

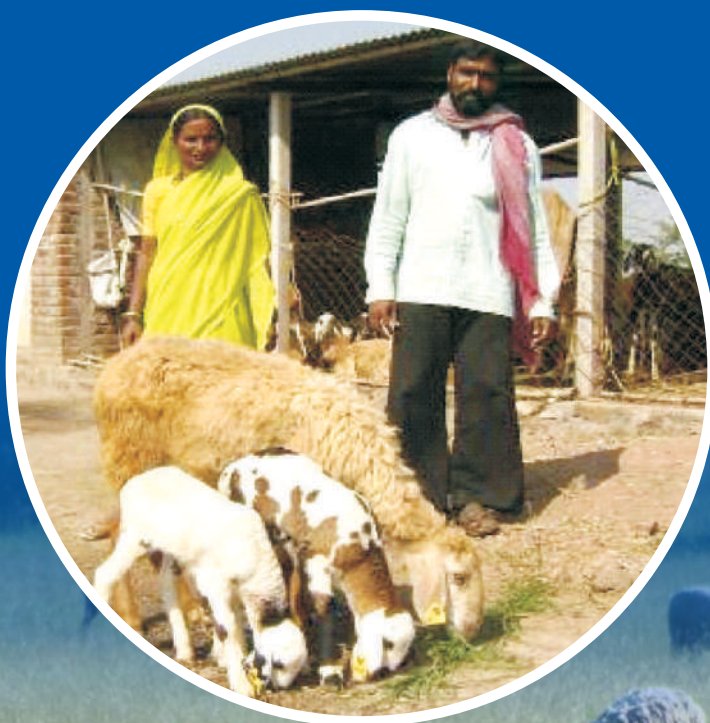


Sixth Dr. C.M. Singh Memorial Lecture

Biotechnology for the benefit of smallholder sheep owners
in the context of a theory of livestock breed improvement

by

Dr. Chanda Nimbkar



Indian Veterinary Research Institute
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Dr. R. Somvanshi

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A-366, Rajendra Nagar, Bareilly (UP)

Ph.: 0581-258 5413, 9837658052

e-mail: dr.rsomvanshi@gmail.com

Cover Photo: NARI Suwarna ewes with a farmer family (Photo courtesy Dr. Chanda Nimbkar)

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Dr. Chanda Nimbkar

Ph.D. (Australia)

Director, Animal Husbandry Division,
Nimbkar Agricultural Research Institute,
Phaltan 415 523, Maharashtra, India
e-mail: chanda.nimbkar@gmail.com

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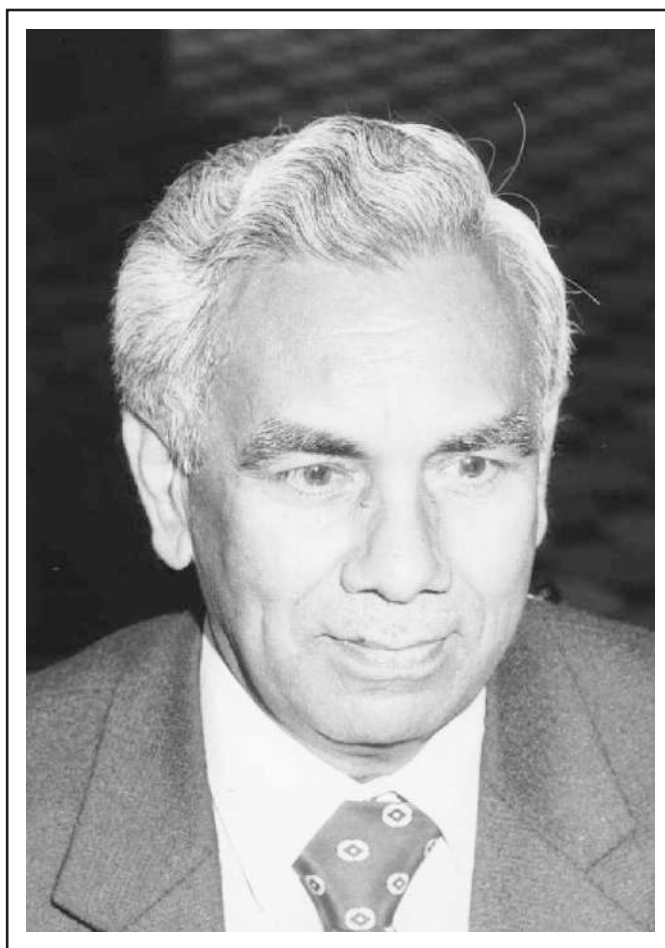
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BAREILLY (UP)



Dr. C.M. Singh

(30.11.1922 - 27.7.2005)

Former Director, I.V.R.I.

