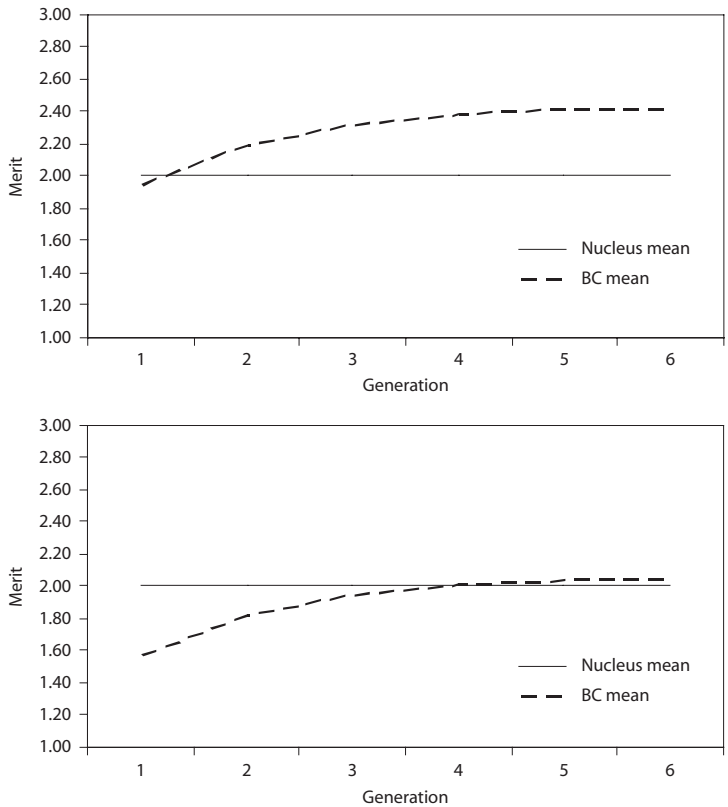

Figure 3. Mean merit of commercial nucleus and backcross (BC) when the major gene effect is large relative to the breed difference (1:2; top) and where it is relatively smaller (1:13; bottom).

to sell homozygous carriers. However, this would first require a generation of crossing BC males and females, which would in fact be intercrossing. Such intercrossing should be of animals not used for nucleus breeding, as the best females of the BC should be backcrossed to males from the recipient breed in order to preserve a nucleus of highest merit BC animals. After all, it is much easier to achieve genetic gain through backcrossing than through selection in an intercrossing phase.

Figure 4 illustrates this for two cases. In case A the gene effect (α) is 2 σP and the breed difference is 4 σP. The recipient line improves with a rate of 0.32 σP per generation (heritability = 0.1; selection intensities are 2 and 0.5 for male and female respectively; and selection accuracies are 0.6 and 0.5 for male and female respectively). It is assumed that the IC generation has a higher rate of improvement, as effectively there is more genetic variation in the cross. The IC improves at a rate of 0.43 σP per

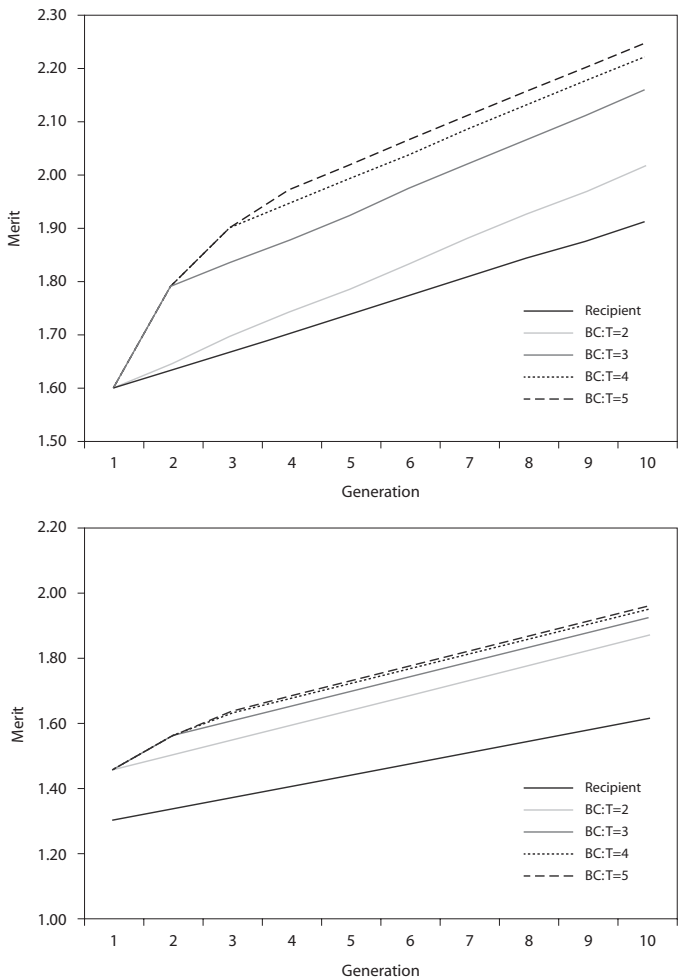

Figure 4. Genetic merit of recipient and backcross (BC) generations when the moment of intercrossing (*T*) is varied from 2 to 5. Case A (top) assumes a breed difference (*D*) of 4 phenotypic standard deviations (σP) and case B (bottom) assumes 2 σP. The allele effect is 2 σP and the response to selection is about 0.3 σP per generation.

generation. This is a 'best case scenario' as selection intensities will actually be lower in initial IC generations. The examples show that increasing the genomic proportion of the recipient line increases merit at a much higher rate than within-line selection, e.g. $0.25D = 1.0\ \sigma P$ from generation 1 to 2; and $0.125D = 0.5\ \sigma P$ from generation 2 to 3, $0.25\ \sigma P$ from 3 to 4, and $0.125\ \sigma P$ from 4 to 5, where D is the breed difference. Only after generation 5 is the difference in selection response higher than the recovery of recipient genome. In case B, D is only $2\sigma P$ and the effect of further backcrossing becomes negligible after four generations of backcrossing.

Visscher and Haley (1999) show that, in the case of selection, the difference between the commercial nucleus and the IC population at generation $T + 2$ can be determined using equation (4) (at foot of page).

The last term in equation (4) is additional compared to equation (1) and describes the genetic lag arising during the first two generations of intercrossing. The difference in selection intensity is larger in the initial generations of intercrossing, as both females and males are now preselected on major gene genotype. In generation $T + 1$ only 25% will be homozygous carriers, and therefore the difference in rate of improvement will be largest. Equation (4) suggests that after several generations of introgression, the difference between the commercial and the introgression lines becomes roughly $2\alpha - 3(\delta - \delta BC)$; when assuming no selection in the BC, this is simply $2\alpha - 3\delta$. Hence, for introgression to be profitable in the long term, the difference between the homozygous major gene genotypes for an additive quantitative trait locus (QTL) should be at least three times the rate of genetic improvement in the commercial nucleus, which, in sheep, would be typically three times 3–6% of the mean.

Introgressing the Booroola gene

The effect of the favourable Booroola mutation is relatively very large. Although the effect under Indian conditions, as described by Nimbkar et al. (2008a, unpublished), is much smaller than that originally described for the Australian Merino (Piper et al. 1985), an increase of 0.3 lambs extra weaned is still large, equivalent to about $2\ \sigma P$. The allele effects are non-additive in the breed genotypes tested in India by Nimbkar et al. (2008a, unpublished) and we should assume the difference between homozygotes (2α) to be about $2\ \sigma P$. These effects need to be related to the variation in overall profit and, more specifically, to the potential genetic gain and the breed difference. Nimbkar (2006) estimated, for Indian conditions, an economic value for litter size of $7.29 per lamb weaned and for 3-month weight of $0.92 per kg, with these two traits being the most important in the overall breeding objective. If approximately one-third of the variation in profit is due to litter size, we could assume that $2\alpha = 0.7\ \sigma P2$, where $P2$ is phenotype for overall profit. In efficient sheep-breeding programs the annual rate of gain that can be achieved is about 0.1–0.2 $\sigma P2$, implying that, in the long term, introgression should be beneficial. The benefit is larger if the rate of improvement due to phenotypic selection is lower. In the short term the difference between donor and recipient breed is relevant as well. Results from Nimbkar et al. (unpublished) show that the polygenic breed difference between Deccani and Garole breeds is small for fecundity. However, the breed effect on body weight, which is an important profit driver, is very large. For an economically sound assessment, D should encompass breed difference for overall profit. Taking 3-month weight as a proxy for 'background genetic merit' would suggest that the financial value of a breed difference of 5 kg at 3 months of age is almost twice the financial advantage of the extra lambs weaned. Hence, in our model, $D \approx 2\alpha$, which is illustrated by case A in Figure 4. Therefore, although the Booroola effect is relatively large, it is recommended to maintain a BC phase of at least four generations.

Dissemination of intercrosses

An important practical aspect of any breeding program, but especially in developing countries, is dissemination of genotypes with improved genetic merit. This is also the case for MAI programs. In this paper I have not attempted a detailed model of dissemination. However, the principles discussed

$$\Delta_{T+2} = 2\alpha - \left(\frac{1}{2}\right)^{T} D + \sum_{i=1}^{t-1} \left(\frac{1}{2}\right)^{t} \delta_{T-i} - \left(1 - \left(\frac{1}{2}\right)^{T-1}\right)\delta_f - (2\delta - \delta_T - \delta_{T+1}) \qquad (4)$$

above are also relevant to dissemination; that is, the merit of the improved population depends on the effect of the introgressed genes as well as on the difference between donor and recipient breed. The latter effect is likely to be small if the major gene has first been introgressed in a nucleus population. Therefore, dissemination strategies can focus primarily on selecting males with the desired homozygous genotype and high polygenic breeding values, and can optimise the multiplier structure where progeny from such males can, in turn, pass on the effects to the commercial population. The multiplier structure is mainly a function of reproductive rate, the method of reproduction and logistical constraints. For example, artificial insemination will create more flexibility and possibly a higher rate of dissemination. There may be additional constraints to account for, such as those due to environment or disease risk. The main challenge will be to create an efficient collaborative structure where there are adequate incentives for all players to use the improved genotypes.

Simple gene flow models can give an indication of the time frame of the resulting increase in genetic merit at the commercial level. Figure 5 gives an example of such a scheme, where the desired alleles are disseminated to a commercial population through males only. In the example we use a typical age structure, with generation intervals for males being 2.5 years and for females 3.5 years, and we introduce only homozygous rams in a self-replacing flock of dams. The figure shows that it takes a substantial number of years (about 25) before the desired allele is fixed in the commercial population. Note that the lag will be even longer if more multiplier layers are needed.

Discussion

The model presented so far has mainly considered improvement per generation, whereas, in reality, selection is across age classes. The rate of improvement will therefore be more gradual, with the different age classes slowly being upgraded. Modelling improvement per year with overlapping age classes will provide more precise predictions of merit, but will be unlikely to lead to different conclusions about the relative merit of the IC generation versus the recipient breed for various levels of breed differences and gene effects. As pointed out by Visscher and Haley (1999), the genetic lag that develops due to limited ability to select in the BC and the initial IC generations ultimately determines the efficiency of the MAI program. Selection based on phenotype or BLUP-estimated breeding values during the BC and IC phase are the best remedies to limit this genetic lag. Gene markers should be used to select for the favourable major genotype. Selection based on genome-wide markers to recover the recipient genome would be mainly useful if polygenic breed differences are large, and would create additional costs. To avoid much reduction in

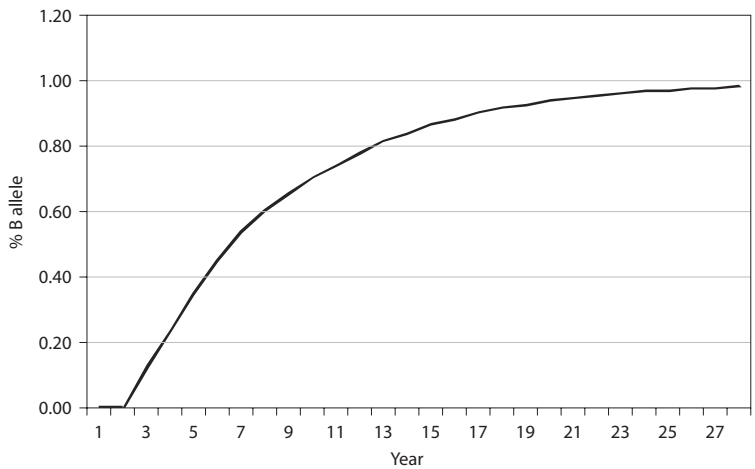

Figure 5. Rate of increase of the desired major gene allele (B) into the commercial population

selection intensity during the BC and IC stages, the reproductive rate could be increased artificially, for example by applying multiple ovulation and embryo transfer to females. Again, this would be a costly exercise, and the benefit would be somewhat negated by the need to maintain sufficient genetic diversity in the IC population.

One important aspect of selection in MAI is to balance the rate of improvement for genetic merit with genetic diversity, especially in the intercrossing phase. Typically, the few animals with the desirable homozygous carrier genotype will have a higher degree of co-ancestry, and short-term gain in merit will need to be compromised for long-term sustainability of the breeding program by selecting sufficient numbers of unrelated sires each generation for the breeding nucleus. Nimbkar (2006) and Nimbkar et al. (2008a) have used a mate selection approach (Kinghorn et al. 2002) to handle this problem in an optimal way. It should be noted that the number of sires used for introgression from the donor breed can be very small, as the genomes contributed by these are supposed to disappear other than in the major gene region. It is the population size of the recipient breed and therefore the number of animals in the BC and IC generations that determine the effective population size of the new breed.

The model presented has ignored the variation that exists among individuals in the different BC generations. In realistic situations appropriate genetic evaluation should be used for BC individuals, possibly with overlapping generations, using BLUP estimates from a mixed animal model with polygenic breeding values, adjusted for differences in breed proportion and major gene genotype. Dominik et al. (2007) found that selection on BLUP estimated breeding value was almost as efficient as MAI, but they used an example of a high heritable trait in sheep. MAI is likely to achieve higher rates of progress than BLUP selection within the commercial population in the case of fecundity, which is a sex-limited and low heritable trait. It would be useful to use stochastic simulation to study optimal MAI strategies when considering the more dynamic aspects of overlapping generations and selection of individuals across BC generations.

Dominik et al. (2007) used stochastic simulation to compare different strategies where either males were chosen from the recipient breed, or females or both (i.e. reciprocal crosses). They found that the strategy where males from the recipient breed were crossed with BC females was generally the most profitable strategy. They did not give a clear explanation of this important practical phenomenon, but equations (1) and (4) in this paper show that if males were selected from the BC line, the term δ_f would be replaced by δ_m. In other words, since selection differentials in males are higher, it is a better strategy to forgo selection in BC females than in BC males.

Conclusion

MAI requires several generations of backcrossing to maximise the merit of the resulting IC most efficiently. The additional gain from MAI depends on the major gene effect, the breed difference and the genetic lag in the IC due to the need to select for desired major genotype at the expense of progress in polygenic effects. In the long term the breed difference is not relevant as the recipient commercial genotype is nearly recovered after five generations of backcrossing. This difference will mainly affect the cost of the MAI program. It is generally most profitable to cross females from the BC generations with males from the recipient breed.

Acknowledgments

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References

Dominik S., Henshall J, O'Grady J. and Marshall K. 2007. Factors influencing the efficiency of a marker-assisted introgression programme in Merino sheep. Genetics, Selection, Evolution 39, 495–511.

Hill W.G. 1993 Variation in genetic composition in backcrossing programs. Journal of Heredity 84, 212–213.

Hospital F., Chevalet C. and Mulsant P. 1992. Using markers in gene introgression breeding programs. Genetics 132, 1199–1210.

Kinghorn B.P., Meszaros S.A. and Vagg R.D. 2002. Dynamic tactical decision systems for animal breeding. In 'Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France. CD-ROM Communication No. 23-07.

Nimbkar C. 2006. Genetic improvement of lamb production efficiency in Indian Deccani sheep. PhD thesis, University of New England, Armidale, New South Wales, Australia.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Ghalsasi P.P., Gupta V., Pardeshi V.C., Maddox J.F., van der Werf J.H.J. and Walkden-Brown S.W. 2008b. Biological and economic consequences of introgression of the *FecB* (Booroola) gene into Deccani sheep. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 90–99. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London.

Visscher P.M. and Haley C.S. 1999. On the efficiency of marker-assisted introgression. Animal Science 68, 59–68.

The economics of litter size in meat sheep

A. Swan[1]

Abstract

Breeding objectives for sheep derived from economic analysis of production systems show that litter size (LS) has a significant impact on profitability. Increasing LS can lead to increased income because more surplus animals are available for sale, but this comes at the expense of higher costs associated with increased feed requirements for ewes during pregnancy and lactation, and for finishing larger numbers of lambs. Poorer lamb survival in large litters also has an impact on costs. For these reasons the economic value for LS should be determined from a realistic bioeconomic model that accounts for the relationship between LS, feed cost and lamb survival, in addition to other economically important traits. This is particularly important when evaluating the importance of LS in harsh environments.

Introduction

Litter size (LS) is an important component of overall reproductive efficiency in sheep which, in turn, has a major impact on the profitability of sheep enterprises. Improving reproductive efficiency leads to more surplus lambs for sale, with the potential to generate higher financial returns. However, these higher returns are associated with higher costs due to various biological compromises. The first of these is that ewes bearing more than one lamb per litter have higher feed requirements during pregnancy and lactation. There will also be extra feed costs incurred because there are more lambs within the flock as average LS increases.

Lambs born in litters are also smaller than lambs born as single offspring, and are either smaller and consequently less valuable at sale age, or take longer to reach a sale weight target. Importantly, lambs born in litters have poorer survival than single-born lambs due to the effects of birthing difficulties, low birth weights, poor maternal behaviour and increased disease susceptibility. Finally, higher LS may have an impact on ewe longevity, although this effect has not been well studied due to the difficulty in observing longevity.

Reproductive efficiency can be improved by both environmental manipulation, including management interventions, and by genetic improvement of traits such as LS. This paper focuses on improvement of reproduction by genetic selection. The aim is to review the issues surrounding the inclusion of reproduction traits in breeding objectives that target increased profitability of sheep enterprises.

Reproduction traits

For the purposes of this paper, reproductive efficiency will be defined as the number of lambs weaned per ewe joined (NLW). This is a composite trait which can be expressed as the product of ewe fertility (ewes lambing per ewe joined), LS (lambs born per ewe lambing) and ewe rearing ability, or lamb survival as a trait of the ewe (lambs weaned per lamb born).

Ewe fertility, LS and lamb survival represent distinctly different but interacting events in overall

[1] Animal Genetics and Breeding Unit, University of New England, Armidale, New South Wales 2351, Australia; andrew.swan@une.edu.au
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reproductive efficiency. Ewe fertility depends on whether or not females are in oestrus at the time of mating, and is affected by nutritional and disease factors, mate fertility, early embryo survival and the length of the mating period. LS is a function of ovulation rate (OR) and embryo survival. With the latter displaying little or no genetic variation, Hanrahan (1980) argued that the genetic correlation between OR and LS is unity. Lamb survival is a complex trait influenced by both direct and maternal genetic effects (Everett-Hincks et al. 2005).

Table 1. Reproduction trait heritabilities (h^2), coefficients of variation (CV) and genetic correlations (r_g) with number of lambs weaned (NLW)

Trait	h^2	CV	r_g NLW
Fertility	0.08	52	0.73
Litter size	0.13	34	0.62
Ewe-rearing ability	0.06	40	0.63
Lamb survival[a]	0.03	46	–
NLW	0.07	64	–

[a] as a trait of the lamb
Source: Safari et al. (2005)

Table 1 shows average estimates of heritabilities and genetic correlations from a review by Safari et al. (2005). Reproduction traits have low heritabilities but high phenotypic variability, and potential responses to selection that are similar to traits with higher heritabilities but lower variability (e.g. body weight). However, responses to selection for reproduction have been slow, due mainly to lack of pedigree performance records in many sheep populations and because of the difficulty of achieving expected selection differentials for traits with few categories of expression. Nevertheless, the results of selection experiments demonstrate that substantial changes can be achieved in the long term. In addition, the use of major genes for reproduction (Davis 2005), including the *FecB* mutation, has the potential to increase the rate of response.

The importance of reproduction in sheep-breeding objectives

Breeding objectives for sheep have been constructed using the classical selection index methodology (Hazel 1943), where the aggregate genotype is a linear function of breeding values for the traits that affect profit, both costs and returns. The breeding value for each trait is weighted by its economic value, which is the monetary value of a unit improvement in the genotypic value of the trait. Selection indexes that maximise response in the aggregate genotype given the measurements available on individual animals can then be constructed.

Economic values for sheep enterprises have been derived from profit equations expressed in terms of the traits that affect costs and returns (Table 2). Examples of this approach have been published by Ponzoni (1986, 1992), Amer et al. (1999), Kosgey et al. (2003), Conington et al. (2004) and Nimbkar (2005), and any of the models developed in these studies can be used as a basis for developing robust breeding objectives.

These studies encompass a wide range of enterprises from high-input intensive to low-input extensive production systems in both developing and developed countries. They show that, for the scenarios studied, LS and/or NLW are important traits of the breeding objective, with significant positive economic values indicating the desirability of an increase in mean level of performance.

However, both Amer et al. (1999) and Conington et al. (2004) showed that the economic value of LS is non-linear depending on the flock mean. It is highest at low LSs, declines as LS increases, reaching zero at the optimal LS for the enterprise,

Table 2. Examples of traits which may be included in sheep-breeding objectives

Trait group	Specific traits
Reproduction and adaptation	Fertility, litter size, age at first mating, lambing frequency, lamb survival, ewe longevity
Output	Live weight / carcass yield of surplus animals, wool weight, manure production
Product quality	Carcass fat, meat tenderness, fibre diameter, staple strength
Input	Feed intake
Disease	Gastrointestinal nematode resistance

and is negative for further increases in the trait when the costs of extra lambs outweigh the returns.

The base populations in the four studies referred to were defined with LSs in the range 1 to 1.5, and NLW ranging from 0.8 to 1.2, typical values for the majority of sheep populations in most countries. At these levels the economic value of increasing LS is clear.

In the case of Conington et al. (2004), three types of UK hill sheep farms were modelled: extensive, semi-intensive and intensive. For the extensive farm, enterprise gross margin plateaued between 0.92 and 0.96 lambs per ewe, indicating that the optimal LS was in this range. For the intensive farm, enterprise gross margin showed a linear increase from the base level of 1.21 lambs per ewe to 1.34 lambs, and then remained relatively constant to 1.46 lambs.

Amer et al. (1999) modelled seven different farm types typical to New Zealand, ranging from low-input 'hill country' farms to intensive finishing systems. Across this range the economic value of LS approached zero at approximately 2.2–2.3 lambs per ewe, with the biggest reductions occurring at the points where triplet and quadruplet litters became more common. Farms with lower levels of lamb survival realised less economic value for increased LS.

These studies highlight the importance of calculating the economic value of LS using accurate data on the production system and at the correct flock mean, and of re-evaluating the breeding objective continually in flocks making genetic progress.

Sheep breeders in Australia have used customised breeding objectives developed using the OBJECT software package for Merino sheep (Atkins et al. 1994) and, more recently, SheepObject, which caters for a greater range of wool, meat and dual-purpose breeding systems (Swan et al. 2007). The importance of reproduction (NLW) compared to other traits in these objectives can be determined following Barwick and Henzell (2005), by scaling the relative economic value (*rev*) for each trait by its genetic standard deviation (σG) and expressing as a percentage of the total for all traits:

$$\frac{rev_j.\sigma_{Gj}}{\sum|rev_i.\sigma_{Gi}|}\times 100 \qquad (1)$$

In objectives for dual-purpose enterprises, the relative importance of reproduction averages 31%, ranging from 21% to 39%; and for enterprises focused on wool the average is 25%, ranging from 14% to 39%. While these figures demonstrate the importance of improving reproduction in Australian flocks, this is not reflected in response to selection in the majority of flocks because they do not record reproduction. Rather, the focus of measurement programs is on meat and wool, with the consequence that these traits dominate in selection indexes.

