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Genomic study for pregnancy loss in Brahman cattle

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Abstract

Reproduction has major influence on productivity of beef cattle operations. Maintaining an animal in the herd for an extended period without producing a marketable product can result in significant economic losses, compromising the efficiency of the production system. Understanding genetic variation's role in pregnancy loss (PL) is crucial for improving reproductive success in cattle. Identifying genomic regions that influence embryo and fetal survival, as well as pinpointing candidate genes associated with PL, can enhance breeding strategies. The objective of this study was to estimate variance components and investigate genetic factors associated with PL in Brahman cattle. Phenotypic records consisted of 29,905 pregnancy (28,691) and abortion (1,214) records from nulliparous, primiparous, and multiparous cows. A total of 921 animals were genotyped using a medium-density SNP chip (~52K markers). Variance components were estimated using a threshold model to assess the binary response to PL through a single-step genomic BLUP procedure. The heritability estimate for PL was low (0.11), but the presence of genetic variance suggests that selection for improved reproductive performance is feasible. Genome-wide association analyses identified 17 candidate regions containing 92 genes. Regions on BTA4, 7, 8, 9, 11, 12, 16, 18, 19, 21, 22, and 29 harbored genes associated with embryonic development and implantation, fertilization, G protein-coupled receptors, embryonic brain development, olfactory receptor activity, and calcium signaling. Orthologous genes were also identified in humans (*Homo sapiens*), rats (*Rattus norvegicus*), and mice (*Mus musculus*). The candidate regions reported in this study provide insights for identifying and selecting animals with improved reproductive performance, ultimately enhancing the productivity of Brahman cattle. Moreover, our findings contribute to a better understanding of the genetic and physiological mechanisms underlying pregnancy retention in beef cattle.

Lay Summary

Pregnancy loss (PL) is a major contributor to reproductive inefficiency in beef cattle operations and carries significant economic implications for producers. Although management and environmental factors are known to influence pregnancy maintenance, the genetic factors contributing to PL remain poorly understood, particularly in *Bos indicus*-influenced breeds. We identified genomic regions and candidate genes associated with PL using genomic and phenotypic data from a Brahman population. Our results revealed several genomic regions potentially involved in reproductive processes essential for pregnancy maintenance. The identified candidate genes are associated with biological functions such as embryo development, immune response, and hormonal regulation—all of which play critical roles during early gestation. By identifying animals with a lower genetic risk of PL, producers may improve calving rates and enhance overall herd productivity through more informed selection and breeding decisions.

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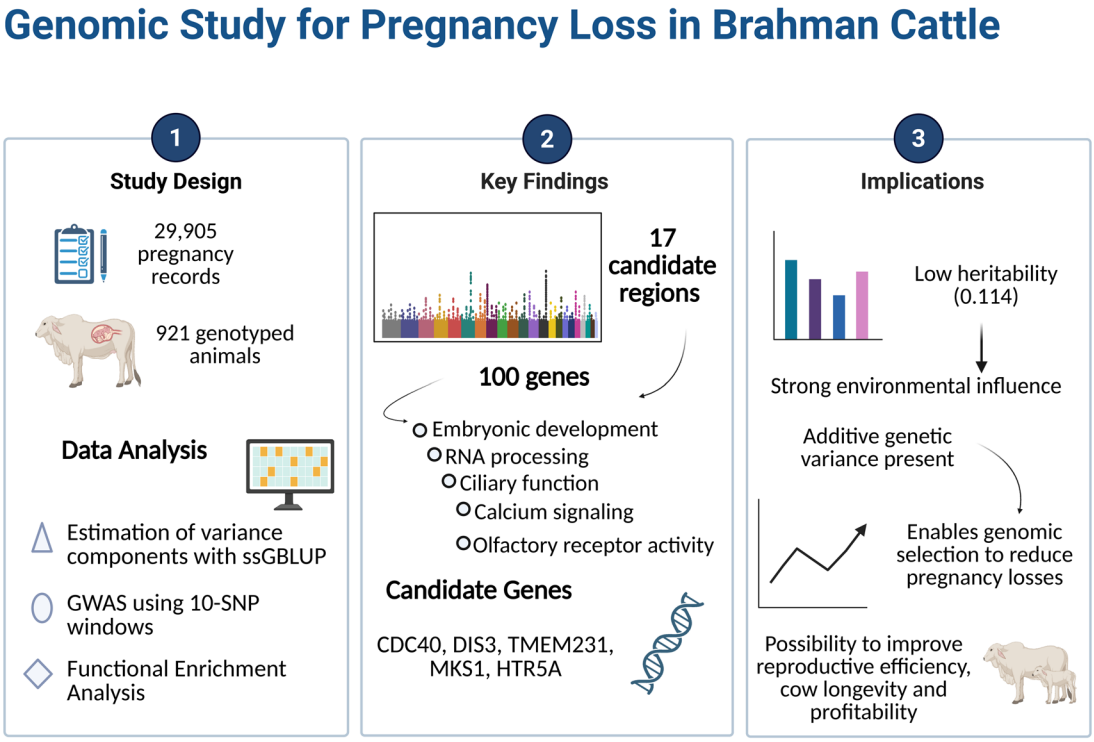
Graphical abstract



