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Weighted Single-Step GWAS Reveals Genetic Mechanisms Underlying Pregnancy Loss in Early Nellore Heifers

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ABSTRACT

Pregnancy loss (PL) affects reproductive efficiency in beef cattle, yet its genetic architecture remains poorly understood in *Bos indicus* populations. Here, PL was defined as failure from pregnancy diagnosis to calving, capturing a window approximately 60 days after the breeding season. This study aimed to identify genomic regions and biological pathways associated with PL in early Nellore heifers using weighted single-step genome-wide association study (WssGWAS). Phenotypic records from 23 507 Nellore heifers were analyzed, including 17 052 genotyped animals (GGP Indicus panel, Neogen) and 44 233 SNPs. Genomic regions explaining more than 0.5% of additive genetic variance were used to identify putative candidate genes and perform functional enrichment analysis. The estimated heritability for PL was low (0.033), indicating strong environmental influence. WssGWAS identified 25 genomic regions across 16 chromosomes, explaining 31.16% of additive genetic variance for PL. The most relevant region was located on BTA16, explaining 6.51% of the variance, although no annotated protein-coding genes were found. Additional regions occurred on BTA6, BTA14, BTA22, BTA25, and BTA29. Putative candidate genes included *IL4R*, *IL21R*, *LRRC4C*, *RBMS3*, *ZFPM2*, *PRSS23*, and *NDST4*, involved in immune regulation, cell adhesion, extracellular matrix remodeling, and transcriptional regulation. Functional enrichment identified Gene Ontology terms and REACTOME pathways related to nucleosome assembly, epigenetic regulation, transmembrane transport, focal adhesion, calcium signaling, immune response, and WNT signaling. These findings inform the genetic architecture of PL in Nellore cattle and suggest roles for embryo–maternal communication, immune regulation, chromatin organization, and placental development in pregnancy maintenance, supporting future functional validation and genomic selection strategies.

1 | Introduction

Beef cattle production plays a crucial role in the Brazilian economy, and improving reproductive efficiency is essential for maintaining productivity and profitability in this sector. Several management and technological strategies have been implemented to enhance beef production, including improvements in animal health and welfare, precision nutrition, genetic selection,

and reproductive management (Terry et al. 2021). Among these factors, reproductive efficiency remains a major determinant of herd productivity.

Reproductive failures compromise calf production, with embryonic mortality representing one of the main factors limiting fertility and economic efficiency in beef production systems. To sustain profitable operations, each cow is expected to produce a

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marketable calf annually. However, besides its economic importance, pregnancy loss (PL) remains relatively underexplored in beef cattle, and estimates of PL rates vary widely across studies (Reese et al. 2020). Although the genetic basis of PL has been investigated in dairy cattle, studies focusing on beef breeds, particularly *Bos indicus* cattle, remain limited.

Genomic studies have begun to provide insights into the genetic architecture underlying PL. In dairy cattle, genome-wide association studies (GWAS) have identified several genomic regions associated with reproductive success and pregnancy maintenance, which harbor genes involved in embryonic and fetal development and placental function (Bamber et al. 2009; Shi et al. 2021; Sigdel et al. 2021). These studies have also highlighted pathways related to fetal and placental development, metabolism, and immune response, emphasizing the complex biological mechanisms underlying successful gestation in dairy cattle.

More recently, genomic studies have also been conducted in *Bos indicus* beef cattle. Rodrigues et al. (2024) performed a GWAS in Nellore cattle and identified genomic windows containing 57 genes across seven chromosomes, with functional annotations related to regulation of hormone secretion and transport and glucagon signaling. Similarly, Amorim et al. (2025) identified 17 candidate genomic regions in Brahman cattle containing 92 genes associated with processes such as embryonic development and implantation, fertilization, G protein-coupled receptors, embryonic brain development, olfactory receptor activity, and calcium signaling. Although these studies provided important insights into the genetic architecture of PL in *Bos indicus* cattle, additional studies are needed to investigate pregnancy loss in distinct Nellore populations. In particular, commercial populations subjected to early reproductive challenges, in which heifers are exposed to their first breeding season at approximately 10 to 14 months of age, represent an important population for investigating the genetic basis of reproductive success because pregnancy outcomes during this first breeding season determine whether heifers enter the breeding herd, directly influencing replacement rates, lifetime productivity, and the overall efficiency of beef production systems. Furthermore, differences in population structure, reproductive management, phenotype definition, and genomic approaches may contribute to the identification of complementary genomic regions and biological mechanisms underlying PL.

From a quantitative genetic perspective, PL is characterized by low heritability estimates, typically ranging from 0.07 to 0.08 in dairy cattle (van Binsbergen et al. 2019; Ask-Gullstrand et al. 2021; Wijma et al. 2022), and from 0.03 to 0.11 in beef cattle (Bis et al. 2026; Rodrigues et al. 2024; Amorim et al. 2025). These low estimates indicate that PL is influenced by many genes with small effects as well as environmental factors. Therefore, identifying candidate genomic regions and the biological pathways involved in PL is essential for improving our understanding of pregnancy maintenance and for supporting the development of selection strategies aimed at improving reproductive efficiency in Nellore cattle.

To further investigate the genetic architecture underlying PL, genomic approaches that integrate pedigree and marker

information can be used to identify regions of the genome contributing to trait variation. The weighted single-step genome-wide association study (WssGWAS), based on the single-step genomic BLUP framework, allows the estimation of SNP effects while accounting for all available phenotypic, pedigree, and genomic information (Wang et al. 2012; Wang et al. 2014). This approach enables the identification of genomic regions that explain a relevant proportion of the additive genetic variance for complex traits in livestock. Therefore, we hypothesized that pregnancy loss in early-challenged Nellore heifers has a polygenic genetic architecture and is influenced by genomic regions harboring genes involved in biological processes essential for pregnancy establishment and maintenance. Thus, this study aimed to identify genomic regions and biological pathways associated with PL in early-challenged Nellore heifers using a WssGWAS approach.

2 | Material and Methods

2.1 | Ethics Statement

The datasets used were obtained from pre-existing databases. Therefore, Animal Care and Use Committee approval was not required for this study.

2.2 | Animals and Phenotypes

The dataset used in this study comprised animals from 44 farms located in the Midwest, Southeast, Northeast, and North regions of Brazil (Nellore GMA Genetics, coordinated by the Animal Genetics and Breeding Program—GMA), developed by the Animal Breeding and Biotechnology Group (GMAB, FZEA—USP, Pirassununga, São Paulo, Brazil). The animals were raised on pasture-based systems (*Urochloa brizantha* cv). Management practices varied across farms, including differences in protein and mineral supplementation intensity, reproductive calendars, and culling criteria. Some farms provided protein and mineral supplementation throughout the dry season, while others offered only urea supplementation, contributing to environmental and nutritional heterogeneity that may influence reproductive outcomes. Regional and seasonal effects, such as differences in temperature and humidity, were accounted for through contemporary group (CG) definitions, which combined animals born on the same farm and year, and month of pregnancy diagnosis. Contemporary groups with fewer than five animals were excluded from the analyses. After data editing, 325 contemporary groups were retained, with a minimum of 5, a mean of 71.8, a median of 34, and a maximum of 615 animals per group. Phenotypic variability for pregnancy loss was observed in 275 CGs, whereas 50 CGs contained only a single pregnancy loss phenotype category (49 with only Category 1 and one with only Category 2).