Methods of including reproduction in breeding objectives

Reproduction has been included in breeding objectives for sheep in different ways. Ponzoni (1986, 1992) used the overall trait NLW, as do the objectives used by Australian sheep breeders. This approach is reasonable for low to moderate levels of reproductive efficiency, as observed in Australian flocks (NLW of 0.7–1.2), and where reproduction is not measured in the flocks under consideration. Under these circumstances, estimated breeding values for reproduction will be determined through genetic correlations with production traits such as body weight, and partitioning NLW into its components will make no difference.

The preferred alternative is to include the components of reproductive efficiency in the breeding objective, modelling LS and lamb survival in particular as separate traits. This was the approach used by Amer et al. (1999), Kosgey et al. (2003) and Conington et al. (2004), allowing a more accurate definition of flock performance for reproductive efficiency.

As discussed above, the component traits represent quite distinct but interacting events. For example, as average LS increases, the frequency of twin, triplet and quadruplet litters also increases, at the expense of single births, and lamb survival shows a substantial decline in larger litters (Amer et al. 1999; Everett-Hincks et al. 2005).

It is important to model these effects adequately in order to accurately assess the profitability of increasing LS. In doing so, predictions of the frequencies of different LSs, given a particular flock mean LS, are required. Amer et al. (1999) used a threshold-liability model for ovulation rate to predict LS, validating on observed data. Conington et al. (2004) used a different approach, calculating expected values from the mean and variances of LS, weighted by scaling factors appropriate for each age of dam class.

A second reason to include LS and lamb survival in the objective as separate traits is that LS has the highest heritability of the component traits, while the heritability of NLW is reduced through the influence of fertility and lamb survival (Table 1). Consequently, greater response in reproductive performance can be expected when LS is the trait in the objective.

In the context of genetic evaluation, reliable data on LS are easier to collect than on lamb survival. For example, ultrasound pregnancy scanning during the last trimester can be a reliable indicator of LS, and is less expensive to collect than birth records. It is also useful to record as a management strategy, allowing differential feeding of ewes of different pregnancy status.

Lamb survival, on the other hand, is poorly recorded in industry flocks because breeders see little value in collecting data on dead animals. However, the combination of ultrasound scanning to estimate LS at birth with electronic tag technology to match surviving lambs with their dams (Richards and Atkins 2007) has the potential to provide estimates of lamb survival as a trait of the ewe.

Feed costs of increased litter size

The approach commonly used to account for feed costs in sheep-breeding objectives is to include relationships of body weight and reproductive status with feed intake in the model (e.g. Australian Agricultural Council: Ruminant Subcommittee 1990), and to assume there are no genetic differences between animals in feed intake and efficiency. These relationships show increased feed consumption with higher body weights and for ewes rearing larger litters.

As the cost of feed increases, the economic value of increased LS decreases (Rae 1988; Ponzoni 1992). The cost of feed affects the economic value of LS due to increased energy demands for ewes during pregnancy and lactation, as well as the need to feed extra lambs. The impact of extra lambs on feed costs may depend on whether they are sold at a constant age or a constant weight. Amer et al. (1999) calculated lower economic values for LS when lambs were sold at a constant weight rather than a constant age. This was because lambs born in larger litters were smaller and took longer to grow to a set finishing weight, requiring more feed.

Higher growth and increased LS can be offset in the model either by assuming a fixed feed base and reducing flock size (Jones 1982), or by purchasing additional feed. One benefit of assuming a fixed feed base is that economic values are then independent of the cost of feed (Ponzoni 1992), which can be difficult to determine accurately.

Reducing flock size as LS increases can have benefits where pasture production is seasonal. For example, in temperate environments having fewer ewes in winter and more lambs in spring can result in better alignment between pasture production and the feed requirements of a more prolific flock.

A fixed feed base may be a realistic assumption in a variety of situations because sheep in many countries are used to graze non-arable land and crop residues. Also, where new grazing areas are limited or expertise in pasture improvement and fodder conservation is poor, the prospects of expanding production may be limited. Nevertheless, at other times it will be possible to buy extra feed by various means including land purchase, and the option to do so should be properly evaluated in the breeding objective model.

Under some circumstances it may be possible to ignore feed costs altogether. This might occur, for example, when pasture is not fully used (Rae 1988), perhaps intentionally by maintaining low stocking rates. With the use of modern grazing practices and competition for land resources in most, if not all, countries with significant sheep populations, this will rarely be a realistic proposition. However, where pasture production is seasonal, it is possible to ignore feed costs for part of the year, for example during a spring flush in temperate climates. The SheepObject software system has the option to assume zero feed costs for part of the year.

The assumption of no variation between animals in feed intake and efficiency is incorrect, as demonstrated by Lee et al. (2002) for fine-wool Merino sheep. Given the importance of feed costs to the overall enterprise, feed intake should ideally be included directly as a trait of the breeding objective model (James 1982). This has been done for sheep by Ponzoni (1986, 1992) and Kosgey et al. (2003). The difficulty with this approach is that the heritability of feed intake and its correlation with other economically important traits are not well known because the trait is very expensive to measure on individual animals and in pasture grazing situations.

One benefit of increasing LS may come from reducing greenhouse gas emissions. Assuming a fixed feed base, ewe flock size must be reduced as the flock becomes more productive. With fewer

ewes and more lambs slaughtered at an earlier age, it may be possible to significantly reduce emissions. This may have a direct effect on profit in countries which introduce emissions trading schemes that include agriculture.

Reduced lamb survival and ewe longevity with increased litter size

Apart from an increase in the feed required, the other main cost associated with increased LS is a reduction in lamb survival. The causes of this relationship are many, with influences from both the lamb and maternal sides. These have been reviewed by Menendez Buxadera et al. (2004). The causes of decreased lamb survival in larger litters include increased competition between foetuses and consequent lower birth weights, poorer maternal–offspring recognition and bonding, a reduction in colostrum and milk production per lamb and a greater increase in ewe parasite load during the lambing period (the periparturient rise).

The relationship between greater lamb mortality and larger litters is the basis for the argument that LS and lamb survival should be treated as separate traits in the breeding objective. Conington et al. (2004) have suggested that, in some situations, improvement of overall reproductive efficiency could focus more on improving lamb survival or rearing ability than on increasing LS. The difficulty with this is that genetic variation in these traits is usually limited (Table 1) and they are not well recorded.

Mysterud et al. (2002) presented evidence that the reduction in LS with older ewes occurred from 5 years of age in a highly domesticated population, compared to approximately 10 years of age in a lightly domesticated population under natural selection, for example the Soay breed from the island of St Kilda in the Outer Hebrides of Scotland. The authors hypothesise that the early onset of reproductive senescence in domestic sheep is related to selection for increased LS.

Although other factors could be involved, and the study does not shed light on variation between animals, a relationship between increased LS and early reproductive senescence could be approached by including ewe longevity as a trait of the breeding objective. This is possible in the SheepObject software system, although the trait accounts for only a small proportion of the economic gain in objectives with typical ewe flock age structures.

In this case the difficulty would be how to model the relationship between LS and reproductive senescence given the lack of experimental evidence. There is also little evidence of genetic variation for longevity in sheep, although in dairy cattle the trait is lowly to moderately heritable (Gonzalez-Recio and Alenda 2007).

The economic value of *FecB*

Amer et al. (1999) calculated the economic value of the *FecB* mutation for different New Zealand farm types assuming an increase in ovulation rate of 1.6 per allele (from Piper et al. 1985). The economic value for a single allele was high, except for farm types in harsh environments, where lamb survival was low. There was very little extra value for two alleles, and in some cases the effect on profit was negative due to increased embryo wastage and reduced lamb survival.

Although the increases in LS with both one and two copies of *FecB* appear to be manageable in the Nimbkar Agricultural Research Institute (NARI) flock under Indian conditions (Nimbkar et al. 2007), the extreme effect of the mutation elsewhere has limited its use by commercial breeders.

Should litter size be increased in harsh environments?

Sheep are often run in environments where feed quality is poor and pasture production is highly variable from year to year. In fact, this is likely to be the rule rather than the exception. Under these conditions both the ewes bearing larger litters and the offspring from those litters are at a greater risk of nutritional deprivation, leading to poorer growth, higher mortality rates and difficulty in rebreeding (Simm et al. 1996).

Inounu et al. (1993) studied the reproduction of Javanese ewes in West Java, a population in which the *FecB* mutation is segregating. Good nutritional conditions led to better ewe gestation weight gain, higher birth weights, improved lactation performance and better lamb survival. For ewes carrying one copy of *FecB*, ewe productivity as measured by total litter weaning weight was increased by 18% under these conditions. By contrast, under poor nutritional conditions, ewe body reserves and lamb survival were poorer and the effect on ewe productivity was negative.

Such findings show that increasing LS will not always be beneficial, particularly for large increases. This highlights the importance of determining the economic value of LS from a realistic bioeconomic breeding objectives model.

Conclusions

Reproductive efficiency is a key driver of the profitability of meat sheep enterprises. Its influence is complex, operating on both costs and returns, and the trait itself is the outcome of a series of interacting events from ovulation to fertilisation, gestation, birth and rearing. The economic importance of reproductive efficiency relative to other traits should be determined using the bioeconomic approach for breeding objectives. The model used should ideally treat LS and lamb survival as separate components of reproductive efficiency. Given that the economic values of these traits are non-linear, depending on the flock mean reproduction in the environment under consideration, they should be derived using the most realistic model possible and re-evaluated as genetic gains accumulate.

References

Amer P.R., McEwan J.C., Dodds K.G. and Davis G.H. 1999. Economic values for ewe prolificacy and lamb survival in New Zealand sheep. Livestock Production Science 58, 75–90.

Atkins K.D., Semple S.J. and Casey A.E. 1994. OBJECT—personalised breeding objectives for Merinos. P. 79 in 'Proceedings of the 5th World Congress on Genetics Applied to Livestock Production', vol. 22, Guelph, Canada.

Australian Agricultural Council: Ruminants Subcommittee 1990. Feeding standards for Australian livestock: ruminants. CSIRO Publishing: Melbourne, Australia.

Barwick S.A. and Henzell A.L. 2005. Development successes and issues for the future in deriving and applying selection indexes for beef breeding. Australian Journal of Experimental Agriculture 45, 932.

Conington J., Bishop S.C., Waterhouse A. and Simm G. 2004. A bio-economic approach to derive economic values for pasture-based sheep genetic improvement programs. Journal of Animal Science 82, 1290.

Davis G.H. 2005. Major genes affecting ovulation rate in sheep. Genetics, Selection, Evolution 37(suppl. 1), S11–S23.

Everett-Hincks J.M., Lopez-Villalobos N., Blair H.T. and Stafford K.J. 2005. The effect of ewe maternal behaviour score on lamb and litter survival. Livestock Production Science 93, 51.

Gonzalez-Recio O. and Alenda R. 2007. Genetic relationship of discrete-time survival with fertility and production in dairy cattle using bivariate models. Genetics. Selection. Evolution 39, 391.

Hanrahan J.P. 1980. Ovulation rate as the selection criterion for litter size in sheep. Proceedings of the Australian Society of Animal Production 13, 405–408.

Hazel L.N. 1943. The genetic basis for constructing selection indexes. Genetics (USA) 28, 476.

Inounu I., Iniguez L., Bradford G.E., Subandryo and Tiesnamurti B. 1993. Production performance of prolific Javanese ewes. Small Ruminant Research 12, 243.

James J.W. 1982. Construction, uses and problems of multitrait selection indices. P. 130 in 'Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production', vol. 5, Madrid.

Jones L.P. 1982. Economic aspects of developing breeding objectives: a specific example; breeding objectives for Merino sheep. P. 119 in 'Future developments in the genetic improvement of animals', ed. by J.S.F. Barker, K. Hammond and A.E. McClintock. Academic Press: Sydney.

Kosgey I.S., van Arendonk J.A.M. and Baker R.L. 2003. Economic values for traits of meat sheep in medium to high production potential areas of the tropics. Small Ruminant Research 50, 187.

Lee G.J., Atkins K.D. and Swan A.A. 2002. Pasture intake and digestibility by young and non-breeding adult sheep: extent of genetic variation and relationships with productivity. Livestock Production Science 73, 185.

Menendez Buxadera A., Alexandre G. and Mandonnet N. 2004. Discussion on the importance, definition and genetic components of the number of animals born in the litter with particular emphasis on small ruminants in tropical conditions. Small Ruminant Research 54, 1.

Mysterud A., Steinheim G., Yoccoz N.G., Holand O. and Stenseth N. 2002. Early onset of reproductive senescence in domestic sheep (*Ovis aries*). Oikos 97, 177.

Nimbkar C. 2005. Genetic improvement of lamb production efficiency in Indian Decanni sheep. PhD thesis, University of New England, Armidale, Australia.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Walkden-Brown S.W., Maddox J.F., Gupta V.S., Pardeshi V.C., Ghalsasi P.P. and van der Werf J.H.J. 2007. Reproductive performance of Indian crossbred Decanni ewes carrying the *FecB* mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 430–433.

Piper L.R., Bindon B.M. and Davis G.H. 1985. The single gene inheritance of the high litter size of the Booroola Merino. Pp. 115–125 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Ponzoni R.W. 1986. A profit equation for the definition of the breeding objective of Australian Merino sheep. Journal of Animal Breeding and Genetics 103, 342.

Ponzoni R.W. 1992. Genetic improvement of hair sheep in the tropics. Paper 101, FAO Animal Production and Health Division, Rome, Italy.

Rae A.L. 1988. Including costs in defining objectives for sheep improvement. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 7, 67.

Richards J.S. and Atkins K.D. 2007. Determining pedigree by association in Merino flocks. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 403.

Safari E., Fogarty N.M. and Gilmour A.R. 2005. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 92, 271–289.

Simm G., Conington J., Bishop S.C., Dwyer C.M. and Pattinson S. 1996. Genetic selection for extensive conditions. Applied Animal Behaviour Science 49, 47.

Swan A.A., van der Werf J.H.J. and Atkins K.D. 2007. Developments in breeding objectives for the Australian sheep industries. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 483.

Potential introgression pathways and strategies for wider use of the *FecB* gene in Maharashtra state and other parts of India

C. Nimbkar[1,2], J.H.J. van der Werf[3], P.M. Ghalsasi[1], B.V. Nimbkar[1] and S.W. Walkden-Brown[3]

Abstract

The *FecB* gene has been introgressed into non-prolific sheep breeds in countries such as Israel, France and India with beneficial consequences. The primary income from most Indian breeds of sheep is earned from the sale of lambs. Because they have single lambs, there is the potential to introgress *FecB* into more Indian breeds, and it is likely to prove profitable. 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 would need excellent institutional infrastructure including a network and extension program among local sheep owners in the surrounding region. The steps to be followed in an introgression program and related issues are discussed.

Introduction

Rate of reproduction is an important determinant of the productivity of sheep reared for meat production. The number of lambs born per lambing contributes much more to the total weight of lamb weaned per ewe than does the growth rate of individual lambs (Bradford 1985). In India 61.5 million sheep were reared according to the 2003 livestock census (http://dahd.nic.in/relcensus.htm, accessed 25 October 2008). The primary income from most of these is from the sale of young (< 6 months age) lambs. Almost all Indian breeds of sheep except the Garole and Kendrapada (Kumar et al. 2009) have single lambs. A moderate increase in their litter size (LS), compatible with available feed resources and management input, is therefore likely to be beneficial. Nimbkar (2006) found LS to be important for the profitability of a Deccani sheep production system, Deccani being one of the most numerous sheep breeds in India. She found that LS had a high positive economic value even after accounting appropriately for feed cost.

Another important factor indicating the need for increasing the biological and economic efficiency of sheep rearing in India is the increasing shortage of sheep grazing areas, while the demand for and price of sheep meat are increasing constantly. Improving reproductive efficiency is one of the best ways to improve the efficiency of sheep production. Increasing the profitability of sheep rearing through this route could also lead to more farmers adopting sheep rearing as complementary to crop cultivation. Experience in developed countries shows that

[1] Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
[2] Corresponding author; chanda.nimbkar@gmail.com
[3] School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia

investment in livestock genetic improvement projects is sustainable and profitable. Because genetic improvement is permanent in nature, it continues to yield returns to farmers year after year.

Introduction of a major gene into the main breeding line by a process of backcrossing is called introgression (Smith 1985). The well-known *FecB* or Booroola gene which increases prolificacy in sheep has been introgressed profitably into the Mérinos d'Arles breed in France, the Assaf breed in Israel, the Malpura breed in Rajasthan (Arora et al. 2009; Gootwine et al. 2009; Teyssier et al. 2009) and many other breeds in other countries. The B allele increases prolificacy while the + allele is the wild type.

FecB was introgressed from the small Garole breed of Sundarbans, West Bengal, into the Lonand strain of the Deccani breed in and around Phaltan in southern Maharashtra state. This work was carried out between 1998 and 2007 at the Nimbkar Agricultural Research Institute (NARI) under two projects funded by the Australian Centre for International Agricultural Research. Two sheep strains with higher productivity were developed—the NARI Suwarna and the NARI Composite (Nimbkar et al. 2009). Ewes of both new strains carrying one copy of *FecB* (heterozygous or B+ ewes) had an average live LS (at birth) of 1.4 and 1.6 in smallholder sheep owners' and NARI's flocks, respectively, compared to the average LS of 1.0 in Deccani ewes. This meant that about 40–60% of the B+ ewes in a flock had twins on average at any given lambing (since the number of triplets was negligible). The number of lambs surviving at 3 months of age per ewe lambing was 0.95 in Deccani ewes and 1.2 and 1.3 in B+ ewes in smallholder flocks and the NARI flock respectively.

Non-carrier twin-bearing ewes in both NARI and smallholders' flocks were found to wean 4.8 kg more weight of lamb than single-bearing ewes without supplementary feeding to ewes or lambs (Nimbkar et al. 2003). With supplementary feeding there appears to be a potential for even larger profit from twins. Twinning increases the efficiency of lamb production since the cost of the dam is spread over two lambs. Crossbred Deccani B+ ewes produced 8–28% higher total lamb weight compared to crossbred Deccani non-carrier ewes (Nimbkar et al. 2009). The increase in lamb production of homozygous BB ewes was similar to that of B+ ewes. It was thus evident that the increase in LS caused by the *FecB* gene was moderate and manageable both in the flock of more than 500 ewes at NARI and in the smaller flocks of 20–150 ewes in smallholders' flocks. This increase in LS led to a significant increase in income earned by smallholder sheep owners from the sale of 3-month-old lambs. According to Bradford (1985), low mortality levels indicate that genetic potential for multiple births could be increased to good advantage. NARI's studies in smallholders' flocks have shown that lamb mortality in these flocks is typically less than 10%. In India lambs are mostly sold on a 'per head' basis. Therefore, the price of two can be expected to be much higher than the price of one even if each of the twins is slightly smaller than a single-born lamb. It is therefore highly likely that smallholders in other parts of Maharashtra state and India would benefit from the introduction of the *FecB* gene.

The process of introgression requires a donor breed that not only carries the desirable *FecB* allele but is also competitive and preferably similar in phenotype to the population that is targeted for introgression. Furthermore, an introgression program requires some level of monitoring, genotyping and recording, and therefore can only be commenced if these tasks are well planned and the program has adequate resources and capability. The population targeted for introgression should be suitable; that is, the local breeders and producers should want the proposed genetic change, and their production systems as well as the environment should be suitable to carry the new genotype profitably. This paper discusses these various aspects in more detail and includes some suitable examples. The paper explores potential introgression pathways, strategies and constraints for wider use of the *FecB* gene in India.

Technical steps in introgression

First step: identify potential donor breeds

In India there are five breed types that could be considered as donor breeds for the *FecB* mutation. The Garole is a natural carrier of *FecB*, the Kendrapada has probably received *FecB* from the Garole through unintentional crossing, and the other three are examples of deliberate introgression of *FecB* from the Garole.

1. Garole: adapted to the hot, humid Sundarbans region of West Bengal state; most animals homozygous for $FecB^{B}$; small-sized breed with

an adult weight of 10–14 kg; 30% lamb mortality up to 1 month of age (Pan and Sahoo 2008)

2. Kendrapada: found in coastal Orissa state, adjacent to West Bengal, 400 km from Sundarbans; *FecB* mutation segregating; adult ewe weight 23–27 kg (Kumar et al. 2009)
3. Garole × Malpura half-bred (F1) and backcross (75% Malpura and 25% Garole proportion) produced at the Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan; adapted to the hot, dry climate of Rajasthan; *FecB* mutation segregating; yearling ewe weight 19 kg (Arora et al. 2009)
4. NARI Suwarna: produced at NARI, Phaltan, Maharashtra state (Nimbkar et al. 2009); adapted to the hot, dry, monsoonal climate and harsh conditions of the Deccan plateau in India; breed composition has contributions from Garole (≤ 25%) and Deccani (≥ 75%) breeds; mixture of B+ and BB genotypes; average adult ewe weight 25 kg; favourable evaluation of B+ and BB phenotypes by sheep owners in the Phaltan area documented (Prior et al. 2009); genotyped B+ and BB rams with estimated breeding values available
5. NARI Composite: adapted to the hot, dry, monsoonal climate and harsh conditions of the Deccan plateau; breed composition has contributions from Deccani (15–75%), Garole (5–25%), Israeli dairy Awassi (0–25%) and Bannur (0–25%) (Nimbkar et al. 2009); mixture of B+ and BB genotypes; average adult ewe weight 27 kg; Genotyped B+ and BB rams with estimated breeding values available.

The Garole would be a suitable donor breed for smaller sized recipient breeds reared in similar environmental conditions to the Sunderbans (i.e. hot, humid weather and swampy terrain). The Garole was found to be an undesirable breed from which to introgress *FecB* into the Deccani at NARI despite its superior internal parasite resistance (Nimbkar et al. 2002, 2003). This was because of its small size compared to the Deccani; poorer lamb rearing ability due to poor milk production; and poor conformation and features of appearance, such as a wide forehead and big horns that are considered undesirable by smallholder sheep owners in the Deccani rearing areas. The Garole proportion therefore had to be reduced to less than 25% to achieve the target of a *FecB*-carrier animal with the desired phenotype which required more generations of backcrossing. The extent of (un)desirability of the donor breed would determine the number of generations of backcrossing required to arrive at a suitable genotype. In general, the larger the difference between donor and recipient breeds, the larger the number of backcrossing generations needed to increase the proportion of recipient-line background genes in the intercross population to a desired level (van der Werf 2009).

The classical backcrossing program involves several cycles (at least three to achieve 88% of the genes of the target breed) through repeated crossing of heterozygous animals (either males or females) with individuals from the recipient breed. The process is greatly facilitated by having a direct DNA test for the trait of interest, enabling early detection of carrier animals, as is the case with *FecB*. The first intercross generation is formed by mating heterozygous backcross males and females, such that individuals are created that are homozygous for the desirable major gene yet have a large proportion of their genome originating from the recipient breed (e.g. Robertson 1985). One such scheme is described in Table 1. In this scheme heterozygous females are backcrossed to local breed males. As many superior local breed rams as possible should be used in cycles two and three to maintain genetic diversity. In practice, breeders may have overlapping generations and fewer cycles of backcrossing, and may not require fixation of the gene in their stocks (Smith 1985). Moreover, only the backcross animals that have a desirable phenotype (i.e. similarity to the recipient breed) can be identified and used for breeding.

