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Article

Study of the Association Between SNPs and External Pelvimetry Measurements in Simmental Cows

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Simple Summary: This study explores how single nucleotide polymorphisms (SNPs) are associated with pelvic conformation in Simmental cows. By examining 33 SNPs located near important genes, the study aims to understand how these genetic variations affect the shape and size of the pelvic bones, including traits such as croup height (CH), buttock height (BH), croup width (CW), rump angle (RA) and croup length (CL). The results proven that certain genetic variants may influence these traits, which could help improve selection strategies for better reproductive performance in cattle.

Abstract: The evaluation of external pelvimetry measurements and the genetic factors influencing them is essential for improving morphological characteristics and reproductive performance in cattle. This study investigates the relationship between single nucleotide polymorphisms (SNPs) and external pelvimetry measurements in Simmental cows, considering traits such as croup height (CH), buttock height (BH), croup width (CW), rump angle (RA) and croup length (CL). A total of 33 SNPs, located on multiple chromosomes, were identified near relevant genes such as *CLSTN2*, *DPYD*, *FBXL7*, *FBXL13*, *SEMA6A*, *RUNX2*, *FSTL4*, *DST*, *DCBLD2*, *FRMD6*, *CAV2.3*, *ABL2*, *SH3BP4*, *RSBN1L*, and *SAMD12*, suggesting that these genetic variants may influence the development and morphology of the pelvic bones. Statistical analysis revealed significant relationships between certain allele variants and croup measurements, highlighting that the presence of alternative alleles can modify their morphological traits.

Keywords: SNPs; external pelvimetry; reproductive traits; Simmental cows

1. Introduction

Single nucleotide polymorphisms (SNPs) represent the simplest form of genetic variation between individuals. These variations can be classified as transitions or transversions and occur in the genome at a frequency of approximately one in every 1,000 base pairs. SNPs play a significant role in the diversity observed between individuals and in genome evolution. They can affect promoter activity impacting gene expression, the stability of messenger RNA (mRNA) conformations, and the subcellular localization of RNA and/or proteins, potentially contributing to the onset of various disorders [1].

SNP genotyping technologies are important in breeding programs because they allow the selection of the best animals based on genetic information. In addition, detailed genetic maps based on the use of SNPs are useful for understanding the genetic variations associated with a specific phenotype. Genome-wide association studies (GWAS) represent an essential method for identifying genetic variants associated with complex traits in different cattle breeds [2,3].

This study uses an innovative design by integrating advanced genotyping technologies, focusing on identifying SNPs associated with external pelvimetry traits in a large population of Simmental cows [4]. A central reason for this research is the impact of pelvic conformation on fertility and reproductive performance, particularly on calving ease, calf survival at birth, and cow recovery after calving. External pelvimetry traits are known as factors that influence the size of the birth canal and, subsequently, calving difficulty.

In this study, we aimed to highlight the relationship between genetic variability and pelvic conformation in Simmental cows. Using association study on genes previously identified as being involved in pelvic conformation, we focused on identifying genetic variants associated with relevant pelvic traits, such as croup width, length, tilt, and rump height, which directly impact the size of the birth canal and, consequently, calving difficulty.

2. Materials and Methods

Animals and Phenotypic Records

During 2023-2024, 152 Simmental cows were measured for external pelvimetry and selected based on the availability of reproductive history and genetic assessments at the Research and Development Station for Bovine – Arad, Romania. All cattle involved in the study were kept in the same housing and feeding conditions and were included in the Official Recording of Milk Production in Romania.

Phenotypic data consisted of 760 measurements. To obtain phenotypic values representing essential points of external pelvimetry (croup height, buttock height, croup length, croup width, and rump angle), we used the Martin pelvimeter, a zoometric rod, and a laser level. The value of the croup height was determined by measuring the distance between the dorsal part of the croup and the floor. For buttock height, the distance between the ischial tuberosity and the floor was measured. The croup length was measured from the iliac tuberosity to the ischial tuberosity. The croup width was measured as the distance between the right and left iliac tuberosities. The rump angle was determined by measuring the angle between the coxal tuberosity and the ischial tuberosity. The laser level shows an angle that indicates the croup's inclination. If the croup is completely horizontal, the angle will be 0°. If the croup is inclined upwards, the angle will be positive, and if it is inclined downwards, the angle will be negative.

Sampling and Genotyping

Biological samples needed for genotyping consisted of whole blood collected by trained veterinarians. The blood samples (n = 152) were extracted from the tail vein in vacutainers containing K3EDTA as an anticoagulant. After collection, the samples were stored in the refrigerator at 4°C prior to sending them to the IFN Schönow GmbH (Bernau bei Berlin, Germany) for DNA extraction and genotyping using an Axiom Bovine v3 microarray (Thermo Fisher Scientific). The used microarray was based on the reference genome Bos_taurus_UMD_3.1.1. Next [5], the data quality control step was performed with the aim of removing the all SNPs (call rates <95%) and animals (call rates <95%) with insufficient genotyping quality. SNPs with minor allele frequency (MAF) < 0.05 and those with genotypes not in accordance with the Hardy–Weinberg equilibrium were eliminated using Plink v1.90b6.21 [6] and the remaining missing genotypes were imputed with BEAGLE v. 5.2 [7].

Investigated Genes and SNPs

A total of 48 genes previously identified as being involved in pelvic conformation were selected across the cattle genome. Based on Axiom Bovine v3 microarray we selected 110 SNPs belong to the 48 genes associated with pelvic conformation. In this study, the term “near” is used to describe the location of SNPs in relation to genes, referring specifically to their presence in introns, exons, or upstream and downstream regions. Details regarding the SNP probe set ID, gene symbol,

chromosome position based on the UMD_3.1.1 genome assembly of *Bos Taurus* [5], alleles and SNP rsID are presented in Table 1.

Table 1. Details of genes, chromosome location and genomic location of the studied SNPs.