The database included records from 23 507 Nellore heifers collected between 2004 and 2023. Animals were managed on pasture and received energy–protein supplementation after weaning. Heifers were exposed to breeding between 10 and 14 months of age for an average breeding season of 3 months. Those that did not conceive during the first breeding season

were re-exposed at approximately 24 months of age. Mating systems included natural mating (NM), artificial insemination (AI), and fixed-time artificial insemination (FTAI).

Pregnancy diagnosis was performed by rectal palpation approximately 60 days after the end of the breeding season. Heifers evaluated for PL were those that had a confirmed pregnancy diagnosis but did not calve. Animals with a confirmed pregnancy but no calving record were classified as pregnancy loss (2), whereas those that conceived and calved successfully were classified as no loss (1). To ensure that PL reflected true pregnancy failures and not inconsistencies in recording, we applied data editing rules based on the timing of pregnancy diagnosis and expected calving intervals. Records were excluded if (1) the interval between birth and pregnancy diagnosis was missing or exceeded 915 days; (2) if the interval between diagnosis and expected calving was shorter than 60 days or longer than 310 days. Because reproductive records from commercial herds may occasionally contain incomplete calving information or inconsistencies in reporting, these editing criteria were designed to reduce the inclusion of animals with uncertain reproductive outcomes. These procedures allowed the dataset to capture PL accurately while minimizing bias from incomplete or inconsistent records, providing robust information for implementation in breeding programs targeting reproductive efficiency. Detailed information on pregnancy loss rates according to farm, mating type, month, and year for this population has been previously reported by Bis et al. (2026).

2.3 | Genomic Data

A total of 17 052 animals were genotyped using the GGP Indicus panel (Neogen, Lansing, MI, USA), which contains 53 492 single-nucleotide polymorphism (SNP) markers. Genomic quality control was performed using the PREGSF90 package (Aguilar et al. 2010). Markers were excluded if they had duplicated genomic coordinates, were located on non-autosomal chromosomes, had a call rate lower than 0.90, or had a minor allele frequency (MAF) lower than 0.05. In addition, markers presenting more than 1% Mendelian conflicts or showing extreme deviations from Hardy-Weinberg equilibrium were removed. After quality control, 17 052 genotyped animals and 44 233 SNPs were retained for subsequent analyses.

2.4 | Estimation of Genetic Parameters

Variance components were estimated using the single-step genomic BLUP (ssGBLUP) methodology through Bayesian inference implemented in the GIBBSF90+ software (Lourenco et al. 2022). A Gibbs sampling chain of 500 000 iterations was generated, with the first 100 000 iterations discarded as burn-in and every 100th iteration retained, resulting in 4000 posterior samples used for inference; heritability and genetic correlations were estimated at each iteration, and the overall means were subsequently obtained. Posterior diagnostics were obtained using POSTGIBBSF90, including posterior means, posterior standard deviations, HPD intervals, Geweke's convergence diagnostic (Geweke 1992), and autocorrelation analysis at lag 50. Convergence of the parameter estimates was further assessed by visual inspection of trace plots.

The ssGBLUP approach extends the traditional animal model by jointly using pedigree and genomic information to estimate breeding values. This is achieved by combining the pedigree-based relationship matrix (**A**) and genomic relationship matrix (**G**) into a single matrix (**H**) (Christensen and Lund 2010; Aguilar et al. 2010). The inverse of the **H** matrix (**H**⁻¹) was computed as follows:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where **G**⁻¹ is the inverse of the genomic relationship matrix and **A**₂₂⁻¹ is the inverse of the pedigree relationship matrix for genotyped animals.

The **G** matrix was constructed following VanRaden (2008):

$$\mathbf{G} = \frac{\mathbf{ZDZ}'}{\sum_{i=1}^m 2p_i(1-p_i)}$$

where **Z** is the centered genotype matrix (with genotypes coded as 0–2p, 1–2p, and 2–2p representing the genotypes **AA**, **Aa**, and **aa**, respectively); *p_i* is the MAF of the *i*-th SNP; **D** is a diagonal matrix containing SNP weights; *P*; and *m* is the total number of SNPs.

2.5 | Statistical Model Overview

Pregnancy loss was analyzed using a threshold animal model to account for the binary nature of the trait. In this framework, the observed categorical outcome is assumed to arise from an underlying continuous liability that follows a normal distribution. The general model can be written as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

where **y** is the vector of observed phenotypes; **X** is the incidence matrix relating observations to the vector of fixed effects (**β**) including CG (year, month of pregnancy diagnosis and farm), mating systems and age at pregnancy diagnosis (covariate); **Z** is the incidence matrix relating observations to the vector of random additive genetic effects (**a**); and **e** is the vector of random residual effects.

In the threshold model, the observed categorical phenotype is assumed to be determined by an unobserved continuous liability. Liability is assumed to follow a normal distribution:

$$\mathbf{U}|\boldsymbol{\theta} \sim \mathbf{N}(\boldsymbol{\theta}, \mathbf{I} \sigma_e^2)$$

where **U** is the vector of underlying liabilities, **θ** = (**β**, **u**) represents the vector of model parameters; **I** is the identity matrix, and σ_e^2 is the residual variance.

2.6 | Weighted Single-Step Genomic Association Study (WssGWAS)

Genomic regions associated with PL were identified using the WssGWAS methodology proposed by Wang et al. (2012). In this approach, SNP effects are derived from the genomic estimated

breeding values (GEBVs) obtained from the ssGBLUP model. Unlike traditional GWAS methods, WssGWAS incorporates all available phenotypic, pedigree, and genomic information simultaneously and iteratively assigns weights to SNPs based on their estimated contribution to the additive genetic variance.

In the first step, all SNPs were assumed to contribute equally to the genetic variance. The $\mathbf{G}$ matrix was constructed using the genotype matrix, and GEBVs were estimated using ssGBLUP. The additive genetic values of genotyped animals can be expressed as:

$$\mathbf{a}_g = \mathbf{Z}\mathbf{u}$$

where $\mathbf{a}_g$ represents the additive genetic effects of genotyped animals, $\mathbf{Z}_g$ is the genotype matrix relating animals to SNP markers, and $\mathbf{u}$ is the vector of SNP effects.

Following Wang et al. (2012), the SNP effects were derived from the GEBVs of genotyped animals as follows:

$$\hat{\mathbf{u}} = \lambda \mathbf{D}\mathbf{Z}'(\mathbf{Z}\mathbf{D}\mathbf{Z}')^{-1}\hat{\mathbf{a}}_g$$

where $\hat{\mathbf{u}}$ is the vector of estimated SNP effects, $\hat{\mathbf{a}}_g$ represents the GEBV of genotyped animals, $\mathbf{Z}$ is the centered genotype matrix, $\mathbf{D}$ is the diagonal matrix of SNP weights, and $\lambda = 1 / \sum 2p_i(1 - p_i)$ is the scaling factor.

