Identification of Genes Targeted in South African Merino and Afrino Sheep Populations Under Long-Term Selection for Reproduction and Body Weight

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Research Article

Keywords: Genome-wide association study, Lambs born, Lambs weaned, Pleiotropic genes, Weight of lamb weaned

Posted Date: December 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-128951/v1>

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Abstract

Background: Reproductive performance and body weight are of the utmost economic importance in determining the efficiency of sheep production. Simultaneous selection for increased reproductive performance and early growth traits is a common strategy in many flocks, but ambiguous results regarding the relationship between reproduction and body weight have been reported. The objective of this study was to perform a genome-wide association study (GWAS) in two South African Merino flocks and an Afrino sheep flock that were selected for both reproduction and body weight over decades. The GWAS aimed to identify SNPs associated with genes affecting the traits number of lambs born (NLB), number of lambs weaned (NLW), total weight of lamb weaned (TWW) and body weight (BW) and thus to ascertain which genes were targeted through directional selection.

Results: In the GWAS, 16 SNP markers associated with reproductive traits were identified among the three populations, while 15 SNPs were associated with body weight. These SNPs were linked respectively to 26 and 21 documented genes in the sheep genome. Most of these genes were previously associated in literature with reproduction related, as well as with growth related traits in various farm animal species. This study, supported by results from previous studies performed on sheep and cattle, identified the following genes that warrant further investigation as to their functions and processes relating to growth and reproduction in sheep: *MAP7D1*, *TRAPPC3*, *THRAP3*, *TRMP8*, *SPP2*, *HDAC9*, *ZFHX3*, *SIX6*, *C14orf39*, *TAF4B*, *TRSP1*, *EYA2*, *RBMS3*, *STL38L*, *BSPH1*, *LIG1*, *CABP5* and *ELSPBP1*.

Conclusions: Long-term selection in the flocks for both body weight and reproductive traits, and especially on the composite trait TWW, have favoured genes with pleiotropic effects influencing both groups of traits. SNPs associated with these pleiotropic genes were detected in the association analyses for the various traits.

Background

In most sheep breeding enterprises, reproductive performance and body weight are two main groups of traits that determine the efficiency of production. Apart from its direct impact (number of marketable lambs), a high reproductive rate also contributes to higher selection intensity. Furthermore, body weight is an important trait considered during selection of both replacement ewes and rams in many wool and mutton sheep production systems. Generally, the aim is to increase reproductive performance and early body weight and growth, while maintaining mature weight to limit maintenance requirements of the ewe flock under extensive conditions [1] (Herselman & Olivier, 2010). Consequently, simultaneous selection for both reproduction and body weight traits have to be performed. Although the genetic relationship between reproduction and body weight in most sheep flocks is positive [2–4] (Snyman et al., 1998; Safari et al., 2005; Olivier, 2014), negative relationships have also been reported [3] (Safari et al., 2005). Within flocks, variation among animals regarding the relationship between reproduction and body weight also exists. Various combinations of positive and negative breeding values for body weight and reproduction are present among animals in a specific flock, which may present difficulties in selecting breeding sires and dams.

One way of incorporating early growth and reproduction in a single selection criterion is through total weight of lamb weaned per ewe (TWW). TWW per ewe lifetime includes ewe fertility, litter size, lamb survival rate and direct growth performance of lambs until weaning and can be used as a biological index to measure reproduction potential in ewes [5, 6] (Zishiri et al., 2013; Matebesi-Ranthimo et al., 2017). TWW was used as reproductive selection criterion, together with body weight, in several governmental experimental sheep flocks in South Africa over many years. The data collected on these flocks will provide insight into the genomic consequences of simultaneous long-term selection for body weight and TWW as reproductive criterion.

Genome-wide association study (GWAS) is an important tool for the identification of candidate genes and molecular variants associated with economically important traits in farm animals. The identification of genes associated with reproduction and body weight in flocks subjected to simultaneous selection for these traits for many years, could provide information to elucidate the underlying mechanisms influencing these traits and the relationship between these traits. During the past decade, the number of GWAS in sheep has increased and various candidate genes have been identified for various body weight, growth and reproductive traits [7, 8] (Xu & Li, 2017; Gebreselassie et al., 2020). The identified genes varied between studies and breeds, and were identified across the entire genome.

The objective of this study was to perform a GWAS in two South African Merino flocks and an Afrino sheep flock that were selected for both reproduction and body weight for many years. The aim of the GWAS was to identify significant SNPs associated with genes affecting reproduction and body weight and thus to determine which genes were targeted when selection was based on body weight and the composite trait total weight of lamb weaned.

Results

Breeding value statistics

The average, minimum and maximum estimated breeding values (EBV) for each trait used in the analyses for the three populations are summarised in Table 1. There was a wide range in EBV among the animals within each flock in all the traits included in the GWAS.

Table 1 The average, minimum and maximum estimated breeding values (EBV) for each trait per population

Trait	Average EBV	Minimum EBV	Maximum EBV
	Afrino (n=152)		
Body weight (kg)	7.48	0.78	14.4
Number of lambs born	0.58	-0.55	1.43
Number of lambs weaned	0.49	-0.36	1.21
Total weight of lamb weaned (kg)	11.73	-7.46	25.50
Grootfontein Merino (n=130)			
Body weight (kg)	4.13	-6.58	12.87
Number of lambs born	0.08	-0.79	0.69
Number of lambs weaned	0.08	-0.40	0.58
Total weight of lamb weaned (kg)	3.22	-9.01	15.51
Cradock Merino (n=129)			
Body weight (kg)	4.29	-4.03	12.51
Number of lambs born	0.23	-0.38	0.82
Number of lambs weaned	0.23	-0.35	0.89
Total weight of lamb weaned (kg)	6.02	-9.92	20.98

Genetic relatedness within and between populations

The relatedness within and between the three populations were investigated and are illustrated in Figure 1. As the three populations clustered according to geographical region and showed genetic differentiation, each was included as a separate population in the GWAS analyses. The relatedness between the two Merino populations could be explained by the use of certain rams as sires in both populations.

Genome-wide association study

Manhattan plots of the four traits for the different populations are illustrated in Figures 2 to 5. Several suggestive SNPs were identified in all the populations for all the traits. These, as well as the SNP effects are summarised in Table 2. For number of lambs born, number of lambs weaned, total weight of lamb weaned and body weight, 10, 16, 9 and 14 SNPs were respectively identified across the three populations.