Biotechnology for the benefit of smallholder sheep owners in the context of a theory of livestock breed improvement

Dr. Chanda Nimbkar

Director

Animal Husbandry Division, Nimbkar Agricultural Research Institute,
Phaltan 415 523, Maharashtra, India

Introduction

The term '**Animal genetic resources (AnGR)**' is used to indicate the **animal species and the populations** within each species, that are, or may be **used, for food production and agriculture** (FAO, 2007). The history of AnGR started around 12,000 to 14,000 years ago, with the domestication of major crop and livestock species. The **domestication** of animals and plants is considered to be one of the **pre-requisites for** the rise of **human civilizations**. However, only a subset of the diversity present in the ancestral species survived in the domestic counterparts (FAO, 2007). Among the world's 148 **non-carnivorous species** weighing more than 45 kg, **only 15** were **domesticated** and only six of these became widespread on all continents (cattle, sheep, goats, pigs, horses, and donkeys) (adapted from Diamond, 1999). The proportion is even lower in the case of birds, with only ten species currently domesticated out of around 10,000 avian species. With the exception of the wild boar (*Sus scrofa*) the **ancestors and wild relatives** of major livestock species are **either extinct or highly endangered** as a result of hunting, changes to their habitats, and in the case of the wild red jungle fowl, intensive cross-breeding with the domestic counterpart (FAO, 2007). In these species, domestic livestock are the only depositories of the now largely vanished diversity of the wild ancestors. This is a major difference from crop species, in many of which the wild ancestors are commonly found at the centres of origin and represent an important source of variation and adaptive traits for future breeding programmes (FAO, 2007). Humankind therefore has a responsibility to ensure that we maintain the existing diversity in the domestic livestock as much as possible.

This lecture will reflect upon some widespread notions about 'pure' breeding in livestock breeds, with a view to encouraging thinking on whether these generally accepted notions lead to an increase or decrease in genetic diversity. This is followed by an account of the development of the prolific 'NARI Suwarna' sheep breed/strain by the Nimbkar Agricultural Research Institute in Phaltan in Satara district of Maharashtra State.

The livestock 'breed' concept

Distinct populations within livestock species are usually referred to as breeds. The concept of '**breeds**'

in livestock is a **convenient means of dividing livestock populations** into groups for ease of describing their genetic diversity and formulating conservation and improvement plans for them.

There are many opinions on what constitutes a 'breed'. The dictionary definition of a breed is 'a stock of animals or plants within a species, having a distinctive appearance and typically having been developed by deliberate selection.' A broader definition at the other end of the spectrum, which brings out the cultural perspective of the term is, 'a breed is a breed if enough people say it is' (Woolliams and Toro, 2007). In a study of the literature, Woolliams and Toro concluded that the question, "What is a breed?" is a simple question but difficult to answer. In India, with its vast diversity of livestock genetic resources, most of which have not yet been characterized, described and grouped into 'breeds', the concept of **'breeds' should be used flexibly** and mainly for ease of organizing sustainable use and conservation programmes. For example, the 'Deccani' breed of sheep is a conglomeration of at least four strains that look different but which are all adapted to the stressful monsoon dependent environment of the Deccan plateau. It is, thus, a good example of the **'inclusive' use of the 'breed' concept**. On the other hand, restrictive use of the 'breed' concept with **excessive emphasis on finer points** of phenotypic features such as colour, type of hair and shape of horns is likely to leave large numbers of animals outside the purview of development projects, leading to a **loss of genetic diversity** (SA PPLPP, 2012).

The FAO's (FAO, 1999) definition of a livestock 'breed', which brings out the cultural aspect of the term 'breed' and is, therefore, more acceptable for India is, "Either 'a subspecific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species' or 'a group for which geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity'.

The first part of this definition indicates the commonly accepted meaning of 'breed'. The second part indicates the **complexity** of this concept **in the context of a developing country situation** where most livestock are maintained by **smallholders on a subsistence** basis, as the sole or a partial source of their livelihoods. More than half of all cattle and buffaloes, almost all sheep and goats, 77% of pigs and 64% of poultry in India belong to smallholders who own <1 ha land or are landless.

Considering this definition, the breed registration process in India needs to be simplified. The need is to have as many of the so far unrecognized breeds registered as quickly as possible, to have **an inventory of existing breeds**. A small amount of overlapping among breeds should not be a matter for grave concern. An alternative is to have a separate list of 'recognized' but 'uncharacterized and unregistered' breeds. This is necessary to obtain at least a workable **estimate of the existing livestock diversity** in India.