The variance of the genetic effects can be expressed as follows:

$$\text{Var}(a_g) = \mathbf{Z}_g\mathbf{D}\mathbf{Z}_g'\sigma_u^2$$

where $\mathbf{D}$ is a diagonal matrix containing SNP weights and σ_u^2 is the SNP variance. In the initial iteration $\mathbf{D}$ is assumed to be an identity matrix, implying equal weights for all markers.

The weight assigned to each SNP was then updated according to:

$$d_i = \hat{u}_i^2 2p_i(1 - p_i)$$

where $\hat{u}_i$ is the estimated effect of the i -th SNP and p_i is the MAF. This procedure increases the weight of SNPs with larger estimated effects and decreases the weight of those with smaller effects. SNP weights were normalized at each iteration to maintain constant total genetic variance. The weighting procedure was repeated for two iterations, following the steps described by Wang et al. (2012). All analyses were conducted using the BLUPF90 software family (Misztal et al. 2002), which allows the integration of genomic information into the genetic evaluation framework (Aguilar et al. 2010).

To identify relevant genomic regions, the proportion of additive genetic variance explained by windows of 10 adjacent SNPs was calculated as follows:

$$\frac{\text{var}(a_i)}{\sigma_a^2} \times 100$$

where a_i represents the genetic value of the i -th genomic window, composed of 10 consecutive SNPs, and σ_a^2 is the total additive genetic variance.

2.7 | Gene Annotation and Functional Enrichment Analysis

Genomic windows explaining more than 0.5% of the additive genetic variance were considered candidate regions for gene annotation and functional enrichment analyses. The 0.5% threshold was used as an empirical criterion to prioritize genomic regions for downstream analyses and does not represent a formal statistical significance test. Rather, it was intended to facilitate the biological interpretation of the WssGWAS results by focusing on regions with relatively larger contributions to the additive genetic variance. Gene annotation was performed using the Ensembl BioMart tool with the Ensembl Genes release 114 and the bovine genome assembly ARS-UCD2.0.

Functional enrichment analysis was conducted using the DAVID v2025_1 platform (Sherman et al. 2022). Gene Ontology (GO) terms (including biological processes, cellular components, and molecular functions) as well as REACTOME pathways were evaluated. Enrichment significance was determined using a threshold of $p < 0.05$, with the bovine genome used as the background reference.

3 | Results

3.1 | Genetic Parameters

The overall PL rate in the dataset was 15.46%. This rate represents heifers with confirmed pregnancy but no calving record and may include losses due to culling, farm transfers, or missing records. When stratified, PL ranged from 10.38% to 21.78% across months, 9.80% to 30.00% across years, 3.19% to 21.62% across farms, and 14.49% to 20.27% across mating systems. Pregnancy loss showed a low heritability estimate of 0.03 (Table 1), with a 95% highest posterior density (HPD) interval ranging from 0.01 to 0.05, indicating that only a small proportion of the phenotypic variation was attributable to additive genetic effects. Visual inspection of the Gibbs sampling trace plots (Figure S1) and posterior density distributions indicated good mixing and convergence of the Markov chains, supporting the stability of the posterior estimates.

3.2 | Genomic Regions Associated With PL

The WssGWAS analysis identified 25 genomic regions across the bovine genome that explained more than 0.5% of the additive genetic variance for PL (Figure 1; Table 2). Together, these regions accounted for 31.16% of the total additive genetic variance for the trait. The identified regions were distributed across *Bos*

TABLE 1 | Genetic parameters for pregnancy loss.

Trait	σ_a^2	σ_e^2	h^2
PL	0.03 [0.01–0.06]	1.01 [0.98–1.03]	0.03 [0.01–0.05]

Abbreviations: σ_a^2 , Additive genetic variance; h^2 , heritability; σ_e^2 , residual variance.

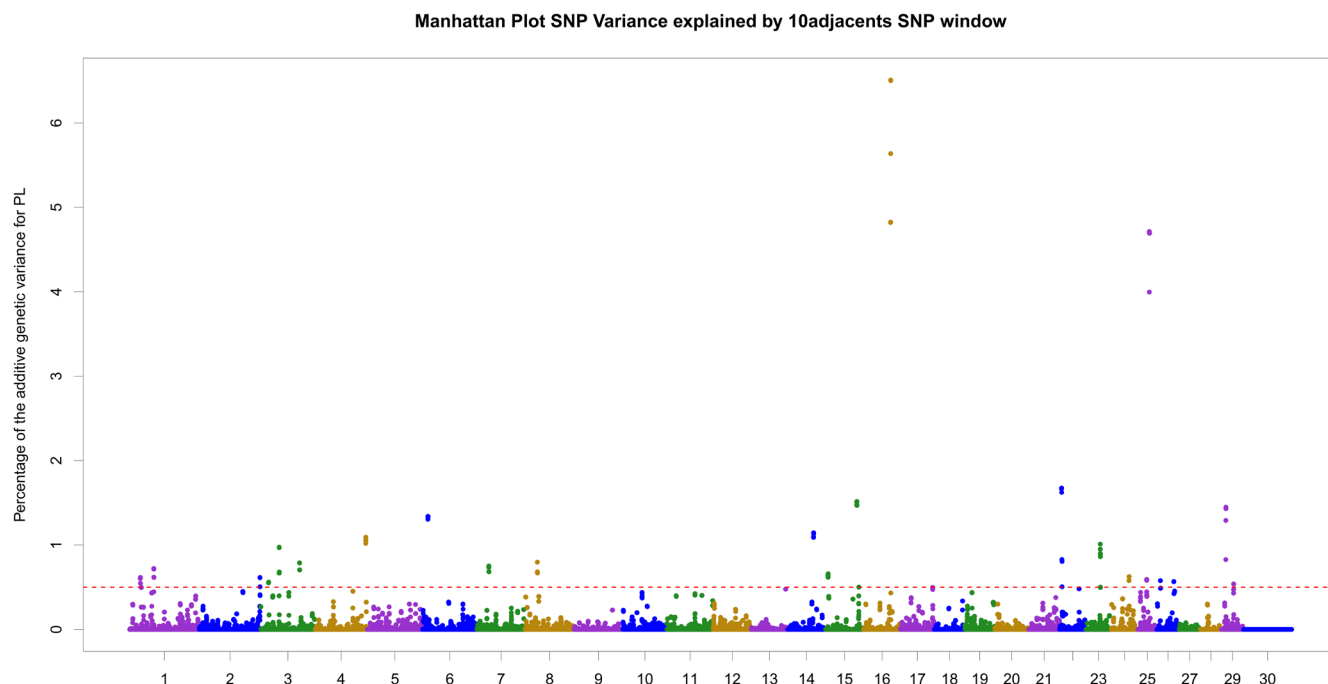


FIGURE 1 | Manhattan plot of the genome-wide association of PL. The X-axis represents the chromosomes, and the Y-axis shows the proportion of the additive genetic variance explained by windows of 10 adjacent SNP. The dots above the red line indicates the genomic regions explaining more than 0.5% of the additive genetic variance for PL.

