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Review Article

## The Need for Phenotypic, Reproductive, Genetic and Genomic Characterization in South African Bosveld Sheep: A Narrative Review

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**Abstract:** The Bosveld sheep is an emerging South African breed developed from crossing the local Bapedi with commercial White Dorper and Van Rooy breeds. This narrative review addresses the necessity for organized phenotypic, reproduction, and genetics characterization of developed sheep breeds such as Bosveld to determine the key areas where it is necessary to improve their reproductive efficiency and productivity. The paper has compiled the existing literature about the phenotypic characteristics, semen, and implementation of the modern techniques such as Genome-wide association studies (GWAS), and single nucleotide polymorphisms (SNP) in sheep. Much attention was paid to the connection between phenotypic and sperm characteristics, genetic diversity, and the identification of the genomic regions or the candidate genes responsible for the reproduction performance. The absence of a well-organized research basis contributed to the fact that the Bosveld sheep is still not officially recognized. This paper covers some of the breeding-related research and targeted breeding programs aimed to achieve the full potential of Bosveld sheep that would be profitable in terms of practical application which would allow adding this breed to the gene pool contributing to the sustainable development and livestock improvement in South Africa.

**Keywords:** Developed sheep, Breeding soundness, Phenotypic traits, Semen quality, Reproductive efficiency.

## Introduction

The developed indigenous ovine species have over time become adapted to different agricultural and production systems under extreme climatic conditions in southern Africa [1]. Their cross breeding could be used as a source of entry to locally bred stocks to improve adaptability and disease resistance traits of some new and commercial breeds [1], thus ensuring developed and sustainable breeding systems [2]. The last count of sheep breed improvement programs was in 1990 and found that they were undertaken in South Africa [3], and had developed new breeds such as the Meatmaster and Dohne Merino sheep with them [1]. Some improved or improved indigenous breeds of sheep include meat quality, fleece, and resistance to common diseases [1]. For example, the Bosveld sheep was adopted from a crossing of the indigenous Bapedi with the commercial White Dorper and Van Rooy sheep types to produce a higher productivity and market competitiveness animal for meat quality and ability to adapt to the tropical heat of the South Limpopo bushveld in South Africa. Thus, the Bosveld enjoys combined traits of different species, namely hardiness (Bapedi sheep), ability to produce meat in terms of weight and quality (White Dorper), and maternal traits and behaviors (Van Rooy sheep) [4].

The Bosveld sheep is managed under the South African Limpopo Bapedi and Bosveld Study Group breeding society. It is, however, not a registered breed, and there is no available recording available on its productivity. The first batch of the first 60 sheep were marketed in the annual Bela Bela sale of the Bosveld organization in February 2018. The highest prices were recorded at R16,500 for a ram and R7,500 for an ewe lamb [4]. Although the Bosveld generated R297 000 in revenue in 2018, this breed has been over-looked despite its potential to considerably boost farm total income, the sustainability of agricultural systems, and human nutrition in South Africa [4]. With all this little information documented about the Bosveld sheep, still not yet recognized as a breed in South Africa making it an essential area for research.

To protect the broad spectrum of genetics, cultural importance and market potential, the genetic diversity provides towards an economically viable and sustainable sheep-based agricultural industry, it maintains both traditional and crossbred methods, such as the Bosveld sheep. As such, this review article will specifically detail the sheep industry in South Africa, specifically, current distribution and classification and breed development based on the success of the Bosveld sheep, and potential use of genomics to define the latter in future. Then, it will cover the need for characterisation, which is an important first step for evidence to officially recognise and register the Bosveld as a unique South African breed influencer in the sheep industry based on the Animal Improvement Act.

### **South African sheep production landscape**

The South African sheep industry is diversified, with a variety of breeds suited to different climates and production processes [2]. Sheep farming is an important part of the South African agricultural sector, mainly through the production of wool and meat, conservation of genetic diversity, and maintenance of rural communities [1]. A report by the National Department of Agriculture of South Africa (2011) indicates that half of the agriculturally viable land for extensive livestock production is utilized for the rearing of sheep and goats. Amongst the nine provinces of South Africa, the Eastern Cape, Northern Cape, and Free State have the highest population of sheep, with the Western Cape having just approximately 11%, Mpumalanga 7%, Northwest 3%, and Limpopo province at 1% [5].