Table 2 Suggestive SNPs associated with reproduction and body weight in the three populations

SNP-Name	OAR	Reference SNP name	P-value	Trait	SNP effect		
					Partial R ²	Estimate	P-value
Afrino Reproduction							
s22463.1	1	rs403392648	5.40E-05	NLW	0.085	0.0200	0.0001
			3.08E-05	TWW	0.085	0.4770	0.0001
OAR7_76295917.1	7	rs411617467	1.95E-05	NLB	0.139	-0.0350	0.0001
			1.54E-05	NLW	0.139	-0.0270	0.0001
			8.93E-06	TWW	0.111	-0.5460	0.0001
s17625.1	20	rs423947737	9.80E-05	TWW	0.163	0.7460	0.0001
OAR23_32551191.1	23	rs423325455	9.17E-05	TWW	0.059	0.4490	0.0002
Grootfontein Reproduction							
OAR1_14315581.1	1	rs402215188	1.67E-05	NLB	0.095	0.0130	0.0001
OAR2_62489834.1	2	rs411314096	7.54E-05	NLB	0.037	0.0160	0.0078
OAR2_150119548.1	2	rs429823566	1.15E-05	NLW	0.116	0.0160	0.0001
			2.50E-05	TWW	0.080	0.3520	0.0005
OAR14_15485140.1	14	rs408741405	4.83E-05	NLB	0.064	-0.0150	0.0008
OAR14_39202046.1	14	rs428477959	2.87E-05	TWW	0.150	0.4010	0.0001
OAR15_36653741_X.1	15	rs399878993	3.11E-05	NLB	0.187	0.0340	0.0001
Cradock Reproduction							
s27280.1	1	rs415733675	7.04E-07	NLB	0.117	0.0120	0.0001
			6.58E-06	NLW	0.123	0.0150	0.0001
			1.08E-05	TWW	0.113	0.3570	0.0001
OAR1_10554666.1	1	rs430430819	5.77E-06	NLB	0.050	-0.0100	0.0056
			2.83E-05	NLW	0.021	-0.0080	0.0700
s20120.1	1	rs416491795	4.42E-05	NLB	0.023	-0.0080	0.0514
OAR2_155832335.1	2	rs413377527	9.85E-05	TWW	0.078	0.3750	0.0007
s61320.1	3	rs423667203	8.06E-05	NLB	0.055	-0.0120	0.0047
OAR4_28811142.1	4	rs418895848	1.49E-05	NLB	0.032	-0.0140	0.0232
			3.53E-05	NLW	0.066	-0.0140	0.0018
			6.51E-05	TWW	0.074	-0.4150	0.0005
Afrino Body weight							
OAR1_267463862.1	1	rs401801089	4.56E-05	BW	0.041	-0.2650	0.0023
OAR3_195698523.1	3	rs411530530	2.73E-05	BW	0.053	0.1410	0.0007
OAR3_195730138.1	3	rs422672684	2.73E-05	BW	0.050	0.1305	0.0006
OAR3_201667351.1	3	rs419550036	4.99E-05	BW	0.024	0.0710	0.0150
OAR3_63334035.1	3	rs400773806	8.10E-05	BW	0.034	-0.1240	0.0043

SNP-Name	OAR	Reference SNP name	P-value	Trait	SNP effect		
					Partial R ²	Estimate	P-value
OAR14_2287469.1	14	rs414746789	4.26E-05	BW	0.108	0.1090	0.0001
OAR14_56900862.1	14	rs420470779	1.09E-05	BW	0.179	2.0320	0.0001
Grootfontein Body weight							
OAR9_64654880.1	9	rs398224229	6.36E-06	BW	0.015	0.1170	0.0663
OAR9_59549818.1	9	rs160659087	3.66E-05	BW	0.253	0.3400	0.0001
OAR13_27572962.1	13	rs408454015	3.72E-05	BW	0.114	0.2520	0.0001
OAR13_80729511.1	13	rs398726943	8.18E-05	BW	0.038	-0.1920	0.0044
OAR19_4811675.1	19	rs418778131	7.58E-05	BW	0.048	0.1750	0.0020
Cradock Body weight							
s49312.1	5	rs405658230	3.24E-05	BW	0.105	0.2440	0.0001
OAR12_2239362.1	12	rs398789428	5.03E-05	BW	0.132	0.2650	0.0001

The Q-Q plots for the various analyses are also illustrated in Figures 2 to 5. These plots for the Afrino body weight (Figure 2) and Cradock Merino NLB (Figure 3) indicate the largest deviations from the distribution under the null hypothesis, suggesting the strongest associations for these traits. Moderate deviations were observed for NLW in the Grootfontein and Cradock Merino populations (Figure 4) and for TWW in the Grootfontein Merino population (Figure 5).

The genes associated with the suggestive SNPs in the three populations are summarised in Table 3. Genes within 50 kb upstream or downstream of the SNP were included for the purpose of this study. Some of the suggestive SNPs were associated with more than one gene. *MAP7D1*, *TRAPPC3* and *THRAP3* are all located within 50 kb up- or downstream from SNP OAR1_10554666.1, while *SPP2* and *TRPM8* were both associated with SNP s20120.1 on OAR1 in the Cradock Merino flock. In the Afrino flock, *RPB1* and *RPB2* were located close to SNP OAR1_267463962.1. Two SNPs on OAR14 were also associated with more than one gene. *ZNRF1*, *LDHD* and *ZFP1* were associated with SNP OAR14_2287469.1 and *BSPH1*, *LIG1*, *CABP5* and *ELSPBP1* with SNP OAR14_56900862.1.

Nine genes were associated with reproduction in the Cradock population. Two of these genes (*GRIK3* and *HDAC9*) were associated with all three reproductive traits, while *MAP7D1*, *TRAPPC3* and *THRAP3* were associated with both NLB and NLW. Eight and nine genes were respectively associated with the reproductive traits in the Grootfontein and Afrino populations. The *SIX6* and *C14orf39* genes in the Afrino population were associated with all three reproductive traits, and *C1orf68* was associated with both NLW and TWW. This illustrates the inter-relationships among these reproductive traits on a genomic basis. Two, six and 12 genes were associated with BW in the Cradock, Grootfontein and Afrino populations respectively.

Table 3 Genes located within 50 kb of the SNPs associated with reproduction and body weight in the three populations

SNP-Name	OAR	Trait	Position SNP	Position gene: From	Position gene: To	Gene position relative to SNP (bytes)	F/R	Gene
Afrino Reproduction								
s22463.1	1	NLW	101 406	101387421	101388583	17 734	R	<i>ENSOARG00000006463</i>
		TWW						
		NLW	101 406	101438553	101440391	-32 236	F	<i>C1orf68</i>
		TWW						
OAR7_76295917.1	7	NLB	69 590	69578590	69580428	9 930	F	<i>SIX6</i>
		NLW	358					
		TWW						
		NLB	69 590	69502230	69553842	36 516	R	<i>C14orf39</i>
		NLW	358					
		TWW						
s17625.1	20	TWW	16 711	16665408	16670167	41 662	F	<i>MRPL2</i>
			829					
		TWW	16 711	16670279	16685193	26 636	F	<i>KLC4</i>
			829					
		TWW	16 711	16649377	16664526	47 303	R	<i>CUL7</i>
			829					
		TWW	16 711	16685438	16748583	Within	F	<i>PTK7</i>
			829					
OAR23_32551191.1	23	TWW	30 815	30763466	30864657	Within	R	<i>TAF4B</i>
			656					
Grootfontein Reproduction								
OAR1_14315581.1	1	NLB	14 442	14431405	14447561	Within	R	<i>PPT1</i>
			508					
		NLB	14 442	14404455	14431336	11 172	F	<i>CAP1</i>
			508					
OAR2_62489834.1	2	NLB	58 161	58139556	58167245	Within	R	<i>PSAT1</i>
			112					
		NLB	58 161	58199853	58237629	-38 741	R	<i>CEP78</i>
			112					
OAR2_150119548.1	2	NLW	141 206	141129472	141205392	835	R	<i>XIRP2</i>
			227					
		TWW						
OAR14_15485140.1	14	NLB	15 189	15047972	15345645	Within	R	<i>ITFG1</i>
			288					
OAR14_39202046.1	14	TWW	37 605	37447871	37613002	Within	F	<i>ZFH3</i>
			578					
OAR15_36653741_X.1	15	NLB	34 773	34787560	34912953	-13 591	F	<i>PIK3C2A</i>
			969					