SNP Probe Set ID	Gene symbol	Chr	Position ¹	Allele A	Allele B	SNP rsID
AX-106734868	<i>MYH15</i>	1	53551656	T	C	-
AX-106732173	<i>MYH15</i>	1	53582877	A	G	-
AX-106720648	<i>MYH15</i>	1	53630189	T	G	-
AX-106750397	<i>MYH15</i>	1	53656600	A	G	-
AX-124378375	<i>MYH15</i>	1	53679183	T	C	-
AX-106735753	<i>DCBLD2</i>	1	42749580	T	C	-
AX-106742670	<i>DCBLD2</i>	1	42771916	T	C	-
AX-115103182	<i>DCBLD2</i>	1	42799445	T	C	rs43229755
AX-106750655	<i>CLSTN2</i>	1	129388513	T	C	rs110780956
AX-106723587	<i>CLSTN2</i>	1	129424205	T	G	rs43263856
AX-106756021	<i>CLSTN2</i>	1	129505485	T	C	-
AX-106723593	<i>ATG4C</i>	3	83306806	T	G	-
AX-106733516	<i>SH3BP4</i>	3	115210793	T	G	-
AX-106751591	<i>DPYD</i>	3	45826189	A	G	-
AX-185119475	<i>DPYD</i>	3	45880538	A	G	rs378500362
AX-106734473	<i>DPYD</i>	3	45996935	T	C	rs43712282
AX-124348371	<i>DPYD</i>	3	46086314	T	G	rs109495108
AX-106735685	<i>DPYD</i>	3	46134907	T	C	rs110776492
AX-106762083	<i>DPYD</i>	3	46318628	A	C	rs109580467
AX-185119477	<i>DPYD</i>	3	46405331	A	G	rs378406199
AX-106762436	<i>DPYD</i>	3	46410484	A	G	rs110001765
AX-115104470	<i>PTPN12</i>	4	43860090	T	G	-
AX-106733848	<i>RSBN1L</i>	4	43710013	T	C	rs110163880
AX-124375871	<i>RSBN1L</i>	4	43763107	A	G	rs29025758
AX-106747158	<i>CCDC146</i>	4	44177870	A	G	-
AX-106757570	<i>FAM185A</i>	4	44359029	T	C	-
AX-171444094	<i>FBXL13</i>	4	44406478	T	C	rs108967918
AX-106736322	<i>FBXL13</i>	4	44469848	A	G	rs43388331
AX-106729600	<i>FBXL13</i>	4	44505761	T	C	-
AX-106763743	<i>FBXL13</i>	4	44542142	T	C	rs42765191
AX-106729600	<i>LRRC17</i>	4	44505761	T	C	-
AX-115104470	<i>PTPN12</i>	4	43860090	T	G	-
AX-106747158	<i>CCDC146</i>	4	44177870	A	G	-
AX-106724406	<i>ARMC10</i>	4	44612810	A	C	rs42767622
AX-106754981	<i>NAPEPLD</i>	4	44671762	A	G	-
AX-106721536	<i>NAPEPLD</i>	4	44694369	A	G	-
AX-106757570	<i>FAM185A</i>	4	44359029	T	C	-
AX-106733848	<i>RSBN1L</i>	4	43710013	T	C	rs110163880
AX-124375871	<i>RSBN1L</i>	4	43763107	A	G	rs29025758

SNP Probe Set ID	Gene symbol	Chr	Position ¹	Allele A	Allele B	SNP rsID
AX-171444094	<i>FBXL13</i>	4	44406478	T	C	rs108967918
AX-106736322	<i>FBXL13</i>	4	44469848	A	G	rs43388331
AX-106729600	<i>FBXL13</i>	4	44505761	T	C	-
AX-106763743	<i>FBXL13</i>	4	44542142	T	C	rs42765191
AX-106729600	<i>LRRC17</i>	4	44505761	T	C	-
AX-106725313	<i>COL1A2</i>	4	11649948	A	G	rs110913617
AX-106747250	<i>CCND2</i>	5	106269362	T	C	rs110421124
AX-106754519	<i>DMP1</i>	6	104305915	A	C	-
AX-115113278	<i>LCORL</i>	6	38845992	A	C	rs110961068
AX-106731669	<i>LCORL</i>	6	38869785	T	C	rs109294917
AX-106754365	<i>CHSY3</i>	7	25210041	A	G	rs41592021
AX-106734626	<i>CHSY3</i>	7	25420525	C	G	rs41657989
AX-106753436	<i>SEMA6A</i>	7	38780609	A	C	-
AX-106727722	<i>FSTL4</i>	7	46477516	T	C	-
AX-185104904	<i>FSTL4</i>	7	46493107	A	G	rs382678897
AX-106722191	<i>EPHX2</i>	8	75919385	A	G	rs43436859
AX-106749022	<i>STMN4</i>	8	75650021	A	G	-
AX-106758983	<i>CHRNA2</i>	8	75874902	A	G	rs110370186
AX-106738750	<i>SMU1</i>	8	76152259	T	C	rs110061505
AX-106721012	<i>GULO</i>	8	76005495	A	G	-
AX-169380681	<i>RFX6</i>	9	34163790	A	G	rs135889862
AX-106741677	<i>RFX6</i>	9	34176604	A	G	rs109252847
AX-115106312	<i>KPNA5</i>	9	34317516	A	G	-
AX-124377038	<i>NEPN</i>	9	33618865	A	G	rs41656796
AX-185123100	<i>ROSI</i>	9	33818808	A	G	rs379828862
AX-117083515	<i>FRMD6</i>	10	44362494	T	C	-
AX-185110014	<i>FRMD6</i>	10	44369986	T	C	rs41712319
AX-124386523	<i>FRMD6</i>	10	44417569	A	G	rs41624350
AX-124381785	<i>FRMD6</i>	10	44445350	T	C	rs42444775
AX-106720769	<i>FRMD6</i>	10	44481175	A	G	rs42138737
AX-106732937	<i>FRMD6</i>	10	44521573	T	G	rs42138402
AX-106737920	<i>FRMD6</i>	10	44556318	A	G	-
AX-106763243	<i>FRMD6</i>	10	44601357	C	G	rs29026819
AX-115104960	<i>TNFRSF11B</i>	14	47438919	T	C	-
AX-106757434	<i>MAL2</i>	14	47141282	A	G	rs41255250
AX-115107525	<i>COLEC10</i>	14	47267133	A	G	-
AX-169390172	<i>SAMD12</i>	14	47826228	A	G	rs110259776
AX-106736159	<i>SAMD12</i>	14	47963739	A	G	rs41735513
AX-124375254	<i>SAMD12</i>	14	47996787	T	C	rs29024078
AX-106764082	<i>SAMD12</i>	14	48021988	A	G	rs41630560
AX-106724218	<i>SAMD12</i>	14	48052916	T	C	rs108968111
AX-117082755	<i>SAMD12</i>	14	48145874	T	C	rs41603886
AX-185112171	<i>SAMD12</i>	14	48191019	A	G	rs110078673