In all livestock species in India, at the most **40-50% of the total population belongs to 'recognized' breeds** while 50-60% does not fall into such accepted categories. All improvement efforts, such as they are, are concentrated on the small proportion of animals of specific breeds. Upgrading of non-descript stock with well-defined breeds is widely advocated for all livestock species and is included in the breeding policies of most State governments. As neither the 'purebreds' nor the non-descript stock are likely to be performance recorded or selected, it is unclear whether such a policy would bring about genetic improvement in production performance. Excessive emphasis on maintaining the phenotypic 'purity

and uniformity' of breeds may be resulting in the neglect of valuable genetic variation in performance and adaptation (Nimbkar and Kandasamy, 2011). Genetically diverse livestock populations provide society with a greater range of options to meet future challenges (FAO, 2007). Traditional livestock keepers are found to be inclined to preserve such diversity. However, scientists are almost always found to be in favour of total uniformity of appearance.

Pure breeding vs cross breeding

These days, generally, 'pure breeding' is considered to be virtuous while crossbreeding is considered as 'wrong'. However, domestic livestock diversity has been continuously evolving. Thousands of years of animal husbandry and controlled breeding, combined with the effects of natural selection, have given rise to great genetic diversity among the world's livestock populations. Reshuffling of genes at each generation, mutation, and crossbreeding or admixture of different gene pools has offered new opportunities for natural and human selection. This has been the basis of the enormous gains in output achieved in commercial breeds, and of the adaptation of indigenous livestock to highly diverse and challenging environments (FAO, 2007). It therefore does not stand to reason that all crossbreeding should be opposed. The indigenous livestock breeds we see today are probably as much a result of crossbreeding as of natural and human selection. We see such crossbreeding being carried out by livestock keepers themselves. Some examples are:

- the crossbreeding of Deccani sheep in Satara, Solapur and Ahmednagar districts with the taller and faster growing Madgyal breed from Sangli district and adjoining parts of Karnataka State,
- the emergence of Kheri sheep in Rajasthan which is considered to be a crossbred with unknown levels of inheritance from the Marwari, Malpura and Jaisalmeri sheep, the crossbreeding having taken place during migration, possibly unintentionally (Gowane and Arora, 2010),
- the Totapuri goat breed in Alwar district of Rajasthan, which is considered by some to be a cross between Jamunapari and Sirohi breeds (SA PPLPP, 2012),
- alleles from *Bos Taurus* (i.e. alleles that have a greater frequency in European than Indian breeds) being found in six Indian zebu cattle breeds and also in Indian Desi cattle (Kumar *et al.*, 2003) and
- Evidence that both taurine and zebu cattle existed in the Harappan civilization of the Indus valley in the third millennium BC.

Detailed molecular studies indicate not only that crossbreeding between livestock populations was common, but also that genetic introgression from wild populations sometimes occurred even after the initial domestication events. Examples include the introgression of local aurochs into European and possibly also Asian cattle (FAO, 2007).

Care must also be taken to ensure that any opposition to crossbreeding is not detrimental to the family income and economics of smallholder livestock keepers, which can be improved through crossbreeding. The population status of different breeds should, however, be monitored. If it happens that the advent of a new crossbred threatens the viability of an existing breed, either that breed should be conserved *ex situ in vitro* or people who continue to rear that breed should be given adequate incentives.

As an example of improvement of livestock productivity for the **benefit of smallholders through gene introgression**, the **history of the *FecB* or Booroola gene** which was introgressed into the Deccani sheep in Maharashtra is given below.

The story of a gene travelling across continents (adapted from Nimbkar, 2002)

Looking at a large-bodied Australian merino with its heavy fleece it is hard to imagine that one of its ancestors may have been a small coarse-wooled sheep from West Bengal in India. But research has shown that this little known Garole sheep, a prolific breeder, may well be the reason why Australia's famous wool industry grew and thrived in the 1800s, making a huge contribution to the establishment of the young Australian colony. These facts were confirmed in the course of a project primarily designed to develop prolific meat sheep for Maharashtra State in India. Mr Bonbehari Nimbkar, founder of the Nimbkar Agricultural Research Institute (NARI), in Phaltan, Maharashtra, dreamt of making sheep-raising in the state more profitable by introducing twinning.