SNP-Name	OAR	Trait	Position SNP	Position gene: From	Position gene: To	Gene position relative to SNP (bytes)	F/R	Gene
Cradock Reproduction								
s27280.1	1	NLB	11 378 621	11 388 286	11 632 622	-9 665	R	GRIK3
		NLW						
		TWW						
OAR1_10554666.1	1	NLB	10 806 824	10 805 167	10 826 896	Within	F	MAP7D1
		NLW						
		NLB	10 806 824	10 788 110	10 800 988	5 836	R	TRAPPC3
		NLW						
		NLB	10 806 824	10 846 957	10 928 374	-40 133	F	THRAP3
		NLW						
s20120.1	1	NLB	6 790 152	6805491	6875514	-15 339	R	TRPM8
		NLB	6 790 152	6746855	6774253	15 899	R	SPP2
OAR2_155832335.1	2	TWW	146 883 202	146744519	147095289	Within	R	SLC4A10
s61320.1	3	NLB	7 147 363	7197777	7199589	-50 414	F	IER5L
OAR4_28811142.1	4	NLB	27 431 427	27257256	27548397	Within	F	HDAC9
		NLW						
		TWW						
Afrino Body weight								
OAR1_267463862.1	1	BW	247 472 170	247420622	247448039	24 131	F	RBP1
		BW	247 472 170	247483250	247516682	-11 080	F	RBP2
OAR3_195698523.1	3	BW	181 460 390	181489920	181581583	-29 530	F	PKP2
OAR3_195730138.1	3	BW	181 491 220	181489920	181581583	Within	F	PKP2
OAR3_201667351.1	3	BW	187 197 623	187116315	187199023	Within	R	STK38L
OAR3_63334035.1	3	BW	59 586 743	59566222	59588647	Within	R	IL1RN
OAR14_2287469.1	14	BW	1 800 770	1738269	1824439	Within	F	ZNRF1
		BW	1 800 770	1830275	1837900	-29 505	R	LDHD

SNP-Name	OAR	Trait	Position SNP	Position gene: From	Position gene: To	Gene position relative to SNP (bytes)	F/R	Gene
		BW	1 800 770	1843122	1879195	-42 352	F	<i>ZFP1</i>
OAR14_56900862.1	14	BW	53 769 443	53763526	53766540	2 903	R	<i>BSPH1</i>
		BW	53 769 443	53813069	53838703	-43 626	R	<i>LIG1</i>
		BW	53 769 443	53799988	53810178	-30 545	R	<i>CABP5</i>
		BW	53 769 443	53772485	53796703	-3 042	F	<i>ELSPBP1</i>
Grootfontein Body weight								
OAR9_64654880.1	9	BW	61 385 044	61350520	61629720	Within	F	<i>TRPS1</i>
OAR9_59549818.1	9	BW	56 557 380	56561778	56770897	-4 398	R	<i>ZNF704</i>
OAR13_27572962.1	13	BW	24 880 781	24853588	24873001	7 780	F	<i>THNSL 1</i>
		BW	24 880 781	24823067	24859309	21 472	F	<i>ENKUR</i>
OAR13_80729511.1	13	BW	75 045 518	75032434	75209764	Within	F	<i>EYA2</i>
OAR19_4811675.1	19	BW	4 564 162	3749181	4558077	6 085	F	<i>RBMS3</i>
Cradock Body weight								
s49312.1	5	BW	38 043 064	38044702	38090981	-1 638	R	<i>ZNF496</i>
OAR12_2239362.1	12	BW	3 445 853	3 225 000	3 520 000	Within	F	<i>ENSOARG00000005343</i>

Gene ontology (GO) categories identified for genes associated with body weight and reproduction in the Afrino, Grootfontein and Cradock populations are presented in Supplementary Tables 1 to 3 respectively. The gene groups identified in the various populations were classified into several main biological processes and molecular functions.

In Table 4 (Afrino population), the genes *ENSOARG00000006463*, *C1orf68*, *C14orf39*, *MRPL2*, *CUL7* and *ELSPBP1* were not included in any category, while the following 15 genes were categorised into the three GO categories: *LIG1*, *SIX6*, *TAF4B*, *ZFP1*, *BSPH1*, *IL1RN*, *CABP5*, *KLC4*, *LDHD*, *PKP2*, *PTK7*, *RBP1*, *RBP2*, *STK38L*, *ZNRF1*. Main biological processes identified were “Metabolic processes” and “Developmental processes”, while “Binding” was the only molecular function identified. Genes in this population that were categorised under biological processes were mostly associated with BW and TWW. All traits were associated with genes in the molecular function and cellular components categories.

Thirteen of the 14 genes identified in the Grootfontein population (*EYA2*, *RBMS2*, *PSAT1*, *TRPS1*, *ZFH3*, *ZNF704*, *PIK3C2A*, *XIRP2*, *CAP1*, *CEP78*, *ENKUR*, *ITFG1*, *THNSL 1*) were categorised into the three GO categories (Table 5). Only the *PPT1* gene was not categorised. Main biological processes identified in the Grootfontein gene group were “Biosynthetic processes”, “Transcription”,

“Metabolic processes” and “Gene expression”. All genes were categorised under “Binding” as molecular function. Similar to the Afrino population, genes categorised under biological processes were mostly associated with BW and TWW. The same applies for the “Binding” molecular function, with the addition of NLW. All traits were associated with genes under the cellular components category.

As summarised in Table 6, in the Cradock population, the following six of the 11 identified genes were categorised into the three GO categories, namely *GRIK3*, *SLC4A10*, *THRAP3*, *TRPM8*, *HDAC9*, *ZNF496*. The genes *MAP7D1*, *TRAPPC3*, *SPP2*, *IER5L* and *ENSOARG00000005343* were not included in any category. Two biological processes, namely “Homeostasis” and “Transcription” and two molecular functions, “Transporter activity” and “Channel activity” were identified. All three reproductive traits were associated with genes categorised in all three GO categories, while BW was associated with genes in the “Transcription” biological process and cellular components category.

Table 4 Gene ontology categories for genes associated with body weight and reproduction in the Afrino population

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Biological process				
<i>Metabolic processes</i>				
Vitamin metabolic process	GO:0006766	2	<i>RBP1, RPB2</i>	BW
Vitamin A metabolic process	GO:0006776	2	<i>RBP1, RPB2</i>	BW
Fat-soluble vitamin metabolic process	GO:0006775	2	<i>RBP1, RPB2</i>	BW
Retinoid metabolic process	GO:0001523	2	<i>RBP1, RPB2</i>	BW
Isoprenoid metabolic process	GO:0006720	2	<i>RBP1, RPB2</i>	BW
Diterpenoid metabolic process	GO:0016101	2	<i>RBP1, RPB2</i>	BW
Terpenoid metabolic process	GO:0006721	2	<i>RBP1, RPB2</i>	BW
Lipid metabolic process	GO:0006629	3	<i>IL1RN, RBP1, RPB2</i>	BW
<i>Development processes</i>				
Regulation of embryonic development	GO:0045995	2	<i>IL1RN, PTK7</i>	BW, TWW
Cardiac ventricle development	GO:0003231	2	<i>PKP2, PTK7</i>	BW, TWW
Cardiac chamber development	GO:0003205	2	<i>PKP2, PTK7</i>	BW, TWW
Anatomical structure development	GO:0048856	6	<i>SIX6, BSPH1, IL1RN, PKP2, PTK7, RBP1</i>	BW, NLB, NLW, TWW
Single-organism developmental process	GO:0032502	6	<i>SIX6, BSPH1, IL1RN, PKP2, PTK7, RBP1</i>	BW, NLB, NLW, TWW
<i>Other processes</i>				
Heterotypic cell-cell adhesion	GO:0034113	2	<i>IL1RN, PKP2</i>	BW
Response to lipid	GO:0033993	3	<i>IL1RN, PTK7, RBP1</i>	BW, TWW
Cell-cell signaling	GO:0007267	3	<i>IL1RN, PTK7, PKP2</i>	BW, TWW
Regulation of hormone levels	GO:0010817	2	<i>IL1RN, RBP1</i>	BW
Molecular function				
<i>Binding</i>				
Binding	GO:0005488	10	<i>LIG1, SIX6, ZFP1, BSPH1, CABP5, LDHD, PTK7, RBP1, RBP2, STK38L</i>	BW, NLB, NLW, TWW
Small molecule binding	GO:0036094	5	<i>LIG1, LDHD, PTK7, RBP2, STK38L</i>	BW, TWW
Cellular component				