According to Maqhashu, et al. [5], nine sheep breeds [Izimvu (Zulu), Namaqua Afrikaner, Bapedi, Persian (Blackhead or Redhead), Ronderib Afrikaner (gladde- or Blinkhaar), Ronderib Afrikaner (steekhaar), Speckled Persian (Black or Red), Vondor and Van Rooy] are in danger of going extinct. The primary usage of these sheep breeds is the production of mutton and wool [6]. Moreover, the introduced/exotic breeds like Merino Spain and Dorper South Africa have also played a significant role in the commercial performance and genetic improvement in South Africa. The Merino has been studied for fine wool, while the Dorper can excel for meat quality and semi-adaptability with many environments [7]. Merino, native to Spain, is said to be widely distributed in the world and has significant populations in Australia, New Zealand, and South Africa. Dorper was developed in South Africa, and now it is widely distributed in the arid and semiarid regions of the world.

### **1. South African Indigenous breeds**

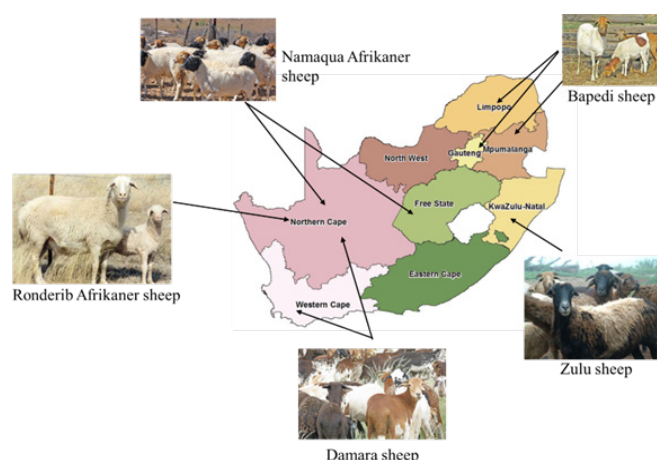
The indigenous or native breeds of sheep of South Africa are Zulu, Damara, Namaqua Afrikaner, Blinkhaar Ronderib Afrikaner, and Bapedi [1]. These breeds are well matched to the local conditions and are appreciated for their tenacity, adaptability, and unique genetic characteristics [1,2]. Besides the local breeds, South Africa has crossbreeds such as Meatmaster and Dohne Merino from the planned crossing programs [1,2]. In addition to the indigenous breeds, South Africa has crossbreeds such as Meatmaster and Dohne Merino which resulted from strategic crossbreeding initiatives [1]. These breeds are known for their improved wool and meat qualities while maintaining productivity and hardiness [8]. The developed breeds are used for the better quality of wool and meat which is not affected by the productivity or the hardiness of the animal [2].

These indigenous sheep in South Africa has various uniqueness among them being well-known for their traits in distinct attributes in most harsh environments [2]. Among this uniqueness one could also include the extraordinary reproduction and production characteristic among the breeds, making them valuable tools made by breeding in the world of agriculture [1,2]. The other unique trait that is evident among these sheep is their ability to adapt to the different weather and climatic regions and conditions [1]. Figure 1 shows the location of the South African indigenous sheep breeds, namely, the geographical map divided by provinces. Additionally, Table 1 describes the indigenous breeds in South Africa, its characteristics and attributes of the breeds including reproduction and production capacities, as well as the adaptation features on the nature of the breed.

### **2. South African cross or developed breeds**

The Meatmaster and Dohne Merino were two other developed breeds which consolidated the meat and wool qualities upon maintain their sturdiness and high production [8]. The Meatmaster was a prominent breed of its developed country, i.e., South Africa, which was developed by selection and crossing highly durable and potential Damara breed [7].