taurus chromosomes (BTA) 1, 2, 3, 4, 6, 7, 8, 14, 15, 16, 22, 23, 24, 25, 26, and 29. The genomic window explaining the largest proportion of additive genetic variance was located on BTA16 (58.68–59.07 Mb), accounting for 6.51% of the total variance, although no protein-coding genes were identified this region, one annotated lncRNAs were present. Other regions explaining relatively large proportions of genetic variance included windows on BTA25 (4.71%), BTA22 (1.68%), BTA29 (1.45%), BTA6 (1.34%), and BTA14 (1.14%). Several genomic regions contained multiple annotated genes, including regions on BTA3 and BTA23. For example, the region on BTA3 (16.05–17.02 Mb) contained multiple putative candidate genes such as *IL6R*, *ATP8B2*, *AQP10*, *HAX1*, *RAB13*, *SLC39A1*, and *CRTC2*, while the region on BTA23 (31.83–32.26 Mb) included histone-related genes (*H1*, *H2*, and *H3* families) and other genes such as *TRIM38* and members of the *SLC17A* gene family.

In total, the candidate regions contained genes distributed across several chromosomes, while two genomic windows (BTA16 and BTA22) did not contain annotated protein-coding genes within the defined intervals; however, they included non-coding RNA genes, including a lncRNA on BTA16 and a snRNA (U6) on BTA22.

3.3 | Functional Enrichment Analysis

A total of 173 genes were identified within the 25 genomic regions associated with PL, including 118 protein-coding genes, 52 non-coding RNA genes, and 3 pseudogenes. Functional enrichment analysis identified significant enrichment ($p < 0.05$) for eight Gene Ontology (GO) biological processes, seven GO cellular components, 18 GO molecular functions, and 92 REACTOME

pathways. The complete list of significantly enriched terms is provided in the Table S1, whereas Figures 2–5 present the most relevant enriched terms associated with PL.

The enriched GO biological processes included terms related to nucleosome assembly, sensory perception of smell, monoatomic ion transport, urate transport, transmembrane transport, sodium-dependent phosphate transport, urate metabolic process, and negative regulation of focal adhesion assembly.

REACTOME pathway analysis revealed several significantly enriched pathways. The most significant pathways were associated with epigenetic regulation of gene expression, including negative epigenetic regulation of rRNA expression, epigenetic regulation of gene expression by MLL3 and MLL4 complexes, and positive epigenetic regulation of rRNA expression. Additional enriched pathways included estrogen-dependent gene expression, WNT signaling pathways, cellular responses to stress, interleukin-7 signaling, hemostasis, and immune system pathways.

4 | Discussion

4.1 | Genetic Parameters

Similar results have been reported in dairy cattle, where Ask-Gullstrand et al. (2021) estimated a heritability of 0.02 (± 0.02) for fetal loss occurring between 42 days after artificial insemination and calving in 1876 Holstein cows using an animal model. Likewise, Sigdel et al. (2022), analyzed 23910 Holstein heifers diagnosed as pregnant at 39 ± 3 days after mating, of which

TABLE 2 | Genomic regions explaining more than 0.5% of the additive genetic variance associated with PL.

BTA	Region (bp)	Putative candidate genes	% Variance explained by SNP windows
1	24476 580–24 776 602	<i>ROBO2</i>	0.62
1	56 134 816–56 487 055	<i>CD96, ZBED2, PHLDB2</i>	0.72
2	134 788 902–135 431 433	<i>ACTL8, ARHGEF10L, RCC2, PADI6, PADI4, PADI3, PADI1</i>	0.61
3	16 047 644–17 023 975	<i>TDRD10, SHE, IL6R, ATP8B2, AQP10, HAX1, UBAP2L, C3H1orf43, CFAP141, TPM3, NUP210L, RPS25, RAB13, JTB, CREB3L4, SLC39A1, CRT2, DENND4B, GATAD2B, SLC27A3, INTS3, NPR1, ILF2, SNAPIN, CHTOP, S100A1, S100A13, S100A14, S100A16, S100A2, S100A4, S100A5, S100A6, S100A7</i>	0.56
3	35 118 298–35 481 227	<i>VAV3</i>	0.97
3	83 486 857–84 130 254	<i>PATJ, TM2D1</i>	0.79
4	116 702 976–116 929 918	<i>DPP6, PAXIP1</i>	1.09
6	10 149 734–10 524 080	<i>NDST4</i>	1.34
7	26 307 891–26 518 740	<i>CTXN3, PRRC1</i>	0.75
8	27 021 166–27 301 766	<i>CNTLN</i>	0.80
14	58 542 748–59 003 493	<i>ZFPM2</i>	1.14
15	49 137 747–54 605 598	<i>DYNC2H1</i>	0.66
15	70 300 271–70 685 457	<i>LRRC4C</i>	1.52
15	74 774 424–75 277 408	<i>TSPAN18, TP53I11, PRDM11</i>	0.50
16	58 677 123–59 075 332 ^a	<i>ENSBTAG00000075980</i>	6.51
22	3 742 292–4 183 194	<i>RBMS3</i>	1.68
22	4 296 398–4 598 006 ^a	<i>U6</i>	0.83
23	31 834 793–32 264 563	<i>H4C3, H1-6, HFE, H1-2, H3C6, H2BC3, H3C6, H1-1, TRIM38, SLC17A2, SLC17A3, SLC17A1, SLC17A4, H2AC1, H2BC1, SCGN</i>	1.01
24	42 098 512–42 571 509	<i>APCDD1, NAPG, PIEZO2</i>	0.63
25	19 081 516–19 652 696	<i>CRYM, ABCA14, ABCA16, UQCRC2</i>	0.59
25	24 916 448–25 235 601	<i>IL4R, IL21R, GTF3C1, KATNIP</i>	4.71
26	7 674 515–8 032 383	<i>PRKG1</i>	0.58
26	40 412 922–40 781 334	<i>PLPP4</i>	0.57
29	8 687 198–8 948 174	<i>PRSS23, ME3</i>	1.45
29	27 095 686–27 614 811	<i>OR8B1, OR8B1AI, TMEM225, OR8G3F, OR8G3E, OR8D6, OR8B1R, OR8B1T, OR8C26, OR8G3B, OR8G3D, OR8G5B, OR8G5, OR8G47, OR8D2, OR8B1U, OR8B1S</i>	0.54

Abbreviation: BTA, *Bos taurus* chromosome.^aNo protein-coding genes were found to be in this genomic window.

11 847 were genotyped, reported a similarly low heritability estimate (0.015) using a threshold animal model.