Key words: abortion, Bos indicus, GWAS, genetic parameters, reproductive efficiency

Abbreviations: AI, artificial insemination; ANCP, Associação Nacional de Criadores e Pesquisadores; BCS, body condition score; BLUP, best linear unbiased prediction; BTA, *Bos taurus* chromosome; BOA, Bayesian output analysis; CNVR, copy number variant region; DAVID, database for annotation, visualization and integrated discovery; eQTL, expression quantitative trait loci; GEBV, genomic estimated breeding value; GO, gene ontology; GWAS, genome-wide association study; KEGG, kyoto encyclopedia of genes and genomes; MAF, minor allele frequency; MAP, mycobacterium avium subspecies paratuberculosis; MeSH, medical subject headings; OMIA, Online Mendelian Inheritance in Animals; OMIM, Online Mendelian Inheritance in Man; PAGs, pregnancy associated glycoproteins; PL, pregnancy loss; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; ssGBLUP, single-step genomic BLUP;

Introduction

The economic sustainability of beef cattle production relies on 4 key pillars: genetic improvement, animal health, nutrition, and reproduction (Herring, 2014; Terry et al., 2021). Among these, reproduction plays the most critical role in determining system efficiency, as it directly impacts the production of the industry's most vital output—the calves.

The establishment and maintenance of pregnancy are critical to reproductive efficiency and the economic sustainability of beef cow-calf herds. Pregnancy losses (PL) are a significant issue, resulting in an estimated $3.7 billion in losses per year for beef cattle operations (Ealy and Seekford, 2019). Causes of PL in cattle are broadly classified into 2 categories, infectious agents- such as reproductive tract infections leading to abortion (Van Loo et al., 2021; Mee, 2023) and noninfectious factors, including genetic defects, nutritional deficiencies, teratogens, and environmental stressors (Poliakiwski et al., 2025).

In beef cattle systems, the primary goal of cow-calf operations is to maximize the number of calves produced by each cow per year. However, reproductive management in beef herds is generally less intensive than in dairy herds, which means many PL go undetected, making it challenging to determine when they occur or identify their underlying causes. More specifically, the Committee on Bovine Reproductive Nomenclature (1972) categorizes PL into 2 stages: (1) embryonic loss, occurring from fertilization to 42 d of gestation, and (2) fetal loss, occurring from 43 to 280 d. Research indicates that embryonic losses can affect up to 54% of beef cattle within the first 45 d post-insemination, with approximately 28% occurring by day 7, 4% between days 7 and 16, 16% from days 16 to 32, and 6% from days 29 to 45 (Reese et al., 2020; Poliakiwski et al., 2025). Early losses, particularly those occurring before maternal recognition of pregnancy (~ day 17) are difficult to detect, as they do not alter the length of the estrous cycle (Poliakiwski et al., 2025). Early embryonic mortality reduce herd profitability, as cows experiencing PL are 3 times more likely to be culled, and if they remain in the herd, they have a 5 times greater risk of late-term abortion compared to cows that have never aborted (El-Tarabany and El-Tarabany, 2015).