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Cell	GO:0005623	14	<i>LIG1, SIX6, TAF4B, ZFP1, BSPH1, CABP5, KLC4, LDHD, PKP2, PTK7, RBP1, STK38L, ZNRF1</i>	BW, NLB, NLW, TWW
Cell-cell junction	GO:0005911	2	<i>PKP2, PTK7</i>	BW, TWW
Cytosol	GO:0005829	3	<i>CABP5, RBP1, ZNRF1</i>	BW

^a BW = body weight; NLB = number of lambs born; NLW = number of lambs weaned; TWW = total weight of lamb weaned

Table 5 Gene ontology categories for genes associated with body weight and reproduction in the Grootfontein Merino population

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Biological process				
<i>Biosynthetic processes</i>				
Biosynthetic process	GO:0009058	6	<i>EYA2, RBMS2, PSAT1, TRPS1, ZFHX3, ZNF704</i>	BW, NLB, TWW
Regulation of biosynthetic process	GO:0009889	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Cellular biosynthetic process	GO:0044249	6	<i>EYA2, RBMS2, PSAT1, TRPS1, ZFHX3, ZNF704</i>	BW, NLB, TWW
Regulation of cellular biosynthetic process	GO:0031326	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Macromolecule biosynthetic process	GO:0009059	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of macromolecule biosynthetic process	GO:0010556	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Cellular macromolecule biosynthetic process	GO:0034645	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of cellular macromolecule biosynthetic process	GO:2000112	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Cellular nitrogen compound biosynthetic process	GO:0044271	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Organic substance biosynthetic process	GO:1901576	6	<i>EYA2, RBMS2, PSAT1, TRPS1, ZFHX3, ZNF704</i>	BW, NLB, TWW
RNA biosynthetic process	GO:0032774	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of RNA biosynthetic process	GO:2001141	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
<i>Transcription</i>				
Nucleic acid-templated transcription	GO:0097659	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of nucleic acid-templated transcription	GO:1903506	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of transcription from RNA polymerase II promoter	GO:0006357	3	<i>TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of transcription, DNA-templated	GO:0006355	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
<i>Metabolic processes</i>				
Nitrogen compound metabolic process	GO:0006807	6	<i>EYA2, RBMS2, PSAT1, TRPS1, ZFHX3, ZNF704</i>	BW, NLB, TWW
Regulation of nitrogen compound metabolic process	GO:0051171	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Regulation of RNA metabolic process	GO:0051252	4	<i>EYA2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
<i>Gene expression</i>				
Gene expression	GO:0010467	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Regulation of gene expression	GO:0010468	5	<i>EYA2, RBMS2, TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Molecular function				
<i>Binding</i>				
Binding	GO:0005488	7	<i>EYA2, RBMS2, PIK3C2A, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLB, NLW, TWW
Cation binding	GO:0043169	5	<i>EYA2, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLW, TWW
DNA binding	GO:0003677	3	<i>TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Ion binding	GO:0043167	5	<i>EYA2, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLW, TWW
Metal ion binding	GO:0046872	5	<i>EYA2, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLW, TWW
Sequence-specific DNA binding	GO:0043565	4	<i>TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Transition metal ion binding	GO:0046914	4	<i>TRPS1, XIRP2, ZFHX3</i>	BW, NLW, TWW
Zinc ion binding	GO:0008270	4	<i>TRPS1, ZFHX3, ZNF704</i>	BW, TWW
Cellular component				
Cytoplasm	GO:0005737	8	<i>EYA2, CAP1, ENKUR, PIK3C2A, PSAT1, THNSL1, XIRP2, ZFHX3</i>	BW, NLB, NLW, TWW
Intracellular	GO:0005622	11	<i>EYA2, CAP1, CEP78, ENKUR, PIK3C2A, PSAT1, THNSL1, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLB, NLW, TWW
Organelle	GO:0043226	12	<i>EYA2, CAP1, CEP78, ENKUR, ITFG1, PIK3C2A, PSAT1, THNSL1, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLB, NLW, TWW
Intracellular organelle	GO:0043229	9	<i>EYA2, CAP1, CEP78, ENKUR, THNSL1, TRPS1, XIRP2, ZFHX3, ZNF704</i>	BW, NLB, NLW, TWW
Intracellular non-membrane-bounded organelle	GO:0043232	5	<i>CAP1, CEP78, TRPS1, XIRP2, ZFHX3</i>	BW, NLB, NLW, TWW
Extracellular organelle	GO:0043230	4	<i>CAP1, ITFG1, PIK3C2A, PSAT1</i>	NLB
Membrane-bounded organelle	GO:0043227	10	<i>EYA2, CAP1, ENKUR, ITFG1, PIK3C2A, PSAT1, THNSL1, TRPS1, ZFHX3, ZNF704</i>	BW, NLB, TWW
Non-membrane-bounded organelle	GO:0043228	5	<i>CAP1, CEP78, TRPS1, XIRP2, ZFHX3</i>	BW, NLB, NLW, TWW
Vesicle	GO:0031982	5	<i>CAP1, ENKUR, ITFG1, PIK3C2A, PSAT1</i>	BW, NLB
Extracellular vesicle	GO:1903561	4	<i>CAP1, ITFG1, PIK3C2A, PSAT1</i>	NLB
Extracellular exosome	GO:0070062	4	<i>CAP1, ITFG1, PIK3C2A, PSAT1</i>	NLB

^a BW = body weight; NLB = number of lambs born; NLW = number of lambs weaned; TWW = total weight of lamb weaned

Table 6 Gene ontology categories for genes associated with body weight and reproduction in the Cradock Merino population