In beef cattle, Rodrigues et al. (2024) also reported a low heritability (0.06 ± 0.03) for PL in a Nellore population from a closed experimental selection program, consistent with both the present results and findings in taurine breeds. Similarly,

in commercial Brahman cattle, low heritability for PL (0.11) was also observed (Amorim et al. 2025; Cardona-Cifuentes et al. 2025) and in the same commercial Nellore population evaluated in the present study (0.033) (Bis et al. 2026). Collectively, these findings indicate that additive genetic variation explains a small proportion of the phenotypic variation in PL, suggesting that direct phenotypic selection is expected

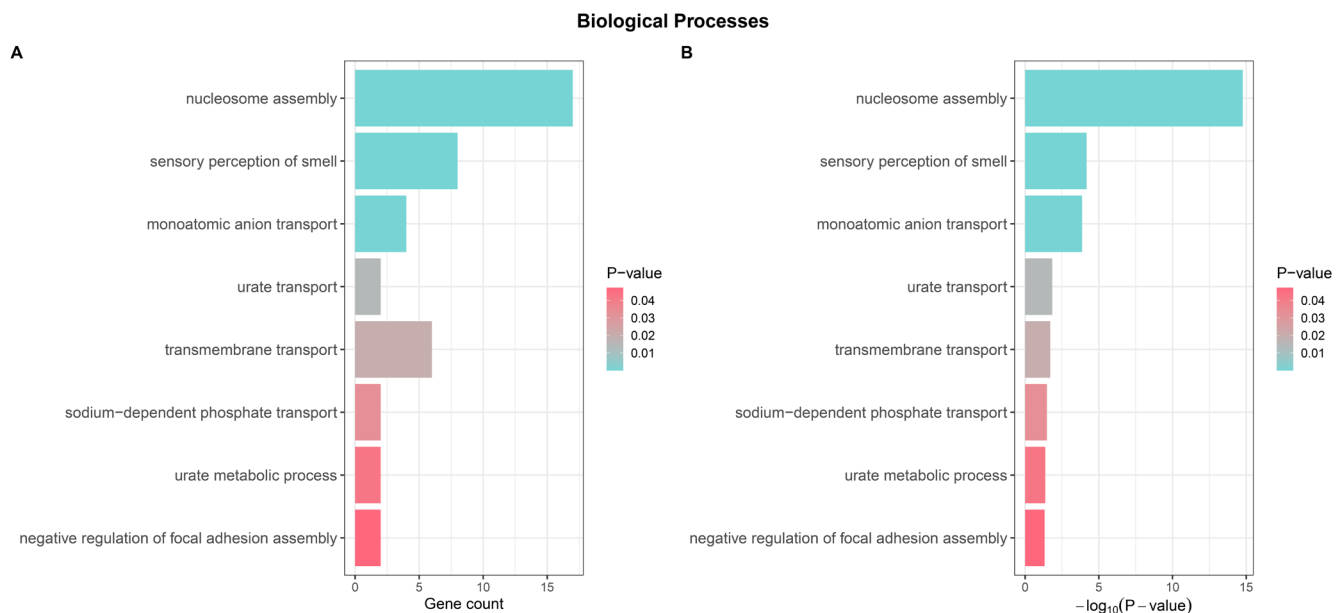


FIGURE 2 | Functional enrichment analysis for GO biological processes of genes associated with PL. A is a bar plot showing the number of genes annotated to each enriched term, colored by p -value. B is the corresponding enrichment scores ($-\log_{10}(p\text{-value})$) for the same terms, reflecting the significance of over-representation.

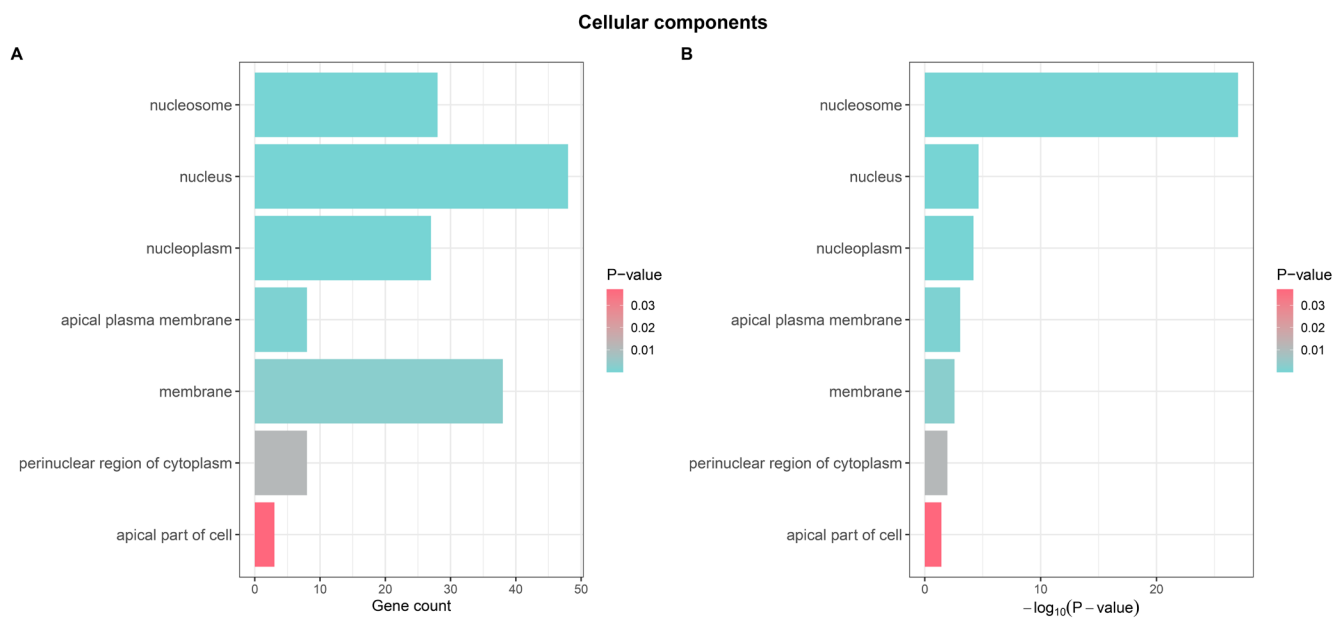


FIGURE 3 | Functional enrichment analysis for GO cellular components of genes associated with PL. A is a bar plot showing the number of genes annotated to each enriched term, colored by p -value. B is the corresponding enrichment score ($-\log_{10}(p\text{-value})$) for the same terms, reflecting the significance of over-representation.

to result in slow genetic progress. Recently, Bis et al. (2026) reported that direct selection for PL showed low expected genetic gain and that indirect selection through genetically correlated reproductive traits may be a more effective strategy. Therefore, although the genomic regions identified in the present study improve our understanding of the biological mechanisms underlying pregnancy maintenance, their practical value for genomic selection has not yet been evaluated. Future studies should investigate whether incorporating this information into genomic evaluations improves the prediction accuracy of breeding values for PL.