It is very important to introgress only those *FecB*-carrier animals into local flocks that are considered superior by the local sheep owners. The best ways to guard against the introduction of inappropriate animals are to ensure that an appropriate level of backcrossing is used prior to dissemination, and to resist the temptation to disseminate animals at an early stage of backcrossing. However, when a case can be made for dissemination at an early stage of backcrossing, a suitable strategy is to use only such animals as the local smallholder flock owners would be willing to buy. In an ideal situation, production traits are measured and genetic evaluation and selection are used during the introgression process; that is, animals are not only chosen based on *FecB* genotype but also on merit according to the breeding objective. The most important selection decisions are those relating to the selection of destination breed rams to be used in the backcrossing program.

Table 1. A suggested program to introgress the *FecB* mutation into a local breed of sheep using suitable donor rams and the direct DNA test for early genotyping

Generation (G)	Objective of breeding	Ram		Ewe		Progeny		Progeny use	
		Breed	*FecB* genotype	Breed	*FecB* genotype	Breed	*FecB* genotype	Male	Female[a]
1	First step of introgression (use at least 500 local ewes)	Donor	BB	Local	++	F1 50% local 50% donor	All B+	Cull	Retain all for G2
2	Backcross to increase local proportion to 75%	Local (selected strongly for superior phenotype)	++	F1	B+	BC1 75% local 25% donor	50% B+ 50% ++	Cull	Retain B+ for G3; retain ++ for G1 mating
3	Backcross to increase local proportion to 88%	Local (selected as above)	++	BC1	B+ (genotyped)	BC2 88% local 12% donor	50% B+ 50% ++	Retain best B+ for inter-se mating (G4); cull remaining B+ and all ++	Retain B+ for G4; retain ++ for G1 mating or cull
4	Inter se mating among backcrosses to produce 88% local homozygous breeding rams and ewes	BC2	B+ (genotyped)	BC2	B+ (genotyped)	BC3 88% local 12% donor	25% BB 50% B+ 25% ++	Retain best BB for inter-se mating; disseminate surplus BB and best B+	Retain BB and B+ for multiplication in nucleus; retain ++ for G1 mating or cull
5 (smallholders' flocks)	Introgression into smallholders' flocks	BC3	BB (genotyped)	Local	++	94% local 6% donor	100% B+	Cull or retain depending on objective	Retain for breeding

BC = backcross

[a] Retain only animals of sufficient quality.

Second step: identify suitable sheep breeds and regions for introgression of *FecB*

The information required to make a decision about breeds and regions suitable for introgression of *FecB* includes:

- the population size of the breed; efforts should focus on the major breeds
- the purpose for which the breed is reared or the product of the breed (meat or wool) from which sheep owners earn the most income, the market price of the product and the approximate margin of profit to the sheep owner for that product; introgression of *FecB* is likely to have the greatest economic impact in breeds used mainly for meat
- the role of sheep in the agricultural production system in the region
- the body size and weight of animals of the breed
- the system of management followed—flock size, whether grazing or stall feeding is followed, how much personal attention is given to the flock by the owner/shepherd, whether breeding is controlled
- the rainfall and climate of the region, and whether there is irrigation available for agriculture
- the fodder resources available in the region, especially in the summer or dry season, the possibility of procuring nutritious supplementary feed such as grain or oil cakes and its price; introgression is likely to be more successful in regions where feed resources are not severely limiting
- whether sheep owners migrate with sheep, and the distance and duration of migration; it needs to be evaluated whether long and arduous migration patterns lead to reduction in the benefits of increased twinning
- the level of education of sheep rearers.

In India information on livestock numbers is available from censuses conducted by the government. Information on the production system of the breed and phenotypic and production parameters is available for some breeds from reports of the 'network' projects of the Indian Council of Agricultural Research. For breeds for which such information is not available, surveys would have to be conducted by the agency interested in an introgression project.

State governments conduct livestock censuses every 5 years. Census data are sent to the central government by the statistical departments of the state governments and collated to arrive at the total number. In most states population data by breed were not collected for sheep up to the last census in 2003. For example, in Maharashtra state sheep numbers are separately counted for three categories—indigenous, crossbred and exotic. It is therefore not possible to obtain sheep population data by breed from official Indian Government statistics. Therefore, these data were obtained from four main sources:

1. the FAO publication 'Sheep and goat breeds of India' by Acharya (1982)
2. the FAO Domestic Animal Diversity Information System (DAD-IS) database (http://dad.fao.org), to which information is supplied by each country's National Coordinator for Animal Genetic Resources
3. publications under the Network Project on Sheep Breeding carried out by the Indian Council of Agricultural Research
4. breed seminar proceedings such as Anthra (2007).

Indian sheep breed population data are presented in Table 2, which gives the names and approximate populations of 24 breeds, 19 of them woolly breeds and 5 hair breeds, which are likely to be suitable for the introgression of the *FecB* gene. This is because the primary income from sheep is earned from the sale of lambs. Although the uses of the woolly breeds are given as 'meat and wool' in the literature, 90% or more of the rearers' income is earned from the sale of animals for meat, as in the Deccani (Nimbkar 2006). There is a trend among smallholder flocks, even in Rajasthan, which has some well-known Indian carpet wool breeds, to increasingly select for meat rather than wool production traits (Bhatia et al. 2005).

Introgression of *FecB* using BB NARI Suwarna rams can be started with sheep of the Lonand strain in other districts of Maharashtra. Introgression can also be started with Deccani sheep in Karnataka and Andhra Pradesh as they are phenotypically similar to the Lonand Deccani. This would be analogous to the Australian situation in the 1970s when the *FecB* in CSIRO Booroola Merinos was introgressed into other major strains of Merino sheep. Differences between strains are likely to be smaller than those between breeds. NARI has about 100 BB adult rams available currently and could supply about 50 BB and 50 B+ adult breeding rams each year in support of such a program. The 75% Malpura *FecB*-carrier animals produced at CSWRI can be used for intro-

Table 2. Indian sheep breeds suitable for introgression of the *FecB* gene

Sheep breed	Location and state of India	Population (millions)	Population reference
Chokla	Bikaner, Jaipur, Nagaur districts of Rajasthan	0.983	DAD-IS[a](1987)
Bellary	East, Central Karnataka (adjoining Deccani area)	0.289	DAD-IS[a] (1982)
Coimbatore	Tamil Nadu	0.250	Kandasamy (2006)
Chhotanagpuri	Bihar and West Bengal	0.647	Acharya (1982)
Deccani	Central Maharashtra, North-east Karnataka, Andhra Pradesh	18.800	Anthra (2007)
Gaddi	Southern Jammu-Kashmir, Central Himachal Pradesh	0.517	DAD-IS[a] (1982)
Ganjam	Koraput, Phulbani part of Puri district of Orissa	0.227	DAD-IS[a] (1977)
Jaisalmeri	Jaisalmer, Barmer and Jodhpur districts of Rajasthan	0.525	DAD-IS[a] (1991)
Jalauni	South-western Uttar Pradesh	0.082	DAD-IS[a] (1982)
Madras Red (hair breed)	North-eastern Tamil Nadu	0.423	DAD-IS[a] (1982)
Magra	East and South Bikaner, Rajasthan	0.599	DAD-IS[a] (1987)
Mandya (Bannur) (hair breed)	Mandya and Mysore districts of Southern Karnataka	0.301	DAD-IS[a] (1987)
Malpura	Eastern Rajasthan	0.767	DAD-IS[a] (1987)
Marwari	Rajasthan and Gujarat	5.018	Acharya (1982)
Mecheri (hair breed)	Erode, Karur, Dindigul and Coimbatore districts of Tamil Nadu	0.917	Acharya (1977)
Muzzafarnagri	Muzzafarnagar, Uttar Pradesh	0.046	DAD-IS[a] (1982)
Nali	Northern Rajasthan and southern Haryana	0.675	DAD-IS[a] (1987)
Nellore (hair breed)	South-eastern Andhra Pradesh	1.600	DAD-IS[a] (1982)
Patanwadi	Mehsana, Kutch, Saurashtra, Northern Gujarat	0.667	DAD-IS[a] (1982)
Ramnad white (hair breed)	South India hair type	0.630	DAD-IS[a] (1982)
Rampur Bushair	Himachal Pradesh and Uttaranchal	0.550	Acharya (1982)
Shahabadi	Bihar	0.596	Acharya (1982)
Sonadi	Southern Rajasthan and Northern Gujarat	1.043	DAD-IS[a] (1987)
Tiruchi Black	Tamil Nadu	0.520	Acharya (1982)
	Total	36.672	

[a] The year in the bracket for references from Domestic Animal Diversity Information System (DAD-IS) is the year for which the population figures are given in DAD-IS.

gression into other phenotypically similar (i.e. comparatively large) breeds in Rajasthan (Arora et al. 2009).

In the meat sheep rearing areas of south India there appears to be a trend for crossbreeding woolly breeds with larger sized hair breeds (Kandasamy 2006; Anthra 2007). The smallholder sheep owners who keep woolly breeds but prefer to crossbreed with hair breeds may not prefer the coarse-wool NARI Suwarna. Therefore, introgression into hair breeds would have to be done while largely maintaining the hairy phenotype. This would require three to four generations of backcrossing followed by a final generation of inter se mating as explained above. NARI has started breeding a small number of *FecB*-carrier ewes each year to rams of the hair breed Madgyal from southern Maharashtra because the flock owners in the Pune and Phaltan regions prefer Madgyal rams.

The *FecB* gene could be introgressed profitably into a breed such as the Mecheri, which is reared in comparatively small flocks (30–50 breeding ewes) for meat production in Tamil Nadu state in southern India. They are typically reared in Korangadu pastures consisting of grass, legumes and trees in a well-structured, time-tested traditional system with sound management practices adapted to local conditions (Vivekanandan 2007).

According to Davis and Hinch (1985), transferring the *FecB* gene into breeds of larger mature size should result in increased lamb birth weight and survival because between-breed comparisons show that lamb birth weight and ewe mating weight are highly correlated. For this reason it might be beneficial to introgress *FecB* into larger sized Indian sheep breeds such as the Sangamneri strain of Deccani and the Muzzafarnagari breed, which has an adult ewe weight of 40 kg compared to the 28 kg of the Deccani. However, smaller breeds with good mothering ability due to better milk yield, such as the Patanwadi (Acharya 1982), would also be suitable for introgression of *FecB*.

In India sheep are traditionally reared by smallholders who may or may not own land. Sheep are grazed on crop residues, fallow lands, road and canal sides, and uncultivated, eroded hillsides. Grazing flocks are always shepherded and supervised closely and the sheep are penned near the owner's house at night. It is common to cross-foster lambs to ewes or goat does that produce more milk. As the price of goat and sheep meat is high and increasing, lambs are valuable, and even very young orphaned lambs fetch a price. Under such circumstances, the profitability of sheep production is highly dependent on reproductive rate and even a modest increase in fecundity would increase the owner's income substantially. Introgression of the *FecB* gene would therefore be a profitable proposition, in many cases outweighing phenotypic differences for other traits such as size and rearing ability. However, an introgression program would be doomed to fail if the phenotype of the intercross was not accepted by the smallholder sheep owners.

Third step: survey sheep owners and analyse the local breeding objective

The third step is to conduct a survey among sheep owners who rear the identified breed in the identified region. This survey should be designed with a view to collecting information about the sheep production system, the role of sheep rearing in the agricultural production system of that region, the economics of sheep rearing, and the social and cultural significance of sheep to the sheep-rearing community. The survey should evaluate prevalent sheep management and marketing practices and the attitude among smallholder sheep owners to twinning in sheep. The possibility of introducing prolificacy into sheep and its likely desirable and undesirable consequences should be explained to smallholder flock owners. Information should also be collected on breeding objectives and ewe and ram selection criteria of sheep owners. This information should be used to evaluate the probable value of increased prolificacy for that breed and the constraints to increasing lambing percentage. A simple cost–benefit analysis could quickly reveal the potential value of introducing twinning.

One of the aims of the survey should also be to identify sheep owners who are receptive to the idea of improving the productivity of their flock by introgression of *FecB*, and who are in a position to adopt the necessary changes in management. Good rearers should preferably be selected for introgression in the first instance.

Concurrent third step in institutional flocks

While the survey and the study of survey results are taking place, the process of *FecB* introgression through backcrossing could be started in a suitable

institutional flock. Measurement and genetic evaluation for production traits is highly desirable. Options for management of *FecB*-carrier ewes and their lambs should also be tested in institutional flocks during this time. Identification of superior males of the recipient breed to use in the backcrossing program is important in determining its outcome.

Fourth step: introgress *FecB* into smallholder sheep flocks

Introgression could be started into smallholder sheep flocks when homozygous *FecB*-carrier rams are available that are competitive with rams from the local breed, similar in phenotype, adapted to smallholders' flock conditions and found desirable by them. All progeny of such rams would be heterozygous, which would lead to earlier and easier selection of their progeny for further breeding without the need to genotype. Alternatively, use of heterozygous rams for introgression could be started before homozygous rams become available, but the further spread of the mutation through heterozygous rams would be slower. Efforts should be made to have highly superior selected carrier rams as this will increase the chances of success of introgression. A scheme of identification and performance recording of animals in these target flocks would help identify the potential improvements. An extension and training program with regular contact between the project managers and flock owners will greatly enhance the understanding of outcomes, facilitate problem solving and lead to improved results. The main focus of such a program needs to be the management of flocks containing significant numbers of twin-bearing ewes and twin-born lambs, and the means of maximising profit from such flocks. Such extension programs could be accompanied by other services offered to sheep owners, such as management and veterinary advice and insurance of animals. This could include arranging vaccinations through the government infrastructure. A reasonable charge should, however, be levied on sheep owners for such services. Monitoring of introgressed flocks and extension and training support to them may need to continue for 5–10 years after initial animal dissemination, as it takes several years before large numbers of twin births occur in these flocks.

Introgression through rams or ewes

An important issue that needs to be addressed is whether only rams, or also ewes, should be introduced in smallholders' flocks. Due to the larger number of progeny they have, dissemination of carrier rams will result in more rapid distribution of *FecB* than dissemination of carrier ewes. Ewes may also become available for dissemination in the later stages of a backcrossing program at an institutional site, having the potential to produce more immediate changes in average LS of the flock. However, there can be adaptation problems associated with introduction of crossbred *FecB*-carrier ewes, as has been experienced with the *FecB*-carrier crossbred Deccani ewes introgressed into local smallholder sheep flocks in Maharashtra (ACIAR 2007). These problems may arise due to change of feed and other management conditions, and ewes under the stress of lambing and suckling are more susceptible to them. Introgression through rams or artificial insemination (AI) is more feasible although it involves a considerable time lag before the effects of introgression are seen.

The first generation to express the trait is that of the daughters of the rams, born at least 5 months after ram introduction into the flocks. The time of expression is when the daughters lamb for the first time, which is likely to be at the age of 21–24 months, as in the Deccani. The additional profit from the extra lambs born would be realised after another 3–4 months when the lambs are sold, thus making a total of about 30 months after ram introduction. There is also the added issue of primiparous ewes having the greatest difficulty in successfully rearing lambs from multiple births, so that it is not until the second and later lambings that the major benefits of the new genotype become apparent to the smallholder sheep owners.

FecB-carrier ewes could be introduced into smallholders' flocks if they are reared in conditions similar to those in the smallholders' flocks. A mixture of rams and ewes could be used. About 300 surplus *FecB*-carrier ewes would be available annually for dissemination from a nucleus flock of, say, 500 carrier ewes. About 400 surplus rams would also be available for dissemination but, initially, many of them will not be homozygous. Although preferably only BB rams are disseminated to increase the impact in the recipient flocks, introgression will be speedier if all available and suitable carrier rams are disseminated. Disseminating heterozygous rams after appropriate backcrossing, but before

homozygous rams are available, will speed up the process of introgression and allow a more gradual increase of the frequency of twinning ewes in smallholder flocks. Each ram could be used to inseminate at least 50 ewes per season.

Should heterozygous or homozygous *FecB*-carrier ewes be the target in smallholders' flocks?

Results so far show that crossbred Deccani BB ewes do not have unmanageably high LSs, being comparable with B+ ewes. If this is confirmed as more data become available, it would simplify the introgression process as there would not be a requirement to reduce homozygosity and maintain heterozygosity using complex schemes such as that detailed by Piper and Bindon (1991). In such a scheme the ram breeding flock would keep B+ ewes, mate them with BB rams, cull all BB female progeny and use only B+ females as replacement and for dissemination. Commercial flocks would maintain B+ females and mate them with non-carrier rams. The resulting progeny would be genotyped and only B+ females would be retained as replacements. This would lead to reduced selection intensity and therefore less genetic progress in other economically important traits in both males and females (Piper and Bindon 1991). Genotyping of all ewes at the *FecB* locus would be necessary to distinguish between ++, B+ and BB females, as only about half the B+ and BB females have twins at a lambing, about 10% have triplets and the rest have singles. Therefore, a simple criterion such as selecting ewes having twins would work only to a limited extent. Such a scheme is unlikely to be practical under Indian conditions and also not necessary.

If homozygous ewes continue to be shown to be as desirable as heterozygous ewes, dissemination of homozygous carriers can be used and matings between *FecB*-carrier animals in flocks can be made with confidence, irrespective of *FecB* genotype. In such a system *FecB* genotyping may be used to maximise the benefits of *FecB* introgression, but it is not necessary to prevent any adverse effects. This is likely to be the situation in India.

Infrastructure required for introgression

The basic infrastructure required for a *FecB* introgression program is an institution with a network among sheep owners, and regular extension and training activities. The institution could procure *FecB* rams of the appropriate phenotype and disseminate them among smallholders' flocks. Training smallholders in feeding and management of twinning ewes and twin-born lambs would be beneficial. If the flock owners find that the *FecB* gene is profitable, they will retain the progeny of carrier rams as replacement ewes and also retain home-bred carrier rams for breeding. The Thoka gene in Icelandic sheep, which increases LS by 0.6–0.7 per copy of the gene, was distributed through AI in the Icelandic national flock from 1980 to 1995 (Jónmundsson and Eythórsdóttir 2003). Farmers were found to have kept the Thoka gene in their flock through selection of daughters of carrier ewes for replacement, as well as use of home-bred carrier rams in 50% and AI in 22% of the flocks. If genotyping is used and a record is kept of the number of lambs born, died and sold in each flock, and of the flock owner's income and expenditure, the benefits of introgression can be assessed.

Animal identification, measurement and recording of economically important traits in smallholders' flocks can be added to the project if possible. This would help to keep track of the lifetime performance of carrier versus non-carrier ewes, and collection of such data would be the first step in the direction of developing a program for genetic improvement in other economically important traits. A genotyping facility need not necessarily be available at each institution involved in introgression. Blood samples can be sent for testing to central facilities such as the National Chemical Laboratory at Pune or the Indian Veterinary Research Institute at Izzatnagar in Uttar Pradesh state. NARI, Phaltan, will also soon have a *FecB* genotyping facility.

An institution interested in producing *FecB*-carrier animals should have the appropriate infrastructure to maintain a large sheep flock of several hundred ewes at least, and to carry out backcrossing, data recording and genetic evaluation. The size of the flock would determine the diversity of the genetic material being dispersed as well as the number of animals available for dispersion. The availability of expertise and infrastructure for implementing reproductive technologies such as AI and embryo transfer would increase the speed of production of competitive homozygous animals. The institution producing carrier animals could also carry out their dissemination or, alternatively, introgression

into smallholders' flocks could be managed by another institution with better links among the flocks. The institution carrying out the production and/or dissemination of *FecB*-carrier animals could be a non-government organisation, a breeders' society or cooperative, or a government research institute or sheep development corporation. Introgression could be a program under the Network Project on Sheep Breeding of the Indian Council of Agricultural Research.

The availability of the direct DNA test for identification of the *FecB* mutation (Wilson et al. 2001) has increased the speed of introgression manyfold. If the DNA test had not become available, genotypes of ewes would have to be identified by measuring their ovulation rate by laparoscopy, and males would have to be progeny tested. The period of time required to produce BB rams with 88% genes from the breed into which the *FecB* gene was introgressed was predicted to be 10–11 years in the absence of a reliable test for early genotyping (Elsen et al. 1985; Piper and Bindon 1991). Now that the DNA test is available, this period can be reduced to about 8.5 years assuming an average generation interval of 26 months for females. The DNA test also makes it possible to carry out backcrossing using B+ males and females.

Constraints and opportunities for introgression of the *FecB* gene into Indian sheep breeds

The major constraint is the unorganised and unstructured nature of the Indian sheep industry, and the fact that sheep rearing is considered a subsistence rather than a commercial activity. This is also the reason why smallholders sometimes sell either their whole flock or a substantial part of it for emergency cash needs. In such a situation it is difficult to implement any kind of systematic genetic improvement. On the other hand the 'family-based' nature of this occupation also makes it more conducive to introduction of a major gene for prolificacy such as *FecB*. This is because of the family labour available for extra care of twinning ewes and their lambs, for example for cross-fostering of lambs to ewes with more milk and to goat does.

Bradford (1985) suggested optimum levels of prolificacy for different production environments in terms of lambs born per mature (second or later lambing) ewe lambing. His suggested goal was 'twins for mature ewes' for seasonal rainfall climates where forage is consistently adequate for at least 3 or 4 months of the year and where there is a potential for supplementation at critical times. The Deccan plateau production environment falls in this category. If the survey of sheep owners and sheep rearing environments in technical step three indicated that such a potential for supplementary feeding did not exist in a region, it may not be appropriate to introgress *FecB* in that region. Supplementary feeding to ewes in the last stage of gestation and to lambs after the age of 1 month is likely to help maximise the benefits of *FecB* introgression.

Results show that if all ewes in a flock had one copy of *FecB*, about half of them would have twins at a lambing (Nimbkar et al. 2009). In reality, smallholder flock owners would probably want to increase the number of *FecB*-carrier ewes in their flocks gradually so that they have time to make changes in flock management. In Iceland it was found, in a questionnaire survey of farmers who deliberately kept Thoka gene carriers in their flocks, that the proportion of ewes with high fecundity in individual flocks varied from 1% to 50%, with most farms having less than 20% of the flock as carriers (Jónmundsson and Eythórsdóttir 2003).

Speed of introgression

The speed of introgression and rate of animal dissemination were modelled for an institutional flock of 1,000 breeding ewes with the sole purpose of maximising the production of 87.5% target breed animals carrying the $FecB^B$ allele, following the scheme laid out in Table 1. The key assumptions based on available data were:

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

As shown in Figure 1, 28 B+ rams would be available for dissemination in the fifth year from the

start, and 149 B+ and 110 BB rams by the tenth year. B+ ewes would be available for dissemination from year 11 onwards, and BB ewes from year 13 onwards.

Introgression from an institutional flock into the wider sheep population was also modelled. If only BB rams are used for mating ewes in the target flocks, the mean frequency of the *FecB*^{B} allele among breeding ewes in recipient flocks would be 0.5 in 7 years, 0.8 in 13 years and 0.95 in 22 years.