SNP Probe Set ID	Gene symbol	Chr	Position ¹	Allele A	Allele B	SNP rsID
AX-106724034	<i>CACNA1E/ CAV 2.3</i>	16	64116934	T	C	rs42663551
AX-124374736	<i>CACNA1E/ CAV 2.3</i>	16	64166847	T	G	-
AX-106762254	<i>CACNA1E/ CAV 2.3</i>	16	64194978	A	G	-
AX-28511035	<i>CACNA1E/ CAV 2.3</i>	16	64246344	A	G	rs109662586
AX-106737399	<i>CACNA1E/ CAV 2.3</i>	16	64359255	A	G	-
AX-106752137	<i>CACNA1E/ CAV 2.3</i>	16	64387234	T	C	rs110799337
AX-106742186	<i>ABL2</i>	16	61960715	A	G	-
AX-117089449	<i>RYR1</i>	18	48538054	A	G	-
AX-106742061	<i>COL1A1</i>	19	37099312	T	C	rs110819475
AX-106731384	<i>FBXL7</i>	20	57301571	A	G	-
AX-124386000	<i>MEF2A</i>	21	7145068	A	G	-
AX-124350023	<i>CNTN4</i>	22	23365188	A	C	rs110926315
AX-106747756	<i>CNTN4</i>	22	23390508	A	G	-
AX-106757157	<i>CNTN4</i>	22	23458863	T	C	-
AX-106750212	<i>CNTN4</i>	22	23502867	A	C	-
AX-106735713	<i>CNTN4</i>	22	23554117	T	C	rs110466322
AX-106742630	<i>CNTN6</i>	22	25056533	T	G	rs41583553
AX-124375974	<i>CNTN6</i>	22	25076967	T	C	rs110331907
AX-106746728	<i>CNTN6</i>	22	25113789	T	G	-
AX-185116096	<i>CNTN6</i>	22	25215190	A	G	rs209258798
AX-106753232	<i>LMOD3</i>	22	32538832	A	T	rs41642469
AX-106726459	<i>DST</i>	23	3468292	T	G	rs109673019
AX-117088037	<i>DST</i>	23	3581582	T	C	-
AX-124384326	<i>RUNX2</i>	23	18695002	T	C	-
AX-106761391	<i>RUNX2</i>	23	18715079	A	G	-
AX-106741590	<i>RUNX2</i>	23	18764200	T	C	-
AX-106739513	<i>CDH7</i>	24	11204777	T	C	rs110351082
AX-115118013	<i>THRB</i>	27	41760090	A	G	rs110994656

¹ Position based on the UMD_3.1.1 genome assembly of *Bos taurus*. Chr: chromosome [5].

Association Analysis

Statistical association analyses were performed using the R programming language v. 4.3.3 [8]. Descriptive statistics were collected using the psych package v. 2.4.12 [9]. Association tests were performed via linear regression models with each pelvimetric trait as dependent variables, the genotypes as independent variables and the first 5 principal components included as covariates, to account for population structure effects. Results were deemed statistically significant at a p-value threshold of 0.05.

3. Results

3.1. Value and Variability of Pelvic Measurements in Simmental Cows

For the females in this population of Simmental cows, the following results were obtained for the external pelvimetric measurements: the average croup height was 143.73 cm ± 0.30, with an almost symmetric distribution and moderate variability. The average buttock height was 126.47 cm ± 0.31, while the croup length was 54.25 cm ± 0.16, with all these traits showing small to moderate variability.

The average croup inclination was $-0.24^{\circ} \pm 0.18$, indicating a slightly inclined croup, and the croup width was $56.68 \text{ cm} \pm 0.19$.

Table 1. Descriptive analysis of external pelvimetry measurements.

Variable	N	Mean	SD	Median	Min	Max	Range	Skewness	Kurtosis	SE
Croup height (CH)	152	143.73	3.73	144	134	152.0	18.0	-0.28	-0.46	0.30
Buttock height (BH)	152	126.47	3.80	126	117	137.0	21.0	-0.01	-0.08	0.31
Rump angle (RA)	152	-0.24	2.17	0	-6	5.5	11.5	0.09	-0.23	0.18
Croup length (CL)	152	54.25	1.92	54	50	58.0	8.0	-0.07	-0.63	0.16
Croup width (CW)	152	56.68	2.35	57	50	62.0	12.0	-0.18	0.02	0.19

Legend: N - number of cases; SD- standard deviation; Range - the difference between the maximum and minimum values in the dataset (Max - Min); Skewness - measures the asymmetry of the data distribution. If the skewness is positive, the distribution has a long right tail (positive skew); if it's negative, the distribution has a long left tail (negative skew); Kurtosis - measures the "tailedness" of the data distribution; high kurtosis indicates heavy tails and more outliers, while low kurtosis suggests a distribution with lighter tails; SE- standard error.