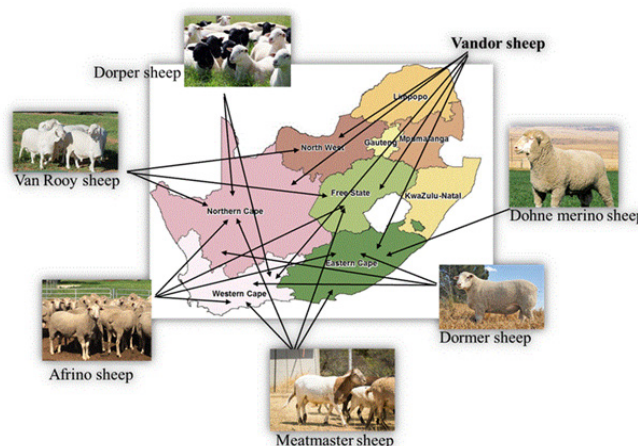
As such, it can be said that the breed developed from crossing were performed to improve characteristics, including carcass quality, meat yield and growth rate only but not sturdi-ness from parents breeds [7]. The second sheep breed evolved by crossing in South Africa was Dohne Merino, which was developed by Peppin-style Merino rams and German Mutton Merino ewes breeds in order to improve the wool and meat quality [7]. The Dohne Merino carries a reputation as a dual purpose breed for producing good quality wool for clothing use and meat conformation [8]. Table 2 represents developed sheep breed in South Africa along with their significant and developers.



**Figure 1:** Geographical map of indigenous Sheep breeds found in South Africa.

**Table 1:** Sheep breeds found in South Africa and their unique attributes, traits, reproduction and production characteris-tics, as well as their adaptation abilities.

Breed Name	Attributes	Reproduction and Production	Adaptation	References
Damara	Heat and drought tol-erant, strong maternal instincts	Seasonal breeders, good milk production, good meat and wool yield	Well-adapted to arid regions, low mainte-nance requirements	Motaung, et al. [1]
Namaqua Afrikaner	Disease resistance, high fertility rates	Year-round breeders, good meat yield	Well-adapted to hot and dry climates, good foragers	Motaung, et al. [1] Dzomba, et al. [9]
Ronderib Afrikaner	Foraging ability, disease resistance, hardi-ness	High fertility, early maturity, good carcass quality	Adapted to arid and semi-arid desert conditions	Motaung, et al. [1]
Bapedi	Good meat conformation	High fertility rates and good mothering instincts	Well-adapted to hot and dry climates, and efficient foragers	Motaung, et al. [1] Ngcobo, et al. [2] Maqhashu, et al. [10]
Zulu	Medium-sized, fat tailed, valued for cul-tural purposes	Prolific breeders and com-monly produce single lambs, though twins do occur	Highly adapted to the harsh, var-iable climates of KwaZulu-Natal and surrounding areas	Ngcobo, et al. [2]



**Figure 2:** South African developed breeds and their geographical location.



**Table 2:** Developed sheep breed in South Africa along with their significant and developers.

Developed breeds	Contributing Breeds	Use of the developed breed	Breed Developer	References
Van Rooy	Ramboillet Merino, Blinkhaar Afrikaner and Somali-type sheep	Meat production and terminal sire crossing	JC Van Rooy	Ngcobo, et al. [2] Seegmuller [7]
Dormer	Dorset Horn and German Merino	Meat production, terminal sires and good quality carcass	Department of Agriculture in South Africa	Seegmuller [7]
Dorper	Dorset Horn and Black-head Persian	Meat production	Department of Agriculture in South Africa	Ngcobo, et al. [2] Seegmuller [7]
Dohne Merino	Peppin Merino and German Mutton Merino	Fine wool and meat	Dohne Agricultural Research Station, Eastern Cape	Dzomba, et al. [8]
Afrino	Merino, Ronderib Afri-kaner and South African Mutton Merino	Meat and wool	Carnavon Research Station	Ngcobo, et al. [2]
Meatmaster	Dorper, Damara, Black-head Persian and Van Rooy	Meat	University of Pretoria's Faculty of Veterinary Sciences	Ngcobo, et al. [2] Dzomba, et al. [8]
Vandor	Van Rooy and Dorper	Wool	CJ Van Vuuren	Ngcobo, et al. [2]

The Bosvelders sheep (Figure 2) is one of the newly developed sheep in South Africa. According to Agriorbit-Magazine [11], the Bosvelders sheep developed in 2009 in the Bush-veld region of South Africa, is well-suited for sheep farming in this area due to its adaptability to harsh conditions, resistance to parasites and heartwater, durability, productivity, maternal qualities, and strong flock instincts. To create a sheep that can withstand the harsh environments of the South African Limpopo and Northwest bushveld as well as yield a quality carcass, the Bapedi (50 %) sheep was integrated with White Dorper (25 %) and Van Rooy (25 %) to develop Bosvelders sheep. This breed offers profitable sheep farming opportunities with adult ewes averaging 45 kg and rams weighing between 80 and 90 kg. It is particularly beneficial for emerging farmers seeking superior carcass qualities compared to Bapedi sheep [11]. However, there is no scientific documentation that has been conducted to confirm all these characteristics.