4.2 | Genomic Windows, Putative Candidate Genes and Biological Mechanisms

The genomic window explaining the largest proportion of additive genetic variance (Table 2) for PL was located on BTA16 (6.51%). No protein-coding genes were identified within this region; however, one long noncoding RNAs (lncRNAs; ENSBTAG00000075980) was detected. Long noncoding RNAs are transcripts longer than 200 nucleotides that do not encode proteins but play key regulatory roles in gene expression and cellular function (Cui et al. 2025). These molecules can influence transcriptional regulation (Chen

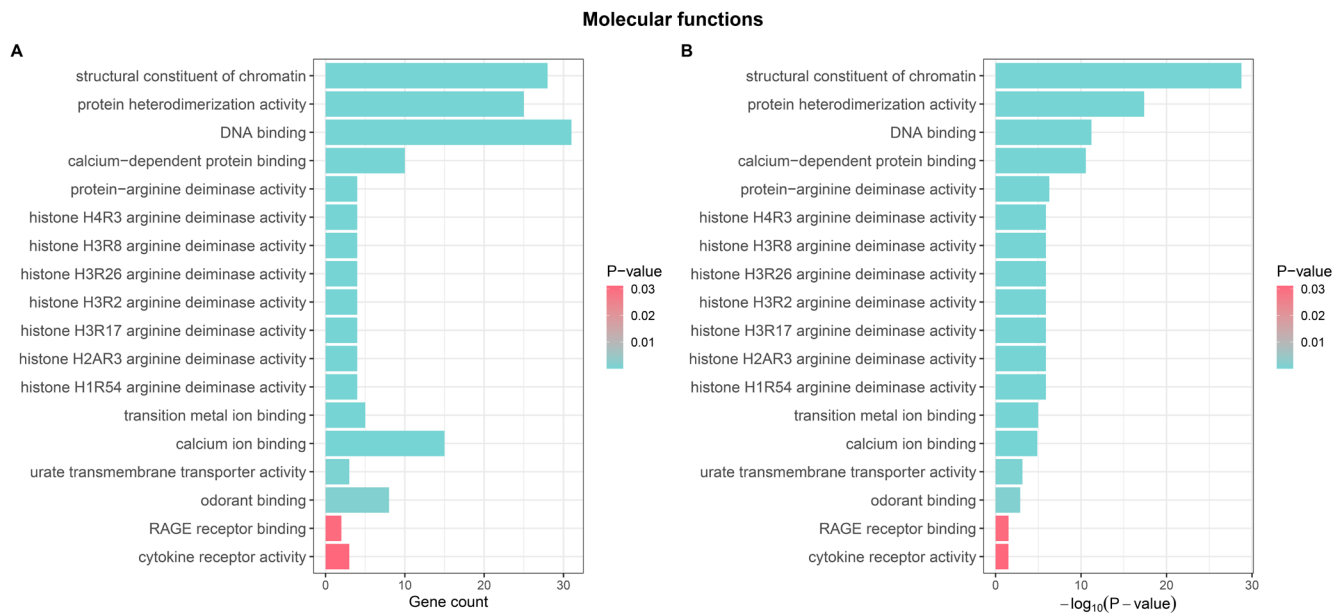


FIGURE 4 | Functional enrichment analysis for GO molecular functions of genes associated with PL. A is a bar plot showing the number of genes annotated to each enriched term, colored by p -value. B is the corresponding enrichment scores ($-\log_{10}(p\text{-value})$) for the same terms, reflecting the significance of over-representation.

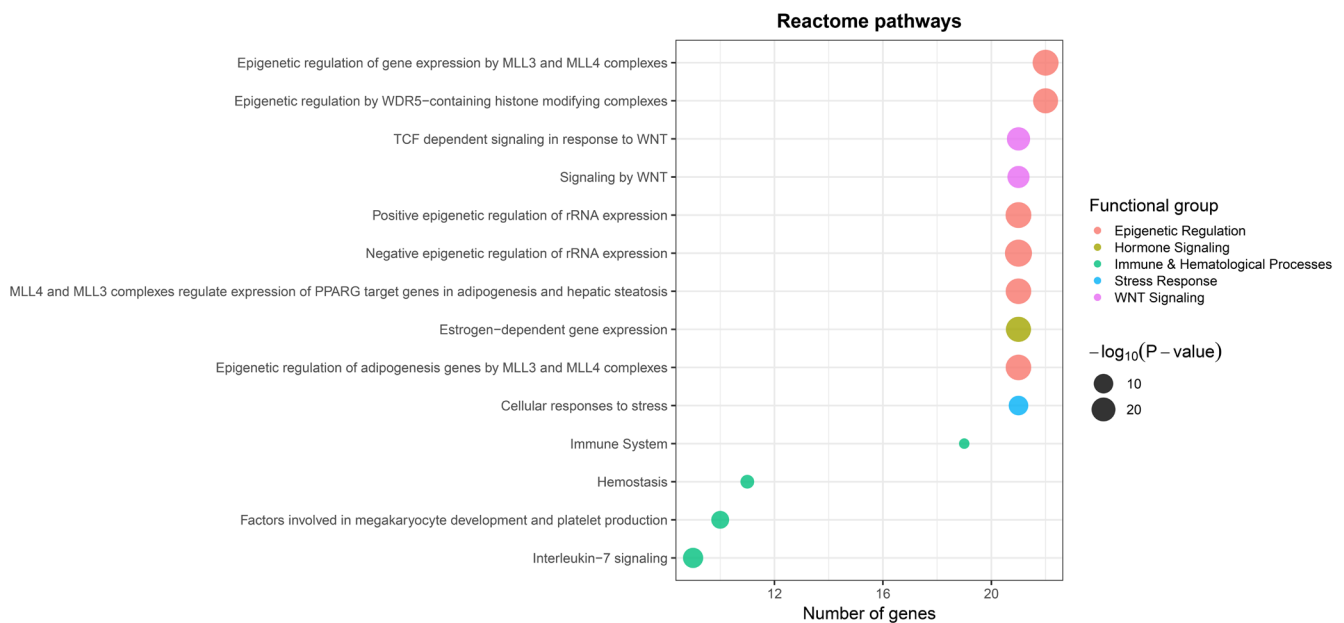


FIGURE 5 | Functional REACTOME pathways significantly enriched with genes associated with PL. The y-axis displays the names of gene sets associated with the studied trait. The size of the dots represents the significance of enrichment ($-\log_{10} p\text{-value}$), and the x-axis represents the number of genes in each functional term.

and Carmichael 2010), alternative splicing (Tripathi et al. 2010), mRNA translation (Aleshkina et al. 2021), and epigenetic regulation (Mercer and Mattick 2013). lncRNAs are also involved in processes such as cell proliferation, apoptosis, pluripotency, and cellular differentiation (Wang et al. 2023), all of which are fundamental for embryonic growth and development (Pauli et al. 2011).

Although the functional roles of lncRNAs in bovine reproduction remain poorly characterized, recent studies suggest their involvement in early reproductive processes. A recent study by

Wang et al. (2023) analyzed lncRNA expression in bovine ovarian follicles and early embryos using a dataset of 468 microarray hybridizations targeting 7724 lncRNA transcripts. Their results showed that lncRNAs participate in regulatory networks controlling folliculogenesis, oogenesis, and early embryonic development. Therefore, the presence of only genes encoding lncRNAs within the genomic window explaining the greatest proportion of PL variance corroborates that noncoding regulatory elements may play an important role during the earliest stages of gestation.