3.2. Analysis of SNPs Associated with Croup Height (CH)

Through genomic analysis, 7 SNPs significantly associated with CH were identified in Simmental cows. The identified genetic markers are located on chromosomes 1, 3, 4, 7, and 14, suggesting that they may influence the morphological development of the pelvis.

On chromosome 1, two SNPs (AX-106723587 and AX-106750655) were identified near the CLSTN2 gene. For the SNP AX-106723587, the reference allele is T, and the alternative allele is G. When comparing homozygotes (TT) with heterozygotes (TG), a decrease in CH was observed with an estimated value of -5.75 ($p=0.0228$) (Table 1). Additionally, the difference observed between homozygotes with the TT genotype and those with the GG genotype was -5.33 ($p=0.0468$).

A third SNP located on chromosome 1, AX-106750655, shows a positive effect on CH, with an estimated value of $+1.83$ ($p=0.0269$). This suggests that heterozygotes (TC) may exhibit a greater CH compared to homozygotes (CC).

On chromosome 4, the SNP AX-106763743 was identified near the FBXL13 gene. This SNP is associated ($+1.52$ ($p=0.0489$)) with CH, suggesting a possible role for the FBXL13 gene in the growth and development of pelvic bones.

The SNP AX-106753436 is located on chromosome 7 near the SEMA6A gene. The estimated value of -3.82 indicates a negative association between the alternative allele (A) and CH, and the p-value of 0.0487 confirms the statistical significance of this result.

Table 2. SNP localization on chromosomes and allele effects on croup height (CH).

SNP	Chr	Reference allele	Alternative allele	Contrast	Estimated value	p-value	Gene
AX-106723587	1	T	G	SNP0 - SNP1	-5,74	0,0227	CLSTN2
AX-106723587	1	T	G	SNP0 - SNP2	-5,33	0,0467	CLSTN2
AX-106750655	1	T	C	SNP1 - SNP2	1,83	0,0269	CLSTN2
AX-185119475	3	A	G	SNP0 - SNP1	-2,86	0,0094	DPYD
AX-106763743	4	C	T	SNP1 - SNP2	1,51	0,0489	FBXL13
AX-106753436	7	C	A	SNP0 - SNP2	-3,82	0,0487	SEMA6A
AX-106724218	14	C	T	SNP0 - SNP2	-3,87	0,0031	SAMD12
AX-106724218	14	C	T	SNP0 - SNP1	-3,77	0,0019	SAMD12
AX-117082755	14	C	T	SNP0 - SNP1	-2,56	0,0261	SAMD12
AX-117082755	14	C	T	SNP0 - SNP2	-3,31	0,0060	SAMD12

The results for SNP AX-106724218, located on SAMD12 gene, suggest a clear association between the presence of the T allele and a decrease in CH. Homozygous individuals for the alternative allele (TT) exhibited a significant reduction in this phenotype (-3.87, p=0.0031) compared to homozygous individuals for the reference allele (CC).

For SNP AX-117082755, located near to SAMD12 gene, the analysis confirms the influence of the T allele on this phenotype. The estimated difference between individuals with the CC and TT genotypes was -3.31 (p=0.0060), highlighting a similar trend of reduction in CH.

3.3. Analysis of SNPs Associated with Buttock Height (BH)

In the analysis, 6 SNPs associated with BH were identified, located on chromosomes 3, 4, 14, and 20.

SNP AX-106733516, located on chromosome 3, near the SH3BP4 gene, shows a significant association with BH. The estimated value recorded (-3.08, p=0.0390) suggests that the presence of the G allele contributes to a decrease in BH compared to the T allele.

Table 3. SNP localization on chromosomes and allele effects on buttock height (BH).

SNP	Chr	Reference allele	Alternative allele	Contrast	Estimated value	p-value	Gene
AX-106733516	1	T	G	SNP0-SNP2	-3,07	0,0389	SH3BP4
AX-106736322	1	A	G	SNP0-SNP2	-2,70	0,0148	FBXL13
AX-124375871	1	G	A	SNP0-SNP1	-2,17	0,0471	RSBN1L
AX-117082755	3	C	T	SNP1-SNP2	-1,77	0,0266	SAMD12
AX-185112171	4	A	G	SNP0-SNP1	2,25	0,0326	SAMD12
AX-106731384	7	G	A	SNP1-SNP2	2,23	0,0098	FBXL7

Analyzing the AA and GG genotypes for SNP AX-106736322, located near the FBXL13 gene, it can be observed that the recorded estimated value of -2.71 indicates a decrease in BH. This result suggests that the presence of the G allele contributes to a reduction in BH compared to the A allele, as confirmed by the p-value of 0.0148. For SNP AX-124375871, located near the RSBN1L gene the estimated value of +2.17 (p=0.0471) indicates an increase in BH. On chromosome 20, SNP AX-106731384 was identified, located near the FBXL7 gene. Regarding the difference between the GA and AA genotypes, the positive estimated value (+2.23) indicates an increase in BH. The A allele is involved in determining the increase in BH, with a more pronounced effect in homozygotes (AA) compared to heterozygotes (GA).

3.4. Analysis of SNPs Associated with Rump Angle (RA)

The statistical genomic analysis highlighted the presence of 9 SNPs located on chromosomes 3, 7, 16, and 23, which were subsequently examined for their potential implications regarding RA.

Table 4. SNP localization on chromosomes and allele effects on rump angle (RA).