**Figure 3:** South African Bosvelders sheep.

### Characterization of Sheep

The characterization of a species is the most important aspect of the definition of a species. There are two types of characterization, the first one is primary, which refers to the collection of information through single field visits. For example: measurement of animals' quantitative features etc. [12]. The second one is advanced characterization, which refers to activities that require repeated visits. For example: measuring productive traits i.e. growth rate, etc [12]. Qualitative and quantitative phenotypic characterisation is fundamental in livestock for the understanding of breed character, genetic diversity and adaptation with the environment [13]. These works will add-value by increasing understanding of the conformation and performance potential of the different sheep breeds [12].

Phenotypic qualitative and quantitative characterization studies in sheep are of high importance because phenotypic characterization is the base information required to rational breed management decisions such as understanding and the management of a South African crossbreeds, such as Bosveld sheep. Investigations of comprehensive phenotypic characterization study on Bosveld sheep give a proper insight to get to know better, the genetic makeup of a breed with high performance ability that are able to help in the programme for breed conservation and preservation of their genetic diversity for sustainable livestock production.

### **1. Phenotypic qualitative characterization**

These characteristics include the external physical form, shape, colour and appearance of the animal, and are recorded as categorical variables [13]. Some of the examples of phenotypic qualitative characteristics include coat colour, horn shape and ear length etc [14]. These characteristics have less direct significance to the production and service functions of an animal [12]. However, they may relate to adaptation to a specific environment. For example: hair coat, size of ears and presence of horns are known to be relevant to the dissipation of excess body heat [14].

Qualitative phenotypic characterizations of sheep consist of detailed traits evaluations that help determine the physical depiction of the breed. These traits include head features, eye pigmentation and shape, skin color and appearance, ears and horns [12]. Understanding and documenting these traits is crucial for breed standardization, genetic diversity analysis, and breed-specific adaptations.

The Food and Agriculture Organization (FAO) of the United Nations has been very influential in the characterization, conservation, and sustainable utilization of animal genetic materials worldwide [15]. FAO has introduced a Global Strategy to assist the countries in finding out, describing, and keeping track of their animal genetic resources. These encompass sheep breeds, particularly indigenous and locally adapted types. The FAO standardizes the methods and supplies the necessary instruments for the phenotypic characterization of Animal Genetic Resources (appearance, productivity, adaptation traits) [15] and for the molecular genetic characterization of Animal Genetic Resources (achieved through DNA markers and genotyping).

### **2. Phenotypic quantitative characteristics**

The phenotypic quantitative characteristics include the animal's body measurements indicated below.

#### **2.1. Body condition score**

Body condition score (BCS) is achieved by palpation of the back and loin areas of the sheep for the estimation of fat and muscle cover [13]. The score for the condition of the body (BCS) is given on a 1-5 scale, where 1 stands for very thin, and 5 indicates overweight [16]. The best BCS interval for rams in the mating period is from 3 to 3.5 [14]. Nevertheless, BCS differences may still exist within a breed due to the impact of nutrition, health, and other management factors.

#### **2.2. Age**

The optimum breeding age of a ram ranges from 6 months to 4 years [16]. One of the things that lead to an increase in spermatogenic activities at a certain age is the significant growth of the seminiferous tubules and the differentiation of Sertoli cells [17,18]. Age has an effect on testicular size [16]. Old age arrives in males at different ages, depending on the health, environment and the use [14]. The development and advancement of old age in male sheep (rams) differs greatly based on breeds and geolocations because of health, environment, and management purpose [13]. For the southern part of Africa, breeds such as dorper and damara rams in some incidence are able to have a maintainable sound reproductive activity with increased age [14]. One of the main reasons of their toughness is, more than anything else, their genetic properties which were deliberately chosen in order to adjust to dry conditions and metabolic stress [1,2].

#### **2.3. Body weight**

Body weight is closely related to age, breed, and phenotypic quantitative characteristics [19]. Past information suggested that body weight should correlate with sperm cell concentration as more weight generally leads to higher sperm cell concentration [14]. Heavier males tend to produce more viable sperm cells [20]. It can be concluded that age and body condition of a male have a strong influence on body weight and conformation traits [19]. Body weight positively correlates with the production of sperm cells [14].

