

REVIEW ARTICLE

Genomic Regions and Candidate Genes Associated With Semen Traits in Rams: A Systematic GWAS Review With Implications for South African Sheep Breeds

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Abstract: Ram fertility is a key determinant of reproductive efficiency in sheep production systems, particularly under extensive and semi-extensive conditions prevalent in South Africa. Semen quality traits, including ejaculate volume, sperm concentration, motility, and cryotolerance, exhibit measurable genetic variation and are increasingly investigated using genomic approaches. This systematic review synthesizes evidence from three eligible Genome-Wide Association Studies (GWAS) analyses that have identified genomic regions and candidate genes associated with semen traits in rams. Across Merino, Assaf, and composite populations, semen traits were consistently shown to be polygenic, involving genes linked to spermatogenesis, energy metabolism, oxidative stress regulation, and membrane integrity. Comparative analysis revealed both shared and breed-specific genomic regions, reflecting differences in genetic architecture and selection history. However, no studies were conducted in South African sheep populations, limiting the direct applicability of these findings. Comparative analysis revealed both shared and breed-specific genomic regions, highlighting variation in genetic architecture across populations. However, the absence of studies conducted in South African sheep breeds limits the direct applicability of these findings and underscores the need for locally relevant genomic research.

Keywords: Ram, Fertility, GWAS, South Africa, Semen

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Introduction

Reproductive efficiency in sheep is strongly influenced by ram fertility [1], particularly in extensive and semi-extensive production systems where a single ram is responsible for servicing many ewes within a defined breeding season [2]. Under such conditions, even modest reductions in semen quality can have disproportionate effects on conception rates, lambing percentages, and overall flock productivity [3]. In South Africa, sheep production relies predominantly on natural mating, often under variable and challenging environmental conditions characterized by fluctuating nutrition, heat stress, and seasonal resource availability [4]. These production realities place increased emphasis on the reproductive soundness of rams and elevate semen quality as a critical determinant of reproductive success at flock level [3, 5].

Semen quality assessment forms a central component of ram breeding soundness evaluation and Artificial Insemination (AI) programs [6]. Traits such as ejaculate volume, sperm concentration, motility, morphology, and post-thaw survival are widely used as indicators of fertilizing potential [3] and are routinely measured in both research and commercial breeding contexts [7]. Sperm motility and viability are strongly associated with fertilization capacity [1, 8], while post-thaw survival is of growing importance as semen cryopreservation is increasingly adopted for dissemination of superior sires, genetic improvement, and conservation of valuable animal genetic resources [9, 10].

Although semen traits are influenced by environmental factors such as age, season, nutrition, health status, and management practices, a substantial proportion of the observed phenotypic variation among rams is attributable to genetic differences [11]. Low to moderate estimates of heritability for semen traits in sheep, indicate that genetic improvement through selection is feasible [12]. Recent advances in molecular genetics, including the availability of high-density Single Nucleotide Polymorphism (SNP) genotyping platforms and Genome-Wide Association Study (GWAS) methodologies, have enabled the identification of genomic regions and candidate genes underlying complex reproductive traits [13]. These approaches have provided insights into the biological pathways regulating spermatogenesis, sperm motility, membrane integrity, energy metabolism, and oxidative stress response [14].

Despite these technological advancements, genomic research focusing on semen traits in rams remains limited when compared to the extensive body of work conducted in cattle. Existing studies are restricted to a small number of breeds, primarily from temperate production systems, and do not adequately represent the genetic diversity or environmental adaptations of sheep populations in regions such as South Africa. Consequently, the extent to which identified genomic regions and candidate genes are applicable to indigenous and locally developed South African sheep breeds remains unclear. This systematic review therefore consolidates and critically evaluates available genomic evidence on semen traits in rams and assesses its relevance to South African sheep production systems, with the aim of identifying knowledge gaps and informing future research priorities.

Materials and Methods

Review Design

This study was conducted as a systematic review following established guidelines for evidence synthesis in animal science research. The review aimed to identify, evaluate, and synthesize peer-reviewed genomic studies investigating semen traits in rams.

Literature Search Strategy

A comprehensive literature search was conducted across Web of Science, Scopus, PubMed, and Google Scholar. The search covered studies published between January 2015 and December 2025, reflecting the period of rapid development in GWAS technologies.