4.2.1 | Maternal-Fetal Immune Regulation

The second most influential genomic region was located on BTA25 and explained 4.71% of the additive genetic variance for PL. This region contained several genes, including *IL4R*, *IL21R*, *GTF3C1*, and *KATNIP*. Among these, *IL4R* and *IL21R* are involved in cytokine signaling pathways associated with immune regulation. Cytokines are small signaling proteins that regulate cell proliferation, differentiation, and immune responses (Orsi 2008). In reproductive tissues, cytokines influence processes such as follicular development, ovulation, and corpus luteum function (Liu et al. 2009), contributing to the establishment of an immunologically permissive environment that supports pregnancy (Orsi and Tribe 2008).

The *IL4R* gene encodes the alpha chain of the interleukin-4 receptor (IL-4R α), which plays an essential role in the differentiation of T helper type 2 (Th2) lymphocytes and the regulation of immune responses (Woods and Judd 2008). IL-4 signaling through IL-4R α activates *STAT6* and promotes the expression of genes involved in Th2 immune responses. In cattle, maintaining a balanced uterine immune environment is essential for maternal recognition of pregnancy and prevention of inflammatory responses that could compromise gestation. Therefore, genetic variation affecting *IL4R* may influence maternal immune tolerance and increase susceptibility to PL.

Similarly, the *IL21R* gene encodes the receptor for interleukin-21 and is expressed in T and B lymphocytes and natural killer (NK) cells (Mehta et al. 2004). In experimental models, IL-21/IL-21R signaling activates components of the JAK/STAT pathway, including *STAT3*, and may influence the proliferation, differentiation, and function of immune cells (Avery et al. 2008). Recent evidence indicates that this signaling axis may play a direct role in the regulation of pregnancy. In humans, this pathway contributes to the differentiation of uterine natural killer cells that participate in spiral artery remodeling during pregnancy, a process required for proper maternal-fetal immune tolerance and placental vascularization (Han et al. 2023). Although the JAK/STAT pathway was not significantly enriched in the present analysis ($p=0.34$), the biological role of *IL21R* suggests a potential contribution to reproductive immune regulation.

Another candidate gene identified in our study was *LRRC4C* (leucine rich repeat containing 4C), located in a genomic window on BTA15 explaining 1.52% of the additive genetic variance for PL. *LRRC4C* is involved in cell adhesion molecule binding and the regulation of neural progenitor cell differentiation (Zhang et al. 2013). Binelli et al. (2015), studied transcriptome signatures in beef cattle and associated this gene with bovine uterine receptivity during early gestation, and this affects subsequent development of the fetus during gestation. Because embryo implantation requires extensive communication between embryonic and maternal tissues, variation in genes related to cell adhesion and signaling may influence early embryonic survival.

The *RBMS3* (RNA binding motif single stranded interacting protein 3) gene, located within a genomic window explaining 1.67%

of the variance for PL, encodes an RNA/DNA-binding protein involved in transcriptional regulation and extracellular matrix remodeling (Górnicki et al. 2022). These biological functions are critical for processes such as angiogenesis, cell migration, and tissue remodeling during embryo implantation and placental development. Supporting its potential relevance for reproduction, *RBMS3* has also been reported as a candidate gene associated with reproductive traits in *Bos indicus* adapted to arid environments (Sukhija et al. 2024).

Another candidate gene identified in this study was *ZFPM2* (zinc finger protein, FOG family member 2), located on BTA14 and explaining 1.14% of the additive genetic variance for PL. *ZFPM2* encodes a zinc-finger protein that interacts with GATA transcription factors, including *GATA4*, which plays a central role in gonadal development and reproductive tissue differentiation. Previous studies demonstrated that *ZFPM2* facilitates the interaction between *GATA4* and *SRY* during early gonadal formation (Conta and Breitbart 2008). Although this mechanism has primarily been studied in humans and mice, these conserved transcriptional interactions suggest that variation in *ZFPM2* may influence reproductive signaling pathways important for pregnancy establishment.

On BTA29, the *PRSS23* (serine protease 23) gene is located within a genomic region explaining 1.45% of the variance for PL. *PRSS23* has been associated with follicular atresia, a process involving the degeneration of ovarian follicles (Wahlberg et al. 2008). Previous studies reported differential expression of *PRSS23* across follicular development stages and increased expression in atretic follicles (Miyakoshi et al. 2006; Wahlberg et al. 2008). In mouse, *PRSS23* could be involved in the uterine luminal epithelium preparation for the embryo attachment (Diao et al. 2013). These findings suggest that *PRSS23* may contribute to extracellular matrix remodeling or apoptosis through loss of cell–matrix adhesion, processes that could influence follicular health and subsequent reproductive success.

Finally, the *NDST4* (N-deacetylase and N-sulfotransferase 4) gene, located on BTA6 and explaining 1.34% of the variance for PL, belongs to the N-deacetylase/N-sulfotransferase family involved in the biosynthesis of heparan-sulfate proteoglycans. These molecules regulate extracellular matrix structure and growth factor signaling. Studies in mice have demonstrated that related enzymes (NDST1-3) are essential for uterine receptivity and embryo implantation (Yin et al. 2018). Although *NDST4* has not yet been directly linked to bovine reproductive processes, its biological function suggests a potential role in early pregnancy events.

Taking together, the putative candidate genes genomic regions associated with PL participate in biological processes related to immune regulation, extracellular matrix organization, transcriptional regulation, and cellular signaling. These mechanisms are essential for establishing uterine receptivity, supporting embryo implantation, and maintaining early placental development. To further explore the biological pathways underlying these associations, functional enrichment analyses were performed.

4.3 | Functional Enrichment Analysis

4.3.1 | Chromatin Organization and Epigenetic Regulation

Among the enriched biological processes (Figure 2), nucleosome assembly (GO:0006334) showed the strongest statistical significance ($p = 1.69 \times 10^{-15}$). This enrichment was driven primarily by several histone genes, including *H2BC1*, *H4C3*, *H1-1*, *H1-2*, and *H1-6*, as well as the histone-modifying enzyme *PADI4* (peptidyl arginine deiminase 4). The *PADI4* gene encodes a transcriptional regulator expressed in embryonic stem cells that mediates pluripotency, a key mechanism for gastrulation (Singh et al. 2023). Nucleosomes are the fundamental structural units of chromatin, formed by DNA wrapped around histone proteins, and their assembly plays a central role in maintaining chromatin integrity and regulating gene expression (Dutta et al. 2001; Fyodorov et al. 2017). It is well known that throughout oogenesis and embryo development the epigenome is regulated through histone modifications (Bozdemir and Uysal 2023).

The results obtained for the REACTOME pathways reinforced this pattern, highlighting pathways related to epigenetic regulation of gene expression, including positive and negative regulation of rRNA expression and epigenetic regulation mediated by MLL3/KMT2C and MLL4/KMT2D complexes. These pathways were largely driven by histone genes and PAXIP1 (PAX interacting protein 1), a component of histone-modifying complexes. Histone modifications regulate chromatin accessibility and transcriptional activity, processes that are critical during early embryonic development and tissue differentiation (Xie et al. 2022). Chromatin remodeling and histone modification have been widely recognized as essential mechanisms during early embryogenesis, when extensive transcriptional reprogramming occurs. Therefore, the enrichment of chromatin-related processes suggests that epigenetic regulation may influence pregnancy success by affecting gene expression programs required for embryo development and early placental formation.