SNP	Chr	Reference allele	Alternative allele	Contrast	Estimated value	p-value	Gene
AX-106751591	3	A	G	SNP1 - SNP2	1,06	0,0393	DPYD
AX-106735685	3	C	T	SNP0 - SNP2	1,89	0,0263	DPYD
AX-124348371	3	G	T	SNP0 - SNP2	2,07	0,0130	DPYD
AX-106727722	7	T	C	SNP0 - SNP2	-1,57	0,0361	FSTL4
AX-106742186	16	G	A	SNP0 - SNP1	1,36	0,0288	ABL2

AX-106724034	16	T	C	SNP0 - SNP2	-1,73	0,0247	CAV2.3
AX-106724034	16	T	C	SNP0 - SNP1	-1,63	0,0317	CAV2.3
AX-124384326	23	C	T	SNP0 - SNP2	-3,89	0,0330	RUNX2
AX-124384326	23	C	T	SNP0 - SNP1	-4,49	0,0119	RUNX2

On chromosome 3, three SNPs associated with significant changes in RA were identified. Depending on the genetic variant present, these SNPs were linked to both positive and negative effects on the analyzed phenotype. For SNP AX-106751591, the difference between the heterozygous genotype (AG) and the homozygous genotype (GG) is reflected by the positive estimated value (+1.06), suggesting an association between the G allele and an increase in RA. This is further confirmed by the p-value of 0.0393, which is below the significance threshold.

When comparing the two homozygous genotypes (CC and TT) for SNP AX-106735685, it can be observed that the positive estimated value (+1.89) indicates an increase in RA. The alternative T allele is associated with this increase, and the p-value of 0.0263 confirms the statistical significance of the relationship between the analyzed variables.

For SNP AX-124348371, the estimated value (+2.07) highlights the significant comparison (p=0.0130) between the homozygous genotypes GG and TT, suggesting an association of the alternative T allele with an increase in RA. These three SNPs were located near the DPYD gene, suggesting a role for this gene in the growth and development of pelvic bones, including influencing RA.

On chromosome 7, SNP AX-106727722 was identified near the FSTL4 gene. Analyzing the homozygous genotypes TT and CC, the estimated value of -1.57 suggests that the alternative C allele is associated with a decrease in RA.

Regarding chromosome 16, SNPs AX-106724034 and AX-106742186 were identified. SNP AX-106724034 is located near CAV2.3 gene and presents the reference allele T and the alternative allele C. Analyzing individuals with homozygous genotypes (TT) and heterozygous genotypes (TC), a decrease in RA was observed with the estimated value of -1.73 (p=0.0247). For SNP AX-106742186, located near the ABL2 gene, the G allele is associated with a significant increase in RA compared to the A allele. Analyzing the two genotypes, it was found that homozygotes (GG) exhibit a greater RA than heterozygotes (GA), with the estimated value being +1.36.

SNP AX-124384326, located near the RUNX2 gene, is associated with RA in the cattle in this study. When comparing the homozygous genotypes CC and TT, the estimated value (-3.89, p= 0.0330) indicates that the alternative T allele is associated with a decrease in RA.

3.5. Analysis of SNPs Associated with Croup Length (CL)

Three SNPs significantly associated with this trait have been identified on chromosomes 16 and 23.

Table 5. SNP localization on chromosomes and allele effects on croup length (CL).

SNP	Chr	Reference allele	Alternative allele	Contrast	Estimated value	p-value	Gene
AX-106752137	16	T	C	SNP0 – SNP1	2,12	0,0105	CAV2.3
AX-106752137	16	T	C	SNP0 – SNP2	2,26	0,0215	CAV2.3
AX-117088037	23	C	T	SNP0 – SNP1	1,41	0,0375	DST

SNP AX-106752137, located on chromosome 16, showed a significant association with CL. Comparing the TT and TC genotypes, it can be observed that the positive estimated value (2.12, p=0.0105) indicates an increase in CL. Additionally, in the comparison between the homozygous TT and CC genotypes, the estimated value of 2.26 and p=0.0215 suggest that the C allele is associated with a more pronounced increase in CL.

3.6. Analysis of SNPs Associated with Croup Length (CL)

In our analysis, 5 SNPs located on chromosomes 1, 10, and 16 were identified and subsequently significantly associated with croup width. On chromosome 1, two SNPs (AX-106742670 and AX-115103182) were identified, located near the DCBLD2 gene. Analyzing the TC and CC genotypes for SNP AX-106742670, it was found that the alternative C allele is associated with an increase in CW. This association is supported by the estimated effect value of 1.19 and a p-value of 0.0236, indicating a statistically significant association. On the other hand, for the CT and TT genotypes for SNP AX-115103182, it was observed that the T allele is associated with a decrease in CW. This is reflected by the estimated value of -1.27 and a p-value of 0.0318.

Table 5. SNP localization on chromosomes and allele effects on croup width (CW).

SNP	Chr	Reference allele	Alternative allele	Contrast	Estimated value	p-value	Gene
AX-106751591	1	T	C	SNP1 - SNP2	1,19	0,0236	DCBLD2
AX-106735685	1	C	T	SNP1 - SNP2	-1,27	0,0318	DCBLD2
AX-124348371	10	C	G	SNP0 - SNP2	1,53	0,0320	FRMD6
AX-106727722	10	G	A	SNP0 - SNP1	-1,63	0,0157	FRMD6
AX-106742186	16	T	C	SNP0 - SNP1	2,16	0,0050	CAV2.3

When comparing the two homozygous genotypes CC and GG for SNP AX-106763243, it was observed that the positive estimated value (1.53, p-value = 0.0320) indicates a significant difference between them. The presence of the G allele is associated with an increase in CW, highlighting its significant effect on the development of the trait.

For SNP AX-124386523, heterozygous genotype (GA) exhibit a smaller CW than homozygous (GG) and the negative estimated value (-1.63, p=0.0157) confirms this aspect. This reflects the fact that the alternative allele A is associated with a decrease in CW.