Search terms included combinations of: “ram”, “sheep”, “semen traits”, “sperm quality”, “motility”, “cryotolerance”, “GWAS”, “genomic regions”, “candidate genes”, and “fertility”. Reference lists of retrieved articles were also screened to identify additional relevant studies.

Study Selection Process

The study selection process followed a structured approach of PRISMA flow as depicted in Fig. 1.

Identification

- Records identified through database searching: n = 85
 - Web of Science: 22
 - Scopus: 25
 - PubMed: 18
 - Google Scholar: 20
- Additional records identified through reference lists: n = 5

Total records: n = 90

Screening

- Records after duplicates removed: n = 72
- Records screened (title and abstract): n = 72
- Records excluded: n = 60 (Reasons: non-genomic studies, not ram-focused, and irrelevant traits)

Eligibility

- Full-text articles assessed: n = 12
- Full-text articles excluded: n = 9 (Reasons: no GWAS/SNP analysis, and no ram semen trait data)

Included

- Studies included in qualitative synthesis: n = 3
- Studies included in quantitative synthesis (meta-analysis): n = 0

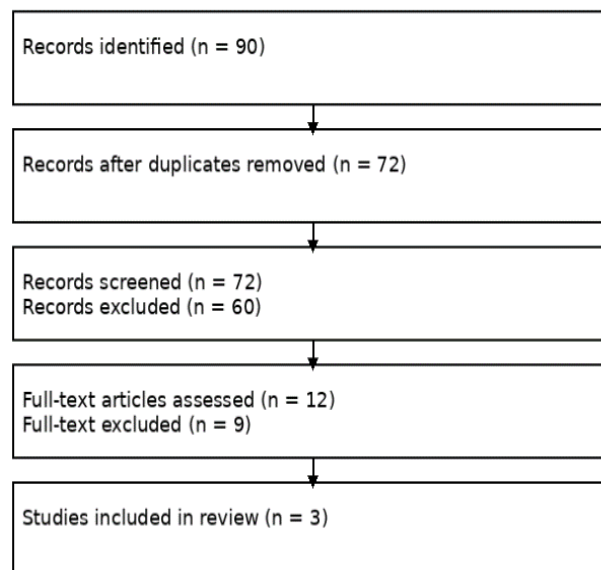


Fig. 1: PRISMA flow diagram of study selection process

Eligibility Criteria

Inclusion criteria:

- Studies conducted in rams
- Evaluation of semen quality traits (fresh and/or frozen-thawed)
- Use of GWAS or SNP-based genomic approaches
- Peer-reviewed original research articles

Exclusion criteria:

- Studies on females only
- Non-genomic studies
- Other livestock (Cattle, Pig and Goat)
- Reviews, editorials, or conference abstracts

Data Extraction and Synthesis

Data extracted included breed, sample size, semen traits analysed, genomic platform, statistical methodology, significant genomic regions, and candidate genes. Findings were synthesised narratively, with emphasis on biological relevance and applicability to South African sheep breeds. A comparative narrative synthesis was performed, focusing on cross-study consistency, breed-specific differences, and biological relevance of candidate genes.

Results and Discussion

Overview of Included Studies

The systematic search identified a limited number of eligible studies, reflecting the emerging nature of ram semen genomics. All included studies employed GWAS methodologies and evaluated semen traits under controlled collection protocols. Breeds represented included Merino, Assaf, and composite ram populations (Table 1).

Table 1: Overview of genomic studies on semen characteristics in rams

Study	Breed	Sample size	Semen characteristics	Genomic approach
[15]	Merino	246	Fresh and post-thaw traits	GWAS
[22]	Assaf	429	Fresh semen traits	GWAS
[25]	Composite	Multiple populations	Fresh and frozen-thawed traits	GWAS

Semen Characteristics Evaluated

Across studies, semen characteristics included ejaculate volume, sperm concentration, mass or progressive motility, total sperm number per ejaculate, and post-thaw motility or viability. More recent study incorporated frozen-thawed semen traits, reflecting growing interest in sperm cryotolerance.