Body length, chest girth, and leg length traits impact body weight in sheep [19]. There are differences in breeds in terms of their potentials for body weight [14].

#### **2.4. Body size**

Body size in sheep is an important trait that influences various physiological and reproductive characteristics [13]. Larger body size is associated with larger testicular size, which is an important factor for reproductive performance [14].

Large-sized sheep are heavier and have bigger testicular measurements than small-sized sheep [16]. Even though the body and testicular measurements of animals increase with age, the age at which domestic animals reach puberty in a commercial set up is postponed until they attain a required body size and weight [19]. Understanding the relationship between body size and reproductive traits can help in selecting animals for breeding programs to improve overall productivity [14].

## 2.5. Scrotal Circumference

Scrotal circumference (SC) is measured to give an indication of a ram's breeding endurance [14,19]. Season, breed, and body condition have an influence on the SC, but it is generally at its highest peak during the mating season [12,13]. A ram lamb whose SC is below 30 cm and a mature ram with less than 33 cm should primarily be considered as a source of no good breeding stock [12]. Males with larger testes tend to sire daughters that reach puberty at an earlier age and ovulate more oocytes during each oestrous period [19].

## 2.6. Skeletal Dimensions

Body height, length, depth, width, pelvic width, length hock length, tail length, heart girth and scrotal circumference are some of the characteristics that are measured in sheep [12,13]. Body Height is often used to estimate overall body size and weight and is positively correlated with heart girth and body weight [14]. Body Length is a measured distance from the shoulder to the base of the tail and the longer body length can indicate better growth and meat production potential [19]. Body depth is vertical distance from the top of the back to the bottom of the chest [12]. Body width is a horizontal distance across the body. Both body depth and width are indicators of body capacity and overall health [13].

## 3. Characterization of semen in sheep

Characterization identifies distinctive traits that are valuable in various populations and offers information on reproductive performance [19]. In the past, a ram breeding soundness was assessed through observation of behavior after being introduced to the ewe flock [20]. The breeding soundness assessment, which considers, fertility, health history, physical fitness, particularly of feet and legs and eyesight, may now be undertaken prior to breeding season thanks to advancements in assisted reproductive technology [20]. Furthermore, breeding soundness assessment also involves a complete examination of the scrotum (because it directly affects the production of sperm), where testicles are being palpated, and the sheath and penis being checked for abnormalities and disorders [21].

### 3.1. Male sheep fertility

Male sheep fertility is one of the factors that influence reproduction and efficiency in a sheep breeding program [22] and the knowledge of the factors affecting male fertility underpins optimum breeding outcomes as well as the maintenance of the sheep genetic resources [22]. Fertility is a complex concept commonly ascertained by conception rate [16,22], the number of services, ejaculate traits, number of lambs born per lambing, lambing interval, non return, and lambing rate [20].

Fertility in male animals is an important characteristic that is used to select sires and it is more economically important than other production characteristics [16]. The characteristic of potential fertility of mature males and its influence on the course of reproductive activity of animals has phenotypic evaluation [21]. It increases the probability of successful breeding during the mating period. Moreover, male fertility depends on development of the testis, growth libido, quality of seminal plasma, ability of sperm cells to penetrate zona pellucida of ova [22]. So it is important for the management of male animals to be done well to optimize their breeding performance [21].

### 3.2. Indicators for male sheep fertility

After examination of the reproductive organs in a male, the collection of semen samples that will then be needed [16]. This is accomplished by collecting semen samples using an approved electro ejaculation method or artificial vagina [22]. After collection, semen samples are tested for volume, color, pH, sperm cell concentration, sperm cell motility, acrosome and membrane integrity, live/dead, and morphology [20]. Semen parameter evaluation is a reliable, objective, rapid, economical, and conventional means of showing the fertility of breeding males, other than explicitly measuring their ability to make females pregnant [16].

A good understanding of semen quality must be possessed so it can be managed and understood. The impact of semen quality on animal reproductive success is crucial for sustainable livestock breeding programs. Assessment of ram breeding soundness is crucial for ensuring the reproductive success in sheep breeding programs. This method has not been documented and practiced in the South African Bosveld sheep breed. This procedure is conducted in other animals to determine their breeding soundness but not yet conducted in Bosveld sheep breed. Conducting such assessment is important for a better performance that leads to genetic conservation and breed specific adaptation.