Conclusions

Results in NARI's and smallholders' sheep flocks indicate that it would be worthwhile to introgress the *FecB* gene into Deccani sheep more widely in other parts of Maharashtra state and into other suitable Indian sheep breeds. In other districts of Maharashtra where the Lonand strain of Deccani sheep is reared, the newly developed NARI Suwarna strain with < 25% Garole genes would be a suitable donor

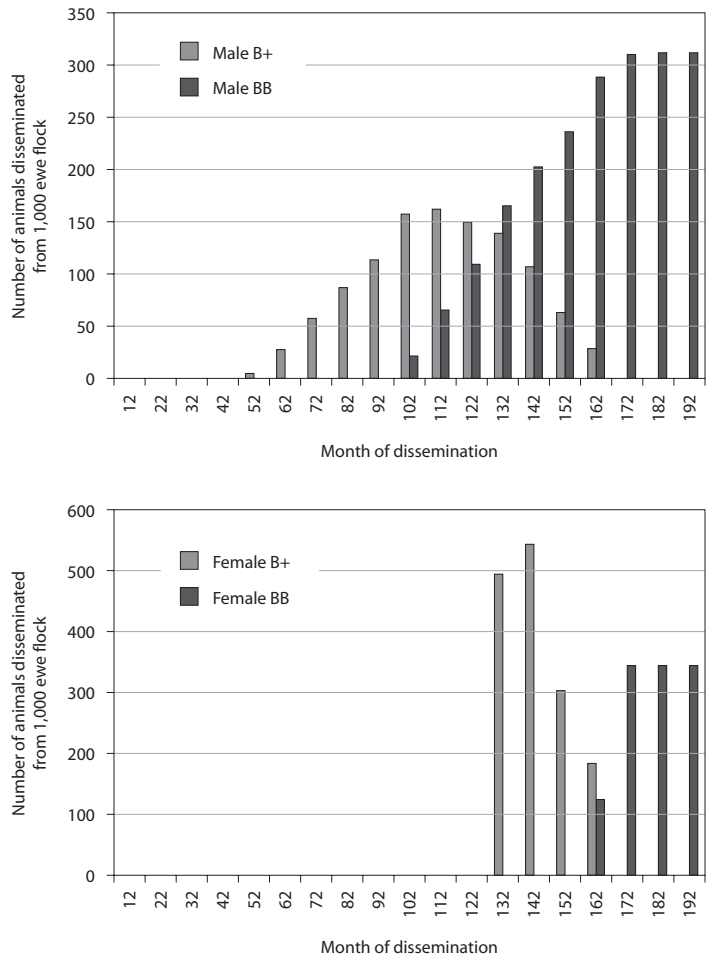

Figure 1. Dissemination of *FecB*-carrier animals from a 1,000 ewe institutional flock following a backcrossing program as outlined in Table 1.
Notes:
1. Model assumptions are in the text.
2. Month of dissemination is measured in months from the first mating in the flock.

breed of *FecB*. In other areas where distinctly different breeds are reared, there would have to be three to four generations of backcrossing so that the *FecB*-carrier animals would have a similar phenotype as the original breed. Smallholder sheep owners are likely to welcome introgression if the *FecB*-carrier animals are phenotypically superior and they find the increased lambing rate to be profitable. Institutions with effective networks among the sheep-rearing community are essential for implementation and success of an introgression program.

References

ACIAR (Australian Centre for International Agricultural Research) 2007. Annual Report January 2006 – December 2006: Project AH/2002/038: Improved productivity, profitability and sustainability of sheep production in Maharashtra through genetically enhanced prolificacy, growth and parasite resistance. ACIAR: Canberra.

Acharya R.M. 1982. Sheep and goat breeds of India. FAO Animal Production and Health Division, Paper no. 30.

Anthra 2007. Summary of the seminar. P. 3 in 'Proceedings of a National Seminar on Sustainable Use and Conservation of the Deccani Sheep', 20–22 February 2007, Hyderabad, India.

Arora A.L., Mishra A.K. and Prince L.L.L. 2009. Consequences of introgression of *FecB* gene into Malpura sheep in Rajasthan. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 111–118. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Bhatia A., Arora R. and Ahlawat S.P.S. 2005. Pastoralists evolved sheep of Rajasthan 'Kheri'. Monograph 17. National Bureau of Animal Genetic Resources: Karnal, Haryana, India.

Bradford G.E. 1985. Selection for litter size. Pp. 3–18 in 'Genetics of reproduction in sheep', ed. By R.B. Land and D.W. Robinson. Butterworths: London, UK.

Davis G.H. and Hinch G.N. 1985. Introduction and management of the Booroola gene in sheep flocks in New Zealand. Pp. 139–148 in 'Genetics of reproduction in sheep', ed. By R.B. Land and D.W. Robinson. Butterworths: London, UK.

Elsen J.M., Vu Tien J., Bouix J. and Ricordeau G. 1985. Linear programming model for incorporating the Booroola gene into another breed. Pp. 175–181 in 'Genetics of reproduction in sheep', ed. By R.B. Land and D.W. Robinson. Butterworths: London, UK.

Gootwine E. 2009. Biological and economic consequences of introgressing the B allele of the FecB (Booroola) gene into Awassi and Assaf sheep. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 119–127. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Jónmundsson J.V. and Eythórsdóttir, E. 2003. Farmers' experience from use of Thoka gene carriers in Iceland. In 'Proceedings of the International Workshop on Major Genes and QTL in Sheep and Goat', Toulouse, France, 8–11 December 2003. CD-ROM communication no. 2-15.

Kandasamy N. (ed.) 2006. Survey, evaluation and characterization of Coimbatore sheep breed. Final Report under the Network Project on Animal Genetic Resources. Veterinary College and Research Institute: Namakkal, Tamilnadu.

Kumar S., Mishra A.K., Prince L.L.L., Paswan C., Arora A.L. and Karim S.A. 2009. Identification of the Booroola mutation in Kendrapada sheep of Orissa, India. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 235–237. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Nimbkar C. 2006. Genetic improvement of lamb production efficiency in Indian Deccani sheep. PhD thesis, University of New England, Armidale, Australia.

Nimbkar C., Ghalsasi P.M., Swan A.A., Walkden-Brown S.W. and Kahn L.P. 2003. Evaluation of growth rates and resistance to nematodes of Deccani and Bannur lambs and their crosses with Garole. Animal Science 76, 503–515.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Ghalsasi P.P., Gupta V.S., Pardeshi V.C., Maddox J.F., van der Werf J.H.J., Gray G.D. and Walkden-Brown S.W. 2009. Biological and economic consequences of introgression of the *FecB* (Booroola) gene into Deccani sheep. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 90–99. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Nimbkar C., Ghalsasi P.M., Walkden-Brown S.W. and Kahn L.P. 2002. Breeding program for the genetic improvement of Deccani sheep of Maharashtra, India. In 'Proceedings of the 7th World Congress on Genetics Applied to Livestock Production', Montpellier, France. CD-ROM communication no. 25-11.

Pan S. and Sahoo A.K. 2008. The Garole sheep – history, management, production and current status. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 32–43. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Piper L.R. and Bindon B.M. 1991. Strategies for utilization of a major gene for prolificacy in sheep. Pp. 349–358 in 'Second International Workshop on Major Genes for Reproduction in Sheep', ed. by J.M. Elsen, L. Bodin and J. Thimonier. Toulouse, 16–18 July 1990.

Robertson, D.E. 1985. Principles and practice for the use of the Booroola Merino in extensive husbandry. Pp. 169–174 in 'Genetics of reproduction in sheep', ed. By R.B. Land and D.W. Robinson. Butterworths: London, UK.

Smith C. 1985. Utilization of major genes. Pp. 151–158 in 'Genetics of reproduction in sheep', ed. by R.B. Land and D.W. Robinson. Butterworths: London, UK.

Teyssier J., Bodin L., Maton C., Bouquet P.M. and Elsen J.M. 2009. Biological and economic consequences of introgression of the FecB gene into the French Mérinos d'Arles sheep. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 128–134. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Van der Werf J.H.J. 2009. Genetic aspects of Booroola introgression in breeding programs. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 160–169. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Vivekanandan P. 2007. Korangadu: a traditional pastureland farming system in the drylands of Tamilnadu, South India. SEVA Foundation: Madurai, India.

Wilson T., Wu X., Juengel J., Ross I., Lumsden J., Lord E., Dodds K., Walling G., McEwan J., O'Connell A., McNatty K. and Montgomery G. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Translating animal breeding research into the real world: use of the sustainable livelihoods framework

K. Marshall[1,2], A.M. Okeyo[1] and N. Johnson[1]

Abstract

The objective of this paper is to introduce the sustainable livelihoods framework as a useful tool in translating animal breeding research into livelihood improvements for the world's rural poor. This framework recognises the interacting components of assets, activities, vulnerability context, institutional context and livelihood outcomes. In essence, it provides a way of thinking about livelihoods and prompts users to ask the right questions in the design and implementation of potential interventions. While the framework is well recognised and used by researchers and development organisations supporting agricultural endeavours such as cropping, the same does not hold for animal breeding. It is proposed that the framework can be similarly used for animal breeding, and that its application will lead to the success of a greater proportion of development interventions around animal breeding, in terms of both their impact and sustainability.

Introduction

In developing countries a considerable number of research and development (R&D) projects have focused on improving livelihoods from livestock. This seems well justified given that livestock form a component of livelihoods for 70% of the world's rural poor (LID 1999). Projects around animal breeding have had varied (and often limited) success in terms of their impact on livelihoods and sustainability, and some explanations for these results have been identified in the literature (e.g. Kosgey et al. 2006; summarised in Table 1).

The purpose of this paper is to offer a means for animal breeding researchers to apply the lessons from Table 1. Terms such as poor, inadequate, simple, cheap, low-risk and incompatible are context specific, and a systematic way to assess this context is useful. This paper advocates the sustainable livelihoods (SL) framework (Carney 1998; Scoones 1998) as a way to ask the right questions in the design and implementation of animal breeding R&D interventions. In brief, this framework recognises the interacting components of assets, activities, vulnerability context, institutional context and outcomes (Ellis and Freeman 2004). It is proposed that using this approach may help animal breeding research—such as evaluating different genotypes or pilot testing novel breeding schemes—translate more successfully into sustained livelihood improvement.

Livestock in relation to the livelihoods of the world's rural poor

The role of livestock in the lives of the world's rural poor is varied, and comprises both economic and

[1] The International Livestock Research Institute (ILRI), Old Naivasha Road, PO Box 30709, Nairobi 00100, Kenya

[2] Corresponding author: k.marshall@cgiar.org

social functions. For example, livestock can contribute to household nutrition and income though the consumption or sale of milk and meat; act as a form of savings and insurance where financial markets don't exist; provide draft power and fertiliser for crop production; provide transportation; and play a role in meeting the sociocultural needs of their owners (Waters-Bayer and Bayer 1992; LID 1999). Further, livestock can contribute to household security by risk diversification (e.g. in crop–livestock production systems) and allow benefits to be gained from access to common-property resources (such as communal grazing areas) independent of individual landholdings (Waters-Bayer and Bayer 1992; Bhende and Venkataram 1994; LID 1999). At the household level there is a negative correlation between ownership of livestock and poverty, although the causal mechanisms are still not well understood (Krishna et al. 2004, 2006).

Livestock assets vary greatly among the rural poor. In many systems livestock ownership increases as household income increases, typically with a move 'up the ladder' from poultry to goats, to sheep, to cattle/buffalo (Dercon and Kirshnan 1996; Dolberg 2001; Ellis and Freeman 2004; Kristjanson et al. 2005; Deshingkar et al. 2008). This is commonly followed by a shift to more non-farm income in comparison to farm-related income (Reardon 1997; Barrett et al. 2001; Ellis and Freeman 2004). For example, in a livelihoods analysis of four African countries by Ellis and Freeman (2004) the highest income quartile of the rural poor was found to have the highest livestock holdings as well as the highest percentage of total income from non-farm sources. These authors stated that 'this illustrates the interlocking nature of relative livelihood success in rural areas. Livestock is a substitutable asset than can be sold in order to invest in land or small businesses and, vice versa, non-farm income can be used to build up herds'.

The constraints to livestock production within developing countries are typically both significant and numerous. These include, for example, the lack of cash or credit schemes to acquire livestock and other production system needs; inadequate support services such as animal health care and extension; restrictions relating to water, feed and available labour; lack of access to innovative production and processing technologies; poor market access at local levels; and higher level trade barriers (LID 1999; Bennett et al. 2006; Kosgey and Okeyo 2007).

In addition, the policy and institutional contexts in many developing countries present challenges to livestock keeping. Institutions can be defined as 'regularized practices (or patterns of behaviour) structured by rules and norms of society which have persistent and widespread use' (Scoones 1998). For example, informal norms about which genders, castes or ethnic groups can own livestock, access communal resources such as pasture or water, or engage in specific management or marketing activities have strong implications. In many countries political instability and lack of rule of law are also problems, especially in rural areas, as they can lead to loss of animals due to conflict or theft (Kristjanson et al. 2009). Even in countries that are relatively stable, inadequate or inconsistent application of health regulations and vaccination campaigns is common, resulting in reduced produc-

Table 1. Issues related to success/failure of small-ruminant animal breeding programs within the tropics

Failure lessons	Success lessons
Needs of farmers not appropriately considered	Programs should be simple, cheap and low-risk
Poor breed choice—breeds not able to fulfil traditional role of farmers	Participation of all stakeholders from the onset
Programs abandoned when incentives are withdrawn	Incentives only to promote initial use of breeding programs
Inadequate support services, such as extension and veterinary	Compatibility with sociocultural aspects of producer
Lack of national scientists to provide technical support	Market-oriented breeding programs
Breeding objectives not well defined	Effective monitoring and evaluation
Lack of incentives for continued performance and pedigree recording at the community level	Institutional issues addressed

Source: Kosgey et al. (2006)

tivity and loss of market access (SDP 2004). Table 2 gives some further examples of institutions affecting livestock keepers.

Given the above, it follows that determining what will constitute an improvement in livestock's contribution to household welfare, as well as identifying appropriate interventions, can be very complex. One methodology that can assist in this regard is the SL framework, as discussed in detail below. This framework was developed to help R&D practitioners better understand the contexts in which their interventions would be implemented and, as a result, improve the probability that they will be beneficial. This approach helps to ensure that beneficiaries of interventions will be both willing and able to adopt them.

The sustainable livelihoods framework

Themes in rural development are constantly evolving. In crude terms the 1960s focused on modernisation, the 1970s on state interventions, the 1980s on market liberalisation, and the 1990s on participation and empowerment (Ellis and Biggs 2001). In this last context, which focuses on people's incentives and capacities to change and adopt improved technologies and practices, the SL approach was developed by Carney (1998) and Scoones (1998).

While there are a number of variations on the definition of the term 'livelihoods', one that is commonly used is 'a livelihood comprises the capabilities, assets (including both material and social resources) and activities required for a means of living. A livelihood is sustainable when it can cope with and recover from stresses and shocks, maintain or enhance its capabilities and assets, while not undermining the natural resource base' (Scoones 1998; DFID 2001). Based around this definition, a number of frameworks for the SL approach have been presented by different organisations such as the United Nations Development Programme (UNDP), CARE and the UK Department for International Development (DFID) (Krantz 2001). All these frameworks share several key principles, as explained below (Carney 1998; Scoones 1998; DFID 2001; Brocklesby and Fisher 2003; Adato and Meinzen-Dick 2007). Figure 1 gives an illustration of the framework used by DFID.

One key principle of SL frameworks is the recognition that, in order to meet their needs, individuals and households engage in a **diverse portfolio** of productive and domestic activities—usually referred to as livelihoods strategies—each of which requires access to specific assets or capitals. In the past rural people were often viewed exclusively as farmers or livestock keepers, and the fact that they dedicated significant amounts of their time and other resources to domestic and non-farm activities was overlooked. Recent studies documenting the importance of non-farm income even to rural households and the unambiguously positive impact that this has on welfare are consistent with this livelihoods

Table 2. Examples of institutions affecting poor livestock keepers

Level applied	Formal institutions	Informal institutions
International	Trade barriers Definition of national boundaries	Historical links and relationships between countries manifested in favourable trading relationships Common professional values
National	Property rights Government policies setting levels of prices, subsidies and taxes	Religious taboos on consumption of meat Suspicion of technology or government
Organisational	Membership rules Terms and conditions	Patron–client relationships Market transactions, trader behaviour
Local	Local movement controls Access to resources of different kinds, e.g. forests, village gazing, private land	Use of communal grazing resources Local power structure

Source: adapted from LID (1999)

approach (Reardon 1997; Barrett et al. 2001; Ellis and Freeman 2004).

A second common aspect of SL frameworks is that people have, and draw upon, a number of **capitals** or **assets** for their livelihood strategies. Five different types of assets are usually considered: social capital including social networks, access to opportunities and membership in associations; natural capital including land, water, forests and biodiversity; physical capital such as transport, shelter, water supply, energy and communications; financial capital such as cash, credit, savings and remittances; and human capital including knowledge, skills and labour.

A third key aspect of SL frameworks is the importance of the **vulnerability context**. Poor rural households face many sources of vulnerability, including both shocks (such as natural disasters) and stresses (e.g. trends over time, seasonal change). As these households have few coping mechanisms available, they often cannot afford to take risks, and may choose low-risk low-return investments over those that offer higher returns but with an unacceptable level of risk. This relates to both real and perceived risks, as both can influence people's decisions and thus their livelihood strategies.

A final common feature of SL frameworks is recognition of the importance of **policies, institutions and processes** in enabling or constraining the choices available to households. Some examples, such as the importance of functioning law enforcement or health regulations, or local sociocultural norms that control access to resources and who is allowed to do which activities, have been mentioned previously. A criticism of the SL framework is that, while it recognises the existence of such rules, it does not make explicit the fact that such policies and institutions are not random or arbitrary but rather are perpetuated precisely because they confer advantage to some (often the powerful few) at the expense of many (the powerless poor). This is important because attempts to introduce change and reform will not be successful if they do not acknowledge the forces supporting the status quo (Adato and Meinzen-Dick 2007).

Under the SL approach the livelihoods of the poor can potentially be improved in multiple ways. These include increasing the quality of, or access to, assets; enhancing the return on those assets by improving the productivity of existing livelihood strategies or the availability of new ones; reducing vulnerability; and changing the policy and institutional context in which people use their assets and strategies. What is important about the SL framework is that it requires looking beyond a specific intervention to see whether there may be something else in the broader context that might prevent potential gain from being realised.

Animal breeding within the sustainable livelihoods framework

Animal breeding programs within an SL framework are emerging, such as several projects supported by the International Livestock Research

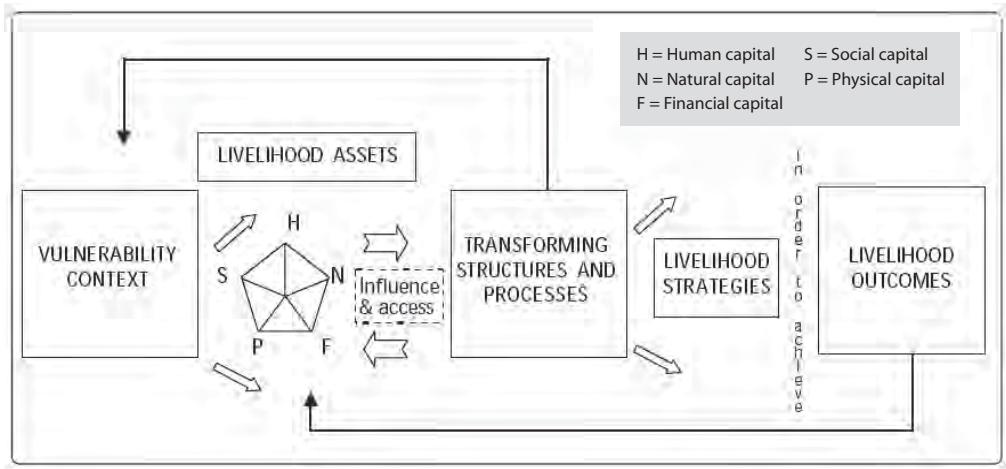

Figure 1. The sustainable livelihoods framework

Institute (ILRI), although at present there are no reports on animal breeding programs that have explicitly used this approach in project development or evaluation. This is in contrast to a number of other agricultural technologies (Adato and Meinzen-Dick 2007). This section thus indicates the *potential* role of the SL framework in relation to the design and implementation of animal breeding programs. Two aspects of animal breeding programs where research is often focused in developing countries will be discussed—the evaluation of genotypes and the evaluation of different breeding program design—both with a view to improved productivity.

Evaluation of genotypes

Matching of genotypes to production systems / environments is an issue that should be highly prioritised in developing countries, given the diversity of indigenous genotypes and the opportunity to capitalise on appropriate exotic genotypes, as well as the diversity in production and marketing environments. One approach to finding the 'optimal' genotype is to compare the net economic value (outputs minus inputs) of a number of promising genotypes within the particular production system / environment where they will perform (to avoid issues associated with genotype by environment interaction). Although this is not straightforward, as it can be difficult to assign economic values to intangible traits such as the keeping of livestock for cultural purposes, such an approach is not uncommon. The SL framework, however, suggests that such an analysis ignores many pertinent issues.

An improved genotype is an asset, which can either replace an existing asset or constitute a new asset within the household's portfolio. Assets are useful as 'stores of value' that can be kept and sold when households require cash. Livestock often serve this purpose, so an improved genotype that has higher market value would be an attractive asset to a household, as long as it is equally or, preferably, more likely to survive and be available at times of need than the old genotype.

The main way in which assets affect livelihoods is through the livelihoods strategies that they enable households to undertake. Livestock-related livelihood strategies are generally distinguished by the types of products and services produced (e.g. milk, meat, cheese, draught power), the cost and complexity of the combinations of inputs required to produce them, and what is done with the products/services (consumed, sold or given away). Other things being equal, the ability to produce more products would be an advantage. However, other things are often not equal, and if an increase in quantity or diversity of production brings with it an increase in requirements for complementary inputs or changes in management practices that do not fit with other household activities, then households may be unwilling (because it is too costly or risky) or unable (because they cannot access credit) to adopt the improved genotype.

Large livelihood gains can be realised if a household can move from subsistence to market-oriented production; however, this requires not only the willingness and ability to bear increased cost and risk, but also the existence of stable and functioning markets. Stability refers not only to demand and price, but also to infrastructure and policy. For example, poor road networks and faulty power supplies can be major deterrents to investing in perishable products, as can changeable or inconsistent enforced environmental or food safety regulations.

Thus, rather than simply evaluate genotypes via an economically based breeding objective (even if it does comprise both tangible and intangible traits), use of the SL framework prompts the researcher to ask additional questions such as those alluding to the issues raised above. A pertinent question may be 'is this genotype associated with increased vulnerability?' This could arise from, for example, a reliance on the availability of supplementary feeds, high-level health care and non-household labour; dependence on a few high-output animals rather than a larger number of low-output but better adapted animals; or a fluctuating market. Another question may be 'what type of assets (human, natural, financial, social and physical) are required to support this genotype, and what type of assets are lost (such as social capital related to prestige) if the old genotype is replaced?'

Evaluation of breeding program design

Looking at the asset value alone of improved genotypes fails to consider what is required to access, maintain and benefit from the improved genotype over time. For smallholders, who are the focus of many international agricultural R&D agencies, the same constraints that can limit their ability and willingness to adopt an improved

genotype can act as constraints to participating in breeding programs.

As the SL framework recognises that households engage in a diverse portfolio of activities, one of the first questions the framework prompts is 'what is the importance of livestock to people's livelihoods?' If livestock represent a minor portion of the household's livelihood strategies, technologies around improving the productivity of livestock, such as breeding programs, may not be of interest. Another question prompted by the framework is 'how would participation in the breeding program affect other livelihood activities?' For example, if the labour requirements of the breeding program limit a household's ability to engage in other activities, such as cropping, then the household may choose not to participate due to concerns that decreasing their number of livelihood activities will increase their vulnerability. On the other hand, breeding programs can help to ensure a multiplicity of livelihood activities via the household becoming engaged in input and service supply, such as provision of feed or sire services.

The asset strengths and weaknesses of households, which will influence the uptake and sustainability of breeding programs, are also an important consideration in breeding program design. For example, sire rotation or loan schemes, aimed at reducing inbreeding, can depend on levels of trust, since animals may be entrusted to the care of others where it may be difficult to monitor that proper care is given. Such a scheme is likely to be more successful where risks are lower and social capital is higher. Another example relates to community-based breeding programs, where smallholders form collective groups to ensure a sufficiently high number of animals. Again, this is likely to be more successful if social capital is high, such as where communal grazing schemes already exist. A further example is nucleus-based breeding programs, which are often located on research stations some distance from the community. Here a higher level of physical capital, related to transport and communication, allows easier dissemination of animals and would likely correlate with higher levels of uptake. A high level of financial capital to purchase or rent animals is also necessary. For farmers to make an investment in a high-return technology package, such as F1 crossbreds (including not just animals but also complementary inputs such as on-farm and off-farm infrastructure), access to the different types of capital is critically important. This is examined further in the case study given in the following section.