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Biological process				
<i>Homeostasis</i>				
Cellular cation homeostasis	GO:0030003	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
Cellular ion homeostasis	GO:0006873	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
Cation homeostasis	GO:0055080	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
Cellular chemical homeostasis	GO:0055082	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
Inorganic ion homeostasis	GO:0098771	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
Ion homeostasis	GO:0050801	2	<i>SLC4A10, TRPM8</i>	NLB, TWW
<i>Transcription</i>				
Regulation of transcription from RNA polymerase II promoter	GO:0006357	3	<i>HDAC9, THRAP3, ZNF496</i>	BW, NLB, NLW, TWW
Transcription from RNA polymerase II promoter	GO:0006366	3	<i>HDAC9, THRAP3, ZNF496</i>	BW, NLB, NLW, TWW
Negative regulation of transcription from RNA polymerase II promoter	GO:0000122	2	<i>HDAC9, THRAP3</i>	NLB, NLW, TWW
<i>Other processes</i>				
Regulation of biological quality	GO:0065008	4	<i>GRIK3, SLC4A10, THRAP3, TRPM8</i>	NLB, NLW, TWW
Cellular response to hormone stimulus	GO:0032870	2	<i>HDAC9, THRAP3</i>	NLB, NLW, TWW
Response to hormone	GO:0009725	2	<i>HDAC9, THRAP3</i>	NLB, NLW, TWW
Molecular function				
<i>Transporter activity</i>				
Transporter activity	GO:0005215	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Substrate-specific transporter activity	GO:0022857	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Transmembrane transporter activity	GO:0022857	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Ion transmembrane transporter activity	GO:0015075	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Substrate-specific transmembrane transporter activity	GO:0022857	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Passive transmembrane transporter activity	GO:0022803	2	<i>GRIK3, TRPM8</i>	NLB, NLW, TWW
<i>Channel activity</i>				
Channel activity	GO:0015267	2	<i>GRIK3, TRPM8</i>	NLB, NLW, TWW
Ion channel activity	GO:0005216	2	<i>GRIK3, TRPM8</i>	NLB, NLW, TWW

Gene ontology category	GO ID	No of genes	Genes in this category	Associated traits ^a
Cellular components				
Nucleoplasm part	GO:0044451	3	<i>HDAC9, THRAP3, ZNF496</i>	BW, NLB, NLW, TWW
Nuclear body	GO:0016604	2	<i>THRAP3, ZNF496</i>	BW, NLB, NLW, TWW
Plasma membrane part	GO:0044459	3	<i>GRIK3, SLC4A10, TRPM8</i>	NLB, NLW, TWW
Plasma membrane region	GO:0098590	2	<i>GRIK3, SLC4A10</i>	NLB, NLW, TWW

^a BW = body weight; NLB = number of lambs born; NLW = number of lambs weaned; TWW = total weight of lamb weaned

Discussion

Extensive reviews on identified candidate genes associated with different growth and reproduction traits in various sheep breeds published by [7] Xu & Li (2017) and [8] Gebreselassie et al. (2020) confirmed that these genes are distributed throughout the sheep genome. There was limited agreement between the previously reported studies in either the identified genes or the broader genomic regions where the genes were located. This could be ascribed to various factors, including breed differences, long-term selection practices followed in the breeds or flocks, sample sizes, models of analyses applied, levels of significance for identification of significant and suggestive SNPs and distance of significant or suggestive SNPs from the associated gene. For example, [9] Al-mamun et al. (2015) identified 39 SNPs associated with body weight in Australian Merino sheep, with a region on OAR6 containing 13 significant SNPs. Several SNPs related to growth and carcass traits in Scottish Blackface lambs were also reported on OAR6 ([10] Matika et al., 2016). However, no significant or suggestive SNPs were found for body weight or growth traits on OAR6 by [11] Zhang et al. (2013) or [12] Almasi et al. (2020), nor in the populations in the current study.

Many of the genes associated with reproduction in the current study were previously associated in literature with reproductive related traits, as well as with growth related traits in different farm animal species. Such genes associated with reproductive traits in the current study are *MAP7D1*, *TRAPPC3*, *THRAP3*, *SPP2* and *HDAC9* in the Cradock Merino population, *ZFH3* and *PIK3C2A* in the Grootfontein Merino population and *SIX6* in the Afrino population.

Previous literature linked many of the genes associated with body weight in the current study to reproduction traits in farm animal species. Some of these genes were associated with both growth and reproductive related traits in literature. In the case of the Afrino population, all the genes associated with body weight in the current study (except for *LDHD*), were previously related to reproductive traits (*RBP1*, *RBP2*, *PKP2*, *STK38L*, *IL1RN*, *ZNRF1*, *ZFP1*, *BSPH1*, *LIG1*, *CABP5*, *ELSPBP1*). The genes *TRPS1*, *ENKUR*, *EYA2* and *RBMS3* in the Grootfontein Merino population and *ZNF496* in the Cradock Merino population were also previously associated with reproductive traits.

Long-term selection in the flocks for both body weight and reproductive traits, especially the composite trait TWW, could have favoured genes with pleiotropic effects influencing both these traits, with the result that SNPs associated with such pleiotropic genes would be detected in the GWAS for the different traits. Furthermore, genes associated with BW and TWW in the Afrino and Grootfontein Merino populations featured in the same GO biological processes identified for these two populations. This confirms that the same underlying physiological processes are involved in these traits.

Previously published literature linked genes associated with either reproduction or body weight in this study to various reproductive processes from oocyteogenesis through conception, implantation and pregnancy to milk production. Genes differentially expressed in or associated with bovine follicles or oocytes were *MAP7D1* and *ZNF496* [13] (Dickinson, 2016), *TRAPPC3* [14] (Donnison & Pfeffer, 2004), *RBP1* [15] (Mamo et al., 2011), *PKP2* [16] (Franchi et al., 2016) and *STK38L*, *ZFH3*, *ZNRF1* [17] (Hatzirodos et al., 2014). In sheep, *LIG1* and *SPP2* [18] (Smith et al., 2019) were differentially expressed between fetal ovaries of fetuses whose dams

were exposed to either maintenance or restricted nutrition. *GRIK3*, *EYA2* and *BSPH1* were part of a group of genes that were differentially expressed between a subset of Finnsheep and F1 crossbred ewes maintained on a flushing diet [19] (Pokharel et al., 2018). *EYA2* was differentially expressed between uniparous and multiparous goat ovaries [20] (Ling et al., 2015). *MAP7D1* and *TRAPPC3* were associated with NLB and NLW in the Cradock Merino population, while *SPP2* was also associated with NLB, supporting the above findings. In the Grootfontein Merino population, the *ZFH3* gene was linked to TWW. The gene *SIX6* has been noted as a regulator of gonadotropin releasing hormone (GnRH) in cattle [21] (Cánovas et al., 2014) and sheep [22] (Mellon et al., 2018). In the Afrino population, *SIX6* was linked to all three reproductive traits, NLB, NLW and TWW. According to [23] Grive et al. (2014) *TAF4B* is expressed in both somatic and germ cells in the ovary, and possibly play multiple roles in primordial follicle formation. It is also required for the initial establishment of the primordial follicle reserve at birth. Furthermore, infertility in *TAF4B* null female mice was associated with defects in early follicle formation and oocyte maturation [24] (Falender et al., 2005a). One of the gene ontology biological processes of *TAF4B* is “Positive regulation of stem cell proliferation” [25] (<https://david.ncicrf.gov/>), which concurs with the associations found in literature. This gene was associated with TWW in the Afrino population in the current study.

The current study only identified one gene that was associated with conception rate in previous literature. *PKP2* was one of the genes identified in a genome-wide association study that modulate conception rates in cattle [26] (Sugimoto et al., 2013). A range of genes have been identified that is associated with embryonic development in cattle (*PKP2* and *IER5L* – [27] Killeen et al., 2014), sheep (*EYA2* – [28] Ahbara et al., 2019) and mice (*CUL7* – [29] Skaar et al., 2005; *EYA2* – [30] Grifone et al., 2007; *PTK7* – [31] Yen et al., 2009). In the current study, *IER5L* was linked to NLB in the Cradock Merino population, while *PTK7* and *CUL7* was associated with TWW in the Afrino population.