## Genetic Diversity in Sheep

Genomic diversity is one of the important characteristics of the natural selection process and is the main factor for the species to be able to adapt and survive over time [23]. Genomic diversity is crucial for breeding programs in which animals are selected, based on certain traits such as disease resistance, quality of wool and, quality of meat [24]. Furthermore, genome-wide association studies (GWAS) have been identified as an effective method of associating genetic features from complex traits in animals [25].

Genetic diversity is important for the adaptability and survival of species, providing a pool of gene variants in a population that increase the likelihood of survival by adapting to changes in the environment and resistance to diseases [23]. In livestock, the genetic variety allows the breeder to enrich the livestock with the desired economically advantageous qualities. South African sheep breeds exhibit varying levels of genetic diversity, with wool breeds showing the highest diversity and fat-rumped breeds the lowest [26]. The South African Bosveld sheep, which is noted for its hardiness and tolerance to the harsh South African climate, is no exception to the value of genetic diversity. Genetic diversity in sheep has been extensively studied using various molecular markers [23,26]. Microsatellites and mitochondrial DNA have been traditional markers for assessing genetic variation. Recently, SNPs have become the marker of choice because of its availability and the development of high-throughput genotyping tools [26]. The studies have shown high genetic diversity both within and among sheep breeds that indicate their adaptability to different environments and artificial selection [23].

The studies in Table 3 that have been discussed which include the use of molecular biology in understanding genetic diversity, population structure, and traits in relation to the sheep. These features play a huge role in the advancement of breeding and production programs of sheep. They have used molecular biology techniques, sifting through GWAS and SNPs data to look at a variety of factors in different sheep viable breeds they could lay their hands on.

**Table 3:** South African studies conducted on sheep using SNPs data technique.

Study title	Molecular biology technique / SNP platform	Sample size	Sheep breed(s) investigated	Traits investigated	References
"Genetics underlying phenotypic diversity in South African sheep breeds"	SNP-based genome analyses	897 genotyped animals	14 African sheep breeds	Phenotypic diversity (body size, conformation, coat/skin traits)	Visser, et al. [26]
"South African sheep breeds: Population genetic structure and conservation implications"	Microsatellite / SNP genotyping	622 animals	20 South African breeds (indigenous, locally developed, introduced)	Population genetic structure and conservation (adaptation and diversity)	Soma, et al. [27]
"Runs of homozygosity analysis of South African sheep breeds from various production systems"	SNP genotyping (Illumina Ovine SNP50K) - ROH analysis	400 SA sheep (13 breeds) and comparative global set	13 South African breeds (mutton, pelt, wool, indigenous)	Autozygosity/inbreeding; production system effects (meat, wool, and pelt)	Dzomba, et al. [28]
"The genetic assessment of South African Nguni sheep breeds using the Ovine 50K chip"	SNP genotyping (Illumina Ovine 50K)	144 sheep (75 Nguni and 69 reference)	Nguni ecotypes (Swazi, Pedi, Zulu) and reference breeds (Namaqua Afrikaner, Dorper, Damara)	Genetic diversity, population structure; adaptation or hardiness traits (communal and indigenous context)	Nxumalo, et al. [23]
"Genetic diversity and differentiation of pelt, mutton and wool sheep breeds of South Africa using genome-wide SNPs"	SNP genotyping (Illumina Ovine SNP50K)	400 SA sheep (13 breeds) and 623 international genotypes	South African pelt, wool, mutton, dual-purpose and indigenous breeds	Production-type differentiation (meat, pelt, and wool)	Dzomba, et al. [8]



A critical aspect of exploring genetic variation in sheep that should be highlighted is the application of genetics in conservation, breeding strategy, and population dynamics. Various molecular markers, especially microsatellites and SNPs, have been implemented extensively to investigate the genetic polymorphisms of the sheep populations [24].

## **1. Molecular markers**

The use of molecular markers has revolutionised the field of sheep genetics, enabling accurate selection, identification, and breeding of superior genotypes. One of the current technologies available for molecular markers in sheep genetics include the microsatellites and single nucleotide polymorphism.

### **1.1. Microsatellites**

Microsatellites, or simple sequence repeats (SSRs), refer to highly variable short tandem DNA repeats [24]. As a virtually limitless source of genetic variation, they have been widely used as a major tool in genetic studies to achieve this goal due to their high number and rapid mutation rate [24]. The applications of microsatellites in sheep include the study of population structure, genetic diversity, and parentage testing.