The time and skill required to record and analyse pedigree and performance information (human capital) should not be underestimated. For this reason easily observable criteria are commonly used, even if they are less precise, as they are more likely to be recorded by farmers and thus have the potential to lead to larger impact. For community-based breeding programs the target group for data collection also requires consideration: men often make decisions, but if women do the daily work (e.g. milk cows), they may be in the best position to gather data. Similarly, children often play a role in livestock keeping, and if they have more education than their parents, involving them in data collection might make sense. This has been done in crop research programs in which high school students play a major role in conducting trials and analysing results (van der Fliert et al. 2001). Further, innovative systems for supporting farmers in data collection and, especially, analysis could also have potentially high impact. For example, use of cell phones or other options based on information and communication technology might have more promise than systems that rely on field agents or others as intermediaries, especially if visits are required.

Commercial orientation often requires more and different types of human and social capital than subsistence production. Producers need to know how to deal with consumers or traders, be able to access price and product quality information, and, in many cases, be able to market collectively. The inability of producers to form and maintain sustainable marketing organisations, particularly for perishable products, is a major reason behind their inability to successfully commercialise. This means that, as well as looking at the availability of additional inputs, interventions that seek to commercialise producers may need to look at business acumen and ability to cooperate.

As suggested by the SL framework, the impact of breeding program design on household vulnerability also requires consideration. For example, a breeding program promoting an increase in herd size in pastoral systems may not be compatible with sustainable natural resource management, and thus increase vulnerability. Alternatively, a breeding program aimed at increasing the feed conversion ratio may lessen pressure on natural resources, and

thus decrease vulnerability. Another question indicated by the SL framework is 'what changes in institutional context or policy are required for people to participate in the breeding technology?' Mechanisms may need to be put in place to strengthen existing institutions, such as formalising traditional forms of cooperatives, or to create new institutions.

As can be gleaned from this discussion, the components of the SL framework are interrelated, with consideration of one issue often leading to consideration of another. The examples given above are not exhaustive, but hopefully serve to illustrate how the SL framework can be used when assessing the relative merits of different interventions.

Case study

One example of a well-known livestock improvement program incorporating a breeding component is FARM-Africa's goat program. While publications describing this program do not stress the use of an SL framework (Ahuya et al. 2005; FARM-Africa 2004; Peacock 2005a), the holistic principles of SL are followed. This case study is given as an example of how taking a livelihoods approach is possible and can improve the impact and sustainability of interventions.

The FARM-Africa goat improvement program has been tested in four East African countries over 19 years, with the most success in Kenya (FARM-Africa 2004; Ahuya et al. 2005; Peacock 2005a). Briefly, the project involves groups of smallholder farmers that crossbreed local goats with higher performing, exotic Toggenburg bucks at community-managed buck stations. Group-managed breeding units ensure the supply of replacement bucks, and inbreeding is avoided by rotating bucks among groups. Overall coordination of the breeding activities is by umbrella goat-breeding associations, with goat pedigrees registered with the Kenyan stud book. In addition, the program has established a community animal health worker program, introduced raised slatted-floor housing, and ensured an all-season supply of high-quality fodder. All activities have been supported by a number of training programs and a series of policy change advocacy programs.

In Kenya the program has been running since 1996, with the national population of improved dairy goats numbering > 160,000, 170 buck stations established and > 8,000 households directly benefiting. Monitoring and evaluation studies have indicated that household nutrition and income have been significantly improved (Peacock 2005b; Peacock et al. 2009).

The reasons for the success of this program were summarised as follows (Peacock et al. 2009):

- inspirational nature of the program with quick, dramatic improvements—the result of using a crossbreeding, rather than a within-breed improvement, approach
- involvement of the community, ensuring that locally appropriate strategies were adopted
- an appropriate and manageable scale, with replication of the project achieved within the community by group members sharing information with non-group members
- comprehensive services—improved breeds, health delivery, housing and fodder production, credit schemes, goat pricing policies
- limited continual external inputs, with plans for withdrawal of support by FARM-Africa.

A number of challenges have also been identified, including the demand for breeding stock exceeding the supply; the need to increase the capacity of the breeding associations in relation to management and leadership; a lower level of performance recording than anticipated; a lack of interest of smallholders to breed indigenous goats due to their lower profitability; and a lack of interest of larger scale private breeders. Activities to overcome these challenges are reported to be ongoing (Peacock et al. 2008).

In relation to the SL framework, this program has addressed many asset-related aspects, including social capital (community-managed breeding program, formation of associations), financial capital (access to credit schemes), human capital (training programs to increase knowledge and capacity), natural capital (improved livestock genotype, better quality forage) and physical capital (improved inputs/services). Aspects of the program such as goat pricing policies have helped address vulnerability. Lobbying of the government resulted in a major policy change such that goat milk was legally recognised as a sellable product. Other institutional and policy issues are also being addressed by dissemination of evidence, networking and advocacy (FARM-Africa 2007).

Conclusion

This paper is the first to address the potential role of the SL approach in influencing the design and implementation of R&D programs around the use of animal genetic resources. As feedback from programs that have explicitly used this approach becomes available in future years, further insights should become apparent. To a large extent this will depend on the quality of monitoring and evaluation, as well as on documentation, of these programs. The SL framework prompts researchers and development organisations to ask the right questions; how to achieve this in practice is at the discretion of the stakeholders (see DFID (2001) for an overview of possible approaches). Given the demonstrated utility of the SL framework to other development programs within agriculture, such as cropping (Adato and Meinzen-Dick 2007), the authors consider the SL framework to be a currently underused tool in relation to translating animal breeding research results into livelihood improvements.

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References

Adato M. and Meinzen-Dick R.S. 2007. Agricultural research, livelihoods, and poverty: studies of economic and social impacts in six countries. Johns Hopkins University Press and International Food Policy Research Institute: Baltimore, Maryland.

Ahuya C., Okeyo A. and Peacock C. 2005. Developmental challenges and opportunities in the goat industry: the Kenyan experience. Small Ruminant Research 60, 197–206.

Barrett C.B., Reardon T. and Webb P. 2001. Nonfarm income diversification and household livelihood strategies in rural Africa: concepts, dynamics, and policy implications. Food Policy 26(4), 315–331.

Bennett A., Lhoste F., Crook J. and Phalen J. 2006. The future of small scale dairying. In 'Livestock report 2006', ed. by S. Henning, A. Costales, J. Rushton, B. Scherf, T. Bennett and D. Hall. FAO: Rome, Italy

Bhende M.J. and Venkataram J.V. 1994. Impact of diversification of household income and risks: a whole farm modeling approach. Agricultural Systems 44, 301–312.

Brocklesby M.A. and Fisher E. 2003. Community development in sustainable livelihoods approaches: an introduction. Community Development Journal 38, 185–198.

Carney D. 1998. Sustainable rural livelihoods: what contribution can we make? Department for International Development (DFID): London.

Dercon S. and Krishnan P. 1996. Income portfolios in rural Ethiopia and Tanzania: choices and constraints. Journal of Development Studies 32(6), 850–875.

Deshingaker P., Farrington J., Rao L., Sharma S.A.P., Freeman A. and Reddy J. 2008. Livestock and poverty reduction in India: findings from the ODI Livelihoods Options Project. Discussion Paper No. 8: Targeting and Innovation. ILRI (International Livestock Research Institute): Nairobi, Kenya.

DFID (Department for International Development) 2001. Sustainable Livelihoods Guidance Sheets. DFID: United Kingdom.

Dolberg F. 2001. A livestock development approach that contributes to poverty alleviation and widespread improvement of nutrition among the poor. Livestock Research for Rural Development 13(5).

Ellis F. and Biggs S. 2001. Evolving themes in rural development 1950s–2000s. Development Policy Review 19, 437–448.

Ellis F. and Freeman H.A. 2004. Rural livelihoods and poverty reduction strategies in four African countries. Journal of Development Studies 40(4), 1–30.

FARM-Africa 2004. Seeking innovation in agriculture: FARM-Africa Annual Review 2003–2004. Waterside Press: London, United Kingdom.

FARM-Africa 2007. From grassroots to government: FARM-Africa's experience in influencing policy in subSaharan Africa. FARM-Africa policy and research series no. 5. Waterside Press: London, United Kingdom.

Kosgey I.S., Baker R.L., Udo H.M.J. and van Arendonk J.A.M. 2006. Successes and failures of small ruminant breeding programs in the tropics: a review. Small Ruminant Research 61, 13–28.

Kosgey I.S. and Okeyo A.M. 2007. Genetic improvement of small ruminants in low-input, smallholder production systems: technical and infrastructural issues. Small Ruminant Research 70, 76–88.

Krantz L. 2001. The sustainable livelihoods approach to poverty reduction: an introduction. Sida, Division for Policy and Socio-Economic Analysis: Stockholm.

Krishna A., Kristjanson P., Kuan J., Quilca G., Radeny M. and Sánchez-Urrelo A. 2006. Fixing the hole at the

bottom of the bucket: household poverty dynamics in forty communities of the Peruvian Andes. Development and Change 37(5), 997–1021.

Krishna A., Kristjanson P., Radeny M. and Nindo W. 2004. Escaping poverty and becoming poor in 20 Kenyan villages. Journal of Human Development 5, 211–226.

Kristjanson P., Krishna A., Radeny M., Kuan J., Quilca G. and Sánchez-Urrelo A. 2005. Dynamic poverty processes and the role of livestock in Peru. FAO/Pro-Poor Livestock Policy Initiative Working Paper.

Kristjanson P., Mango N., Krishna A., Radeny M. and Johnson N. 2009. Understanding poverty dynamics in Kenya. Journal of International Development (in press).

LID 1999. Livestock in poverty-focused development. Livestock in Development (LID): Crewkerne, United Kingdom.

Peacock C. 2005a. Goats: unlocking their potential for Africa's farmers. FARM-Africa Working Paper series. Paper presented at the 7th Conference of Ministers Responsible for Animal Resources, Kigali, Rwanda.

Peacock C. 2005b. Goats: a pathway out of poverty. Small Ruminant Research 60, 179–186.

Peacock C., Ahuya C.O., Okeyo A.M. and Ojango J.M.K. 2009. Practical breed improvement – an example of success: FARM-Africa's goat model. Livestock Science (submitted).

Reardon T. 1997. Using evidence of household income diversification to inform study of the rural nonfarm labour market in Africa. World Development 25, 735–-747.

Scoones I. 1998. Sustainable rural livelihoods: a framework for analysis. IDS Working Paper no. 72. Institute of Development Studies (IDS): Brighton.

SDP (Smallholder Dairy (R&D) Project) 2004. The policy environment of Kenya's dairy sector. SDP Policy Brief no. 6.

van de Fliert E., Johnson N., Asmunati R. and Wiyanto. 2001. Beyond higher yields: the impact of sweetpotato ICM farmer field schools in Indonesia. Pp. 331–342 in 'Scientist and farmer: partners in research for the 21st century'. Program report 1999–2000, Centro Internacional de la Papa (CIP): Lima, Peru.

Waters-Bayer A. and Bayer, W. 1992. The role of livestock in the rural economy. Pp. 30–49 in 'Livestock production in rural development: development of livestock policies', ed. by C.P. Gootjes, G. Hertog, R. de Jong and A.J. Nell. Proceedings of the International Workshop on livestock production in rural development, Wageningen, The Netherlands.

Smallholder sheep owners' views on the value and management of Deccani crossbred *FecB*-carrier ewes with a higher twinning percentage: implications for a future introgression extension program

J. Prior[1,2], P.M. Ghalsasi[3], S.W. Walkden-Brown[1], K.M. Chavan[3], S.R. Kulkarni[3] and C. Nimbkar[3]

Abstract

Deccani sheep are reared on the Deccan plateau of India by smallholder sheep owners whose main source of income is lamb production. Deccani ewes usually give birth to only one lamb. The *FecB* mutation, which increases prolificacy, was introduced into the Deccani breed from the Garole breed of West Bengal by the Nimbkar Agricultural Research Institute (NARI), to increase lamb production and incomes of sheep owners. In 2002 NARI began to disseminate *FecB*-carrier rams and ewes into 26 local sheep owners' flocks to enable field testing of *FecB*-carrier progeny born in those flocks. In order to assess their opinions of the *FecB* rams and ewes and the resulting twin lambs, surveys were conducted with 23 of the participating sheep owners. They were asked to assess the perceived advantages, disadvantages and risks associated with twin lambs, to outline their management responses to twin lambs, and to describe their preferred ram phenotype. In general, 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.

Introduction

The aims of this paper are to report on a socioeconomic analysis of the impacts of the *FecB* gene dissemination program implemented in local smallholder sheep flocks in 2003–06, and to use this analysis to contribute to the design of an extension strategy to facilitate the dissemination of the *FecB* mutation beyond the current project area. In order to inform the design of a future dissemination strategy, a survey was undertaken with the sheep owners whose flocks participated in the dissemination of the *FecB* gene. This paper deals with the results of the survey and the implications of these results for the development of a future introgression extension program.

[1] School of Environmental and Rural Sciences, University of New England, New South Wales 2351, Australia
[2] Corresponding author; jprior@une.edu.au
[3] Animal Husbandry Division, Nimbkar Agricultural Research Institute (NARI), Phaltan, Maharashtra, India

Project history

Sheep rearing is an important income-generating activity for traditional smallholder sheep owning communities in India. The sheep population in India is estimated to be around 61.5 million and, of this, 19.1 million (31%) are considered to be of the Deccani breed (Anthra 2007). Of the total Deccani sheep, 8.5 million are in Andhra Pradesh, 7.2 million in Karnataka and 3.4 million in Maharashtra state. It is estimated that close to half a million families depend completely or partially on Deccani sheep rearing for their livelihood. Deccani sheep flocks are maintained by smallholders (flock size ranging from 20 to 200) by grazing them on crop residues, fallow lands and hilly areas.

Like most other breeds of sheep in India, Deccani sheep have a comparatively low reproductive rate, producing one lamb every 10–12 months. The sale of 3.5–4-month-old male lambs for meat is the major source of income. Female lambs not needed for replacement are also sold. The small quantity (600 g/year) of coarse, hairy fleece produced by Deccani sheep is of low value—insufficient to cover the cost of shearing. Given the close personal supervision of ewes while grazing through the day and vigilance at night, a 'personalised' system of management, and the high and increasing demand for and market price of lambs, a moderate increase in the litter size (LS) of Deccani ewes would lead to an increase in the number of saleable lambs and in the incomes of sheep owners. An increase in sheep productivity and meat production would also conform with Indian national agricultural priorities and may help to address the serious problem of protein malnutrition, especially among women and children. Provision of an increased supply of meat would potentially lead to reduced prices, making it possible for more families to buy meat.

The Australian Centre for International Agricultural Research (ACIAR) has funded two collaborative research projects in India to increase the efficiency and profitability of sheep production and to reduce losses due to gastrointestinal parasitism. The first project 'Prolific, worm-resistant meat sheep for Maharashtra, India' was initiated in 1998. As a continuation of this project, ACIAR funded another 3-year project (2002–05) 'Improving productivity, profitability and sustainability of sheep production in Maharashtra, India, through genetically enhanced prolificacy, growth and parasite resistance'. This project was extended by another 2 years to 2007.

In the first project local Deccani ewes were crossbred with Garole, a small but prolific sheep breed from West Bengal. The Garole is the only known prolific breed in India, and the project established in 2001 that its prolificacy is associated with the Booroola fecundity (*FecB*) mutation (Davis et al. 2002). The mode of action of the *FecB* gene is additive for ovulation rate, and partially dominant for LS at birth. However, in the Australian Booroola Merino the *FecB* homozygote has a lower LS than the heterozygote. This single gene for prolificacy was introgressed from the Garole into the Deccani breed. The project scientists also discovered that the Garole sheep have superior resistance to internal parasites compared to the Deccani (Nimbkar et al. 2003). Epidemiological studies of internal parasite infestation in sheep were carried out to establish sustainable parasite control protocols using a minimum quantity of deworming medicines. The small size of the Garole, its lack of adaptation to the hot, dry climate of the Phaltan area in Maharashtra, its inferior mothering ability and its physical appearance were not acceptable to the sheep owners. In order that the crossbred ewes would retain the capacity to have twin lambs but have the conformation and appearance of the Deccani, it was necessary to backcross them to the Deccani while ensuring that the *FecB* mutation was not lost.

Backcrossing commenced at NARI in 2000. At that time, however, it was not confirmed that the prolificacy of the Garole breed was due to the *FecB* mutation, nor was there a direct test to identify carriers of the putative fecundity gene. This problem was solved in 2001 when the *FecB* mutation was identified almost simultaneously by researchers in New Zealand, France and the UK (Mulsant et al. 2001; Souza et al. 2001; Wilson et al. 2001), and a direct DNA test was developed. This test was established at the National Chemical Laboratory in Pune in 2002 to provide rapid and early identification of carrier lambs for use in the breeding program. Ram and ewe lambs from each generation with the desired characteristics were selected systematically and retained for further breeding to ensure that the crossbred sheep retained the toughness and resilience of the Deccani and the high reproductive ability of the Garole. These desired characteristics included higher body weight, and the conformation, colour and phenotype preferred by local sheep

owners in the case of the 'fecund Deccani' strain; and absence of deformities or defects in the case of the 'Nari Suwarna' strain.

In the follow-on project lambs were selected for faster growth, adaptation to a harsh environment and appearance preferred by local sheep owners. Two new more productive *FecB*-carrier strains were developed at NARI—the 'NARI Suwarna' or 'fecund Deccani', comprising around 25% Garole and 75% Decani, and the 'NARI Composite', which includes about 25% Israeli dairy Awassi breed. At NARI *FecB*-carrier ewes give birth, on average, to twin lambs every alternate lambing. Although twin lambs are slightly smaller than single-born lambs (their weight at 3 months of age being about 2 kg less), their combined weight is at least 25% higher (Nimbkar et al. 2009). The increase in average LS of the flock is only about 0.5 per ewe lambing, which is a modest and manageable increase for a small flock but substantial enough to increase income and profit. The sale price per lamb is substantial, namely Rs1,200–1,500, while the cost of feed per lamb up to 3.5 months of age is likely to be only about Rs250.

Following a socioeconomic survey of 87 sheep owning families in 2001–02, which indicated a strongly positive attitude towards the few Deccani ewes which bear twins (S. Khot, C. Nimbkar, P.M. Ghalsasi and S.W. Walkden-Brown, unpublished data), NARI started, in 2003, to disseminate *FecB*-carrier rams into local smallholder sheep owners' flocks for breeding. This was to enable field testing of *FecB*-carrier progeny born in those flocks to be carried out. Winning the confidence of deeply traditional smallholder sheep producers was a difficult task. The concepts of introducing twinning in sheep and ongoing genetic improvement were new to them. They did not like the horns and wide foreheads of the first few generations of rams because of the high proportion of Garole in them. Project scientists learnt gradually about the sheep owners' preference for certain facial features, colour and other physical characteristics, and included those preferences in selecting breeding animals. The results have been encouraging—some flock owners have sold their own rams in preference for using the NARI breeding rams. Others are rearing male progeny of NARI's rams carrying the *FecB* mutation to use as breeding rams in future. It was recognised that carrier animals with 25% Garole origin would meet resistance from sheep owners on account of both their size and appearance, but it was important to determine whether the encouraging results being observed in the NARI flocks would also be observed in the traditional smallholder sheep owning environment.

Methodology

The 26 sheep owners into whose flocks ram and ewe introductions were made were selected on the basis of their contact with NARI over the previous few years through NARI's extension and research activities. These activities included demonstration of sustainable parasite control methods, a trial assessing the cost–benefit results of deworming, and carrying out emergency veterinary treatment on demand in sheep owners' flocks. Fourteen of the flocks were from Wadjal village, where NARI's office is located; six were from Bhilkati, Bandalwasti and Kawalban villages, 3–5 km from the NARI office; three were from Bhadali and Nandal villages, 12–18 km from the NARI office; and two were from Waghoshi and Ahire villages, about 30 km from the NARI office. Sheep owners' willingness to participate in the project was the only criterion used for selection.

In 2003 and 2004, 48 B+ NARI Suwarna (fecund Deccani) ewes, 40 ++ ewes consisting of only Deccani and Garole genotypes, 12 B+ NARI Composite ewes and 20 ++ Composite ewes were introduced into 14 smallholder flocks. In addition, *FecB*-carrier rams were distributed by NARI in 2003–07 into the flocks of 26 participating sheep owners. Rams were introduced for periods of 35–90 days and, after joining, were returned to NARI as these same rams were used in NARI's own breeding program. Oestrus synchronisation and artificial insemination were also carried out in some flocks in 2004. Homozygous $FecB^{BB}$ rams were introduced for the first time in 2005, and by 2007 all 18 rams introduced were BB (Table 1).

Of the 26 participating flocks, one belonged to the Baramati Agricultural Development Trust. Since the flock belonged to an organisation and not to an individual owner, and was managed quite differently from the management by local sheep owners, an interview was not taken for this flock. Of the remaining 25 flocks, 10 left the study due to various reasons before the conduct of the first interviews. Three flocks were sold due to family circumstances external to this project, while the owners of seven did not want to cooperate with the project and follow the contract entered into at the start. Some did not

want to introduce NARI rams into their flocks, some wanted to sell and not retain B+ ewe lambs, and some did not want their animals ear tagged.

Table 1. Ram introduction into participating sheep owners' flocks

Year	Number of rams introduced ($FecB^{BB}$ rams)	Participating flocks
2003	10 (0)	12
2004	21 (0)	18
2005	16 (2)	14
2006	12 (10)	8
2007	18 (18)	13

All participating flocks were visited by NARI's extension staff at least once a week and all lamb births, deaths and sales were recorded. All animals in these flocks were ear tagged and weighed every 2 months. Some of the results of the analysis of data from these flocks are presented in the paper by Nimbkar et al. (2009) in these proceedings. Individual faecal samples were collected from all sheep at the start of and during the monsoon rains, and they were treated with anthelmintic if necessary. NARI staff also provided free vaccinations against enterotoxaemia and peste de petit ruminants (PPR), and treated sick animals for free. Additionally, all animals in the participating flocks were insured by the project, and NARI staff helped to prepare insurance claims for animals that died.

The limited number of participating sheep owners required that a case study methodology be adopted, with the unit of analysis being the individual owner and their flock. Case study research seeks analytic generalisation (theory-connected) rather than statistical generalisation (Yin 2003). However, within case study methodology it is important to emphasise that the 'cases', that is the sheep owners, are not sampling units. Each 'case' is essentially an individual experiment. While an attempt may be made to generalise from the observations made on these cases, the results obtained relate to each case alone and cannot be extrapolated to other populations in the same way one might do with the statistical generalisations involved in more quantitative research.

A draft survey instrument was piloted with the NARI extension staff, and necessary modifications were made to a final survey design prior to its use with the case study sheep owners. A long version of the survey was used in the initial interviews, while a shortened version was used in the second set of interviews. The final survey instrument containing the questions common to both interviews is illustrated in the appendix. This paper deals primarily with the responses to Section B of this survey (questions 1–14), although reference is made to irrigated land data (question 3). In addition, the general implications of the extension support provided to participating sheep owners (Section C) are also canvassed, although the data are not presented or analysed here.

Conduct of the survey

All sheep owners were initially informed that their participation within this project also involved interview surveys. Two sets of interviews were conducted by NARI extension officers of the 15 sheep owners remaining in the study. In the first set (conducted November 2006 to February 2007) the participants answered a long questionnaire and the sessions were audiotaped. The interviewees felt, however, that the formality of this approach inhibited them from answering freely, and that the length of the survey resulted in fatigue. Thus, for the second set of interviews (conducted April–May 2008) audiotaping was not used, and the length of the survey was shortened. The final survey instrument used is illustrated in the appendix. Eight of the 10 sheep owners who had exited the NARI study were also interviewed once in August 2007 using the same survey instrument. Translating the interviews into English proved to be difficult as the sheep owners used many traditional local Marathi words that have very specific meanings in their lexicon.