Fig.1. The Booroola merino, which came to prominence in Australia for its high prolificacy, with many multiple births recorded and an average of 2.6 lambs per litter. The gene for prolificacy was traced back to the Garole sheep from West Bengal

With funding assistance from the Australian Centre for International Agricultural Research (ACIAR) and AusAID from 1998, the genetics of the Garole's prolificacy was determined and research was done to formulate an appropriate breeding strategy to improve the efficiency of Deccani sheep. Collaboration involved the **Nimbkar Agricultural Research Institute working with the University of New England and CSIRO Livestock Industries in Armidale NSW in Australia, the National Chemical Laboratory in Pune, India and the University of Melbourne, Australia.**

Back in 1982 the late **Dr. Helen Newton Turner and her CSIRO team** established that a **single gene** was **responsible for the high prolificacy** of a group of sheep on the Seears brothers' farm near Cooma, New South Wales. This gene was named the Booroola gene after the name of the farm, and **Australian Booroola Merino** became known as a celebrated highly fecund strain of sheep. Dr. Turner suspected that the Booroola gene could be **traced back to** an early Australian flock known to contain **prolific 'Bengal' sheep**, which many believe gave Australia's sheep population a much needed boost in the 19th century.

She found that two lots of 'Bengal' sheep had been imported to Australia from Calcutta—ten ewes and two rams in 1792 and a further shipment of 100 in the following year. However, it wasn't known exactly where the sheep came from. It was surmised they could be from Bangladesh or from Tibet, as sheep from there were shipped through Bengal. The definitive FAO publication 'Sheep and Goat Breeds of India' did not list a prolific sheep breed from Bengal at all. Mr. Bon Nimbkar, having recently started the Animal Husbandry Division of NARI, corresponded with Dr. Turner about his search for a source of prolificacy in sheep to increase the efficiency of meat production of the Deccani sheep of Maharashtra. He learnt from her about the 'Bengal' sheep and started looking for them. In 1992, he heard about the **Garole sheep of Sunderban**, the hot, humid and swampy delta area of the river Ganga, in Indian West Bengal and Bangladesh. This culminated in the **arrival in Phaltan in 1993**, from West Bengal in the northeast to Maharashtra in the southwest (a distance of 2200 km), of the first lot of **Garole sheep** (two rams and two ewes), 200 years after the 'Bengal' sheep had reached Australia. Forty more Garole sheep (10 rams and 30 ewes) were brought to Phaltan in 1994 and 75 sheep (three rams and 72 ewes) in 1999. Performance recording of Garole sheep started at NARI in 1994. The flock of Garole at NARI produces almost two lambs per lambing on average and has three lambings in two years, in contrast to the average of slightly more than one lamb per year for the local Deccani breed. The Garole are truly 'wonder' sheep. They have their origin in Sunderban, living in a swampy habitat that most sheep would abhor, and yet they appear to be relatively untroubled by diseases such as footrot and helminth infection that such conditions would favour. Experiments conducted under the ACIAR/AusAID project have shown that lambs sired by Garole rams were considerably **more resistant to** the dreaded roundworm *Haemonchus contortus* than lambs of Deccani and Bannur rams (Nimbkar *et al.*, 2003).

Prior to the ACIAR/AusAID project scientists knew little about the genetics of prolificacy in the Garole breed. They did not know whether prolificacy was due to a single major gene or multiple genes. As a part of the project, the project team established appropriate crosses for investigating segregation of a putative single gene for prolificacy in the Garole breed. The project plan was to test for the presence of genetic markers closely linked with the Booroola gene and to see if these segregated with prolificacy as determined by **measuring ovulation rate**, using a laparoscope. Early results of ovulation rates supported the hypothesis that prolificacy in the Garole is due to a single major gene. However, the effect of the Garole gene on ovulation rates appeared to be less than that reported for the Booroola gene.

The turning point came in early 2001 when AgResearch New Zealand **identified the mutation** that causes the super-prolificacy of Booroola ewes (Wilson *et al.*, 2001). Groups in France and the U.K. subsequently confirmed it. Blood samples from 10 Garole ewes that had borne at least one set of triplets all tested homozygous for the Booroola mutation. This confirmed that Dr. Turner's theory was correct—it was the **'Bengal' sheep that carried the Booroola gene to Australia**. The AgResearch study also confirmed that the Booroola gene is present in the Garut strain of the Javanese thin-tailed sheep and in some Chinese sheep breeds, adding another link to an already international story. Interestingly, like the Garole, Javanese thin-tailed sheep have been shown to be relatively resistant to *Haemonchus contortus* as well as to the tropical liver fluke.