### **1.2. Single Nucleotide Polymorphisms (SNPs)**

Single Nucleotide Polymorphisms (SNPs) are the most frequent type of gene variation in the genome, and they are used more frequently as markers in all areas of genetic research due to the development of technology and the decrease in the cost of analysis [25]. SNPs of sheep were also used to perform GWAS, search for the genes correlating with valuable phenotypes, and to conduct research in the field of population genetics.

Kijas, et al. [29] were among the first authors to use high-density SNP chip in sheep, and consequently could achieve the large-scale survey of genetic diversity in sheep populations and verify the associative observations with regard to wool quality, meat yield, disease resistance etc. Davenport [24] further fetched data on population genetics using SNPs and the data described the genetic structure, breed differentiation, and evolutionary history of sheep.

The Ovine SNP50K BeadChip created a revolution in sheep genomics with its approximately 50,000 single nucleotide polymorphisms (SNPs) as it enabled the execution of genome wide association studies (GWAS), analysis of population structure, and determination of various aspects of genetic diversity and linkage disequilibrium (LD) [30]. It has been frequently used in numerous studies to characterize both indigenous and commercial sheep breeds globally [26]. For instance, Kijas, et al. [29] utilized the chip to assess global patterns of genetic diversity across more than 74 sheep breeds. The International Sheep Genomics Consortium (ISGC), along with other international collaborators, has put considerable effort into the promotion and fabrication of this chip to unveil the underpinning genetics of economically important traits and conservation strategies for sheep genetic resources [24].

## **2. Genetic diversity parameters**

Minor allele frequency (MAF) inbreeding, homozygosity, and heterozygosity are the criteria that are used to estimate the genetic diversity in sheep [31]. Genetic diversity parameters, such as MAF, inbreeding, homozygosity, and heterozygosity are significantly important in forming the genetic structure and health of the respective sheep population [32]. Knowing and monitoring these criteria enables breeders and scientists to take appropriate actions for maintaining and enhancing the genetic diversity of the sheep population for future generations.

### **2.1. Minor Allele Frequency**

Minor allele frequency (MAF) is a vital feature for measuring genetic variability within sheep populations [32]. Minor allele frequency is explained by [33] that it refers to frequency amongst population for allele being least common at a locus. This is essential for determination of sheep breed genetic diversity and eco-evolutionary dynamics for a breed [32,33]. MAF is varied between different breeds of the sheep due to their corresponding genetic origin and history of evolution [32].

This feature has been examined in minor allele frequency in an attempt to understand the levels of allelic diversity between different sheep breeds. The research work includes the investigation undertaken by Giovannini et al (2024) who carried out a genome-wide association study (GWAS) in Tunisian Barbarine sheep and observed varying levels of minor allele frequencies in distinct regions indicating the presence of common and rare alleles responsible for a given phenotypic trait.

### **2.2. Inbreeding**

Inbreeding within sheep breeding is worrisome since it reduces genetic diversity and makes it more prone to genetic diseases [32]. Quantifying the level of relatedness or genetic similarity between individuals in populations is the other usage of inbreeding coefficient [33].



Inbreeding coefficient is a well-documented phenomenon that occurs when closely related individuals are mated, resulting in a decrease in fitness and overall health of the offspring [32,33]. High levels of inbreeding can lead to reduced genetic diversity and increased expression of deleterious recessive traits [32].

### 2.3. Homozygosity and Heterozygosity in Sheep

Homozygosity and heterozygosity are crucial parameters that influence the genetic diversity and fitness of sheep populations [34]. Homozygosity is a presence of the identical alleles on the homo-logous chromosomes in the given locus [34] that show homozygosity in its individual in genomics [35]. Amandykova, et al. [35] investigated homozygosity patterns in Kazakh Meat-Wool Sheep and the variation in the levels of homozygosity witnessed in study showed the variation in levels of genetic drift and selection pressure on different breeds. High level of heterozygosity is thought to correlate with genetic adaptability due to genetic diversity, a possibility for asses to adjust to the host resulting in diverse group of pathogens [32].

A study by Gaspar, et al. [34] corroborates with several of the correlations in heterozygosity levels with fitness traits. According to the information, heterozygosity also positively correlated with growth rate traits, higher competitiveness, and resistance to diseases which suggest that it is essential to maintain a high level of genetic diversity within sheep populations.

