

Journal of the Hellenic Veterinary Medical Society

Vol 75, No 4 (2024)



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doi: [10.12681/jhvms.37204](https://doi.org/10.12681/jhvms.37204)

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To cite this article:

Antonopoulou, D., Giantsis, I., & Avdi, M. (2025). Utilisation of genes and SNPs in selective breeding of small ruminants for anoestrus period reduction. *Journal of the Hellenic Veterinary Medical Society*, 75(4), 8389–8394. <https://doi.org/10.12681/jhvms.37204>

Utilisation of genes and SNPs in selective breeding of small ruminants for anoestrus period reduction

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ABSTRACT: Small ruminants are characterised by large anoestrus periods in temperate latitudes due to the influence of photoperiodism. This fact presents a major challenge in sheep and goat farming, especially in countries where milk production from these animals is a vital component of the national economy. In an effort to reduce the usage of exogenous hormones administration for oestrus induction, genetic improvement aimed at achieving non seasonal reproduction could provide a promising alternative. Keeping this in mind, this mini review presents the most commonly studied genes that have been associated with reproductive seasonality in small ruminants. Furthermore, it discusses successful selective breeding schemes that aimed at eliminating reproductive seasonality. Despite the identification of numerous Single Nucleotide Polymorphisms (SNPs) associated with seasonality, their utilization in selective breeding programs has been limited in practise. This raises questions about their effectiveness as selective breeding targets. Future research should, hence, evaluate chronic data from implementation of selective breeding schemes in order to facilitate their validity in various breeds.

Keywords: *small ruminants; selective breeding; reproduction; genes;*

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Date of initial submission: 13-3-2024

Date of acceptance: 15-7-2024

INTRODUCTION

Seasonality in reproduction has always posed a major challenge in small ruminant breeding. Both sheep and goats exhibit seasonal cycles of reproductive activity. The evolutionary goal of these species' seasonal pattern of reproduction in the wild was to ensure that offspring were born at the optimal time of year in terms of temperature and vegetation, typically in spring. Domestication was not capable to change this feature entirely and therefore seasonality still exists in numerous small ruminant breeds. Sheep and goat species undergo a series of oestrous cycles during their breeding season, which typically starts in the summer or early autumn in response to shortened daylight hours and ends in late winter or early spring. The transition phase extends from late spring until the onset of the ovulatory period, while the anoestrous period spans from late winter/early spring to early- or mid-summer. This seasonal breeding pattern results in a corresponding seasonal pattern of milk production and a distinct lambing/kidding time (Abecia et al., 2012). However, sheep reproductive performance is hindered by extended inter-lambing intervals, seasonal and post-partum anoestrus periods, and low ovulation rates. While ovulation rate is affected by seasonality, it does not affect prolificacy (Avdi and Chemineau, 1998). In order to overcome seasonality and synchronize the estrus and lambing peaks of the ewes, conventional dairy sheep farms frequently use hormone treatments. Hormonal treatments ensure successful reproduction and simplify the farmer's job planning. These treatments enable ewes to enter estrus at any time of the year, regardless of their natural reproductive seasonality. By synchronizing the reproductive and production cycles within the flock, these methods enable a more efficient response to market demands for milk throughout the year, making hormone therapies a standard practice in the majority of intensive and semi-intensive dairy sheep farming systems worldwide (Laclef et al., 2023).

However, consumer preferences are evolving, with increasing demand for products produced in accordance with agreed-upon standards for human health, environmental management, and animal welfare. While hormones play a crucial role in maintaining important bodily functions, the presence of external hormone residues in meat and other animal-based foods can act as endocrine-disrupting chemicals (EDCs). These EDCs can have harmful effects on consumers' health and disrupt the body's hormonal and homeostatic systems (Kamaly et al., 2023). This

shift in consumer attitudes challenges for animal businesses all over the world. This is especially visible in profitable, discerning markets driven by discretionary purchasing power (Scaramuzzi and Martin, 2008). Thus, the discovery of genotypes are less responsive to changes in reproductive seasonality may offer a viable option for breeding polyestrous animals capable of reproductive activity year-round, thereby eliminating the need for hormone treatments (Giantsis et al., 2016). Notably, similar outcomes to hormone treatments, have also been accomplished by utilizing the ram effect, according to which rams are kept separate from ewes and introduced into the female herd after a specific period of isolation. It is important to acknowledge that the duration of the anoestrous phase is a significant concern in certain breeds, impacting the consistent production of sheep milk (Antonopoulou et al., 2023).