Genomic Associations With Semen Characteristics in Rams

Merino Rams

Genome-wide association study conducted in Merino rams by [15] represent the most comprehensive genomic investigations of semen traits in sheep to date. Using high-density SNP genotyping platforms and repeated semen records collected under controlled conditions, this study [15] identified multiple Quantitative Trait Loci (QTL) associated with key semen quality parameters, including ejaculate volume, sperm concentration, gross and progressive motility, and post-thaw motility (Table 2). Significant associations were distributed across numerous ovine autosomes, reflecting the complex and polygenic genetic architecture underlying semen traits in rams [15].

The wide chromosomal distribution of QTL indicates that no single genomic region exerts a dominant effect on semen quality [16], rather, phenotypic variation arises from the cumulative contribution of multiple loci with small to moderate effects [13]. Candidate genes located within or near significant QTL regions were predominantly associated with biological processes critical for sperm function [15] (Table 3). These included genes involved in glycolysis and mitochondrial energy metabolism, which are essential for ATP production required to sustain sperm motility [17, 15]. Other genes included were implicated in cellular signalling pathways that control spermatogenesis, sperm maturation, and motility activation [14, 15].

Importantly, the identification of genomic regions associated with post-thaw motility highlights the genetic basis of sperm resilience to cryopreservation-induced stress [18, 19]. This finding has practical relevance for artificial insemination programs, as post-thaw semen quality is a major limiting factor in the widespread use of frozen ram semen [20, 21]. Collectively, results from Merino GWAS provide robust evidence that semen quality traits are heritable and amenable to genetic improvement through genomic selection, although the magnitude of genetic effects varies across traits [15].

Assaf Rams

The GWAS conducted by [22] constitutes the first systematic genomic investigation of semen traits in Assaf rams, a dairy sheep breed selected primarily for milk production. In this study, ejaculate volume, sperm concentration, mass motility, and total number of spermatozoa per ejaculate were evaluated using repeated semen records, and animals were genotyped using SNP arrays followed by imputation to higher density [22]. Several chromosome-wide significant associations were identified

across multiple ovine chromosomes, with sperm motility displaying the strongest and most consistent genomic signals (Table 2).

Candidate genes identified within significant genomic regions were functionally associated with sperm structure and function (Table 3). Moreover, genes related to sperm flagellar development and maintenance were found, providing a mechanistic explanation for observed variation in motility traits [22]. Additional candidate genes were involved in ion transport and intracellular signalling pathways, which are known to regulate sperm capacitation, motility activation, and fertilisation competence [14, 23].

The findings from Assaf rams reinforce the polygenic nature of semen traits and demonstrate that genetic determinants of semen quality are not restricted to meat-type or wool breeds. Moreover, the identification of motility-related candidate genes across independent populations suggests partial conservation of biological pathways regulating sperm function [17], whereas differences in genomic signals among breeds highlight the importance of population-specific genetic architecture [24].

Composite Ram Breeds and Cryotolerance

The study by [25] represents a significant advancement in ram semen genomics by explicitly incorporating semen cryotolerance into a genome-wide association framework. [25] conducted the study in composite ram breeds exhibiting divergent responses to cryopreservation, evaluating both fresh and frozen-thawed semen traits such as sperm motility, viability, membrane integrity, and post-thaw performance. By comparing rams with contrasting cryotolerance phenotypes, the study provided insight into the genetic mechanisms underlying sperm survival during freezing and thawing [25].

Multiple genomic regions were identified as significantly associated with variation in post-thaw semen traits, indicating that cryotolerance is under genetic control and influenced by multiple loci (Table 2). Candidate genes located within these regions were primarily involved in apoptosis regulation, oxidative stress defence, mitochondrial function, and membrane stability [25] (Table 3). These biological processes are central to sperm survival during cryopreservation, which imposes osmotic, oxidative, and thermal stress on spermatozoa [3].

The identification of genes related to antioxidant defence highlights the role of oxidative stress management in maintaining sperm viability [26], whereas genes involved in mitochondrial energy production underscore the importance of ATP availability for post-thaw motility [15]. The involvement of apoptosis-related pathways further suggests that genetic differences in cell survival mechanisms contribute to variability in cryotolerance among rams [27]. This study provides compelling evidence that genetic factors influencing fresh semen quality and frozen-thawed semen performance are partially overlapping but not identical, emphasising the need to consider cryotolerance as a distinct, genetically influenced trait.