The Brahman breed (*Bos indicus*) was developed in the United States through crossbreeding Nelore, Gir, Guzerá, and Krishna Valley cattle (Sanders, 1980). These animals are highly adapted to tropical climates and well known for superior thermoregulation under heat stress (Hammond et al., 1996). Notably, Hernández-Cerón et al. (2004) reported that Brahman embryos demonstrate greater resistance to elevated temperatures compared to embryos from other heat-sensitive breeds. Limited data exists on embryonic mortality differences between *Bos taurus* and *Bos indicus* cattle. Interestingly, a meta-analysis by Reese et al. (2020) reported that *Bos indicus* animals had higher rates of early embryonic mortality (losses between days

7 and 32 of gestation) compared to *Bos taurus*. However, no differences were observed between the subspecies during the late embryonic/early fetal period (gestational days 32–100).

While the genetic influence on PL has been extensively studied in dairy cattle, there is a limited body of research focused on PL in beef cattle, particularly in *Bos indicus*-influenced breeds. Several genome-wide association studies (GWAS) have identified genetic variants linked to PL traits, but these efforts have largely focused on dairy breeds (Bamber et al., 2009; Sigdel et al., 2021; Kelson et al., 2024; Suarez et al., 2024; Kiser et al., 2025). Identifying genomic regions associated with PL would provide valuable insights for genetic improvement strategies in beef production systems. Therefore, the objectives of this study were to estimate variance components and identify genomic regions and biological pathways associated with PL in Brahman cattle.

Material and Methods

Ethics statement

Institutional Animal Care and Use Committee approval was not required for this study because all data were obtained from the Associação Nacional de Criadores e Pesquisadores (ANCP, Ribeirão Preto, Brazil) computer database.

Animals and phenotypes

Pregnancy Loss data (n = 29,905) were collected for Brahman cattle raised in 2 herds in Bolivia—Estancias Espiritu in Beni and San Judas in Santa Cruz de la Sierra, covering mating seasons from 1998 to 2021. Records were categorized into 3 pregnancy stages: nulliparous heifers (first pregnancy), primiparous cows (second pregnancy), and lactating multiparous cows (third pregnancy).

Both herds followed reproductive disease prevention protocols. Female cattle received vaccinations for bovine viral diarrhea, infectious bovine rhinotracheitis, and *Leptospira* at the beginning of the breeding season, with a booster administered 20–30 d later. A third dose was given at pregnancy diagnosis by rectal palpation. Bulls were vaccinated annually 15–30 d before the breeding season. In addition, the RB51 vaccine for brucellosis was administered to females between 3 and 8 months of age, and annual serological testing for brucellosis was performed on all breeding males.

Heifers entered the breeding season at approximately 2 years of age with a minimum weight of 280 kg. The breeding season lasted 90–100 d from October to January, with artificial insemination (AI) used for the first 45–60 d, after which non-pregnant cows were moved to controlled natural mating. Pregnancy was confirmed by rectal palpation approximately 60 d after the breeding season ended. Pregnancy Loss was defined as the failure to calve following a confirmed pregnancy diagnosis, either due to observed abortion or the absence of calving. Cows with successful pregnancies were assigned a phenotype of 1, while cows experiencing PL were assigned a phenotype of 2.

