

GENOME WIDE ASSOCIATION STUDY AND HERITABILITY ESTIMATES FOR RAM SEMEN TRAITS

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SUMMARY

Ram semen traits influence conception outcomes, which in turn, may influence reproductive efficiency in sheep. As such, this study aimed to estimate genetic parameters and identify Quantitative Trait Loci (QTLs) associated with ram semen traits including volume (VOL), gross motility (GM), concentration (CONC), and percentage post thaw motility (PPTM) in a resource population consisting of five sheep breeds common to Australia. Over 11,000 semen collection records were used to estimate the heritability of semen traits ($h^2 = 0.081-0.170$). Genome-wide association (GWA) analysis was subsequently performed using a subset of genotyped animals with 5,363 semen collection records. A total of 34 QTLs located on 16 chromosomes were found to be significantly associated with semen traits. Several candidate genes that have previously been linked to male fertility were identified within these QTLs.

INTRODUCTION

Ram semen traits like GM (David *et al.* 2015), CONC (D'Alessandro *et al.* 2001), and PPTM (Morris *et al.* 2001) may influence conception outcomes in sheep following artificial insemination (AI). Moreover, in natural mating, litter size has been reported to be significantly influenced by the ram (Holler *et al.* 2014). Therefore, assessment of semen quality or breeding soundness should be widely practiced by sheep breeders. However, the genetic and physiological drivers contributing to variability in ram semen traits are not fully understood. Identifying genetic determinants that underlie variability in these traits, could aid in better understanding of these traits, and help devise novel strategies to improve conception outcomes.

Past sheep studies using Spanish dairy sheep (Pelayo *et al.* 2019) and Ethiopian rams (Rege *et al.* 2000) have found semen traits which are routinely assessed for use in artificial breeding to be lowly heritable. Comparable studies have not yet been performed in Australian sheep populations. Similarly, only one GWA study has been previously undertaken to identify genomic regions associated with semen traits such as volume, gross motility, and concentration in Assaf rams (Serrano *et al.* 2021). Therefore, the aim of this study was to estimate heritability and identify QTLs associated with ram semen traits in an Australian population comprising of five sheep breeds.

MATERIALS AND METHODS

Phenotypic data. Semen phenotypes for VOL, GM, CONC, and PPTM were provided by an artificial breeding facility for Dohne, Dorper, Merino, Poll Dorset, and White Suffolk rams. Semen collection, assessment, and initial quality control have been previously described (Hodge *et al.* 2022).

Genetic parameter estimation. A total of 11,470 semen collection records from 864 rams were used to estimate genetic parameters, which has been previously described (Hodge *et al.* 2022).

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GWA. Genotype data was available for 330 rams, as such, a subset of 5,363 semen collection records were used to perform a GWA study via R package RepeatABEL (Rönnegård *et al.* 2016). Quality control for genotype data was performed as previously described (Moghaddar *et al.* 2015). Modified Bonferroni was used to identify significant single nucleotide polymorphisms (SNPs), and genes within ± 0.5 Mega base (Mb) of these SNPs were identified via the National Centre of Biotechnology Information (NCBI) Genome Data Viewer (Rangwala *et al.* 2021), using the *Ovis aries* genome assembly (Oar_v3.1). SNPs with overlapping ± 0.5 Mb regions were considered to represent the same QTL region.

RESULTS AND DISCUSSION

Semen traits were found to be lowly heritable (0.081-0.170) (Table 1), and genetic and phenotypic correlations ranged from -0.630 to 0.321 and -0.074 to 0.347, respectively.

Table 1. Estimated heritability, genetic and phenotypic correlations along with standard errors (heritability in bold on the diagonal, and genetic and phenotypic correlations on upper and lower of the diagonals, respectively)

	VOL	GM	CONC	PPTM
VOL	0.161 (0.041)	-0.071 (0.206)	0.153 (0.227)	-0.262 (0.274)
GM	0.215 (0.020)	0.170 (0.058)	0.321 (0.282)	-0.630 (0.238)
CONC	0.228 (0.022)	0.347 (0.019)	0.089 (0.051)	-0.351 (0.286)
PPTM	0.113 (0.024)	-0.074 (0.024)	0.100 (0.024)	0.081 (0.040)

Overall, the heritability estimates of semen quality traits were found to be low, indicating that environmental variance significantly contributed to phenotypic variance. This is consistent with the fact that the data used in this study was collected over a 20-year period. Furthermore, heritability estimates in the present study largely align with past studies in livestock (Wolf 2009; Berry *et al.* 2019). Ultimately, results of genetic parameter estimation indicate that semen traits have the potential to be improved by selective breeding, as variability in semen traits is partially due to genetics.

A total of 34 QTLs were significantly associated with semen traits, including 8 QTLs for VOL, 9 for GM, 12 for CONC, and 5 for PPTM (Figure 1).