Comparative Analysis of Genomic Regions Across Studies

Although all included studies identified multiple genomic regions associated with semen traits, there was limited overlap in specific chromosomal locations across breeds. For example, several chromosomes (e.g., OAR1, OAR2, and OAR6) were repeatedly implicated, however, the exact loci and associated traits varied. This partial overlap suggests that while core biological pathways regulating semen traits may be conserved, the underlying genetic architecture is influenced by breed-specific selection history and population structure. Differences in SNP density, statistical models, and phenotyping protocols further contributed to variability in detected associations.

Table 2: Genomic regions associated with semen characteristics in rams

Breed	Chromosomes	Semen characteristics	Reference
Merino	OAR1, OAR2, OAR3, OAR4, OAR5, OAR6, OAR7, OAR8, OAR9, OAR10, OAR11, OAR12, OAR14, OAR15, OAR17, OAR20	Volume, concentration, motility, post-thaw motility	[15]
Assaf	OAR1, OAR2, OAR4, OAR6, OAR16, OAR18, OAR26	Volume, motility, concentration, total sperm number	[22]
Composite	Multiple OARs	Fresh and frozen-thawed motility, viability, cryotolerance	[25]

Functional Interpretation of Candidate Genes

The candidate genes identified across studies are involved in key biological processes underlying sperm function (Table 3). ALDOA plays a central role in glycolysis, contributing to ATP production required for sustained sperm motility [15]. Given

that spermatozoa rely heavily on glycolytic pathways for energy, variation in this gene may directly influence motility efficiency [15]. MAPK3 is involved in intracellular signalling pathways regulating spermatogenesis and sperm maturation [15]. Its role in cell proliferation and differentiation suggests importance in testicular development and sperm quality. SLC9C1 encodes a sperm-specific ion exchanger essential for pH regulation and capacitation, a process required for fertilization [22]. Alterations in ion transport mechanisms can significantly affect sperm motility and fertilizing ability [22]. CAT (catalase) and PRDX6 (peroxiredoxin 6) are key antioxidant enzymes that protect spermatozoa from oxidative damage [25]. Given the susceptibility of sperm membranes to lipid peroxidation, these genes are particularly important for maintaining viability during cryopreservation. Collectively, these genes highlight the biological links between energy metabolism, oxidative stress regulation, and sperm motility, reinforcing the polygenic nature of semen traits.

Table 3: Candidate genes associated with semen quality and cryotolerance in rams

Gene	Associated characteristics	Biological role	Reference
<i>ALDOA</i>	Motility	Glycolytic energy metabolism	[15]
<i>MAPK3</i>	Motility	Cell signaling and spermatogenesis	[15]
<i>SLC9C1</i>	Motility	Ion exchange and capacitation	[22]
<i>SPEF2</i>	Concentration	Flagellar development	[22]
<i>CAT</i>	Cryotolerance	Antioxidant defense	[25]
<i>PRDX6</i>	Post-thaw viability	Membrane protection	[25]

Relevance to South African Sheep Breeds

Although none of the genomic studies identified in this systematic review were conducted in South African sheep breeds, the biological pathways and candidate genes associated with semen traits are highly relevant to local populations. South African sheep production is characterized by diverse agro-ecological zones, ranging from arid and semi-arid regions to higher rainfall areas [28], with corresponding variability in temperature, feed availability, and disease pressure [29]. Indigenous and locally developed sheep breeds have undergone long-term natural and artificial selection for survival and productivity under these challenging conditions, resulting in distinct adaptive genetic backgrounds [30, 28].

Adaptation to heat stress, nutritional fluctuation, and endemic diseases may directly influence reproductive physiology [31], including spermatogenesis, sperm membrane composition, and mitochondrial efficiency [4]. Consequently, genes involved in oxidative stress regulation, cellular energy metabolism, and membrane stability identified in genomic studies conducted in Merino, Assaf, and composite ram populations are of particular interest for South African sheep. For example, candidate genes associated with antioxidant defence and mitochondrial function may play a critical role in maintaining sperm motility and viability under thermal stress [15], whereas genes involved in glycolysis and ATP production are likely important for sustaining sperm function in environments characterised by variable nutritional inputs [15, 22].