Genotyping of animals

A total of 921 animals were genotyped using 53,492 SNP (Bovine medium-density chip, Illumina, San Diego, CA, USA). The quality control of the SNP markers consisted of excluding those with unknown genomic position, located on sex chromosomes, with minor allele frequency (MAF) less than 0.05, out of Hardy-Weinberg equilibrium (P-value <10^{-6}), with excess heterozygosity, and highly correlated with each other (SNP pairs with a small difference in allele frequency). Also, samples with a call rate less than 90% were removed. After quality control, 46,342 SNP were used for analyses. PREGSF90 software was used for SNP quality control (Misztal et al., 2002). Missing genotypes were imputed using allele frequency estimates derived from a binomial distribution based on observed SNP data. The imputation was performed using a custom R script, which replaced missing values with the most probable genotype given the estimated allele frequencies. The average missing genotype rate prior to imputation was ~13%. All SNP markers were assigned an ARS-UCD1.3 bovine genome build position.

Estimation of variance components

The variance components were estimated using a single-trait animal model by the single-step genomic BLUP (ssGBLUP) procedure. The ssGBLUP is a modified version of the animal model (BLUP), that consists of integrating additive relationship matrix$(\mathbf{A})$ and genomic relationship matrix$(\mathbf{G})$ into a single matrix $(\mathbf{H})$ (Legarra et al., 2014). The inverse of the **H**matrix ($\mathbf{H}^{-1}$) 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 $\mathbf{G}^{-1}$ is the inverse of the genomic relationship matrix (**G**1) as in VanRaden (2008), and $\mathbf{A}_{22}^{-1}$ is the inverse of **A** for genotyped animals. The pedigree traced back up to 3 generations and consisted of 14,949 animals (23.86% missing parents).

Statistical model overview

To analyze the incidence of PL, we employed a threshold repeatability animal model to account for the binary nature of the trait and repeated measurements within individuals. The general model is specified as:

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

where **y** is a vector of the dependent variable (PL), with binary values indicating success (1) or failure (2); **X** is the incidence matrix for fixed effects, linking observations to systematic effects; $_{\rho}$ is a vector of fixed effects including contemporary groups (animals born in the same farm, year and month of pregnancy diagnosis and age at pregnancy diagnosis as a covariable; **Z** is the incidence matrix for additive genetic effect; **a** is the vector of additive genetics effects; **W** is the incidence matrix for permanent environmental effect; **pe** is the vector of permanent environmental effects, and **e** is a vector for random residual effects associated with the observations.

According to the threshold model, the (co)variance components and genetic parameters for PL were estimated that assuming an underlying scale (**U**) with normal distribution determined by:

$$\mathbf{U} \sim \mathbf{N}\left(\mathbf{X}\beta + \mathbf{Z}\mathbf{a} + \mathbf{W}\mathbf{pe} + \mathbf{I}\sigma_e^2\right)$$

The prior distributions for the random effects in the model, additive genetic effect, permanent environmental effect, and

residuals follow multivariate normal distributions, defined as follows:

$$P(\mathbf{a}|\sigma^2_a) \sim N(0, \sigma^2_a)$$
$$P(\mathbf{pe}|\sigma^2_{pe}) \sim N(0, \sigma^2_{pe})$$
$$P(\mathbf{e}|\sigma^2_e) \sim N(0, \sigma^2_e)$$

Where σ^2_a is the additive genetic variance, σ^2_{pe} is the permanent environmental variance, and σ^2_e is the residual variance.

Since the underlying liability distribution (**U**) is not directly observed, a link between the continuous latent variable **U** and the observed categorical outcome (***y***) is established through a threshold. The probability that an observation falls into a specific category (for example success or failure) is given by:

$$P(y_r = 0|t,) = P(U_r < t|t,) = \varnothing((t - W_r)/\sigma_e)$$

In this model, y_r is the observed response for the *r*th observation, with possible values of 1 (success) or 2 (failure). The threshold **t** is an arbitrarily assigned value since the true threshold is unobservable. $U_r$represents the underlying liability for the *r*th observation, which determines the binary outcome. The function ∅ denotes the cumulative distribution function of a standard normal variable; W_r represents the row in the incidence matrix associated with the *r*th observation, linking the latent variable to the observed outcome. Finaly, $\varnothing = (b', a')$is the vector of parameters, combining systematic (fixed) effects (*b*) and random effects (*a*).