4.3.2 | Transport Processes Embryonic Development

Several enriched GO biological processes were related to transmembrane transport, including monoatomic anion transport, urate transport, sodium-dependent phosphate transport, and transmembrane transport. These terms were mainly driven by genes belonging to the *SLC17* family (*SLC17A1*, *SLC17A2*, *SLC17A3*, and *SLC17A4*) and ATP-binding cassette transporters (*ABCA14* and *ABCA16*).

Membrane transporters play an essential role in maintaining the uterine environment required for early embryonic development. In cattle, the uteroplacental tissues regulate the expression of multiple nutrient transporters during early gestation to ensure adequate nutrient supply to the developing conceptus (Crouse et al. 2020). Disruptions in these transport mechanisms may alter nutrient availability and metabolic signaling, potentially affecting embryo viability.

4.3.3 | Cell Adhesion and Embryo–Maternal Interaction

Another enriched biological process was negative regulation of focal adhesion assembly (GO:0051895), driven by genes such as *RCC2* (regulator of chromosome condensation 2) and *PHLDB2* (pleckstrin homology like domain family B member 2), which are involved in regulating cell adhesion and migration. Focal adhesions are dynamic protein complexes that mediate interactions between cells and the extracellular matrix and play important roles in cell migration, tissue remodeling, and intracellular signaling. *PHLDB2* is also involved in the regulation of embryonic implantation and development (Sabri et al. 2014; Poh et al. 2024).

These mechanisms are particularly relevant during early pregnancy, when endometrial remodeling and embryo attachment require coordinated interactions between maternal tissues and the developing conceptus. Studies in cattle have demonstrated the importance of adhesion signaling pathways at the maternal–conceptus interface, including integrin-mediated signaling and focal adhesion kinase pathways involved in placental development and uterine remodeling (Bridger et al. 2008; Luo et al. 2024). Disruptions in these adhesion processes may impair embryo implantation and increase the risk of PL.

4.3.4 | Calcium Signaling and Early Embryonic Development

The enrichment analysis of molecular function terms also highlighted calcium-dependent protein binding and calcium ion binding, largely driven by the *SCGN* (secretagogen, EF-hand calcium binding protein) gene and members of the S100 protein family. Calcium signaling plays a central role in oocyte maturation, fertilization, and early embryonic development by regulating intracellular signaling cascades and protein–protein interactions (Chen et al. 2024).

Proteins of the S100 family participate in multiple cellular processes, including cytoskeletal dynamics, cell signaling, and immune responses. In addition, those proteins play a critical role in embryo adhesion and implantation, endometrial receptivity, prolactin secretion, immune tolerance, and endometrial epithelial cell apoptosis, to prevent embryo rejection (Singh and Ali 2022). These processes are essential for embryo development and maternal–embryo communication during early gestation. Disruptions in calcium-dependent signaling pathways have been associated with reproductive disorders and increased risk of PL in mammals (Ibrahim et al. 2016; Paudel et al. 2018; Liu et al. 2021).

4.3.5 | Immune Regulation and Pregnancy Maintenance

The enrichment of the pathways related to the immune system was driven by genes such as *IL6R*, *IL21R*, *S100A1*, *S100A7*, *VAV3*, *ARF1*, and *ILF2*, which participate in cytokine signaling and immune-cell activation (Orsi 2008; Liu et al. 2009). Proper regulation of maternal immune responses is essential for the

establishment and maintenance of pregnancy, as the maternal immune system must tolerate the semi-allogeneic embryo while maintaining protection against pathogens. Disruptions in cytokine signaling or immune regulation may therefore increase the risk of embryo loss. The identification of immune-related genes among the enriched pathways supports the importance of immunological balance in early pregnancy success.

4.3.6 | Implications for Reproductive Biology and Breeding

Several enriched pathways, including WNT signaling and cellular responses to stress further support the involvement of regulatory networks controlling embryonic development and placental formation. WNT signaling is known to regulate trophoblast differentiation, placental angiogenesis, and chorion–allantois fusion, processes that are critical for placental development and fetal growth (Sonderegger et al. 2010). In cattle, proper activation of these pathways is essential during early gestation, when communication between the developing conceptus and maternal tissues establishes uterine receptivity and supports placental attachment and vascularization.

Taken together, the enrichment results suggest that PL in cattle is influenced by biological processes involving epigenetic regulation, immune signaling, nutrient transport, cell adhesion, and intracellular signaling pathways. These mechanisms play fundamental roles in establishing uterine receptivity, supporting embryo implantation, and maintaining early placental development in beef cattle. Disruptions in these regulatory networks may compromise embryo–maternal communication during the earliest stages of gestation, increasing the risk of PL.

From a breeding perspective, the identification of genomic regions and biological pathways associated with PL contributes to a better understanding of the genetic architecture underlying reproductive efficiency. Because reproductive traits typically present low heritability, integrating genomic information and functional knowledge may help improve the accuracy of genetic evaluations and support the development of selection strategies aimed at reducing pregnancy loss and improving reproductive performance in Nellore cattle.

5 | Conclusion

Twenty-five genomic regions distributed across 16 chromosomes were identified as explaining a meaningful proportion of the additive genetic variance associated with PL. These regions contain putative candidate genes involved in biological processes that are essential for pregnancy establishment and maintenance, including epigenetic regulation, immune signaling, cellular communication, and placental development. Functional enrichment analyses further supported the involvement of these pathways, highlighting mechanisms related to embryo–maternal communication and early gestational success.

Overall, our results provide new insights into the genetic architecture underlying PL in Nellore cattle. The genomic regions and biological pathways identified represent promising targets

for future functional studies and may contribute to the development of genomic selection strategies that aim to improve reproductive efficiency in beef cattle.

Author Contributions

Flávia C. Bis: writing – review and editing, writing – original draft, visualization, software, formal analysis, conceptualization. **Nedenia Bonvino Stafuza:** writing – review and editing, formal analysis. **Elisângela Chicaroni de Mattos:** data curation, software, resources. **Angélica Simone Cravo Pereira:** writing – review and editing, resources. **José Bento Stermann Ferraz:** writing – review and editing, supervision. **Sabrina T. Amorim:** writing – original draft, writing – review and editing, supervision, project administration. **Fernando Baldi:** writing – review and editing, project administration, methodology, conceptualization, formal analysis.

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Disclosure

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Ethics Statement

This study was exempt from evaluation by the Animal Ethics Committee (CEUA), as established by Law No. 11794 of 08/10/2008 and Normative Resolution No. 51 of 05/19/2021 from the National Council for Animal Experimentation Control (CONCEA) because the data was obtained from an existing database.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are commercially sensitive and are available under reasonable request from the Animal Genetics and Breeding Program—GMA, developed by the Animal Breeding and Biotechnology Group (GMAB, FZEA—USP, Pirassununga, São Paulo, Brazil). Data will only be available after signing a Material Transfer Agreement with restrictions on the use of the data, such as confidentiality.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Trace plots for heritability (h²). **Table S1:** Functional enrichment analysis of genomic regions associated with pregnancy loss in Nellore cattle.