The new test greatly speeded up the breeding program at NARI by allowing rapid detection of sheep with one or two copies of the Booroola gene. This made it possible to transfer just the prolificacy gene into local Deccani sheep without the other characteristics that make the Garole unsuited to the dry, semi-arid and harsh environment of the Phaltan area. The origin of Australia's world-renowned merino wool industry has largely, but wrongly been attributed to the skill and foresight of John and Elizabeth Macarthur who imported small numbers of Spanish fine-wool merinos from Spain, South Africa and England. The reproduction rate of these early merinos was extremely low and it was the importation of the 'Bengal' (Garole) sheep and their subsequent crosses that heralded the **rapid increase in sheep numbers through multiple lambing**. Sheep numbers, under the care of shepherds similar to that practised in India today, grew from about **6000 in 1800 to 61 million in 1891**. The fecundity gene *FecB* apparently **survived about 40–50 generations of breeding** from the imported sheep without specific selection for prolificacy, until the first ewe with triplets was observed in the Seears brothers' 'Booroola' flock about the mid 1940s. The Garoles have now made a contribution to the sheep industry in Maharashtra. Commercially viable prolific sheep have been produced that are suited to local conditions. The Garole are now poised to make a similar contribution to the sheep industries in Andhra Pradesh and Tamil Nadu through the NARI Suwarna.

An account is presented below of the **sustainable improvement in sheep productivity using the *FecB* (Booroola) mutation**, brought about by the Nimbkar Agricultural Research Institute. This project has recently been **selected by the FAO in a world wide competition** for 'case studies of use of livestock biotechnologies in developing countries'. Only five such case studies in livestock were selected in this competition. The selected case studies will be included in a short publication of the FAO on 'use of agricultural biotechnologies in developing countries'.

Development and dissemination of the 'NARI Suwarna' (adapted from Nimbkar *et al.*, 2010)

The Deccani sheep production system

Deccani sheep are reared by smallholders on the Deccan plateau having a dry, monsoonal climate. They are reared in Maharashtra, Karnataka and Andhra Pradesh States of India. The Deccani sheep **population** in the three States is estimated to be 3.4 million, 7.2 million and 8.5 million respectively, thus making a total of **19.1 million** (Anthra, 2007). The 18th Livestock Census (2007), however, reports the Deccani population to be 4.3 million, of which 2.6 million are in Andhra Pradesh, 1.5 million in Karnataka and 2.5 lakh in Maharashtra. They are traditionally reared by communities such as the Dhangar in Maharashtra, Kuruba in Karnataka and 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 and one to four breeding rams. Rams are always with the flock and lambs are not artificially weaned by separating them from their mothers. Eighty per cent of the **flocks migrate** for about eight months of the year – from November to June - to areas of higher rainfall or irrigated areas, over distances of 100 to 500 km.

More than **90% of the income** from rearing Deccani sheep is earned **from the sale of 3-4 months 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, vegetables and other crops. Sheep are penned at night either on a field for manuring or near the owner's house. Sale of manure collected from the pen and manuring of fields are sources of cash income. Deccani ewes usually have only a single lamb (Acharya, 1982). The average litter size of Deccani sheep is 1.02 and the average lambing interval is 10 months (Nimbkar *et al.*, 2002, Waghmode, 2007). Lambs are reared with careful personal attention. 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 ground nut 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 litter size 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. It would therefore be profitable to improve the reproductive performance of Deccani sheep by genetic means.

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 Sundarban in West Bengal State (Nimbkar *et al.*, 2002). Garole ewes are small weighing only 15 kg compared with the 28 kg adult weight of a Deccani ewe. They are also prolific with an average litter size of 1.74 in the management system at NARI (Nimbkar *et al.*, 1998). Garole sheep are likely to be the 'diminutive' Bengal sheep which were taken to Australia from Calcutta, India in 1792-93 and were postulated as the source of the *FecB* 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 (Davis *et al.*, 2002). *FecB* (*Fec* for fecundity and *B* for Booroola) is an autosomal dominant gene having a large effect on ovulation rate 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 was introduced into the Lonand strain which is one of four strains within the Deccani breed based on geographic separation: Lonand, Sangamneri, Sangola and Kolhapuri (Gokhale 2003). The ***FecB* carrier Deccani strain has been named 'NARI Suwarna'**. The next section describes the development of the NARI Suwarna, its benefits and how it has been disseminated.

Introgression of the *FecB* gene into Deccani sheep

The beginning

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 Sundarban, West Bengal State (21°N 88°E) (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.*, 2003). 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 four years, **290 Deccani ewes and 265 Bannur ewes were inseminated with the semen of eight Deccani, nine Bannur and 15 Garole rams and the resulting progeny 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, three and six months and thus 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 gastro-intestinal 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.* 2003).