Genes and single nucleotide polymorphisms (snps) associated with reproduction

According to recent scientific findings, genetic composition plays a crucial role in seasonal reproduction (Luridiana et al., 2020; Cosso et al., 2021). Identification of quantitative trait loci (QTL, Table 2) that influence seasonality and employing marker-assisted selection can aid in reducing the duration of seasonal anestrus and improving reproductive performance in accelerated lambing programs (Notter and Cockett, 2005). Sheep's reproductive physiology is strongly influenced by seasonal changes, primarily controlled by exposure to daylight length, with melatonin being the primary hormone responsible for this process. Nevertheless, variations in melatonin secretion and reproductive activity in ewes has been associated with the structure of the melatonin receptor subtype 1A (MNTR1A) gene (Giantsis et al., 2016). While various mutations of the MTNR1A gene have been identified in sheep breeds worldwide, some mutations have minimal effects on reproductive capacity, while others can significantly affect ovulation rates, litter sizes, or male reproduction (Abuzahra and Wijayanti, 2024).

In an effort to achieve maximum possible outcomes in oestrus synchronization for milk production throughout the year, the molecular analysis of the MTNR1A gene is an excellent tool for marker assisted selection (Antonopoulou et al., 2023). However, research on the MTNR1A gene in goats has not been extensively studied. Notably, Italian goat breeds exhibited a lack of variation in the MTNR1A gene, as

revealed by the restriction enzymes *MnII* and *RsaI*. The observed outcome may be attributed to the distinct spatiotemporal development of goat breeds worldwide, resulting in genetic variations among major breed groups (Agradi et al., 2023). In Egyptian sheep and goats, genetic variations of the *GDF9* gene were identified as effective genetic markers. Additionally, these advantageous gene variations could be utilized in the breeding program through the utilization of genotype assisted selection (GAS), with the objective to achieve the possible improvement of reproduction and production in such tiny ruminants when employed (Aboelhassan et al., 2021).

In Luzhong mutton sheep, the complete open reading frame (ORF) region of *BMP15* was sequenced. *BMP15* is a crucial gene for sheep reproductive. Most of the differences have been observed in European sheep. Six of the 13 discovered variants were new. Four SNPs (ENSOART00000010201.1: c.352+342C>A, c.352+1232T>C, c.352+1165A>G, and c.353-2036T>A) have been shown to be strongly linked with litter size, presenting potential genetic markers for enhancing litter size (Di et al., 2021). The extension of these investigations from circadian to circannual rhythms is challenging. The clock genes involved in circadian timekeeping may also influence seasonal reproduction. Mammals have eight primary clock genes, including *Clock*, *Per1*, *Per2*, and *Per3*, *Bmal1*, *Timeless*, and *Cry1* and *Cry2* (Notter and Cockett, 2005). In recent studies in China, potential SNP markers in the *BMPR1B* gene was suggested for enhancing litter size in Mongolia sheep populations. This data offers important genetic indicators for sheep breeding and new mutations for studying the function of the *BMPR1B* gene (Gao et al., 2021). In addition to the *FecB* mutation at *BMPR1B*, a substantial association between a missense mutation, rs406686139, in the *TSHR* gene was correlated with seasonal lambing and litter size (Tao et al., 2021). In the Bamei mutton sheep population, high-throughput sequencing revealed various SNPs and indels, including *JUN*, *ITPR3*, and *PLCB2*, associated with the complex processes from estrus to lambing. Additionally, a nonsynonymous mutation p.S936A at *KDM4B* was identified, a crucial regulator in the Estrogen Receptor (ER) signaling cascade, controlling the expression of *ER* and *FOXA1* genes, which preserve the estrogen-dependent phenotype. The sequencing data provided insights into the genetic diversity and demographic differentiation of the Bamei mutton sheep population, which was subject to selection for litter size. A number

of intriguing candidate genes and pathways subjected to artificial selection were discovered by the genomic selection scan. These genes and pathways may have contributed to the higher litter size (Yao et al, 2021).