SNP AX-106724034 on chromosome 16, located near the CAV2.3 gene, shows an estimated value of 2.16 and a p-value of 0.0050, indicating a significant influence on CW. Analyzing the TT and TC genotypes, it was found that heterozygous show a smaller CW than homozygous, emphasizing the effect of the C allele on the analyzed phenotype.

4. Discussion

The CLSTN2 gene (Calsyntenin 2) is involved in lipid metabolism and plays an important role in the proliferation of adipocytes in both visceral and subcutaneous adipose tissue. The expression of the CLSTN2 gene is associated with glucose and insulin metabolism, contributing to their regulation and the onset of metabolic disorders. Fluctuations in insulin and glucose levels can influence the regulation of the endocrine axis, directly impacting reproductive processes and the onset of sexual maturity [10,11]. It has been previously reported that the CLSTN2 gene plays a role in influencing hip width [12]. We found that in the case of SNP AX-106723587, located near the CLSTN2 gene, the presence of the G allele is associated with a reduction in CH.

Our findings suggest that although the CLSTN2 gene does not have a direct effect on CH, the proximity of these three SNPs suggests a possible indirect influence on the trait through pleiotropic mechanisms.

The DPYD gene (Dihydropyrimidine Dehydrogenase) encodes the enzyme dihydropyrimidine dehydrogenase, which is responsible for the degradation of pyrimidines, particularly uracil and thymine, when they are no longer needed. The enzyme initiates the pyrimidine degradation process by converting uracil into 5,6-dihydrouracil and thymine into 5,6-dihydrothymine. The products resulting from this process are either eliminated from the body or redistributed into other metabolic pathways [13]. In cattle, the DPYD gene contributes to maintaining energy balance, supports

oxidative metabolism, and participates in the efficient use of nutrients in the body. According to the study conducted by Jourshari (2023), SNPs located near the DPYD gene have been associated with variation in hip width in cattle [12]. The authors also suggest that this gene may be involved in meat quality and fatty acid composition in local cattle breeds in China [13]. Our results emphasize the fact that the G allele for SNP AX-185119475, located near the DPYD gene, is associated with a reduction in CH, possibly by influencing the growth and development of the bone structures in this region.

In the study reported by Guzman et al. (2020), the FBXL13 gene was identified in a region associated with the width of the ilium and body length in heifers [14]. The FBXL13 gene is a protein from the F-box family, characterized by a specific domain of about 40 amino acids. It is integrated into SCF (SKP1-CUL1-F-box) complexes that act as E3 ubiquitin ligases, playing an essential role in ubiquitination and degradation of target proteins [15]. According to our results, the analyzed SNPs, AX-106763743 and AX-106736322, were located near the FBXL13 gene, which had previously been discussed in the context of CH. Although the genotypes differed in the case of CH, it appeared that SNPs located near the FBXL13 gene played an important role in the development of pelvic bones [14].

The SEMA6A gene, also known as semaphorin 6A, is involved in various essential processes such as cell migration, axon guidance, and synaptogenesis. In studies on mice, mutations in the SEMA6A gene have been associated with defects in cell migration and axon guidance in different regions of the brain, including the thalamocortical system, hippocampus, and cerebellum. These defects lead to improper development of neural networks [16]. In the study by Zhang et al. (2024), SNPs located near the SEMA6A gene were associated with CL in Xinjiang Brown cattle, explaining approximately 0.093% of the variation in this trait in the context of their research [17]. Our results highlight that the contrast between SNP0 and SNP2 for SNP AX-106753436, located near the SEMA6A gene, highlights the difference between homozygotes for the reference allele (CC) and those for the alternative allele (AA), indicating a reduction in CH in individuals carrying the A allele.

Genomic analysis highlighted the presence of four SNPs on chromosome 14 associated with this phenotype, all located near the SAMD12 gene. Guzman et al. (2020) identified a genomic region on chromosome 14 that includes the SAMD12 gene and is associated with CL in Murrah buffaloes [14]. This emphasizes the pleiotropic effect of the SAMD12 gene on pelvic conformation.

The SAMD12 gene encodes a protein involved in the tyrosine kinase receptor signaling pathway, being active on the inner surface of the plasma membrane. Zhuang et al. (2020) found significant association of SNPs located on this gene with body weight in 18-month-old Simmental cows [18]. Later, Mancin et al. (2022) confirmed the relevance of this region, identifying a SNP near the SAMD12 gene associated with carcass traits [19]. In our results, for SNP AX-106724218, heterozygous individuals (CT) showed an estimated value of -3.77 ($p=0.0019$), falling between the values observed in homozygotes, suggesting a progressive effect of the T allele on this trait. Similarly, for SNP AX-117082755, heterozygous (CT) individuals showed a less pronounced reduction of -2.56 ($p=0.0262$), indicating that the effect of the T allele becomes more evident in homozygous individuals due to the presence of two copies of this allele.

In Nellore cattle, Machado et al. (2022) associated the SEMA6A gene with muscle development. The authors suggest that the SEMA6A gene is involved in regulating biological processes, including the development and growth of muscle tissue. Furthermore, it plays a role in RNA synthesis, energy metabolism, and response to external stimuli [20]. Given the close connection between muscle and bone structures, the SEMA6A gene could indirectly influence CH as well.

The SH3BP4 gene (SH3 domain binding protein 4) acts as an inhibitor of Rag GTPase activity, an important component of the mTORC1 complex. This protein complex controls essential cellular processes such as cell growth, protein synthesis, and autophagy, responding to nutritional, hormonal, and stress signals. Thus, by controlling the activity of the mTORC1 complex, the SH3BP4 gene influences the maintenance of energy balance and the adaptation of cells to stress conditions [21]. In the study conducted by Lu et al. (2021), the SNP identified near the SH3BP4 gene was associated with croup slope, suggesting an influence on the development of pelvic bones [21]. Butterfield et al. (2021) identified the SH3BP4 gene as having a functional role in the pathogenesis of osteoarthritis in mice.