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 ovulation rate of the resulting ¼ 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

Fig. 2. Artificial insemination in ewes on NARI's farm using fresh, diluted ram semen



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 ovulation rate of one while the other half **had a mean ovulation rate of two**, indicating **single gene inheritance of prolificacy** in the Garole.

After the 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).



Fig. 3. Laparoscopy to measure ovulation rate in ewes at NARI.

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 X 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 X Deccani and Bannur X 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 to increase the frequency of the *FecB^B* allele. Additionally, Awassi-Garole B+ crossbred rams were mated to Deccani-Bannur crossbred ewes.



Fig. 4. Garole rams at NARI

The NARI Suwarna at NARI and in smallholder flocks

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 proportion 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 (EBV) for reproductive and weight traits was used. The ratio of index weights on the different traits was 1:4:12 for fertility, 3 months weight and live litter size at birth respectively. This was based on economic values for a breeding objective calculated by Nimbkar (2006) for the animals in this introgression program.



Fig. 5. Awassi ram at NARI

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 maximize genetic merit, increase frequency of the *FecB^B* allele up to a target of 0.5 to 0.6 and control inbreeding by keeping the predicted progeny mean inbreeding coefficient around 0.02 and the mean coancestry 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 to 90 days and then brought back to NARI as the same rams were used for breeding in NARI's flock. There are now 20 BB ewes and more than 300 B+ ewes in these smallholder flocks which were born there. Flock owners manage twin lambs just like their single-born lambs. In some cases, they provide extra care and attention to twin lambs such as cross-fostering them to ewes other than their dams if the dams do not have enough milk. Sometimes they also provide nutritious fodder such as Lucerne and grain such as wheat or maize to lambs. In January 2010, 94 early pregnant heterozygous and 24 non-carrier lambed ewes and their lambs were sold to 12 smallholder shepherds belonging to Birdev Farmer Shepherds' Club in Bhadali village in the rainfed area of Phaltan taluka, who got loans from the State Bank of India to purchase sheep.

Open nucleus flock

This open nucleus flock at NARI, continued since 2000, now has 421 ewes: 97 homozygous BB, 243 B+ and 81 non-carrier ++ ewes. Phenotypically superior Lonand Deccani rams from shepherds' flocks were earlier brought regularly to the NARI flock for breeding to improve the appearance of NARI's animals, increase genetic diversity in the NARI flock and control inbreeding. Over the last few years, Madgyal rams have been introduced into the breeding programme to make the *FecB* carrier rams more desirable to the local smallholder sheep owners. Non-carrier ewes are maintained with a view to generate heterozygous rams and ewes for dissemination. All matings are done by **cervical artificial insemination (AI) with fresh, diluted semen**. Ewes are inseminated on **natural oestrus detected by vasectomized rams**. Insemination programs are carried out three to four times a year, for 32-34 days or over the length of two oestrus cycles. Oestrus is detected early in the morning and in the evening. Ewes found to be on oestrus in the morning are inseminated at about 2 p.m. in the afternoon while ewes exhibiting oestrus in the evening are inseminated at 10 a.m. the next morning. Ewes that don't conceive after the first insemination within the 32-day period are inseminated again. Detailed records are kept. In the AI program carried out from 25 June to 25 July 2009, 243 ewes conceived out of 274 ewes inseminated, yielding a conception rate of 88.7% to first AI. In 2011, 331 ewes of the three *FecB* genotypes were inseminated, out of which 290 conceived, yielding a **conception rate of 81%**.



Fig. 6. A Garole ewe with its twin lambs

Fig. 7. A FecB gene carrier NARI Suwarna ewe with twin lambs



Fig. 8. A FecB gene carrier NARI Composite ewe with twin lambs



Fig. Shepherds feed Lucerne to lambs for fast growth

Nucleus flock ewes are maintained on NARI's Lundy farm at Rajale village, 15 km east of Phaltan. The farm is 19 ha. and there is canal irrigation facility available only for 8 months in the year. The farm has pastures of *Stylosanthes hamata*, *Stylosanthes seabrana*, *Cenchrus setigerus*, *Desmanthus variegatus* and Mulato (a *Brachiaria* hybrid). Additionally, silage of sorghum fodder is prepared for summer feeding and loppings of *Leucaena leucocephala* (Subabhul) are given to lambed ewes and young lambs. As NARI's own pastures are not adequate to graze all nucleus flock sheep year round, sheep are grazed on road and canal sides, on common grazing lands and on other farmers' lands in return for manure. A **sustainable symbiotic relationship** has been established **with farmers from Rajale village** such that they invite NARI's sheep for grazing on their crop residues, fallow plots, weeds on plots that are going to be ploughed and in orchards. In return, NARI provides them the agreed quantity of sheep manure at times convenient to them. This arrangement has allowed NARI to maintain the nucleus flock for the last ten years and because of the similarity of management, NARI's animals adapt easily when they are introduced into local smallholder flocks. Since NARI's sheep often share grazing grounds with other sheep from the village, no anthelmintic resistance has developed yet in NARI's flock to any class of anthelmintics, probably because of their exposure to refugia larval populations from non-drenched sheep.