Concerning the Chinese Hu (Hu) and Chinese small-tailed Han sheep (StH) breeds, nine candidate genes located at intergenic regions of QTLs have been proposed to provide mechanistic insights into their high-fecundity. These genes include *ADIPOQ*, that encodes adiponectin, *ABCG1*, which plays important roles in regulating macrophage cholesterol levels, *BRWD1*, which is essential for during female meiosis and sperm differentiation, *GRIN2B*, which encodes Glutamate (NMDA) receptor subunit epsilon-2 and is expressed in the brain and testes, *METTL6*, that encodes an enzyme likely involved in methyltransferase activity, *SIAH2*, which is expressed in all but primordial oocytes in sheep, *SLCO2A1*, that encodes an important prostaglandins transporter in ruminants that is involved in the process of luteolysis, *TNIK*, that plays important roles in embryonic development and *UMODL1*, each playing crucial role in fecundity (Cao et al., 2016). In male reproduction among small ruminants, 35 QTL were identified on 16 OARs, significantly associated with Merino ram semen characteristics, including volume, gross motility, concentration, and percent post-thaw motility. Genes such as *SORD*, *SH2B1*, and *NT5E*, have been suggested to enact significant roles in the processes of spermatogenesis, spermatozoal motility, and high motility after cryopreservation. In addition, genes such as *PADI2*, *RAB3B*, and *ALDOA*, have been linked in the past to the promotion of spermatozoal maturation, the acrosome reaction, and successful conception were discovered. Overall, the findings suggest that Merino semen characteristics are not highly heritable and that there are multiple QTLs that influence the variability of these characteristics (Hodge et al., 2023).

Ascertainment biases occur when genetic markers, such as microsatellites or single nucleotide polymorphisms, are deliberately selected to maximize genetic homogeneity in the studied breeds considering high performance, typically focusing on industrial ones. As a result, estimations of genetic diversity for traditional breeds employing such markers may be biased toward low values, particularly impacting heterozygosity estimation. This bias has also been observed in wild species, where only the most polymorphic microsatellites are chosen for further analysis. While genome-wide analyses using SNPs are prevalent in farm

animals, they are still susceptible to ascertainment bias. Sequencing numerous sections of the genome can provide a more realistic assessment of genetic diversity and provide accurate characterization of genetic resources (Taberlet et al., 2011). Table 1 shows the most commonly associated genes with reproduction seasonality and the level that they have been used in previous studies.

Table 1. Genes associated with reproduction seasonality success in small ruminants and the extent to which they have been researched.

Gene	Number of SNPs	Studied level / importance
MNTR1A	4	*****
GDF9	2	****
BMP15	4	****
BMPR1B	2	***
TSHR	3	**
KDM4B	3	*
ADIPOQ	2	*
TNIK	1	*
UMODL1	1	*
SLCO2A1	1	*
SIAH2	1	*
METTL6	1	*
GRIN2B	1	*
BRWD1	1	*
ABCG1	1	*

Seasonal breeding with the usage of genes and spns

Selective breeding involves the deliberate determination of desired traits, followed by the mating of animals that exhibit those traits, with the aim of producing offspring that demonstrate those traits at increased rates. Since the initial domestication of animals, farmers have been able to enhance the performance of their livestock through selective breeding. A study conducted in China from 2006 to 2013 successfully bred a new type of sheep with enhanced fertility and meat production. The researchers used 110 ewes from the Small-tailed Han sheep breed and crossed them with 18 Dorper sires. The breeding program specifically targeted the B allele of the BM-PR1B A746G mutation, which was identified using a created restriction-site PCR method. The impact of genotype on litter size was assessed by grouping experimental ewes from different flocks based on their genotypes. Among the ewes with genotypes B+, BB, and ++, the BB individuals indicated the highest number of lambs (2.13 ± 0.03), significantly greater than the number of lambs produced by B+ and ++ ewes ($P < 0.01$). Additionally, the B+ ewes (1.71 ± 0.02)

had a significantly higher number of lambs compared to the ++ individuals (1.28 ± 0.06) ($P < 0.01$). The results of the aforementioned study demonstrated that the BM-PR1B gene had similar effects on litter size in both Han sheep and its crossbreeds, with significantly enhanced effects observed with the presence of the B allele (Chen et al., 2015). In another study conducted in Tunisia over a two-year period (2019-2020), two distinct electrophoretic profiles were observed in the genotyping results: one for wild individuals (FR+) and another for heterozygous individuals (FR+/FR-). During this two-year timeframe, a total of 92 female lambs were produced through artificial insemination. It is important to note that all of these lambs were heterozygous, specifically carrying the FecXBar+ gene. They were chosen as prospective breeders with the aim of increasing litter size and producing highly productive males in the F2 offspring. Choosing heterozygous F1 females as the initial phase offers the advantage of enhancing litter size and generating highly productive males. These recent findings require the implementation of a breeding program and the use of a breeding method known as Marker-Assisted Selection (MAS) to enhance the rate of prolificacy (Arari et al., 2022).