They found that the absence of the gene led to the early development of osteoarthritis, manifested by the degeneration of articular cartilage. The SH3BP4 gene is also involved in the regulation of cellular signaling by influencing the processes of transferrin receptor transport and other pathways essential for cellular homeostasis [22]. These processes highlight the role of the SH3BP4 gene in the proper functioning of bone and cartilage tissues. Furthermore, the way it regulates cell growth and development could contribute to the formation of the pelvic structure, including traits such as BH. Based on statistical analyses and estimated value recorded for SNP AX-106733516, we concluded that homozygotes (GG) exhibited a lower value for this trait compared to homozygotes (TT).

The RSNB1L gene (Round Spermatid Basic Protein 1 Like) encodes a protein involved in spermatogenesis and oogenesis processes. It shares a common origin with the RSNB1 gene. Available information about RSNB1 highlights its expression in the testis, brain, and ovary. Given the structural and functional similarity between the two genes, these findings may also be relevant for the RSNB1L gene [23]. RSNB1L is a protein involved in chemical reactions that implies oxygen and interacts with metal ions, being active in the nucleus. It also acts as a specific demethylase, removing methyl (-CH₃) groups from lysine residues in proteins, which can influence their structure and function [23]. Guzman et al. (2020) identified a SNP in buffaloes located on chromosome 4, on the RSNB1L gene, associated with ilium width and body length [14]. The results indicate that SNPs located on the FBXL13 and RSNB1L genes can influence both BH and other structures that form the pelvis. Our results show that in the case of SNP AX-124375871 on RSNB1L, a positive association was found, which is attributed to the A allele. This allele causes a more pronounced manifestation of BH growth in heterozygotes (GA), compared to homozygotes (GG).

On chromosome 4, two SNPs were identified, both located near the *SAMD12* gene [24]. Rothhammer et al. (2013) suggest that the *SAMD12* gene plays an important role in Creole cattle breeds in adapting to environmental conditions and in their performance related to milk and meat production. The authors state that certain alleles of this gene have been selected, suggesting that *SAMD12* could be essential for improving performance in cattle [24].

Regarding the values we have obtained, the G allele of SNP AX-117082755 is associated with a decrease in BH, and its effect is more pronounced in heterozygotes (AG). These observations suggest that SNPs located near the *SAMD12* gene may influence BH. Additionally, the T and G alleles of SNP AX-117082755 and SNP AX-124375871 have different effects on this phenotype, indicating a possible role of the *SAMD12* gene in determining pelvic structures.

In the study by Abdalla et al. (2021), the SNP located on the *FBXL7* gene was associated with a trait that influences the positioning of the animal's hind limbs when viewed from behind. This trait is important for mobility because the position of the hind limbs influences the efficiency of movement, and the *FBXL7* gene plays a role in the development and functioning of the musculoskeletal system [25].

The *FBXL7* gene (F-Box and Leucine Rich Repeat Protein 7) is a protein from the F-box family that regulates mitotic cell cycle progression and apoptosis [26]. Wu et al. (2018) identified an SNP located on chromosome 16, on the *FBXL7* gene. The authors suggest that the *FBXL7* gene may influence the reproductive performance of sows from the Landrace and Large White breeds, impacting the total number of piglets born, the total number of live piglets, and their total birth weight [27]. The *FBXL7* gene also impacts mitochondrial function and the cellular energy stability [26].

Our obtained p-value of 0.0098 suggests that this association is statistically significant, indicating a possible role of the *FBXL7* gene in determining BH.

Our results align with those obtained by Lu et al. (2021) in Holstein cows in China. Similarly, they identified a SNP on chromosome 7, located near the *FSTL4* gene, which they associated with RA [21]. The *FSTL4* gene (Follistatin-like 4) is a member of the follistatin family and an inhibitor of growth and TGF- β . *FSTL4* is involved in regulating the functions of mesenchymal cells and in the early development of the nervous and ocular systems [28]. In cattle, the *FSTL4* gene is involved in regulating ovarian function, playing a role in ovulation and corpus luteum formation [29]. Dewison

et al. (2023) suggest that the *FSTL4* gene is a potential indicator of oocyte quality due to its increased expression in fertilized oocytes that have reached the blastocyst stage [30]. We found that the p-value of 0.0361 confirms that the association for SNP AX-106727722 is statistically significant.

The *CAV2.3* gene encodes the protein responsible for producing type R calcium channels. These channels are involved in conducting calcium ions into nerve and muscle cells. Calcium channels are also essential for neuronal and muscular excitability, and mutations in the *CAV2.3* gene can be associated with various neurological disorders [17]. SNPs located this gene suggest a possible influence on the formation of bone structures, including RA, an essential morphological trait that depends on the proper development of the pelvic bones and musculature. Our results are consistent with those observed by Zhang et al. (2024) [17], who associated the SNP located near the *CAV2.3* gene with CL, indicating a similar influence on this trait [16]. In our study, the decrease in RA was recorded in individuals with the *TT* and *CC* genotypes, with an estimated value of -1.63 and a p-value of 0.0317, indicating a statistically significant association. These results suggest that the *C* allele might be associated with a reduction in RA.

ABL2 is a proto-oncogene with tyrosine kinase activity and is not associated with specific membrane receptors. It plays a crucial role in regulating the actin cytoskeleton, influencing cell morphology and motility, as well as cell adhesion to the extracellular matrix. The gene contains a tyrosine kinase domain and two domains that bind to F-actin, which are essential for its functions [31,32]. The *ABL2* gene is involved in the innate immune response, influencing processes such as cell proliferation, migration, and differentiation. It also regulates important metabolic processes, such as food intake in cattle and the thickness of dorsal fat in pigs. These aspects influence the ability of animals to adapt to different food sources and respond to metabolic stress or challenging environmental conditions [34].