### Genome-Wide Association Study

GWAS has become very helpful for breeds of interest, like sheep in our case, for studying the genetic architecture of economically important traits [30]. Kijas, et al. [29] resolved the setup of a high-density SNP chip and thus found several genetic divergences in relation to different phenotypic traits in sheep. This SNP chip has covered the required length of the ovine genome to provide the required modifications to preserve and make polymorphic alleles record for economic and agricultural traits [25] preservations, and for genetic variation to be conserved and developed for any upcoming challenge in newly getting null breed like Bosveld sheep.

Further investigations have expanded the range of GWAS in sheep, exploring the influence of diverse factors including the quality of meat, the quality of wool, the productivity traits of sheep, the resistance of sheep to diseases and the traits of sperm [25,30,34,36,37]. For example, Serrano, et al. [37] utilized GWAS to uncover genetic markers linked with sperm traits in Assaf sheep breed, whereas Sato, et al. [38] investigated the genetic determinants of semen volume, sperm concentration, testis size, and plasma inhibin B levels. These studies have enabled researchers to unravel the fundamental genetic relationships between the most important characteristics of sheep and have opened the way to genomic selection in breeding programs aimed at increasing the desirable traits of interest.

Integrating GWAS data with functional genomics has also contributed morosoly to decipher sheep genetics. For example: gene expression studies are used over gene pleiotropy to validate biological pathways and mechanisms associated gene variability, in addition to screening candidate genes identified through GWAS [39]. This comprehensive method has allowed researchers to not only identify genomic regions of interest, but also to better understand how these regions influence phenotype via gene function and regulation.

### 1. Genomic Regions and Candidate Genes for Semen Traits

The literature on genomic regions and candidate genes for semen characteristics in sheep, as revealed by Genome-Wide Association Studies (GWAS) is expanding as researchers seek to understand the genetic basis of reproductive efficiency. In the realm of semen traits, earlier genome-wide association studies (GWAS) have pinpointed numerous QTLs and candidate genes across various livestock species, such as dairy sheep, goats, cattle, and pigs [36]. For example, a GWAS in Assaf sheep has identified 23, 20, 32, and 76 single-nucleotide polymorphisms (SNPs) on chromosomes 17, 3, 1, and 4, respectively, levels correlating with semen volume, gross motility, concentration and sperm count per ejaculate [37]. Additionally, the present study identified FUT10 and SLC9C1 as potential candidate genes for gross motility, due to their position within the QTL regions identified and previous reports associating them with sperm hyperactivation, motility and fertilization [37].

Even though multiple GWAS have been conducted to identify semen variants across a range of livestock animals, only the Assaf and Merino rams utilized in sheep, whereas no data of Bosveld sheep or other breeds has been available to date. Moreover, the underlying physiology of semen traits may be conserved across species, the genes that contribute to variable traits may be different depending on the genetics of the given animal breed [36].

### Challenges and Future Directions

One of the main challenges pointed out in this review is the lack of structured and all-inclusive research on the South African Bosveld sheep especially the phenotypic, semen, and genomic characteristics. Although the breed still holds promise in terms of adaptive traits and genetic blend between the indigenous and commercial breeds, it is poorly represented both on the scientific and the national genetic resource base levels.

This makes it impossible to include the breed in research and conservation programs. Additionally, some limiting factors such as the lack of breed identification, inadequate data on productivity, and minimal application of advanced molecular tools such as GWAS in this particular breed population lead to challenges that limit possible breeding strategies to enhance reproductive and meat yield efficiencies.

It is anticipated that the genetic and phenotypic data of Bosveld sheep could be identified through extensive field survey and molecular profiling. Coordinated field survey, and molecular profiling should be integrated in future research to develop breed-specific genetic and phenotypic databases of the Bosveld flock. The integration of high throughput genomic tools with transcriptomics and epigenomics will facilitate the identification of key genes regulating phenotypic responses to improve reproductive and productive traits in Bosveld sheep. Functional confirmation of the candidate genes mediated through GWA mapping is needed to validate the association of candidate genes with semen and phenotypic traits in sheep.

## Conclusion

Therefore, we need association and functional validation of all candidate genes identified through quantitative trait loci for reproduction and production traits. Additionally, governmental, research institutions and breeding communities have to collaborate to implement and uniformly validate the breed characterization protocol and guidelines for the official identification and registration of Bosveld.

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