Furthermore, South African composite breeds which incorporate both indigenous and exotic genetic contributions, may exhibit unique genomic architectures influencing semen quality and cryotolerance. The relevance of findings from composite populations studied [25] elsewhere suggests that similar genomic regions may contribute to variation in semen traits in locally developed breeds. However, the extent to which these associations are conserved across populations adapted to different environmental pressures remains unknown. This underscores the importance of validating identified genomic regions and candidate genes within South African sheep breeds to ensure biological relevance and practical applicability.

Genotype Environment Interactions

An important limitation of existing GWAS studies is the limited consideration of genotype-environment interactions. Environmental stressors such as heat stress, nutritional variability, and diseases common in South African production systems can significantly influence semen quality [32]. Genes involved in oxidative stress regulation and energy metabolism may exhibit environment-dependent expression, affecting sperm function under challenging conditions [26]. Therefore, the interaction between genotype and environmental stressors is likely to influence gene expression and sperm function, suggesting that genomic associations identified under controlled conditions may not fully translate to extensive production systems such as those in South Africa.

Research Gaps Identified

This systematic review highlights several critical gaps in the current body of knowledge regarding the genomics of semen traits in rams, particularly in relation to South African sheep production systems. Notably, there is a complete absence of genome-wide association studies investigating semen quality traits in South African indigenous sheep breeds. Given the genetic distinctiveness and adaptive importance of these populations, this represents a significant limitation in understanding the genetic determinants of ram fertility under local conditions.

In addition, no genomic studies to date have focused on semen traits in locally developed composite breeds, despite their growing importance in South African sheep production. These breeds present a valuable opportunity to investigate the genetic architecture of semen traits in populations that combine adaptive resilience with production efficiency. The lack of genomic data for such breeds restricts the development of evidence-based breeding strategies aimed at improving reproductive performance.

Another major gap identified is the limited investigation of genotype-environment interactions affecting semen traits. Environmental stressors common in South African production systems such as heat stress, seasonal feed scarcity, and disease challenges, are likely to interact with genetic factors to influence semen quality. However, existing studies conducted under relatively controlled or temperate conditions do not account for these interactions. The absence of such analyses limits the transferability of genomic findings to South African contexts and constrains the effective implementation of genomic selection programmes for ram fertility.

Collectively, these gaps impede the integration of genomic information into ram breeding and selection programmes in South Africa. Addressing these limitations through targeted genomic studies that incorporate locally adapted breeds, comprehensive semen phenotyping, and environmental data will be essential for improving reproductive efficiency, supporting sustainable sheep production, and conserving valuable animal genetic resources.

Implications for Genomic Selection

The identification of genomic regions associated with semen traits provides a foundation for incorporating fertility traits into genomic selection programmes. However, the absence of validation in South African breeds limits immediate application.

To ensure effectiveness, genomic markers must be:

- Validated in locally adapted populations
- Integrated with phenotypic and environmental data

Evaluated for economic relevance in breeding programmes.

Conclusion

This systematic review demonstrates that semen traits in rams are controlled by a complex polygenic architecture involving multiple genomic regions and biologically relevant candidate genes. While studies in Merino, Assaf, and composite populations provide valuable insights, the absence of genomic research in South African sheep breeds represents a critical knowledge gap. Future research should prioritise genome-wide association studies in indigenous breeds such as the Bapedi, Zulu, and Namaqua Afrikaner and locally developed composites, incorporating longitudinal phenotyping of both fresh and frozen-thawed semen traits. In addition, integrating environmental data to model genotype-environment interactions will be essential for understanding fertility under local production conditions. Such targeted research will be essential for developing robust and locally adapted genomic selection strategies that enhance reproductive efficiency and support sustainable sheep production in South Africa.

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Author's Contributions

Nhlakanipho Zulu: Conceptualization, writing original draft preparation, and writing review and edited.

Khoboso Lehloeny: Validation, formal analysis, and data curation. All authors have read and agreed to the published version of the manuscript.

Ethics

The corresponding author confirms that all the authors read and approved this study and no ethical matters involved.

Conflict of Interest Declarations

The authors declare no conflict of interest.

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