Moreover, *ABL2* influences the proliferation and fusion of myoblasts, allowing for the proper development of muscle fibers. In the study conducted by Lee et al. (2017) on mice, it was found that the absence of the *ABL2* gene leads to excessive proliferation and fusion of myoblasts, which can cause changes in the structure and size of muscle fibers [33]. Vanvanhossou et al. (2020) identified an SNP located near the *ABL2* gene, which they associated with hip width in Holstein cattle. This suggests that the *ABL2* gene may also influence RA, impacting pelvic conformation [33].

The resulted p-value of 0.0288 we have obtained for AX-106742186 confirms the statistical significance of this association, indicating that the *G* allele plays an important role in determining RA.

RUNX2 is essential not only for osteoblast differentiation but also for chondrocyte maturation [35]. The results previously obtained demonstrate that the influence of the *RUNX2* gene on SNP AX-124384326 could play an important role in modifying the shape of the croup, being a relevant genetic factor in the development of pelvic structure in cattle [16]. Deletion of the *RUNX2* gene impairs the development of both oocytes and spermatozoa and hinders the development of intramembranous and endochondral bones. *RUNX2* expression is present in all cells of the osteoblast lineage, including osteoprogenitors, preosteoblasts, immature osteoblasts, mature osteoblasts, osteocytes, and chondrocytes [35].

Based on our results, regarding the contrast between the *CC* and *CT* genotypes, the recorded estimated value (-4.49, $p = 0.0119$) suggests a more pronounced decrease in RA, and the *T* allele continues to be associated with this significant modification.

The *DST* gene encodes the protein dystonin, which is essential for maintaining the structure of the cellular cytoskeleton. It is part of the Plakin family and plays a role in maintaining the stability of the cytoskeleton by facilitating the connection between its different components, such as actin and microtubules. It is also involved in the stability and physiological function of muscular, nervous, and epidermal tissues [36]. Zhang et al. (2024) identified an SNP located on chromosome 23, near the *DST* gene, which they associated with CL in Xinjiang Brown cattle [17].

According to our obtained p-value of 0.0375, which is below the 0.05 threshold, supports the hypothesis of a significant association between this SNP and CL.

The *DCBLD2* gene (Discoidin, CUB, and LCCL domain containing 2) is located on the cell surface of the plasma membrane and is involved in cell migration and interaction. It also plays an important role in regulating the activity of signaling receptors, influencing how cells respond to signals from their environment [36]. In the study conducted by Zhang et al. (2024), a significant association was highlighted between an SNP located on chromosome 1 and CL [17]. The authors suggested that the *DCBLD2* gene, located near this SNP, may influence the development of pelvic bones. These results are relevant to our study, providing an example of how SNPs located near certain genes can influence important morphological traits.

In our study, we found that depending on the SNP location, *DCBLD2* gene might increase the CW (when it is close to SNP AX-106742670, depending on C allele) or decrease (when it is located on SNP AX-115103182, influenced by T allele).

SNPs AX-106763243 and AX-124386523, located on chromosome 10, are significantly associated with CW. Both SNPs are near the *FRMD6* gene, highlighting its influence on croup development. Yu et al. (2023) obtained similar results to ours, identifying an SNP located on chromosome 10 near the *FRMD6* gene, which they associated with CL [38].

The *FRMD6* gene (FERM domain containing 6) is located in the cytoplasm and at the plasma membrane. This gene is involved in cellular signaling and regulates cellular senescence processes. *FRMD6* stimulates the Hippo signaling pathway, activating the MST kinase and inactivating the YAP/TAZ proteins, which are essential for controlling cellular growth and development.

Through the Hippo-YAP-CCN3 signaling axis, the *FRMD6* gene controls the cell fate toward senescence, regulating markers such as p21 and p16. Additionally, *FRMD6* is influenced by the transcription factors p53 and SMAD, which control cellular responses to external signals, including TGF- β .

Concerning the SNPs located near *FRMD6* gene, the alternative allele A (on SNP AX-124386523) is associated with a decrease of CW, while the presence of G allele accompanies an increase of CW meaning that SNP AX-106763243 might influence the development of the trait.

Our studies highlights the association between single nucleotide polymorphisms (SNPs) and external pelvimetry traits in Simmental cows, analyzing 33 SNPs across multiple chromosomes to understand their influence on croup height (CH), buttock height (BH), croup width (CW), rump angle (RA), and croup length (CL) [4].

5. Conclusions

This study focuses on five croup traits such as: croup height (CH), buttock height (BH), croup width (CW), rump angle (RA) and croup length (CL) which were selected as parameters of reproductive capacity in Simmental cows.

The identified SNPs are associated with the measurements involved in external pelvimetry. A total of 33 SNPs were identified that affect the development and morphology of the pelvic bones. The found genetic markers are located on chromosomes 1, 3, 4, 7, 10, 14, 16 and 23, suggesting that they may influence the morphological development of the pelvis. We also found some genes associated with CH, BH, CW, RA, and CL that play an important role in these traits in cows. On certain situations, the proximity of SNPs suggests a possible indirect influence on the trait through pleiotropic mechanisms.

The diversity in the localization of the SNPs highlights the complexity of the genetics of pelvic traits and provides the possibility of interactions between different chromosomal regions that contribute to the development of pelvic traits. These results can be applied in genetic selection programs to improve the pelvic conformation of cows, while also reducing the risks associated with calving difficulties and enhancing reproductive performance.

Further investigation and research of these SNPs is necessary to better understand their role in the development of the pelvis. Additionally, future research could explore how these genetic markers interact with other genetic and environmental factors that influence pelvic traits.

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Abbreviations

BH	Buttock height
CH	Croup height
Chr	Chromosome
CL	Croup length
CW	Croup width
F-actin	Filamentous actin
GWAS	Genome-wide association studies
RA	Rump angle
SMAD	Suppressor of Mothers against Decapentaplegic
SNP	Single nucleotide polymorphism
TGF-β	Transforming growth factor β

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