Several of the identified genes in the current study were previously associated with the uterus and placenta, as well as maintenance of pregnancy. *CABP5* (sheep – [32] Burns et al., 2018), *CAP1* (cattle – [33] Fortes et al., 2018), *RBP1* and *RPB2* (pig – [34] Ma et al., 2018) were associated with the uterus, while *CUL7* was involved in mice placental development [35] (Tsunematsu et al., 2006). According to [25] DAVID (<https://david.ncicrf.gov/>), one of the GO biological processes of *CUL7* is “Placental development”. *CABP5* and *ELSPBP1* were part of a list of genes in genomic windows that explained more than 1% of the additive genetic variance for early pregnancy in Nelore heifers [36] (Oliveira Júnior et al., 2017). The *IL1RN* gene was involved in maternal recognition of pregnancy in cattle [37] (Mamo et al., 2012) and early pregnancy in sheep [38] (Pokharel et al., 2020). *PKP2* was upregulated in the endometrium during the preimplantation stage of pregnancy in Finnsheep [38] (Pokharel et al., 2020) and, together with *STK38L*, was expressed in the endometrium of pigs at day 12 of pregnancy [39] (Kim et al., 2012).

Genes previously associated with milk production in cattle were *ITFG1* [40] (Mei et al., 2018), *ZNF496* [41] (Golik et al., 2011) and *TRPS1* [42] (Do et al., 2017), while *ZNRF1* was identified as a candidate gene for milk production in Valle del Belice dairy sheep [43] (Sutera, 2018).

Some of the identified genes in this study were also previously linked to spermatogenesis and male reproductive efficiency. *TAF4B* is important in spermatogonial stem cell maintenance, with high expression in spermatogonia, and some expression in spermatids [44, 45] (Falender et al., 2005b; Cooke et al., 2006). In mice lacking *C14orf39*, a complete arrest of spermatogenesis, as well as reduced testis size were observed [46] (Gómez-H et al., 2016). In the Afrino population, *C14orf39* was associated with all three reproductive traits, NLB, NLW and TWW. *RBMS3* was identified as one of the putative genes related to sperm fertility in Assaf sheep rams [47] (Serrano et al., 2019), while *ELSPBP1* was differentially expressed in rams with high and low sperm motility [48] (Zhu et al., 2020). *ELSPBP1* was associated with dead spermatozoa in cattle [49] (D’Amours et al., 2012) and consequently associated in various studies with spermatozoa in sub-fertile bulls [50, 51] (D’Amours et al., 2010; Kumar et al., 2016). The gene *BSPH1* was indicated as a marker of sperm fertility in mice [52] (Heidari-Vala et al., 2020) and linked to sperm capacitation [53, 54] (Plante & Manjunath, 2015; Vala et al., 2018). Apart from being classified under the GO “Development processes” in the Afrino gene DAVID analysis, “Sperm capacitation” is another GO biological process of the *BSPH1* gene [25] (<https://david.ncicrf.gov/>). The *HDAC9* gene was downregulated in 90-day old male goat kids and this was conducive to the vigorous development of spermatogenesis in this period [55] (Bo et al., 2020). *TRPM8* is a testosterone receptor [56, 57] (Asuthkar et al., 2015a; Asuthkar et al., 2015b) and *TRPM8* channels may be implicated in various physiological processes regulated by androgens [58] (Sutton et al., 2018). *TRPM8* was also part of the genes classified under the molecular function “Channel activity” in the Cradock Merino DAVID gene analysis [25] (<https://david.ncicrf.gov/>).

Several genes identified in the current study were previously linked with body weight and various growth related traits in sheep, cattle and pigs. The *HDAC9* and *EYA2* genes were reported to be involved with myogenesis in these species. *HDAC9* was associated with myogenesis and muscle development in sheep [59] (Cheng et al., 2020), cattle [60] (De Vos, 2018) and pigs [61] (Zhang et al., 2014). The *EYA2* gene was linked to muscle differentiation and development in cattle [62, 63] (Heanue et al., 1999; Hudson et al., 2013), goats [64] (Ling et al., 2019) and pigs [65] (Pérez-Montarelo et al., 2012). *EYA2* was also associated with BW in the Grootfontein Merino population. Downregulated expression of the *HDAC9* gene has been observed in callipyge animals relative to non-callipyge sheep [66] (Vuocolo et al., 2007). Furthermore, *EYA2* was reported to be associated with muscle hypertrophy in goats [64] (Ling et al., 2019).

Some genes were specifically associated with muscle or fat in the live animal. For example, *MAP7D1* (cattle – [67] Sweeney et al., 2016), *SPP2* (sheep – [68, 69] Trukhachev et al., 2016a; Trukhachev et al., 2016b) and *LIG1* (pigs – [70] Da Costa et al., 2004) were associated with or expressed in muscle, while *TRAPPC3* (sheep – [71] González-Calvo et al., 2017), *THRAP3* (cattle – [72] Perez et al., 2010), *PIK3C2A* (pigs – [73] Kim et al., 2015) and *STK38L* (cattle – [74] Lim et al., 2013) were associated with intramuscular or subcutaneous fat. From the genes mentioned here, *LIG1* and *STK38L* were associated with BW in the Afrino population, while *XIRP2* was linked to BW and TWW in the Grootfontein Merino population.

Genes identified in the literature to be generally associated with growth are *ZFHX3* (cattle – [75] Xu et al., 2017; goats – [76, 77] Zhang et al., 2015; Wei et al., 2018), *SIX6* (cattle – [78] Huai et al., 2011; goats – [79] Pan et al., 2011), *EYA2* (cattle – [80] Somavilla, 2015) and *RBMS3* (cattle – [81] Widmann et al., 2013). Genes associated with body weight at specific ages were the *TRPS1* gene which was linked to fetal [82] (Xu et al., 2014) and post weaning weight in sheep [11] (Zhang et al., 2013) and the *HDAC9* and *LIG1* genes which were associated with birth weight in pigs [61] (Zhang et al., 2014) and cattle [83] (Cole et al., 2014) respectively. Most of these genes were associated with BW in the Afrino or Grootfontein Merino populations. *SPP2* was differentially expressed in the loin muscle of Merino sheep with high and low body weight [68, 69] (Trukhachev et al., 2016a; Trukhachev et al., 2016b), while *PIK3C2A* was associated with body weight in pigs [84] (Bovo et al., 2020). *IL1RN* was associated with carcass weight in cattle [85] (Daetwyler et al., 2012). Two of the GO biological processes of *IL1RN* are “Lipid metabolic process” and “Response to glucocorticoid” [25]. This gene was categorised under the “Response to lipid” biological process in the Afrino DAVID gene analysis.