Results

The survey results presented below are grouped according to common themes.

Irrigation profile and relative wealth of sheep owners

Each sheep owner was asked to indicate the area they had available for irrigated crops and pastures, where 'irrigated' did not mean perennially irrigated, rather, that irrigation is available for about 8 months of the year, depending on rainfall. In the absence of complete household income data from the partici-

pants, this parameter provides some indication of the relative wealth of individual sheep owners, and the extent to which alternative employment and income was available to family members on the family farm. An increased irrigation area means that the family is able to plant labour-intensive crops such as vegetables, earning them more income, and crops in the summer. Larger areas of irrigation provide sheep owners with a broader range of feeding strategies for their flocks. This is important for the management of flocks during vulnerable periods, as well as allowing the supplementary feeding and management of larger numbers of lambs due to increased twinning percentages. Access to irrigation also provides sheep owners with an increased range of risk management strategies during periods of limited rainfall.

All participants had some form of irrigation available to them, although there was a substantial range within the group. Sheep owners' irrigation holdings are illustrated in Figure 1. The range of irrigation holdings from 30 acres down to less than 1 acre spans a reasonably wide spectrum of livestock grazing resource access and wealth among the sheep owners. NARI extension staff also undertook a brief assessment of the relative wealth of the 25 owners based on the criteria of the type of housing they own; whether other family members have salaried jobs; and whether they own cattle, motorbikes and household items such as television sets. On this basis it was assessed that nine sheep owners could be classed as relatively wealthy, nine as of moderate means and seven as poor. There was a general correlation between area under irrigation and the assessment of wealth criteria. Thus, this brief analysis indicated a reasonably wide range of relative wealth among the case study participants.

Flock profile and desired number of *FecB*-carrier ewes

Sheep owners were asked about their existing flock numbers, the current number of *FecB* ewes within their flock, and their desired 'ideal' number of *FecB* ewes. Flock numbers ranged from 28 to 227 animals, with a mean of 65. Four sheep owners had larger numbers of *FecB* ewes in these flocks, namely 15, 18, 25 and 50; while the 13 remaining had more modest numbers (1–6) of ewes. All sheep owners wished to increase their numbers of *FecB*-carrier ewes. Their desired 'ideal' number of *FecB*-carrier ewes was in the range 8–100, with a mean of 25 ewes. As the mean of all the existing flock sizes was 65 animals, the mean desired ideal number of 25 *FecB*-carrier ewes represents 38% of the total flock. Those who desired the largest numbers of *FecB*-carrier ewes tended to have larger areas of irrigation (Figure 2). This perhaps reflects a recognition among the sheep owners of the importance of accessible sources of supplementary feed for twin lambs, in particular.

Twin lamb management

Participants were asked to list the management strategies they were presently using in response to increased numbers of twin lambs. They mentioned a broad range of strategies designed to ensure the

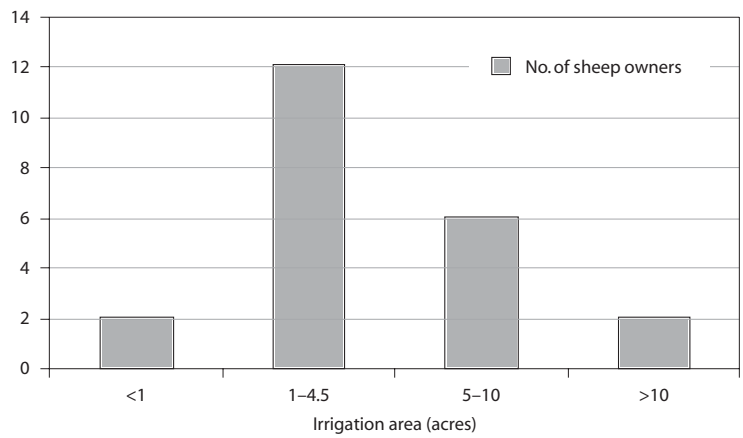

Figure 1. Sheep owners' irrigated land holdings

growth and survival of twin lambs. Out of 21 sheep owners responding to this question, all but one had used supplementary feeding of twin lambs or ewes, or had taken some form of additional management action.

Currently used management strategies mentioned by sheep owners included:

- keeping lambs behind when their dams go grazing and feeding them at home during the day
- supplementary feeding of lambs with concentrate (pellets, groundnut cake, wheat dough) or with extra grain (maize, sorghum, pearl millet etc.) or pasture, acacia trees or onion residue fodder
- planting of additional areas or new fodder crops, e.g. lucerne
- obtaining extra labour for lamb management, either from relatives or purchased from other sources
- supplementary feeding of *FecB*-carrier ewes both pre and post lambing
- migration of the flock to new grazing areas
- lambing during the rainy season
- retention of twin ewe lambs from sale.

An additional strategy of cross-fostering of twin lambs on other ewes and goats was not listed as being currently used, but was cited by one participant as being a future planned strategy. This broad range of management strategies represents the choice of both routine and opportunistic alternatives to sheep owners, the balance of which may vary markedly both between owners and between seasons.

Perceived risk and mortality of twin lambs

Sheep owners were asked their opinions as to the likely risks associated with the raising of twin lambs, and their perceptions as to whether twin lambs were likely to suffer increased mortality relative to single lambs. All those responding to the question of risk (21) felt that twin lambs tended to be smaller than single lambs, but the majority felt that this disadvantage would be overcome by supplementary feeding. Only three specifically stated that they believed that twin lambs suffered higher mortality than singles. There was no opinion expressed among the remaining sheep owners that twin lambs had higher mortality. However, 11 mentioned that twin lambs were 'weaker' in the absence of supplementary feeding. Two mentioned that an advantage of twin lambs was that, if one died, one still remained to raise, and the economic risk of twin lambs was therefore perceived to be less than with single lambs. Thus, in general, no clear perception of a higher risk with twin lambs emerged among the sheep owners interviewed, so long as supplementary feeding was undertaken.

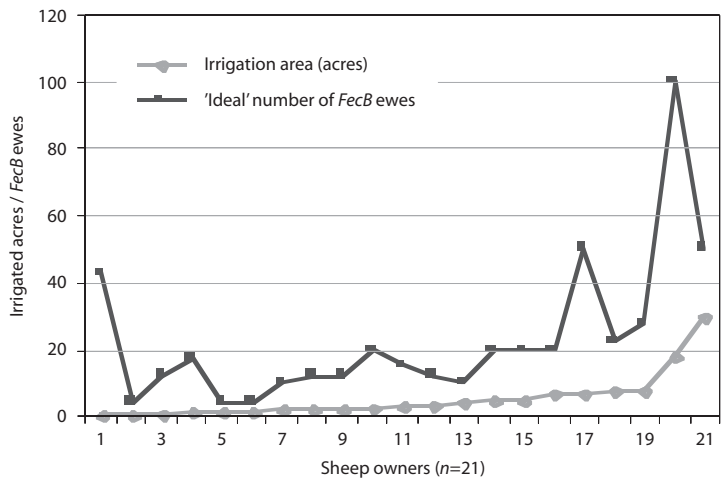

Figure 2. Irrigation area (acres) for each sheep owner compared with the sheep owner's expressed desired 'ideal' number of *FecB*-carrier ewes in the flock

Perceived advantages, disadvantages and profitability of twin lambs

Sheep owners were asked about the perceived advantages and disadvantages of twin lambs. Disadvantages mentioned were the extra workload associated with lamb management, the need for supplementary feeding of twin lambs and ewes, and the slow growth of twin lambs. Seven sheep owners mentioned the small size of twin lambs, and three that twin lambs may be more susceptible to illness. Eleven said that supplementary feeding would overcome any likely disadvantage.

Among the advantages mentioned were the extra profits from additional lambs (cited by 20 sheep owners). However, the fact that NARI's lamb management extension DVD (claiming 1.5 times the income from twin lambs) was shown to participants shortly before the first interview may have influenced their responses in relation to perceived profits. Five owners cited actual sale prices that supported their claims of extra profits—for *single* lambs in the range Rs1,200–1,500, and for a *pair of twins* in the range Rs2,000–2,500. One owner quoted the weight of a single lamb at 3 months of age as 12 kg, while the collective weight of twins was 18 kg—the extra 6 kg was perceived as 'profit'. However, the actual assessment of net profit is unknown without a more detailed flock tracking and analysis. This analysis would include the relative growth rates and survival rates between singles and twins, and an assessment of the extra costs incurred in feeding and the labour inputs for the management of twin lambs.

Sheep owners' preferred ram phenotype and assessment of *FecB*-carrier rams

Participants expressed clear preferences in relation to preferred ram phenotype. This is likely to provide a barrier to their acceptance of *FecB*-carrier rams that have remnants of the Garole phenotype or of breeds other than the Deccani. General ram phenotype preferences mentioned included a 'Roman' nose, tall rather than short, coloured white or black and white, long ears, a narrow forehead and no neck wattles. Fifteen sheep owners specifically mentioned that they did not like *FecB*-carrier rams as they were small (and therefore cannot join effectively with tall ewes) and did not exhibit a Roman nose, with one stating they were not 'good looking'. Five owners expressed a qualified liking for *FecB*-carrier rams, stating that some exhibited a more desirable phenotype than others, or that the 'later' rams were an improvement on earlier versions. Three also changed their views on *FecB*-carrier rams between the first and second interviews: two went from a positive to a negative view of the rams, while one went from negative to positive.

From these responses it appears that ram phenotype will provide a barrier to the sheep owners' acceptance of *FecB*-carrier rams if the introgression pathway does not produce animals of the desired local phenotype. In other regions it is possible that different phenotypes will be preferred.

Discussion: issues for the development of an introgression extension program

The response of sheep owners to twin lambs was generally positive. This reinforced the findings of the wider survey of 87 sheep owner families in 2001–02 prior to the introduction of fecund genotypes (S. Khot, C. Nimbkar, P.M. Ghalsasi and S.W. Walkden-Brown, unpublished data).

There are four key issues, discussed below, which emerged from the survey that have implications for the development of an extension program for the introgression of the *FecB* gene beyond the study area. Some of the general implications of the findings of Section C of the survey that dealt with extension support to sheep owners are also discussed, even though these results are not presented here.

Financial analysis of cost-effective management and supplementary feeding of twin lambs

Sheep owners clearly adopted the view that supplementary feeding and additional management were required to ensure the adequate growth and survival of twin lambs. This understanding was, in part, probably due to the success of the NARI extension program that accompanied the project. Issues that arise, therefore, include the cost of and access to additional fodder reserves, the cost of and access to additional labour requirements, and the risk management strategies necessary during drought or extended dry periods. The participants range from relatively wealthy to relatively modest

means. Thus, the question arises as to whether extra twinning rates will be suitable for those very poor sheep owners who do not have access to at least some area of irrigation, or who practise more strongly nomadic transhumant pastoralism and are less settled for longer periods in a home area.

An analysis of the economics of supplementary feeding of ewes and lambs at NARI was found to be favourable (ACIAR 2006), and first principles suggest that benefits are greater in ewes carrying twins. Indeed, in Australia it is a common management practice to subject ewes to ultrasound scanning for LS, and apply nutritional supplementation only to that portion of the flock carrying multiple births. A gross margins economic analysis of the participants' flocks reported in these proceedings (Nimbkar et al. 2009) showed that *FecB*$^{B+}$-carrier ewes had gross margins between 37% and 50% higher than non-carrier ewes, indicating that current sheep owner management practices resulted in increased profit from twin-bearing ewes. Nevertheless, further investigation is required into optimising management and supplementary feeding strategies for twin lambing flocks to make them even more cost-effective. This research should include financial appraisal of the net profit accruing to sheep owners once the extra costs of supplementary feeding and labour are included, and may, in part, be based on an analysis of the data collected from case studies of their flocks. The result of this research could then contribute to the development of the extension program regarding appropriate feeding and management strategies.

'Improved' phenotype of *FecB*-carrier rams

The first few generations of *FecB*-carrier rams produced at NARI had a 25% proportion or higher of Garole. Also, rams with two copies of *FecB* were not selected on the basis of appearance since NARI had too few at the beginning of the program (ACIAR 2007). Sheep owners' strong perceptions of the inferiority of the smaller Garole-type *FecB*-carrier rams may pose a significant constraint to the future use of rams as a vehicle for *FecB* introgression, particularly during the early stages of introgression into a new breed or type. At NARI efforts need to be made to breed larger bodied *FecB*-carrier rams that more closely resemble the phenotype of local ram breeds. However, the fact that some participants mentioned that the later *FecB*-carrier rams were superior in appearance to the earlier versions suggests that this trend is already occurring. This is a natural consequence of producing larger numbers of animals carrying the *FecB* gene over time, with a greater range of phenotypes to select from.

Other important implications of these findings include the following:

- Where possible, the Garole breed should be avoided as the source of the *FecB* mutation for introgression into other large Indian breeds. It has many undesirable and distinctive features that increase the probability of rejection of carrier rams by sheep owners. As the availability increases of homozygous carrier rams with high proportions of Deccani or Malpura blood, these should be used as the source of *FecB* genes rather than the Garole.
- Consideration should be given to backcrossing to a level of 87.5% local breed, rather than the 75% used in this study. However, if introgressing from one breed or strain to a similar one (e.g. between different strains of the Deccani), a level of 75% local breed may suffice.

The need for a strong, supportive extension program and use of demonstration sheep owners

Extension activities with the participating sheep owners included a package of supply of breeding rams and ewes carrying the *FecB* gene, veterinary treatment and insurance cover for all animals in the flocks. This extension program appeared to be popular with the participants. The fact that increased twinning rates require that sheep owners adopt a higher level of management and higher cost feeding strategies means that ongoing extension support must be a priority. Case studies of sheep owners who have been successful and profitable in integrating *FecB*-carrier animals and associated management strategies into their flocks will provide useful demonstration value for potential adopters.

Discussions with the NARI extension staff indicate that participants highly valued the veterinary health support they received in managing their flocks. It is likely that the adoption of the *FecB* gene will be enhanced among sheep owners where the accompanying extension program provides support in several key areas. These areas would include advice regarding feeding and management strate-

gies for *FecB*-carrier ewes and lambs, veterinary health support, market and marketing advice, and insurance of sheep.

Due to the cost of providing these services, however, they would need to be offered strategically, possibly only for a restricted period around the lambing of the first ewes carrying the *FecB* mutation. This could be 1.5–2 years after the introduction of the mutation via a carrier ram. The project is currently working with 10 sheep owners' flocks, to whom none of these services is offered except veterinary advice and making *FecB*-carrier rams available for free for breeding in their flocks. This suggests that support levels may be able to be reduced as the project expands beyond the demonstration phase.

The need for 'targeting' of sheep owners and the use of local information/knowledge networks within the development of an extension program

'Targeting' relates to two elements of technological change. First, it is important to recognise that the value of new technology will vary depending upon the characteristics of the recipient of the technology (Roling 1988). Some recipients will benefit substantially from new technologies, while others may need to significantly modify the technology or the way it is used to reap its benefits, and still others may not be able to use the technology in a beneficial way at all. Targeting in this sense recognises that both the extension message and the use of the technology itself may need to be modified according to the nature of each farmer or sheep owner group. For example, sheep owners will exhibit some degree of socioeconomic heterogeneity. It is important that a *FecB* extension program is targeted at those groups who are likely to benefit from gene introgression and who can take advantage of increased lamb numbers, for example those with irrigation or access to extra labour, and those who are more settled and less nomadic.

The second element of targeting relates to the mechanisms/networks by which sheep owners exchange information and knowledge (Black 2000; Leeuwis 2004), sometimes termed 'agricultural knowledge and information systems'. This targeting ensures that innovation concepts and learning experiences are shared between information groups. However, targeting strategies should not assume that the information conveyed to one network will necessarily diffuse to another, as they may be based upon kinship relationships, geographical location, commodity producer groups or some other purpose-formed group. For this form of targeting to be effective, it is essential that extension agents understand and take advantage of the information networks that exist within the district. Identifying 'knowledge leaders' within each network is also crucial. Any future extension program must be clear in its objectives as to the socioeconomic and behavioural-production profiles of the sheep owners being targeted, as well as the information networks used and the communication strategies employed.

An additional issue that will require ongoing assessment will be to monitor whether the introduction of the *FecB* gene results in net increases in district animal numbers and, if so, to assess if there is an increase in land degradation through overgrazing. Early indications are that this is not likely to be a problem. Lambs are sold at around 3.5–4 months age weighing around 15 kg, and thus are likely to be undertaking only moderate levels of grazing. In addition, the introduction of *FecB* is expected to increase the efficiency of the sheep production system. The impact of increased twinning numbers on flock (especially ewe) numbers will also be of interest. It is possible that, with increased twinning rates, flock (ewe) numbers may decrease as sheep owners may be satisfied with the higher production levels achieved with fewer breeding ewes.

Conclusion

The survey of the 25 case study sheep owners suggests that their view of the introduction of twinning lambs into their flocks was generally positive. There was a universal recognition among them of the need for supplementary feeding and additional management. Further research is needed involving a financial analysis of cost-effective management and supplementary feeding of twin lambs. This can be conducted both as desktop analysis and case study analysis of the practices of successful sheep owners. Twin lambs were not associated with perceptions of increased risk. There was, however, some resistance to the Garole phenotype of the *FecB*-carrier rams. This concern is likely to dissipate as selection pressure by NARI reduces the proportion of Garole and increases the

proportion of local Maharashtra breeds, with resulting phenotypes being more acceptable to local owners.

Ongoing extension will need to build on the lessons learnt from the NARI project. Strong support for participating sheep owners during the early adoption and demonstration phase of the new twinning technology may be necessary, and should be coupled with the use of locally relevant knowledge networks among the owners. Monitoring of district flock numbers, grazing impacts and any resulting land degradation should also be undertaken as part of any project expansion.

References

ACIAR (Australian Centre for International Agricultural Research) 2007. Annual Report January 2006 – December 2006: Project AH/2002/038: Improved productivity, profitability and sustainability of sheep production in Maharashtra through genetically enhanced prolificacy, growth and parasite resistance. ACIAR: Canberra.

ACIAR (Australian Centre for International Agricultural Research) 2006. Annual Report January 2005 – December 2005: Project AH/2002/038: Improved productivity, profitability and sustainability of sheep production in Maharashtra through genetically enhanced prolificacy, growth and parasite resistance, ACIAR: Canberra.

Anthra 2007. Summary of the seminar. P. 3 in 'Proceedings of a National Seminar on Sustainable Use and Conservation of the Deccani Sheep', 20–22 February 2007, Hyderabad, India.

Black A.W. 2000. Extension theory and practice: a review. Australian Journal of Experimental Agriculture 40(4), 493–502.

Davis G.H., Galloway S.M., Ross I.K., Gregan S.M., Ward J., Nimbkar B.V., Ghalsasi P.M., Nimbkar,C., Gray G.D., Subandriyo, Inounu I., Tiesnamurti B., Martyniuk E., Eythorsdottir E., Mulsant P., Lecerf F., Hanrahan J.P., Bradford G.E. and Wilson T. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (*FecB*) mutation. Biology of Reproduction. 66, 1869–1874.

Leeuwis C. 2004. Communication for rural innovation. Blackwell Science: Oxford.

Mulsant P., Lecerf F., Fabre S., Schibler L., Monget P., Lanneluc I., Pisselet C., Riquet J., Monniaux D., Callebaut I., Cribiu E., Thimonier J., Teyssier J., Bodin L., Cognie Y. and Elsen J.M. 2001. Mutation in bone morphogenetic protein receptor-1B is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences USA 98, 5104–5109.

Nimbkar C., Ghalsasi P.M., Nimbkar B.V., Ghalsasi P.P., Gupta V.S., Pardeshi V.C., Maddox J.F., van der Werf J.H.J., Gray G.D. and Walkden-Brown S.W. 2008. Biological and economic consequences of introgression of the *FecB* (Booroola) gene into Deccani sheep. In 'Use of the *FecB* (Booroola) gene in sheep-breeding programs', ed. by S.W. Walkden-Brown, J.H.J. van der Werf, C. Nimbkar and V.S. Gupta. ACIAR Proceedings No. 133, 90–99. Australian Centre for International Agricultural Research: Canberra. [These proceedings]

Nimbkar C., Ghalsasi P.M., Swan A.A., Walkden-Brown S.W. and Kahn L.P. 2003. Evaluation of growth rates and resistance to nematodes of Deccani and Bannur lambs and their crosses with Garole. Animal Science 76, 503–515.

Roling N. 1988. Extension science: information systems in agricultural development. Cambridge University Press: Cambridge.

Souza C.J., MacDougall C., Campbell B.K., McNeilly A.S. and Baird D.T. 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. Journal of Endocrinology 169, R1–R6.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. and Montgomery G.W. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64,1225–1235.

Yin, R. 2003. Case study research: design and methods. Sage Publications Inc.: Thousand Oaks, USA.

Appendix

Smallholder sheep owner survey questionnaire

NIMBKAR AGRICULTURAL RESEARCH INSTITUTE
ANIMAL HUSBANDRY DIVISION

Name of the person filling out the questionnaire: .....

Date of filling out the questionnaire: .....

Sheep owner's name: .....

Section A: Social profile questions to be asked of the sheep owners participating in the extension program

Q. No. 1: Regarding family:

Sr. No.	Name	Age	Education	Business	Relationship
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Q. No. 2: Regarding his flock:

No. of animals in the flock last year	No. of animals in the flock today	Reasons for increase/decrease in the flock

Q. No. 3: What areas of land do you have?

a) Irrigated:

b) Rainfed:

c) Eroded—hilly area / poor soil:

Q. No. 4: Which crops do you usually grow?

Section B: Questions to be asked regarding twin lambs, *FecB*-carrying ewes and *FecB*-rams and their actual performance in the flock

1. How many ewes have given birth to twin lambs? Whether any lamb(s) died? How many?

2. How many *FecB*-carrying ewes are there in your flock? Do you have a target number of *FecB*-carrying ewes which you would prefer not to exceed? How many?

3. What current management strategies are you specifically using to raise lambs (in general) and particularly twin lambs (e.g. supplementary feeding, using extra labour for husbandry)?

4. If supplementary feeding is being used, what are your primary sources of supplementary feeding (e.g. planting fodder crops, extra grazing, purchased fodder)?

5. What management strategies will you specifically use to raise twin lambs during dry period or droughts?

6. As the proportion of *FecB*-carrier ewes increases over time within your flock, what changes, if any, will you make to your flock structure, composition or size (e.g. increase/decrease the number of animals in the flock, decrease the number of ewes in the flock / maintain the same number of ewes in the flock, earlier sale of twin lambs / keeping twin lambs for breeding, any other type of change)?

7. Do you believe that you will face any problems in relation to increasing twin-born lambs in your flock? If so, what are they, and what potential management strategies would you use to avoid or deal with such problems?

8. What do you believe are the advantages and disadvantages of twin lambs? Why?

9. Did you get higher profits from twin lambs last year?

10. Do you talk to other sheep owners about twin-bearing ewes (e.g. twin lambs are very profitable, profitable, don't know, unprofitable or very unprofitable)? What do they think?

11. How do *FecB*-carrier ewes compare with your traditional ewes (e.g. in terms of performance, size, conformation, size of lambs, milk yield, hardiness of ewes, profitability)?

12. What do you believe is the twin lamb mortality and growth performance as compared with single lambs? Is there any difference? If so, what?

13. How would you describe your preferred type of ram? What criteria do you use when buying a ram?

14. How does the *FecB* ram from NARI compare against these criteria? Are they similar to your 'Deshi' breeding rams?

Section C: Other extension support provided to sheep owners by NARI

15. What other types of extension and advisory support have been provided to you by NARI in addition to the *FecB* program (e.g. disease diagnosis and treatment, subsidised livestock insurance)?