Sheep are grazed in two flocks of about equal size. They are given a concentrate supplement of 100-200 g per day for one month during breeding and from one month before to three months after lambing. Lambs are grazed separately on NARI's pastures after the age of one month. Lambs are given supplementary feed up to the age of six months which consists of maize grain at 1% of their body weight and cotton seed cake at 0.5% of body weight. Young ewes are given concentrate feed of 100-200 g per day up to the breeding age of 12-14 months.

Among the ewes that lambed during September 2011 to May 2012, the **live litter size at birth was 1.48 and 1.67 for heterozygous B+ and homozygous BB ewes respectively** while the **live litter size at 3 months per ewe lambed was 1.39 and 1.59 respectively**.

PCR-RFLP DNA Test for *FecB* mutation detection established at NARI

Under a new project funded by the **Department of Biotechnology**, “Increasing profitability of sheep production by genetic improvement using the *FecB* (Booroola) mutation and improved management”, a molecular biology laboratory has been established at NARI and the **PCR-RFLP DNA test for the detection of the *FecB* mutation** in DNA isolated from blood samples on FTA paper has been standardized successfully in our laboratory. About 1500 Garole and crossbred sheep have been genotyped at the *FecB* locus in this laboratory so far. The DNA test is necessary for this breeding program because

- The phenotype of *FecB* carriers (increased number of ovulations and lambs) cannot be measured in males and before the age of puberty in females and
- The phenotype is not completely associated with genotype in females (a female with two lambs is more likely to carry the *FecB* mutation but often will not be a carrier and carrier ewes do not have twins at every lambing).

Benefits of *FecB* introgression

One copy of *FecB^B* led to an increase in ovulation rate from 1.0 to 2.0 eggs and an increase in live litter size at birth from 1.0 to 1.6 in the NARI flock and from 1.0 to 1.4 in smallholder flocks (Nimbkar *et al.*, 2009). Thus only about 40% of the *FecB* carrier ewes had twins and less than 5% of the litters of carrier ewes were triplets in smallholder flocks. The increased litter size was found to be moderate and manageable under the existing production system of smallholders. The small changes in management with increased twinning in smallholder flocks were keeping young lambs behind in the pens when ewes went grazing and providing lambs with a small amount of supplementary feed. The data from 2009 to 2011 from 10 smallholder flocks of about 40 ewes each, showed that **115 ewes carrying one copy of the *FecB^B* allele had an average live litter size of 1.42 lambs per lambing at birth and 1.29 lambs at 3 months age**, in 242 lambings. This was **37% higher productivity** than that recorded in 546 lambings of 277 ewes in the same flocks, not carrying the *FecB^B* allele and producing 1.0 live lamb at birth and 0.94 at 3 months age. A higher gain in productivity and income is expected from progeny of the more recent batches of *FecB* carrier rams sent to smallholder flocks as they are the products of more generations of backcrossing, leading to a smaller Garole proportion, larger size and more of the phenotypic features desired by smallholders. Smallholders were given free veterinary care and sheep insurance for the first four years. Training in ewe and lamb management and health care is, however, an integral part of the project since the beginning.

In NARI's flock in 2011, 18% of the litters of 90 homozygous BB ewes comprised of triplets and 6% of quadruplets. NARI has established a lamb rearing system whereby the survival of most of the triplet and quadruplet-born lambs is ensured. Studies have to be made on restriction of feeding at the time of breeding to control the number of ovulations and thereby litter size.

Benefits of genetic improvement are permanent as improved animals and their progeny keep giving the benefits generation after generation so long as they are used for breeding. It is therefore the most

sustainable form of livestock development. Such an increase in 'per ewe productivity' implies that the same level of lamb production and income can be achieved with a reduced number of ewes, thereby reducing the pressure on grazing lands. Crop farmers have now started showing an interest in rearing NARI Suwarna and NARI Composite ewes under a stall-feeding regime.



Fig. Mr. and Mrs. D.S. Pisal of Bhadali village with one of their NARI Suwarna ewes

Conclusions

The 'breed' concept should be used flexibly for ease of organizing sustainable use and conservation programmes. Crossbreeding with more productive breeds adapted to stressful conditions could prove beneficial to smallholder livestock keepers.