From the results of this study, as well as evidence from other studies performed on sheep and cattle available in literature, some suggestive SNPs and genes with pleiotropic effects were identified that warrant further investigation. The following SNPs were either linked to more than one trait or gene, or the genes associated with these SNPs were previously associated with both reproductive and body weight traits in sheep and cattle. Such a SNP in the Cradock Merino population is OAR1_10554666.1, which is associated with both NLB and NLW and the genes *MAP7D1*, *TRAPPC3* and *THRAP3*. *MAP7D1* is expressed in bovine oocytes [13] (Dickinson, 2016) and bovine muscle [67] (Sweeney et al., 2016), *TRAPPC3* in bovine oocytes [14] (Donnison & Pfeffer, 2004) and subcutaneous fat of lambs [71] (González-Calvo et al., 2017), while *THRAP3* is involved in the fat profile of bovine muscle [72] (Perez et al., 2010). SNP s20120.1 on OAR1 is associated with NLB and the genes *TRMP8*, a testosterone receptor [58] (Sutton et al., 2018), and *SPP2*, previously linked with body weight and expressed in ovine muscle [68, 69] (Trukhachev et al., 2016a; Trukhachev et al., 2016b) and sheep fetal ovaries [18] (Smith et al., 2019). The *HDAC9* gene (SNP OAR4_28811142.1), associated with NLB, NLW and TWW in the Cradock Merino population, was previously linked to spermatogenesis in goats [55] (Bo et al., 2020). This gene is involved in myogenesis and muscle development in sheep, cattle and pigs, was observed in callipyge sheep [66] (Vuocolo et al., 2007) and was associated with birth weight in pigs [61] (Zhang et al., 2014).

ZFHX3 linked to OAR14_39202046.1 and associated with TWW, was expressed in bovine follicles [17] (Hatzirodos et al., 2014) and previously linked to growth in cattle [75] (Xu et al., 2017) and goats [77] (Wei et al., 2018). SNP OAR7_76295917.1 in the Afrino population was associated with NLB, NLW and TWW, as well as with two genes, *SIX6* and *C14orf39*. *SIX6* is a regulator of GnRH in cattle and sheep [21, 22] (Cánovas et al., 2014; Mellon et al., 2018) and involved in puberty [86] (Fortes et al., 2016) and growth [78] (Huai et al., 2011) in cattle. *C14orf39* was previously linked to spermatogenesis [46] (Gómez-H et al., 2016). The *TAF4B* (SNP OAR23_32551191.1) gene warrants further investigation due to its association with TWW in the Afrino population and evidence from literature linking it to primordial follicle formation [23] (Grive et al., 2014) and sperm stem cell maintenance [45] (Cooke et al., 2006).

SNPs associated with body weight in the current study are OAR9_64654880.1, OAR13_80729511.1 and OAR19_4811675.1 in the Grootfontein Merino population and OAR3_201667351.1 and OAR14_56900862.1 in the Afrino population. The gene *TRSP1* (SNP OAR9_64654880.1) was previously associated with milk production in cattle [42] (Do et al., 2017) and fetal and post weaning weight in sheep [11, 82] (Zhang et al., 2013; Xu et al., 2014). SNP OAR13_80729511.1 might be an important marker, due to its associated gene, *EYA2* being involved with various reproductive and growth traits. *EYA2* is expressed in the ovaries of sheep [19] (Pokharel et al., 2018) and goats [20] (Ling et al., 2015) and involved in embryonic development in sheep [28] (Ahbara et al., 2019). It is also linked to myogenesis [62] (Heanue et al., 1999) and growth [80] (Somavilla, 2015) in cattle. Another gene associated with body weight in the Grootfontein Merino population, *RBMS3* (SNP OAR19_4811675.1), was also previously linked to growth in cattle [81] (Widmann et al., 2013) and to sperm fertility in sheep [47] (Serrano et al., 2019). *STL38L* (SNP OAR3_201667351.1) in the Afrino population was associated with marbling score in cattle [74] (Lim et al., 2013) and expressed in bovine follicles [17] (Hatziriodos et al., 2014) and the porcine endometrium [39] (Kim et al., 2012). The most important SNP associated with body weight in the Afrino population was OAR14_56900862.1, linked to four genes (*BSPH1*, *LIG1*, *CABP5*, *ELSPBP1*). These genes were associated with sperm fertility in sheep and cattle and pregnancy in sheep and cattle, expressed in sheep ovaries and muscle and involved in birth weight of pigs.

Conclusions

In the current GWAS, 16 SNP markers associated with the reproductive traits were identified among the three populations, while 15 SNPs were associated with body weight. These SNPs were respectively linked to 26 and 21 documented genes in the sheep genome. These genes were previously reported to be associated with reproductive related, as well as with growth related traits in different farm animal species. Continuous long-term selection for both body weight and reproductive traits in these flocks have thus favoured pleiotropic genes influencing both these traits, with the result that SNPs associated with such genes were detected in the association analyses for both groups of traits. Selection for the composite trait TWW most probably especially favoured genes with pleiotropic effects on growth and reproduction.

From the results of this study, supported by other studies performed on sheep and cattle, some genes were identified that warrant further investigation as to their functions and processes relating to growth and reproduction in sheep. These include the genes *MAP7D1*, *TRAPPC3*, *THRAP3*, *TRMP8*, *SPP2*, *HDAC9*, *ZFH3*, *SIX6*, *C14orf39*, *TAF4B*, *TRSP1*, *EYA2*, *RBMS3*, *STL38L*, *BSPH1*, *LIG1*, *CABP5* and *ELSPBP1*. Further investigation into these results are necessary, maybe with the inclusion of flocks with different long-term selection objectives and criteria regarding reproduction and body weight, to substantiate these findings. Results will elucidate the metabolic pathways underlying economically important traits, and shed light on the impact of current selection strategies on a genetic level.

Methods

Animals and data

Phenotypic data, pedigrees and genotypes available on animals of the Afrino flock at the Carnarvon Experimental Station, the fine wool Merino stud at Cradock Experimental Station and the Merino stud at Grootfontein Agricultural Development Institute (GADI) were used in this study. These resources were obtained from the GADI-biobank.

The Carnarvon Afrino flock is kept under natural veld conditions at the Carnarvon Departmental Experimental Station (30° 59' S, 22° 9' E) near Carnarvon in the north-western Karoo region of South Africa. The Grootfontein Merino stud is kept at Grootfontein Agricultural Development Institute near Middelburg (31° 28' S, 25° 1' E) in the Eastern Cape Province, while the Cradock fine wool Merino stud is run on irrigated pastures at the Cradock Experimental Station near Cradock (32° 13' S, 28° 41' E) in the Eastern Cape Province.

Selection practices followed in the three flocks since their establishment were discussed in detail by [87] Süllwald (2020). The Afrino flock has been selected for reproductive traits and body weight since breed development started in 1969 at the Carnarvon Experimental Station. Since 1985, ewes were selected on total weight of lamb weaned, and body weight was one of the main selection objectives of ram selection.

The Grootfontein Merino stud was established at the Grootfontein Agricultural Development Institute in 1955. During the early years, selection was based on subjectively assessed traits. From 1985 onwards the main selection objectives were amended to increase body weight, maintain clean fleece weight and decrease mean fibre diameter and pleat score, while the ewes were selected on total weight of lamb weaned. In 2004, selection objectives were changed, and breeding sires and dams were selected on the basis of the national selection index for relative economical value including reproduction [1] (Herselman & Olivier, 2010).

The Cradock Merino flock was established in 1988 as a genetic fine wool Merino stud at the Cradock Experimental Station. Initially, selection was aimed at improving body weight and maintaining fibre diameter. Until 1999, no selection for reproduction was carried out, while ewes with poor reproductive performance were culled since 2000. As for the Grootfontein Merino flock, selection objectives were changed in 2004 and selection was done on the basis of the national selection index for relative economical value including reproduction [1].

Phenotypic traits included in the study were body weight recorded at selection age at 14 months of age (BW), lifetime number of lambs born (NLB), lifetime number of lambs weaned (NLW) and lifetime total weight of lamb weaned (TWW). Estimated breeding values (EBV) of all traits for the individual animals were obtained as back solutions with the ASReml program [88] (Gilmour *et al.*, 2014). Animal models including direct and maternal additive genetic random effects were fitted for body weight, while only a random direct genetic effect was fitted for the reproductive traits.