In a recent study, where the lambing times were recorded for two consecutive years, sequencing of the nucleotides of exon II of the MTNR1A gene revealed the presence of SNPs in three specific breeds of domestic sheep from Slovenia. The mutation at position g.17355452G > A consistently co-occurs with the mutation at position g.17355358G > A, leading to a modification in the amino acid sequence of the encoded protein. Specifically, the amino acid Valine is replaced by Isoleucine in the amino acid chain that plays a crucial role in transmitting the melatonin signal. The Bovška breed of animals exhibits a correlation between the G/G genotype at location g.17355452 and increased fertility as well as a reduced duration of anestrus following lambing. Animals of the Istrian Pramenka breed with the T/T genotype of the g.17355458C > T locus have a higher reproductive rate and a shorter duration of anestrus after giving birth. The Jezer-sko-Solčavska breed of animals shows a correlation between the G/G genotype at location g.17355452 and a higher frequency of lambings during the period of the year when there is a decreasing photoperiod. It can be inferred that these female sheep primarily mated at the time of year when the duration of daylight is gradually getting longer. While it is clear that the g.17355452G > A and g.17355458C > T SNPs play

a role in controlling reproductive performance, additional research is required to fully comprehend the impact of these changes (Starič et al., 2020).

CONCLUSION

Small ruminant farming in temperate latitudes has long faced difficulties due to reproductive seasonality. For a long time, this issue was resolved by utilizing external hormones administration. Nevertheless, there is now a growing need in the market for environmentally friendly alternatives. Utilizing genetic markers for selective breeding in small ruminants represents a sustainable, efficient, and enduring approach. Nevertheless, despite the relatively large number of alleles and genes potentially associated with reproduction seasonality, research conducted so far is limited to the investigation of the frequency of these genes in different breeds and populations. However, chronic data from the implementation of selective breeding towards reduction of reproduction seasonality are scarce. This is largely on account of the difficulty of conducting such studies in terms of time longevity and strict compliance requirement. Future research should aim to incorporate the utilization of MAS or GAS in a chronic population level to determine the improvement achieved in respect to seasonality of reproduction on various breeds.

FUNDING

The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 'First Call for HFRI Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant' (Project Number: HFRI-FM17-2987).

Table 2. List of abbreviations.

Abbreviation	Definition
ABCG1	ATP binding cassette subfamily G member 1
ADIPOQ	Adiponectin, C1Q and collagen domain containing
ALDOA	Aldolase, fructose-bisphosphate A
BMP15	Bone morphogenetic protein 15
BMPR1B	Bone morphogenetic protein receptor type 1B
BRWD1	Bromodomain and WD repeat domain containing 1
Clock	Clock circadian regulator
Cry1	Cryptochrome circadian regulator 1
Cry2	Cryptochrome circadian regulator 2
EDCs	Endocrine-disrupting chemicals
ER	Estrogen Receptor
FecB	Fecundity gene, Boorla, of sheep
FOXA1	Forkhead box A1
GAS	Genotype assisted selection
GDF9	Growth and differentiation factor 9
GRIN2B	Glutamate ionotropic receptor NMDA type subunit 2B
ITPR3	Inositol 1,4,5-trisphosphate receptor type 3
JUN	Jun proto-oncogene, AP-1 transcription factor subunit
KDM4B	Lysine demethylase 4B
MAS	Marker-Assisted Selection
METTL6	Methyltransferase 6, tRNA N3-cytidine
MNTR1A	Melatonin receptor subtype 1A
NMDA	N-methyl-D-aspartic acid
NT5E	5'-nucleotidase ecto
OAR	Otp, aristaless, rax
ORF	Open reading frame
PADI2	Peptidyl arginine deiminase 2
PCR	Polymerase Chain Reaction
Per1	Period circadian regulator 1
Per2	Period circadian regulator 2
Per3	Period circadian regulator 3
PLCB2	Phospholipase C beta 2
QTL	Quantitative trait loci
RAB3B	Member RAS oncogene family
SH2B1	SH2B adaptor protein 1
SIAH2	Siah E3 ubiquitin protein ligase 2
SLCO2A1	Solute carrier organic anion transporter family member 2A1
SNPs	Single Nucleotide Polymorphisms
SORD	Sorbitol dehydrogenase
Timeless	Timeless circadian regulator
TNIK	TRAF2 and NCK interacting kinase
TSHR	Thyroid stimulating hormone receptor
UMODL1	Uromodulin like 1

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