16. What is your opinion of this support? What has been the most valuable / least valuable?

Sheep owners' opinions about extension strategies:

17. What extension activities of NARI did you participate in? How many times did you participate in each activity?

18. What did you become aware of, or learn, from each of these extension activities?

19. What did you like or not like about each activity? Do you think these extension strategies are effective?

20. What would you recommend as the more effective extension strategies that should be used in future? Why?

20. What are the existing information and knowledge networks which operate within and between communities (e.g. kinship network, village network, discussions at sale yards and markets, annual fairs)?

22. What are your information needs in relation to flock management?

23. What do you think your ideal flock should be like in future?

24. In your opinion what is to be done to make your flock an ideal flock (e.g. improvement in the flock, increase in profit from the flock)?

Session 5:
Poster papers

The effect of the Booroola gene on meat production efficiency in Texel sheep

A.H. Visscher[1]

Introduction

The fecundity of the dam and the growth and slaughter quality of her progeny determine the efficiency of a lamb meat producing system. The Texel (T) breed has positive slaughter traits; however, its fecundity is mediocre compared to some other breeds. The Booroola Merino (BM) has a major gene *(Fec^B)* for ovulation rate (OR) and litter size (LS). Hence, the *Fec^B* was introgressed into a Texel population through systematic backcrossing to improve the economic efficiency of the Texel breed further.

From 1986 to 1995 the Booroola gene was introduced into a Dutch flock of Texel sheep by crossbreeding Texel dams with heterozygous F_1 (BM × T) sires, the sons of BM sires, proven homozygous by a female progeny test. Female progeny were systematically backcrossed with purebred Texel sires. From 1995 onwards DNA genotyping was practised, and males carrying the *Fec^B* gene, of backcross generation R_2 (T ((BM × T)T)) or higher, were used for breeding too.

Research

The research focused on reproduction traits and meat production traits.

OR at 8 months of age and LS at 1 and 2 years of age were determined in about 700 females from 1987 to 1993 (Nieuwhof 1998). Gibbs sampling was applied for inference in a mixed inheritance model. Estimates for the gene effect in heterozygous females were +1.5 corpora lutea and +1.3 lambs at 2 years of age. The average phenotypic LS of non-carriers at 2 years of age was 1.7 lambs. The gene effect on LS at 1 year of age was small (+0.1). There were significant lamb carrier effects on testes weight, with male carriers having 7.5% heavier testes relative to non-carriers. Farmers claim that Booroola-gene-carrying Texel sires have more libido than purebred Texel sires.

In the experimental flock the increase in LS in carrier dams was accompanied by, on average, a doubling of total lamb mortality at birth (stillborn and within 24 hours) to 20–25%. So the effect of *Fec^B* sires on lamb mortality at birth was tested in a mixed-breed crossbred flock (about 1,200 non-carrier dams, average LS 2.2, all single-pen mated) for 5 years. The direct effect of the gene on lamb mortality was tested with 66 homo- and heterozygous sires of R_2 and higher generations, and 15 fat lamb sires in 3,682 litters from 1999 to 2001. It showed that the fat lamb sires had a significantly higher chance of lamb mortality in their progeny than *Fec^B*-carrying sires. The indirect effect of the gene on lamb mortality was tested in the progeny of daughters of the carrier and non-carrier sires from 2000 to 2003. Data from 2,117 litters of three parities were tested in a restricted maximum likelihood (REML) analysis where sire was a random term and the chance on mortality was the *y* variable. It showed that, apart from LS, only the interaction between parity and genotype of sire of the daughter was significant. It was decided in 2003 that the experimental flock could be transferred to the sheep industry. The industry claims that the additional profit of the Booroola *Fec^B* gene is about €50 per ewe present.

Possible correlated or pleiotropic effects of the gene on food intake, growth rate and carcass traits

[1] Animal Breeding & Genomics Centre, ASG of Wageningen UR, Holland

were investigated (Visscher 2000). In a 3-year experiment (1995–97) 273 spring-born male lambs, offspring of 19 sires, were offered concentrates ad libitum after weaning. The different traits were analysed by regression analysis of variance. The results indicated that the Fec^B gene is accompanied by positive lamb carrier effects on dressing percentage (+1.15%), and longissimus muscle depth (+0.26 to +0.75 mm) and cross-sectional area (+0.98 cm^2), but the gene also increased overall fatness by 11.9%. Dam carrier effects of the Fec^B gene were negative on feed efficiency and longissimus muscle depth (–1.38 to –2.25 mm). Overall, the increased fatness resulted in higher dressing percentage and reduced feed efficiency. So the higher LS of carrier dams is counterbalanced by increased fatness in the carrier progeny. This could imply that, for optimal use of Fec^B, non-carrier terminal sires should be used on carrier dams.

References

Nieuwhof G.J., Visscher A.H. et al. 1998. Identification of early predictors of carriers of the Booroola gene in sheep using a mixed inheritance model. Animal Science 67, 317–325.

Visscher A.H., Dijkstra M. et al. 2000. Maternal and lamb carrier effects of the Booroola gene on food intake, growth and carcass quality of male lambs. Animal Science 71, 209–217.

The distribution, morphology and genotypes of Garole sheep in Bangladesh

M.O. Faruque[1], M.Y.A. Khan[1], M.M. Rahaman[1] and M.I. Hussain[2]

Characterisation of sheep in Bangladesh, to identify the existing breeds and to determine their phylogenetic origin, is a recent endeavour (Faruque 2008). There is a breed known as Garole and three populations of indigenous Garole sheep: Bangladeshi North (BGN), Bangladeshi Central (BGC) and Bangladeshi East (BGE). Garole, BGN, BGC and BGE are native Bangladeshi sheep.

Garoles are found in the extreme south-west of Bangladesh adjacent to the Sundarbans forest in the coastal area (Figure 1). With a small population of around 40,000, the animals are small in size with short ears and a thin tail. The coat is usually light brown in colour, with some animals having black spots on the legs and the head region (Figure 2). The ears are either short (less than 3.0 cm) or medium (3.0–8.0 cm), the average being 6.42 cm (n = 30). The average heights at wither and hip for adult sheep at 2 years of age or more are 47.60 and 48.75 cm, respectively, with a range of 44–57 cm (n = 33). Males are larger than females. The weight of females at 1.5–2.0 years of age is 18.25 kg (n = 12), and of those more than 2 years of age 19.40 kg. The males are a little heavier, being 22.00 kg (n = 4) at 2 years of age or older.

This is a preliminary study; attempts are being made to record weight from more animals including both males and females. The present findings are similar to those of Sharma et al. (1999).

Microsatellite genotyping of the Garole is being conducted at mtDNA and SNP levels. Eighteen microsatellite markers have been tested so far. All the loci were polymorphic. The average number of alleles per locus was 4.72 ± 1.71. The observed heterozygosity (0.5810 ± 0.0187), on average, was less than expected (0.6262 ± 0.0424). A chi-square test indicated a non-significant difference (P > 0.01)

Figure 1. Site for Garole breed

Figure 2. A flock of Garole sheep

[1] Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2] Breed-up Gradation Through Progeny Testing Project, Savar, Dhaka

from Hardy-Weinberg equilibrium for genotypic frequencies within the population. A dendrogram drawn from Nei's genetic distant value shows a genetic difference between Garole and indigenous sheep. The data on mtDNA and SNP will be available by the end of 2008. Productive and reproductive data are being collected to use in future improvement programs, and in a quantitative trait loci study of the Garole for prolificacy, internal parasite resistance and meat quality. Preliminary studies indicate that the Garole has an earlier puberty age, produces twin kids and has a high resistance to internal parasites. They can survive better in saline water than other sheep populations and the Black Bengal goat. However, their existence is in threat due to shrimp cultivation in the area inhabited by them.

References

Faruque M.O. 2008. Progress report of CRP of IAEA no. BDG 13005. Gene-based technologies in livestock breeding: Phase I–Characterization of small ruminant genetic resources in Asia. Bangladesh Agricultural University: Mymensingh.

Sharma R.C., Arora A.L. et al. 1999. Characteristics of Garole sheep in India. Animal Genetic Resources Information 26, 57–64.

Experience with use of Booroola sheep in Poland

E. Martyniuk[1], J. Klewiec[2] and M. Gabryszuk[2]

The introduction of Booroola sheep (B) to Poland was aimed at improving reproduction performance in the Polish sheep population without compromising wool production. In 1988, 121 B embryos were imported into Poland from New Zealand (Klewiec et al. 1991). All embryos were the progeny of two sires and eight dams, some of which were related. Following the embryo transfer, 31 ram lambs and 29 ewe lambs were born. The certificates provided by the New Zealand Booroola Sheep Society indicated that all embryos were homozygous carriers of a single Fec^B gene (Klewiec et al. 1991). The average ovulation rate (OR) observed over 5 years in purebred B ewes was 4.25 (SD = 1.23), ranging from 2 to 8 (Klewiec and Gabryszuk 1998). However, among 28 B females, 9 did not record an OR of 5 or more during at least three laparoscopic observations. The average prolificacy observed in up to five lambings was 228%, with the largest litters consisting of five lambs.

The crossbreeding of Polish Merino (PM) ewes with B rams resulted in a substantial increase in the OR in 66 F1 ewes, with an average 2.68 corpora lutea (CL) (SD = 0.96, range 1–6) (Klewiec and Gabryszuk 1998). The mean litter size (LS) in F1 ewes was 1.98, compared to 1.46 in purebred PM ewes (Klewiec and Gabryszuk 1996). The F1 dams reared, on average, 0.5 more lambs (Klewiec and Gabryszuk 1996). At birth, F1 lambs were heavier by 1.1 kg than pure B lambs and the difference increased to 4 kg by the 16th week of age (Klewiec et al. 2001). At the same time, the body weight at 12 months of age in F1 ewes was 14% lower than in the purebred PM (Klewiec and Gabryszuk 1996). Further backcrossing with PM was expected to improve growth rate and body weight in F2 lambs (25% of B genotype). F2 lambs out of PM dams and by F1 sires had a similar body weight during the first 8 weeks of age as F1 lambs. The F2 out of F1 dams and by PM sires were lighter by 1.4 kg at 8 weeks of age, confirming a significant maternal effect. However, the adult body weight was similar in F1 and F2 ewes (62 kg) and significantly higher than in B ewes (45.5 kg) (Klewiec et al. 2001). As expected, the mean LS in F2 ewes was lower than in F1 ewes (1.94 vs. 2.27); also F2 Fec^B carriers had a lower LS (2.09). However, the prolificacy of F2 non-carriers (1.59) was significantly higher than the purebred PM (Klewiec et al. 2001). The comparison of growth rate of progeny out of F1 ewes mated with PM or Suffolk (S) sires in comparison with pure PM showed that lambs of F1 dams have significantly lower body weight at birth (3.4 kg and 3.8 kg respectively) in comparison with pure PM (4.4 kg) (Janiuk et al. 1998). During the rearing period (2–56 days of age) the daily gain of S progeny (255 g/day) and pure PM (212 g/day) were significantly higher than F1 PM backcrosses (181 g/day). The best fattening results were obtained in F1 S lambs (372 g/day), compared to PM (309 g/day) and F1 PM lambs (301 g/day) (Janiuk et al. 1998), resulting in significant differences in the fattening period (50, 55 and 67 days respectively).

In spite of quite encouraging results obtained in the two-stage crossbreeding of PM with B and S sires, the Booroola sheep did not gain popularity in Poland. The lower body weight and very poor conformation were responsible for rejection of the breed in the context of prime lamb production for the export market. Also, profitability of the sheep sector was not sufficient to allow long-term backcrossing experiments to fix the Fec^B gene in local sheep populations.

[1] Department of Animal Genetics and Breeding, Warsaw University of Life Sciences, Poland
[2] Institute of Animal Genetics and Breeding, Polish Academy of Sciences, Jastrzebiec, Poland

References

Janiuk W., Baranowski, A. Klewiec J. 1998. Performance and slaughter value of Polish Merino, Booroola and Suffolk crossbred lambs. Journal of Animal and Feed Sciences 7, 161–170.

Klewiec J. and Gabryszuk M. 1996. The effect of different shares of merino sheep in the genoytpe of Merino × Booroola crossbreds. Prace i Materialy Zootechniczne 48, 7–16.

Klewiec J. and Gabryszuk M. 1998. Variation in the ovulation rate in Booroola and Polish Merino × Booroola crosses. Animal Science Papers and Reports 16(1), 35–40.

Klewiec J., Gabryszuk, M., Slowak M 1991. Preliminary information on the Booroola sheep in Poland. Pp. 39–40 in 'Second International Workshop on Major Genes for Reproduction in Sheep', ed. by J.M Elsen, L. Bodin and J. Thimonier. L'Institut Scientifique de Recherche Agronomique (INRA): Paris.

Klewiec J., Martyniuk E. and Gabryszuk M. 2001. Effect of different shares of the Booroola genotype on growth rate and reproduction performance of crosses with Polish Merino. Animal Science Papers and Reports 19(2), 123–130.

Genetic polymorphism of the Booroola fecundity (*FecB*) gene in Garole sheep

P.K. Ranga[1] and R.V. Singh[1]

Indian sheep mostly have a litter size of one lamb; the exception is Garole sheep, which are renowned for high reproductive efficiency and prolificacy due to the presence of the Booroola fecundity (*FecB*) gene (Pardeshi et al. 2005). The Garole breed of sheep originated from the Sunderbans delta in West Bengal. The *FecB* gene increases ovulation rate and litter size in Garole sheep. It has been mapped to the sheep chromosome $6q_{23\text{-}31}$, which is syntenic to the human chromosome $4q_{21\text{-}23}$. Bone morphogenetic protein receptor 1B (BMPR1B), also known as activin-like kinase-6 (ALK-6), is located in the region containing the *FecB* locus. Two point mutations responsible for the Booroola phenotype were found in the highly conserved kinase signalling domain of this receptor, one at base 746 of the coding region (A746G), leading to a change from glutamine to arginine at position 249 in the amino acid sequence (Q249R), and the other at base 1113 position (C1113A) of the coding region (Wilson et al. 2001; Davis et al. 2002).

In order to screen these point mutations, the present study was carried out on 53 Garole sheep maintained at Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, and 10 Muzaffarnagari sheep at the Indian Veterinary Research Institute, Izatnagar, using the PCR–RFLP technique. The 223 bp and 153 bp fragments harbouring the mutations were synthesised and amplified by PCR from genomic DNA of Garole and Muzaffarnagari sheep, and digested with HaeIII and XhoI restriction enzymes. Digestion of the 223 bp fragment with HaeIII revealed 61 bp and 162 bp fragments, and digestion of the 153 bp fragment with XhoI revealed 18 bp and 135 bp fragments, in all 53 Garole and 10 Muzaffarnagari sheep. No polymorphism was found in these fragments and genotypic frequency for the *FecB* allele was found to be unity (1.0). The XhoI (+ve) restriction site was reported to be linked with A→G substitution on exon 8, which causes the Booroola phenotype.

In order to detect a change in nucleotide and amino acid sequence, the 223 bp fragment (exon 8) of Garole sheep was cloned into a pGEM-T easy vector and sequenced, and the 153 bp fragment of both breeds was directly sequenced. On sequencing, the 223 bp fragment exhibited A→G transition in Garole sheep, whereas the 153 bp fragment revealed C→A transition in both Garole and Muzaffarnagari sheep. The XhoI +ve can be used as a marker for the Booroola phenotype (Souza et al. 2001). On phylogenetic analysis, it was found that the *FecB* gene of Garole sheep showed a very high percentage of identity with that of other sheep breeds, especially Muzaffarnagari sheep. Phylogenetic tree analysis also revealed that the Garole sheep is closely related to the caprine (goat), followed by the cow, human, chimpanzee, rat, mouse and chicken.

[1] Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar- 243122, Uttar Pradesh, India

References

Davis G.H., Galloway S.M. et al. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Pardeshi, V.C., Sainani M.N. et al. 2005. Assessing the role of FecB mutation in productivity of Indian sheep. Current Science 89(5), 887–890.

Souza C.J.H., MacDougall C. et al. 2001. The Booroola (FecB) phenotype is associated with a mutation in the

bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology 169**,** R1–R6.

Wilson T., Xi-Yang Wu J. et al. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. BioIogy of Reproduction 64**,** 1225–1235.

Evaluation of the Booroola (*FecB*) gene in Muzaffarnagari sheep

R.V. Singh[1], A. Sivakumar[1], S. Sivashankar[1] and G. Das[2]

The Booroola fecundity gene refers to an autosomal mutated allelic variant of the bone morphogenetic protein receptor 1B (BMPR1B). It governs ovarian follicular number and follicle growth in sheep. The gene has been renamed *FecB* by the Committee on Genetic Nomenclature of Sheep and Goats (COGNOSAG 1989). The symbol B was assigned to the putative high prolificacy allele and + for the wild type. It is a single major gene and is inherited as a single autosomal locus. The effect is additive for ovulation rate but not litter size, for which it exhibits incomplete or partial dominance. The *FecB* gene is probably derived from the Indian sheep breed, the Garole (Turner 1982; Montgomery et al. 2001; Davis et al. 2002). The Muzaffarnagari breed is one of the largest mutton breeds in India and, like most Indian sheep breeds other than the Garole, is monotocous (Sharma et al. 1999).

The present investigation was conducted to evaluate the FecB gene in Muzaffarnagari sheep. A total of 115 purebred Muzaffarnagari sheep were sampled randomly, comprising 64 from the Indian Veterinary Research Institute, Izatnagar, and 51 from the Central Institute for Research on Goats, Makhdoom. Genomic DNA was isolated from a 3.1-mm punch taken from blood samples on FTA paper (Whatman Bioscience Cambridge, UK) as per the manufacturer's instructions. The FecB gene was amplified using forward and reverse primers designed to amplify 140 bp of the BMPR1B gene using the forced PCR–RFLP technique (Wilson et al. 2001). The amplified product was digested using Ava-II enzyme. This digestion yielded one fragment (110 bp) in the homozygote, two fragments (140 bp, 110 bp) in the heterozygote carrier and a single fragment (140 bp) in non-carrier sheep, visualised using 3% metaphor agarose gel electrophoresis.

In the present study the genotypes at the *FecB* locus in 115 Muzaffarnagari sheep were ascertained as follows: 4 (3.47%) were homozygous ($FecB^{BB}$), 48 (41.73%) were heterozygous carrier ($FecB^{B+}$) and 63 (54.78%) were non-carrier ($FecB^{++}$). Mean litter sizes were available from only 32 of these animals (Table 1).

Table 1. Mean litter size of Muzaffarnagari ewes of different *FecB* genotypes

Genotype	Mean litter size	*n*	Std. deviation
$FecB^{BB}$	1.00	2	0.00
$FecB^{B+}$	1.00	7	0.00
$FecB^{++}$	1.04	23	0.21
Total	1.03	32	0.18

The litter size of Muzaffarnagari ewes reported in the literature is also 'mostly single'. Therefore, finding the *FecB* mutation in the Muzaffarnagari breed is unexpected, and needs to be confirmed by validation of genotyping from independent laboratories and recording of ovulation rates or litter sizes of genotyped ewes over several parities.

References

COGNOSAG (Committee on Genetic Nomenclature of Sheep and Goats) 1989. Proceedings of the 1987 workshop, Lavoisier, Paris, p. 49.

[1] Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar -243 122, Uttar Pradesh, India
[2] Sheep Farm, Central Institute for Research on Goats, Makhdoom-281 122, Mathura, Uttar Pradesh, India

Davis G.H. et. al. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Montgomery G.W. et. al. 2001. Genes controlling ovulation rate in sheep. Reproduction 121, 843–852.

Sharma R.C. et al. 1999. Characteristics of Garole sheep in India. Animal Genetic Resources Information 26, 57–64.

Turner H.N. 1982. The Booroola Merino. Pp. 1–7 in 'Proceedings of a workshop, Armidale, 24–25 August 1980', ed. by L.R. Piper, B.M. Bindon and R.D. Nethery. CSIRO: Victoria.

Wilson T. et al. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Identification of the Booroola mutation in Kendrapada sheep of Orissa, India

S. Kumar[1], A.K. Mishra[1,2], L.L.L. Prince[1], C. Paswan[1], A.L. Arora[1] and S.A. Karim[1]

The Kendrapada is a local sheep found mainly in Kendrapara and Puri districts of coastal Orissa state, India, which is about 400 km from the Sunderbans delta of West Bengal (home tract of the Garole). This sheep produces 62.8% twins and 2.3% triplets (Patro et al. 2006). Because of its high prolificacy, there might be segregation of a major fecundity gene in this sheep, as reported in several prolific sheep breeds across the world. The high prolificacy of Booroola Merino, Garole, Javanese, Hu and Small Tail Han sheep is due to mutation in the BMPR1B gene, also known as the Booroola fecundity gene (*FecB*) (Wilson et al. 2001; Davis et al. 2002, 2006). The $FecX^G$ (Galway) mutation in the BMP15 gene also causes high prolificacy in the Belclare, Cambridge and Small Tail Han sheep breeds (Hanrahan et al. 2004; Chu et al. 2007).

The aim of the study was to investigate the presence of *FecB* and $FecX^G$ mutations in Kendrapada sheep. Forty-six Kendrapada sheep (5 males and 41 females) were selected from their native tract (19 from Kendrapara district and 27 from Nimapara, Puri district). Subsequently, another 19 Kendrapada animals (5 males and 14 females) were selected randomly from both locations. The *FecB* and $FecX^G$ genotyping was carried out using the forced PCR–RFLP technique (Wilson et al. 2001; Hanrahan et al. 2004). The Booroola mutation was observed in the Kendrapada sheep, which is the sixth reported sheep strain carrying this mutation naturally. Out of 65 Kendrapada animals, 27 were homozygous (BB), 30 heterozygous (B+) and 8 non-carriers (++). The majority of animals (~87.7%) were carriers (BB and B+) for the *FecB* mutation. The overall frequency of the *FecB* allele was about 0.65 in both selected and random samples. None of the Kendrapada sheep carried the $FecX^G$ mutation.

Results indicated that the frequency of the *FecB* allele is high but the gene is not fixed in the population. The occurrence of the *FecB* mutation in Garole and Kendrapada sheep may be separate events. Alternatively, Garole sheep might have travelled into the adjoining parts of northern Orissa many generations ago, and the farmers later developed the Kendrapada sheep through selection or the Garole were outcrossed with local available sheep (i.e. Ganjam). Kendrapada and Ganjam ewes are polled and males are horned like the Garole. The fleece of the Garole is light brown, while the fleece of Kendrapada sheep ranges from light brown to dark brown (Patro et al. 2006) and that of Ganjam sheep from brown to dark tan (Mishra et al. 2004). The adult body weight of Kendrapada sheep is much higher (~23–27 kg) (Patro et al. 2006; Mishra et al. 2007) than Garole sheep (~10–14 kg). This theory needs to be tested further by physical and molecular characterisation of these breeds. The discovery of the *FecB* mutation in Kendrapada sheep is an exciting development because they are much bigger than the Garole, and therefore likely to prove more acceptable to sheep rearers as a source of *FecB*.

[1] Central Sheep and Wool Research Institute, Avikanagar, Rajasthan -304501, India
[2] Central Avian Research Institute, Izatnagar, Bareilly, Uttar Pradesh, -243122, India

References

Chu M.X., Liu Z.H. et al. 2007. Mutations in BMPR1B and BMP15 genes are associated with litter size in small tailed Han sheep (*Ovis aries*). Journal of Animal Science 85(3), 598–603.

Davis G.H., Balakrishnan L. et al. 2006. Investigation of the Booroola (FecB) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science 92, 87–96.

Davis G.H., Galloway S.M. et al. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Hanrahan J.P., Gregan S.M. et al. 2004. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biology of Reproduction 70, 900–909.

Mishra P.K., Barik N. et al. 2004. Production potentiality of Ganjam sheep under extensive management. The Indian Journal of Small Ruminants 10(2), 171–172.

Mishra A.K., Kumar S. et al. 2007. Indian Society for Sheep and Goat Production and Utilization (ISSGPU) Newsletter, July, pp. 1–2.

Patro B.N., Mallick C.R. et al. 2006. Production performance of indigenous meat type sheep in Kendrapada district of coastal Orissa. The Indian Journal of Small Ruminants 12 (1), 42–47.