For each flock, animals with a range of high and low EBVs for all traits were selected amongst the animals with available genotypes in the GADI-Biobank. Genotypic data were obtained with the Illumina® Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA). A total of 411 genotypes, comprising 152 Afrino, 129 Cradock Merino and 130 Grootfontein Merino animals, were included.

Genomic analyses

Quality control

The individual genomic datasets for each population were updated with Oar v4.0 SNP Chimp that was downloaded from the SNPchip v.3 database [89] (Nicolazzi *et al.*, 2015). Only the 26 autosomal chromosome pairs were used for downstream data analysis. Each dataset's information was updated for individual identification number, breed, parentage and sex using PLINK v1.07 software [90] (Purcell, 2017). Individual and marker-based quality control (QC) measures were performed on each individual dataset using PLINK v1.07 software [90] (Purcell, 2017). All non-informative SNPs and individuals with missing genotypes were removed at the following parameters: individual call rate of below 90%, a SNP call rate lower than 95%, minor allele frequency of less than 0.02 (MAF <2%) and violation of Hardy-Weinberg equilibrium ($p < 0.001$). Five animals, three from the Grootfontein Merino dataset, and one each from the Afrino and Cradock Merino datasets, were excluded from downstream analyses. The total number of SNPs retained was 42117 for the Afrino, 46196 for the Cradock Merino and 43655 for the Grootfontein Merino datasets.

Principal component analysis (PCA)

Principal Component Analysis was performed to investigate the genetic relatedness of individuals within and between the populations using the merged dataset comprising all three populations. The genomic relationship matrix and estimated principal components were generated with the use of the Genome-wide complex trait analysis v1.24 (GCTA) software [91] (Yang *et al.*, 2011).

Genome-wide association study (GWAS)

The Afrino, Cradock Merino and Grootfontein Merino datasets were analysed separately for each population and each trait, using the software, efficient mixed model association eXpedited (EMMAX) [92] (Kang *et al.*, 2010). EMMAX software was favoured for the analysis as it controls genome-wide error rate successfully compared to other genomic software and it is more suited to calculate the kinship matrix for smaller populations [93] (Eu-ahsunthornwattana *et al.*, 2014).

Results from the association analyses were visualised by creating manhattan plots in R-studio [94] (R Core Team, 2017). Quantile-quantile (Q-Q) plots for each GWAS were also created in R-studio to indicate any deviation from the distribution under the null hypothesis of no association. From the results of the association analyses significant ($p < 10^{-7}$) and suggestive ($p < 10^{-4}$) SNP

markers were identified on a chromosome-wide level and investigated further on a molecular level. Each SNP marker's reference SNP name and position were identified from the database Oar v4.0 SNP Chimp that was downloaded from the SNPchiMp v.3 database [89] (Nicolazzi et al., 2015). SNP effects were determined by including all suggestive SNPs for each trait per population in a stepwise regression analysis.

Gene ontology

The Ensembl database [95,96] (Zerbino et al., 2016; Hunt et al., 2018) was used to identify genes that are linked to or in close proximity to the respective SNP markers. To determine the functions of and possible relationships between the identified genes, the genes for each population were analysed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) platform [97,25] (Dennis et al., 2003; <https://david.ncifcrf.gov/>), with the sheep genome OAR_v4, to categorise the genes in the three gene ontology (GO) categories: biological process, molecular function and cellular component.

Declarations

Ethics approval

Samples for genotyping were collected under approval numbers AP10/3/3 and AP10/3/4 of the Animal Research Ethics Committee of the Grootfontein Agricultural Development Institute. Approval for the use of external data was granted by the ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (NAS125/2019). I hereby confirm the study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the current study are not publicly available due it being part of the Grootfontein Biobank resources. The datasets used are, however, available from the corresponding author on reasonable request for specific projects.

Competing interests

The authors declare that they have no competing interests.

Funding

Funding for some of the genotypes was provided by Cape Wools South Africa under project AP1/17/4.

Authors' contributions

MAS - Concept of the study, assist with writing of project proposal, assist with interpretation of results, co-supervisor of the study, writing of the manuscript

SS - Writing of project proposal, analysis of data, interpretation of data for purpose of MSc thesis, assist with writing of the manuscript

WJO - Assist with writing of the manuscript

CV - Concept of the study, assist with writing of project proposal, assist with interpretation of results, supervisor of the study, assist with writing of the manuscript

All the authors have read and approved the final manuscript.

Acknowledgements

The GADI-Biobank, the Eastern Cape Department of Rural Development and Agrarian Reform and the Northern Cape Department of Agriculture, Land Reform and Rural Development are acknowledged for the use of the resources.

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Figures

Figure 1

Genetic relationships among the animals in the three populations for the first and second principal components

Figure 2

Manhattan plots of $-\log_{10}(p\text{-values})$ for body weight in the Afrino (A), Grootfontein Merino (C) and Cradock Merino (E) populations. The red line corresponds to a significant chromosome-wide threshold of 10^{-7} , while the blue line corresponds to a suggestive chromosome-wide threshold of 10^{-4} . Quantile-quantile plot for $-\log_{10}(p\text{-values})$ for the association analysis for body weight in Afrino (B), Grootfontein Merino (D) and Cradock Merino (F) populations. Green dots represent the $-\log_{10}(p\text{-value})$ of this analysis and the red line represents the expected values under the null hypothesis of no association

Figure 3

Manhattan plots of $-\log_{10}(p\text{-values})$ for number of lambs born in the Afrino (A), Grootfontein Merino (C) and Cradock Merino (E) populations. The red line corresponds to a significant chromosome-wide threshold of 10^{-7} , while the blue line corresponds to a suggestive chromosome-wide threshold of 10^{-4} . Quantile-quantile plot for $-\log_{10}(p\text{-values})$ for the association analysis for number of lambs born in Afrino (B), Grootfontein Merino (D) and Cradock Merino (F) populations. Green dots represent the $-\log_{10}(p\text{-value})$ of this analysis and the red line represents the expected values under the null hypothesis of no association

Figure 4

Manhattan plots of $-\log_{10}(p\text{-values})$ for number of lambs weaned in the Afrino (A), Grootfontein Merino (C) and Cradock Merino (E) populations. The red line corresponds to a significant chromosome-wide threshold of 10^{-7} , while the blue line corresponds to a suggestive chromosome-wide threshold of 10^{-4} . Quantile-quantile plot for $-\log_{10}(p\text{-values})$ for the association analysis for number of lambs weaned in Afrino (B), Grootfontein Merino (D) and Cradock Merino (F) populations. Green dots represent the $-\log_{10}(p\text{-value})$ of this analysis and the red line represents the expected values under the null hypothesis of no association

Figure 5

Manhattan plots of $-\log_{10}(p\text{-values})$ for total weight of lambs weaned in the Afrino (A), Grootfontein Merino (C) and Cradock Merino (E) populations. The red line corresponds to a significant chromosome-wide threshold of 10^{-7} , while the blue line corresponds to a suggestive chromosome-wide threshold of 10^{-4} . Quantile-quantile plot for $-\log_{10}(p\text{-values})$ for the association analysis for total weight of lambs weaned in Afrino (B), Grootfontein Merino (D) and Cradock Merino (F) populations. Green dots represent the $-\log_{10}(p\text{-value})$ of this analysis and the red line represents the expected values under the null hypothesis of no association