The NARI Suwarna and NARI composite strains of Deccani sheep, carrying the *FecB* mutation, have the potential to be biologically and economically more efficient than single-bearing Deccanis. They are poised to make the sheep production system more sustainable in the changing agricultural, demographic and economic milieu.

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Dr. Chanda Nimbkar

Dr. Chanda Nimbkar has a Ph.D. in 'Farm animal breeding and genetics' from the University of the New England, Australia. She received the 'John Allwright Fellowship' from the Australian Centre for International Agricultural Research for her Ph.D. studies. She is the Director of the Animal Husbandry Division (AHD) of the Nimbkar Agricultural Research Institute (NARI) in Phaltan, Maharashtra. Her team of dedicated workers is engaged in projects for genetic improvement of local goats and sheep which are reared mainly by the poorer sections of rural society. The AHD which was started in 1990 by the founder of NARI Mr. B.V. Nimbkar has many outstanding achievements to its credit. Briefly they are;

- Development of the NARI Suwarna strain of Deccani sheep which have >50% twins compared to the Deccani which nearly always have singles. As these sheep are reared to earn an income from selling lambs, the higher frequency of twinning can increase the sheep owners' incomes by 40 to 50%. The proportion of twinning was increased by the introduction of the FecB (Booroola) gene from the Garole sheep of Sunderban in West Bengal in a 10 year long breeding programme and evaluation project.
- Dissemination of the Southern African Boer breed of meat goat (considered to be the world's best meat goat breed) and the Middle Eastern Damascus breed of dual purpose goat through crossbreeding of small-holders' goats. Crossbreeding has led to substantial increases in body weights of crossbred kids, leading to an increase in the incomes of goat keepers.
- Establishing the technology of artificial insemination in goats using frozen buck semen. This technology became necessary as there is a severe shortage of good quality breeding bucks in villages.
- Training of farmers and professionals in goat management and artificial insemination
- Publication of information booklets on goat and sheep management in easy Marathi

Dr. Chanda Nimbkar's team won the 2007 award for 'Science and Technology Innovation for Rural Development', jointly with the National Chemical Laboratory, Pune, given by the Council for Scientific and Industrial Research of the Government of India for 'Use of the FecB gene in Deccani sheep to improve lamb production and thereby the incomes of shepherds'.

She has published more than 20 refereed scientific papers and one book chapter. She has been invited by the FAO several times to write papers and present them at international conferences.

Dr. Nimbkar was a member of the National Commission on Farmers, under the chairmanship of Prof. M.S. Swaminathan from 2006 to 2008. She won the 'Illustrious Alumnus Award' for 2011 from her college, the Ness Wadia College of Commerce in Pune, where she studied for her Bachelor's degree in Commerce.

She is a member of the governing body of the Indian Council of Agricultural Research (ICAR) Society until 2014, as a member representing rural interests. She is also a member of the Research Advisory Committee of the National Bureau on Animal Genetic Resources, Karnal, Haryana. She is a non-official Member of the Central Advisory Committee for the Development of Sheep, Goats and Rabbits, reconstituted by the Government of India, Ministry of Agriculture, Department of Animal Husbandry and Dairying for four years from January 2009.

She is a reviewer of papers for the international scientific journals 'Tropical Animal Health and Production', 'Animal Genetic Resources Information' published under the joint auspices of the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP) and the Journal of Animal Breeding and Genetics, a Wiley-Blackwell Journal.

She was the convener of the "Helen Newton Turner International Workshop on 'Using the FecB (Booroola) gene in sheep breeding programs' held on 10-12 November 2008 at NCL, Pune. The Workshop was convened jointly with NCL and UNE, Australia. Nineteen invited scientific papers were presented at the Workshop including 12 by international scientists and 55 registered delegates attended the Workshop. She edited the final Workshop Proceedings published in 2009 as an ACIAR monograph, together with Prof. J. H. J. van der Werf, Prof. S.W. Walkden-Brown and Dr. V.S. Gupta.

Dr. Chanda Nimbkar and one of her colleagues Dr. Pradip Ghalsasi were appointed as National Consultants in the field of 'Small ruminant breed conservation and improvement' by the Food and Agriculture Organisation (FAO) of the United Nations under the South Asia Pro-Poor Livestock Policy Programme (SA PPLPP), a joint initiative of FAO and the National Dairy Development Board, for 40 days in the second half of 2011. The report written by her will be soon be published and available on the SA PPLPP web site.

Dr. Nimbkar has her own farm where she grows grain, fruits and trees using as little chemicals as possible. Her favourite recreation is reading, watching movies, trekking and bird watching.