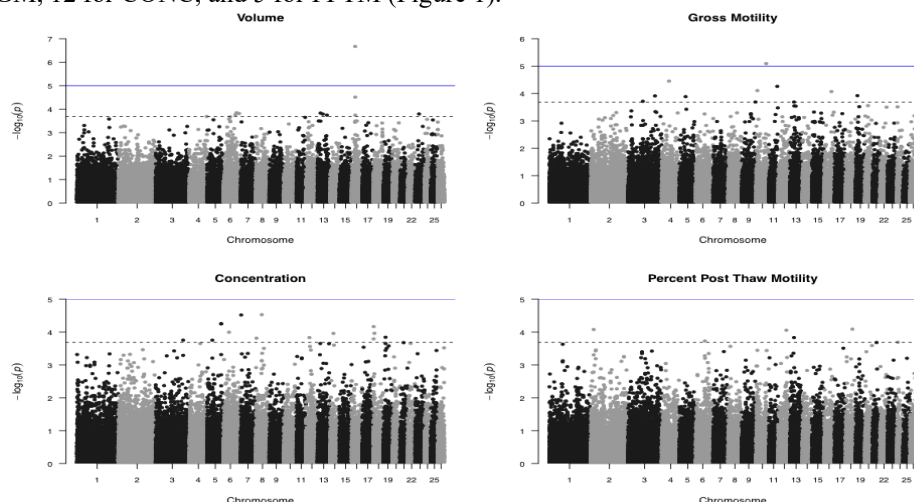


Figure 1. Manhattan plot for VOL, GM, CONC, and PPTM

Several candidate genes were identified within QTLs associated with semen traits (63 for VOL, 105 for GM, 60 for CONC, and 21 for PPTM). Table 2 presents candidate genes identified in the top two most significant QTLs associated with each semen trait. Noteworthy candidate genes found within QTLs associated with VOL, GM, CONC, and PPTM include *ANKRD17*, *LAMB1*, *YTHDC2*, and *CYLC2*, respectively. Proteomic analysis of pig semen found ANKRD17 to be significantly expressed in the post sperm-rich fraction (one of three parts which constitutes pig semen) of higher fertility boars compared to lower fertility (Martine *et al.* 2022). *LAMB1* was identified as a candidate gene associated with progressive motility following a GWA study in pigs (Gao *et al.* 2019). *YTHDC2* is reported as significantly upregulated in murine testes during spermatogenesis (Wojtas *et al.* 2017). Furthermore, *YTHDC2* null mice have significantly smaller testes, which ultimately resulted in infertility (Hsu *et al.* 2017). Finally, *CYLC2* was abundantly expressed in cryopreserved semen from bulls with high conception rates proven by AI (D'Amours *et al.* 2019). Several key positional candidates identified within genomic regions associated with semen traits have also been reported in past studies to have functional roles influencing semen quality, spermatozoal motility, and conception outcomes following AI. Thus, such genes may be important putative candidates for semen quality and potentially influence to conception outcomes in sheep.

Table 2. Top two most significant QTLs and candidate genes associated with different semen traits

Trait	Position (Chromosome:Mb)	Candidate Gene
VOL	6: 87.69 - 88.69	<i>ANKRD17</i> ^A , <i>ALB</i> , <i>AFP</i> , <i>AFM</i> , <i>RASSF6</i> , <i>CXCL8</i> , <i>CXCL5</i> , <i>PPBP</i> , <i>PF4</i> , <i>CXCL1</i> ^A , <i>TRNAS-GGA</i> ^A , <i>TRNAC-GCA</i> ^A
	16: 23.63 - 25.23	<i>PPAP2A</i> ^A , <i>SKIV2L2</i> ^A , <i>DHX29</i> , <i>CCNO</i> ^A , <i>MCIDAS</i> ^A , <i>CDC20B</i> ^A , <i>GPX8</i> ^A , <i>GZMK</i> ^A , <i>ESM1</i> , <i>SNX18</i> , <i>HSPB3</i> , <i>ARL15</i> ^A
GM	4: 48.58 - 49.58	<i>COG5</i> , <i>DUS4L</i> , <i>BCAP29</i> , <i>SLC26A4</i> , <i>CBLL1</i> , <i>SLC26A3</i> ^A , <i>DLD</i> ^A , <i>LAMB1</i> ^A , <i>LAMB4</i> , <i>NRCAM</i>
	10: 68.09 - 69.09	<i>GPC6</i> , <i>TRNAE-UUC</i>
CONC	7: 2.20 - 3.20	<i>YTHDC2</i> ^A , <i>KCNN2</i>
	8: 39.75 - 40.75	<i>KLHL32</i> ^A , <i>NDUFAF4</i> ^A , <i>GPR63</i> ^A , <i>FHL5</i> , <i>UFL1</i> , <i>FUT9</i> , <i>TRNAH-</i> <i>GUG</i> , <i>TRNAF-GAA</i>
PPTM	2: 19.27 - 20.27	<i>CYLC2</i> ^A
	18: 45.66 - 46.66	<i>PAX9</i> , <i>SLC25A21</i> , <i>MIPOL1</i> , <i>FOXA1</i>

Note: Genes with superscript ^A were previously found to be associated with male fertility

There has only been one past GWA study identifying QTLs associated with semen traits published in sheep. This study involved the analysis of semen traits like VOL, GM, and CONC collected from Assaf rams (Serrano *et al.* 2021). There was no overlap in QTLs significantly associated with any of the semen traits identified in the present study and those genomic regions significantly associated with semen traits in Assaf rams. Unlike the present study, which used Modified Bonferroni, only QTLs associated with GM in Assaf rams passed significance (10% false discovery rate), which is likely due to use of a more stringent significance threshold as well as using semen collected only from Assaf rams. Given Australian sheep breeds exhibit higher rates of linkage disequilibrium (LD) decay (Al-Mamun *et al.* 2015), and single breed resource populations exhibit higher rates of LD (van de Berg *et al.* 2016), the present study used a multi-breed population to identify QTLs significantly associated with ram semen traits in breeds common to Australia.

CONCLUSIONS

Semen quality traits including VOL, GM, CONC, and PPTM are lowly heritable, and as such, may be improved via selective breeding. Furthermore, several of the candidate genes identified in the present study have been previously found to influence spermatogenesis and normal morphological development and may be putative candidates influencing ram semen traits. Thus, validating such genes would be beneficial to determine their impact on semen traits, and in turn potential subsequent influence on conception outcomes and reproductive efficiency in sheep.

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