Wilson T., Wu X.Y. et al. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction 64, 1225–1235.

Use of nutritional restriction at mating to dampen reproductive performance of *FecB*-carrier merino ewes

D.H. Wolfenden[1] and S.W. Walkden-Brown[2]

The nutritional milieu strongly influences ovulation rate in both carriers and non-carriers of the Booroola fecundity gene (*FecB*) (Montgomery et al. 1983; Kleemann et al. 1991). On the property 'Allandale' both homozygous ($FecB^{BB}$) and heterozygous ($FecB^{B+}$) merino ewes have large litter sizes (LSs) (2.1 and 2.3 respectively) and excessive losses from ovulation to weaning (> 64%) (Walkden-Brown et al. 2007). This study reports an attempt to inhibit the expression of *FecB* by a period of nutritional restriction, resulting in weight loss at mating.

[1] 'Allandale', Rand, New South Wales 2642, Australia
[2] Animal Science, School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia

The experiment used 2002-, 2004- and 2005-born ewes from a special flock established in 2002 to enable unbiased comparisons of the three *FecB* genotypes as determined by DNA test (Walkden-Brown et al. 2007). Between 20 February 2007 (20/2/07) and 5 May 2007 (5/5/07), groups of approximately 100 ewes containing the three genotypes were maintained on normal (N) or restricted (R) grazing. Unadjusted mean weights (± SEM) of ewes in the N and R treatments at different times were: 20/2/07, 39.4 ± 0.57 and 39.2 ± 0.49; 24/3/07, 42.2 ± 0.56 and 35.1 ± 0.45; and 5/5/07, 42.2 ± 0.53 and 33.1 ± 0.43 respectively. Mating commenced on 31/3/07 and rams were removed on 4/5/07. Ewes were ultrasound scanned for LS on 18/6/07 and separated into LS groups for lambing on 6/8/07. Lambs were marked on 8/10/07

Table 1. Least squares means of key variables by nutritional group (NUTR) and *FecB* genotype (GEN), including overall *P* values for effects and their interaction (significant values in bold)

GEN	NUTR	*n*	CR	OR1	OR2	LS1	LS2	Mark	Wean	LL
$FecB^{++}$	N	37	0.84	1.38	1.28	1.27	1.08	0.63	0.63	0.43
	R	38	0.79	1.13	1.12	1.12	1.06	0.69	0.69	0.21
$FecB^{B+}$	N	34	0.82	2.99	2.72	2.06	1.71	1.14	1.08	0.36
	R	34	0.58	2.50	2.11	1.99	1.17	0.80	0.77	0.35
$FecB^{BB}$	N	31	0.77	3.86	3.88	2.27	1.84	0.77	0.74	0.57
	R	31	0.45	3.41	3.10	2.12	1.05	0.66	0.63	0.28
P value	GEN		0.150	**<0.001**	**<0.001**	**<0.001**	**0.005**	**0.040**	0.077	0.518
	NUTR		**<0.001**	**0.002**	**<0.001**	0.334	**<0.001**	0.237	0.274	**0.014**
	GEN × NUTR		0.253	0.683	0.194	0.958	0.224	0.291	0.348	0.234

CR = conception rate, i.e. ewes pregnant at scanning / ewes scanned (mean not LSM); OR1 = ovulations / ewe ovulating; OR2 = ovulations / ewe available to ovulate; LS1 = LS / ewe in lamb at scanning; LS2 = LS / ewe scanned; Mark = lambs marked / ewe scanned; Wean = lambs weaned / ewe scanned; LL = proportion loss between scanning and lambing for ewes in lamb at scanning (LS not fitted as covariate). N = normal; R = restricted.

and weaned on 12/12/07. Ovulation rate (OR) on the cycle resulting in conception was determined by laparoscopy. Data were analysed using appropriate linear models in JMP 6.0 (SAS Institute, NC), fitting the fixed effects of ewe birth year, *FecB* genotype, nutritional treatment and significant interactions. Key results are summarised in Table 1.

Both CR and OR were reduced by the R treatment, particularly for *FecB* carriers. LS was also reduced by the R treatment when expressed per ewe scanned, but not per ewe in lamb. Lamb losses between scanning and weaning were significantly lower in the R treatment, in line with our objective. However, the marked reduction in CR seen in ewes in the R treatment makes this approach unsatisfactory. Wool quality could also be expected to suffer (Kleemann et al. 1991).

References

Kleemann D.O., Walkley J.R.W. et al. 1991. Effect of premating nutrition on reproductive performance of Booroola Merino × South Australian Merino ewes. Animal Reproductive Science 26, 269–279.

Montgomery G.W., Bray A.R. and Kelly R.W. 1983. Ovulation rates of first cross Booroola compared with local breed ewes following differential feeding. Animal Reproduction Science 6, 209–215.

Walkden-Brown S.W., Wolfenden D.H. et al. 2007. Expression of reproductive and production traits in commercial Merino ewes having 0, 1 or 2 copies of the *FecB* mutation. Proceedings of the Association for the Advancement of Animal Breeding and Genetics 17, 426–429.

Assessment of the *FecB* mutation in three Indian sheep breeds, including Garole in its native tract, and its effect on prolificacy

R. Banerjee[1,2], A. Gupta[1] and K. Ray[1]

The *FecB* fecundity gene is an autosomal dominant gene with large and additive effects on ovulation rate and litter size (LS) in sheep. Mutation (746 CAG > CGG; Q249R) in the highly conserved intracellular kinase domain increases corpora lutea by 1.6 and LS by about one extra lamb for each copy ($FecB^B$) in the Booroola Merino (Davis et al. 2002). Incorporation of this mutation was from the Indian Garole to the Australian Egelabra Merino (Turner 1982). The native tract of Garole sheep is the coastal Sundarbans delta of West Bengal.

We analysed the status of the *FecB* mutation and its correlation with LS in the Garole in and away from its native tract, and in two other Indian breeds, the Shahabadi and the Muzaffarnagari. In addition, we studied the effect of introduction of $FecB^B$ from the Garole to the Muzaffarnagari. The *FecB* mutation was screened following a method with modifications in the polymerase chain reaction and gel (polyacrylamide) electrophoresis protocols (Davis et al. 2002). The mutation was found in the Garole and Shahabadi but not in the Muzaffarnagari. However, its frequency in the Garole was less compared to previous reports (Davis et al. 2002). Interestingly, out of a total of 200 Garole samples, 100 were collected from a government-organised farm where the percentage of mutant homozygous Garole sheep was significantly lower compared to samples from the native tract (Table 1). The farm was started in 1968 at Kalyani, around 160 km away from the Garole tract, with four different flocks of Muzaffarnagari, Shahabadi, Nellore and Correidale breeds at separate locations. Crossbreeding was carried out among the breeds, maintaining pure parent stock. Garole sheep were introduced from

[1] Molecular & Human Genetic Division, Indian Institute of Chemical Biology, Kolkata, India
[2] Present affiliation: Animal Resources Development Department, Government of West Bengal, India

Table 1. Average genotype percentage, mean (± SE) litter size and mean (± SE) lambings by breed in *FecB* genotypes in Garole (G), Shahabadi and Muzaffarnagari (M) sheep

FecB mutant genotypes	Genotype percentage				
	Garole (native tract)	Garole (organised farm)	Shahabadi	Muzaffarnagari	G × M (F1)
$FecB^B/FecB^B$	80 (2.65 ± 0.3) [2.9 ± 0.13]	40 (2.39 ± 0.3) [3.0 ± 0.16]	20 (2.50 ± 0.2) [2 ± 0.2]	Not found	–
$FecB^B/FecB^+$	17 (1.86 ± 0.2) [3.1 ± 0.22]	45 (1.59 ± 0.41) [3.0 ± 0.21]	70 (1.70 ± 0.2) [3.0 ± 0.32]	Not found	100 (1.42 ± 0.2) [3.0 ± 0.3]
$FecB^+/FecB^+$	3 (1.6 ± 0.15) [2.0 ± 0.3]	15 (1.5 ± 0.23) [3.0 ± 0.2]	10 (1.30 ± 0.12) [3.0 ± 0.4]	100 (0.80 ± 0.1) [3.2 ± 0.2]	–

Note: value in first brackets ( ) denotes mean litter size and in second brackets [ ] mean number of lambings.

1997 and housed at another unit of the farm for ex-situ selection and conservation. Outbreeding to non-Garole sheep was not allowed.

The mean (± SE) LS of $FecB^{B}/FecB^{+}$ and $FecB^{B}/FecB^{B}$ genotypes were significantly ($P \leq 0.01$) higher in the Garole reared in its native tract (Table 1). Probably, the coastal saline agroclimate naturally selected the *FecB* mutant allele in Garole sheep, with a near complete loss of the homozygous wild-type genotype. In all observations LS (live birth) was measured at birth. In this study planned crossings were made at the farm between tested Garole and Muzaffarnagari sheep. The *FecB* mutation increased the mean (± SE) LS from 0.8 (± 0.1) to 1.42 (± 0.2) in 15 heterozygous Muzaffarnagari × Garole F_1 crossbreds, with a mean number of lambings of 3.0 (± 0.3). A higher percentage of mutant homozygotes in the Garole population than the Shahabadi population indicates the onward transmission of $FecB^{B}$ from Garole to Shahabadi, both breeds being found in close geographical proximity. The increased mean LS in Muzaffarnagari × Garole F_1 crossbreds indicates that the $FecB^{B}$ allele could be introduced into other poorly fecund breeds, enhancing LS in the eastern Indian agroclimate.

References

Davis G.H. et al 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction 66, 1869–1874.

Turner H.N. 1982. Origins of the CSIRO Booroola: the Booroola Merino. Pp. 1–7 in 'Proceedings of a workshop held at Armidale, New South Wales', ed. by B.M. Bindon, R.D. Nethery and L.R. Piper. CSIRO, Victoria.

A socioeconomic study of smallholder sheep owners/rearers in Phaltan taluka, Satara district, Maharashtra, India

B.V. Nimbkar[1], P.M. Ghalsasi[1], K.M. Chavan[1], C. Nimbkar[1], B.M. Pawar[1] and S. Khot[2]

A pilot study of sheep owners in Phaltan taluka was done in 2001, and a more extensive study was carried out from March 2002 to October 2003. For the extensive study 5% of the approximately 2,000 sheep-owner families residing in Phaltan taluka were randomly selected. Of these families, 80% reside in 18 villages in the taluka only during the monsoon season.

The study had three objectives. The first was to ascertain the social status of sheep owners. Of the sheep-owner families studied, 94% belonged to the Dhangar caste. We found that sheep-owners' habitations lacked basic amenities; however, this may be because these locations were distant from the main villages. The average family size in the sample of sheep-owner families studied was 4.6, with an average 1.7 children per family. The number of females per 1,000 males was 890, which was considerably lower than the state and national averages. Literacy rate in these families was only about half the state and national rates. This low rate may be due to their migratory lifestyle, which precludes them from sending children to school.

The second objective was to ascertain the economic status of the community and study their sheep-rearing practices. Sheep rearing was the major source of income for the families studied. Most of these families had small landholdings which allowed subsistence farming. The land was used to grow the family's grain requirements, rather than to grow fodder for their sheep. Of the families studied, 80% were above the poverty line, with the average income per family being Rs38,000/year, 90% of which came from their sheep. During the period of study, Phaltan taluka was in the grip of a severe drought, and it was observed that sheep rearing could cope better with drought conditions than any other agricultural activity. This may explain the sheep owners' higher economic status compared to other poor villagers. In addition to their annual long-distance migration when the dry season set in, the sheep rearers ranged fairly widely in search of grazing and other food and water for their sheep, which is why they were not badly affected by drought. The major factors preventing improvement of their economic conditions were continuous reduction of grazing areas, high incidence of disease among the animals and lack of marketing facilities.

The third objective of our study was to ascertain whether the introduction of prolificacy into the flocks (wherein a large number of ewes would produce twins) would be welcome or would pose problems. Of the sheep owners interviewed, 87% felt that twin lambs were profitable, 18% were willing to buy sheep that produced twins, 74% said they would retain ram lambs born to prolific sheep for breeding, 85% thought that prolificacy was a genetic trait, 14% said that sheep with twins gave more milk, while 74% felt that some twin lambs would have to be cross-fostered as some ewes don't have enough milk to feed twin lambs. It was found that 4% of their sheep produced twins and 10% of

[1] Animal Husbandry Division, Nimbkar Agricultural Research Institute, Phaltan, Maharashtra, India
[2] 1104 Woodland Avenue, Gananjay Society, Pune 411 029, Maharashtra, India

these had twins at every lambing, and 5% of the sheep owners had ewes which had given triplets. It appeared that introduction of twinning would be a welcome innovation, resulting in increased profitability without increasing flock size. A summary of steps needed to improve the social and economic status of this neglected community was drawn up.

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Social scientist Ms Jyoti Datar helped with the survey, village studies and focus group discussions.

Panel discussion

Panel discussion

At the end of the workshop there was a 2-hour panel discussion on 'The policy implications for wider dissemination in India of sheep containing the *FecB* gene arising from the ACIAR projects'. The discussion was facilitated by Prof. Stephen Walkden-Brown and the members of the panel were:

- Dr Venkateswaralu, Additional Director, Animal Husbandry Department, Government of Andhra Pradesh
- Dr A.D. Deo, Technical Officer, Punyashlok Ahilyadevi Maharashtra Sheep and Goat Development Corporation
- Dr David Notter, Professor, Animal and Poultry Science, Virginia Tech University, USA
- Dr Elisha Gootwine, Volcani Centre, Israel
- Mr Ramesh Shendge, Member of the Legislative Council of Maharashtra, and Chairman, Maharashtra Sheep and Goat Corporation
- Dr Neelkantha Rath, an eminent economist of the Indian School of Political Economy.

The panel was asked to address four broad questions, as follows:

1. What is our breeding objective?
   – Is the need for prolificacy established?
   – What level of prolificacy is desirable?
2. Are we confident that we can use the *FecB* mutation to achieve the breeding objective?
   – Are there issues still to be resolved?
   – Are they of sufficient magnitude to stop or slow the process of introgression?
3. What regions and breeds of sheep should be targeted for *FecB* introgression?
   – On what basis should these be selected?
4. What are the pathways and policies for further introgression?
   – What methods should be employed?
   – What institutions should be involved?

After the panel had discussed each topic, the discussion was opened to the wider audience.

Question 1: What is our breeding objective?
– Is the need for prolificacy established?
– What level of prolificacy is desirable?

Dr Venkateswaralu pointed out that the breeding objective depends on the production system, and the benefit will vary between different cases.

Dr Gootwine thought it was clear that there is no alternative other than increasing sheep productivity and efficiency of production. He also said that, at first, only 10–15% of sheep owners might opt for the introduction of prolificacy, and that others would follow later.

Dr Rath thought that, if sheep can be made moderately prolific and their milk production can be increased so that they give about 250 mL milk per day over and above that suckled by lambs, it may very well change the ownership pattern of sheep. In other words, poorer people might start to keep sheep and in smaller flocks of four to five or less per household, the way goats are reared currently.

Dr Notter pointed out that there may be some possible exceptions, but we should not ignore that, generally, there is ample evidence that increasing lambing rate leads to more profit. This is more likely to be the case where lambs are produced for sale and where sheep rearing is more 'commercial'. He also pointed out that breeding objectives are generally hard to set in stone, and genetic improvement should proceed according to our best attempt to set objectives but that such objectives might evolve over time.

Dr Notter also pointed out that increasing twinning rates is desirable, but that the incidence of triplets should be kept at a minimum as triplets have a much lower survival rate. He had worked out that the frequency of triplets becomes significant and therefore too high when average lambing rates exceed 160%.

Dr Tandale was of the opinion that we should consult the sheep owners as they will know best what is good for them. It was pointed out that such

consultation had happened in the Nimbkar Agricultural Research Institute (NARI) region during the course of the ACIAR project and had led to the same conclusion—that sheep owners prefer a moderate level of twinning in their flocks.

Dr Bodin informed the meeting that, in the prolific Lacaune sheep in France, selection for prolificacy started at an average litter size of 1.3, which has now increased to 2.0. He said that there were no changes in the environment to sustain this higher level but that sheep owners' education and awareness had improved significantly.

Dr Arora urged that other important traits also needed to be considered, such as growth rate and feed conversion efficiency. He advised a thorough economic analysis for 2–3 years before advocating widespread dissemination of prolificacy.

Dr Kandasamy suggested dissemination of heterozygous rams to keep the average flock prolificacy moderate, and advised conducting an assessment of the level of milk production in the flock.

In summary, there was broad support for the view that there is a positive benefit in increasing fecundity in most meat sheep production systems. The value of the benefit depended on the environment provided to sheep and on system management. It was recognised that these benefits have to be established for individual cases and that there may be exceptions. The benefit is likely to be highest when the current level of fecundity is low (around 100% lambing rates) or where conditions are adverse and the feeding situation is very problematic. There was also general support for the view that lambing rates above 160% are, in most cases, becoming problematic as the proportion of triplets increases and there is associated lamb mortality.

Question 2: Are we confident that we can use the *FecB* mutation to achieve the breeding objective?
- **Are there issues still to be resolved?**
- **Are they of sufficient magnitude to stop or slow the process of introgression?**

Dr Gootwine was of the opinion that a full introgression program is justified if we are confident that we can aim for the $FecB^{BB}$ genotype (i.e. that BB animals are found to be desirable for commercial flocks). However, this is not yet the case, as several studies have shown that the BB genotype has significantly more lamb mortality and is overall of lesser benefit than the heterozygous genotype. He suggested that more experimental data are needed to establish that the BB genotype is not undesirable in the Deccani case—that data on at least 1,000 BB animals over 3–4 years would be needed for a convincing case.

He continued by making the point that, in spite of the uncertainty about the BB genotype, there is no need to wait with introgression. While waiting for further results on $FecB^{BB}$, there is no reason not to provide B+ rams to commercial sheep owners' flocks. There is an obvious benefit to introducing the gene into flocks at a moderate level, and this will also provide further information about benefits. Similarly, BB rams could be provided to other centres, where introgression in new breeds could commence with the appropriate level of measurement and monitoring. Dr Marshall agreed with Dr Gootwine and said that it was better to start early rather than causing unnecessary delay by resolving all technical issues at the outset.

Dr Gootwine suggested not encumbering the introduction of rams with a plea for sheep owners to collect data, as that may well hold back further uptake.

Dr Venkateswaralu suggested that conducting a trial at the institutional level would raise confidence levels and that commercial farmers would adopt the innovation voluntarily if it was shown to be profitable. He preferred the introduction of BB rams since the genotype of the resulting progeny can be guaranteed.

It was pointed out that it is important that the first experience of sheep owners with the introduced gene should be positive, and therefore a cautious approach is warranted. In other words, there should be a high degree of certainty about the effect before unleashing it into sheep owners' flocks. Dr Notter thought it was not important whether heterozygous or homozygous rams were introduced, but that there should be monitoring to see if the BB genotype was always advantageous.

Dr Rath suggested starting the process of further introgression in more regions so that, in the meantime, further experience can be obtained. As the benefits clearly outweigh the potential risks, such a technology should not be withheld and further experience should be obtained during the introduction process, as is generally the case when introducing new technologies. There is always the

possibility to scale up or down the process of introduction, depending on the experience.

In summary, it was generally recognised that the homozygous $FecB^{BB}$ genotype may be undesirable in many cases. Results at NARI seem to indicate problems in the Deccani case to be of a lesser extent; however, there is clearly a need to confirm this with a lot more evidence. The heterozygous genotype seems to be advantageous. The uncertainty about the homozygous genotype should not be an impediment to use carrier rams in sheep owners' flocks or to start using BB rams in other centres. In fact, it is better to start early as introgression is a long process. Further collection of data is warranted at research or nucleus centres and, where possible, from commercial sheep owners' flocks.

Question 3: What regions and breeds of sheep should be targeted for *FecB* introgression?
– On what basis should these be selected?

Dr Venkateswaralu said that state and federal governments should support introgression. As an example of a recipient breed, he cited the Nellore breed in Andhra Pradesh, of which there are 10 million animals.

Dr Deo commented that introgression should start in regions where ample grazing and veterinary aid were available. Dr Notter suggested targeting breeds with very low frequency of twinning and similar or superior maternal ability to the Deccani, and also those breeds in which rearing was reasonably commercialised.

Dr Rath suggested starting in regions with significant sheep populations, and he gave the example of the Mahanadi, Godavari and Kaweri river valleys on the eastern coast of India.

In summary, factors that should be considered are the size of the targeted sheep population and the various aspects of the production system, such as feed availability, extent of migratory activity and current fecundity levels, that determine the fit of the improved technology. Market stability was also seen as an important condition.

It was also pointed out that there should be a willingness among the various players in the introgression process to cooperate or participate in the program. Participants include sheep owners, breeders, researchers and extension people, and their enthusiasm is a critical success factor. If the conditions are favourable but there is local resistance or an uncooperative attitude, the chances of success will not be high. On the other hand, if conditions are maybe doubtful but there is a lot of interest and enthusiasm to participate, then one should not be afraid to try.

Question 4: What are the pathways and policies for further introgression?
– What methods should be employed?
– What institutions should be involved?

Dr Gootwine questioned the feasibility of developing two synthetic lines at NARI. Since NARI is a relatively small institute with limited resources, maintaining a breeding nucleus for two breeding lines is simply too much of a demand on these resources. More important than new breed formation is that introduced carrier rams should be acceptable to breeders of local stock. Hence, it is important to maintain a process of upgrading such that most of the local genome is restored, and dissemination can commence when this 'acceptable level' has been achieved. Dr Gootwine suggested introgression of *FecB* into the Madgyal breed, which is preferred by local sheep owners in many regions of Maharashtra, and that emphasis should be placed on developing *FecB*-carrier sheep of uniform size.

It was pointed out that appropriate extension and training is required before introducing carrier rams into sheep owners' flocks. Some good examples of engagement of farmers, where local farmers had been strongly involved in the process, were presented to the conference by Dr Gootwine. The case at NARI is another good example of engagement.

It was suggested that government farms can be suitable centres to develop an introgression program where the *FecB* gene is introduced in a local breed. Mr Shendge of the Maharashtra Sheep and Goat Development Corporation suggested using the corporation's farms together with the association of 2,700 sheep owners established by the corporation. Dr Venkateswaralu suggested that the farms of the Andhra Pradesh Government could also be used for this purpose. Some people questioned firmly the track record of (government) institutes to deliver to

commercial producers—the dissemination process through such institutions could be a challenge as there is no history of strong engagement.

It was pointed out that it is important to market the improved genotypes. Other people commented that it is up to the producer of upgraded animals to take care of the quality of the improved genotype and to clearly communicate that to potential users.

Dr Notter commented that genetic improvement can be undertaken but is often not successful via government farms. But he suggested that a gene with a clear effect of 'twinning' is a relatively easy case for genetic improvement, and there is therefore a higher likelihood of success. The introduction of new technologies or improved genetics always needs to be supported with sufficient *research capacity*, as there will be new questions in relation to the effects in new local breeds. At the same time it is important to have sufficient *outreach capacity.*

There was general consensus that the last question was the most difficult one. Pathways to further introgression should be mapped out by teams of researchers and policymakers, including technical and political people. There should be a clear model and pathway mapped out, and it is important to keep the aims and the process simple. While plans may be developed at a regional level, experiences can be exchanged between regions or even states. An introgression plan could be peer reviewed to ensure that plans are realistic and optimised.

It was generally agreed that a mix of private and public funds is needed for introgression programs. Private investors and entrepreneurs need to be identified to engage in the process. It is important that there is strong governmental support, both morally and in resources, but the programs should not rely only on such inputs.

It was agreed that a process is already underway and can be further expanded. There are mechanisms in place that can be used to seek further funding of activities, but the process needed to be identified by governments as a priority area for rural development. An enabling policy framework would accelerate the process.