The analyses were conducted using Bayesian inference with the Gibbs sampling algorithm implemented in the GIBBSF90+ software (Lourenco et al., 2022). Gibbs chains of 1,000,000 iterations were generated, with a burn-in period of 200,000 iterations and a sampling interval of every 10 cycles. Convergence was assessed through visual inspection and the Geweke diagnostic test (Geweke, 1991) which compares the means of the early and late segments of the chain to detect lack of stationarity. All diagnostic analyses were performed using the Bayesian Output Analysis (BOA) package in R software (R Core Team, 2023), and convergence was confirmed when Z-scores from the Geweke test fell within the range of −1.96 to 1.96 for all parameters.

Genome wide association study (GWAS)

Genome-wide association study analyses were conducted using the single-step method proposed by Wang et al. (2012), applying the previously described threshold animal model to estimate variance components. The animal effects were partitioned into genotyped ($\mathbf{a}_g$) and non-genotyped ($\mathbf{a}_n$) animals. For genotyped animals, the animal effect is defined as:

$$\mathbf{a}_g = \mathbf{Mu}$$

Where **M** is a matrix relating genotypes at each locus, and **u** represents a vector of marker effects. The variance of the animal effect for genotyped animals was assumed as:

$$var(a_g) = var(\mathbf{Mu}) = \mathbf{MDM}'\sigma^2_u = \mathbf{G}^*\sigma^2_a$$

Where **D** is a diagonal matrix of weights for marker variances (**D** = **I** for GBLUP), σ^2_u is the additive genetic variance captured by each SNP marker when no weights are applied and $\mathbf{G}^*$is the weighted genomic relationship matrix.

Marker effects were estimated as:

$$\hat{\mathbf{u}} = \mathbf{DM}'\mathbf{G}^{*-1}\hat{\mathbf{a}}_g = \mathbf{DM}'[\mathbf{MDM}']^{-1}\hat{\mathbf{a}}_g$$

where »is a variance ratio defined by VanRaden et al. (2009):

$$\lambda = \frac{\sigma^2_u}{\sigma^2_a} = \frac{1}{\sum_{i=1}^{m} 2p_i(1-p_i)},$$

where m is the number of SNPs, and p_i is the allele frequency of the second allele for the *i*th SNP.

Wang et al. (2012) iterative process was used to estimate SNP effects. The genomic estimated breeding values (GEBV) were updated for all animals across 3 iterations. The proportion of additive genetic variance explained by the *k*th region was calculated as:

$$\frac{Var(u_k)}{\sigma^2_a} x\,100\% = \frac{Var\left(\sum_{j=k}^{n} Z_j ß_j\right)}{\sigma^2_a} \times 100\%$$

where u_k represents the genetic value of the *k*th region, consisting of 10 continuous adjacent SNPs, $\sigma^2_a$is the total additive genetic variance, Z_j is the vector of SNP content for the *j*th SNP across all individuals, and $ß_j$ is the marker effect of the *j*th SNP within the *k*th region. The GWAS analyses were performed using the BLUPF90 family software (Aguilar et al., 2010) modified to include genomic information. Chromosome regions that explained more than 1.0% of the additive genetic variance were selected to explore and determine possible quantitative trait loci (QTL).

Gene annotation and functional enrichment analysis

The Ensembl Biomart tool with the Genes 113 database and cow genes ARS-UCD1.3 dataset was used to identify the gene content of genomic regions displaying more than 1% of the additive genetic variance for PL.

The search for significant ($P < 0.05$) pathways (KEGG—Kyoto Encyclopedia of Genes and Genomes) and Gene Ontology terms (GO biological processes, GO cellular components, and GO molecular functions) was performed by DAVID v.2024q4 tool (Sherman et al., 2022) using all genes present in genomic regions that explained more than 1% of the additive genetic variance and the bovine genome as background.

Orthologous genes had their functions investigated in MeSH (Medical Subject Headings, https://www.ncbi.nlm.nih.gov/mesh/), OMIN (Online Mendelian Inheritance in Man, https://omim.org/), and OMIA (Online Mendelian Inheritance in Animals, https://www.omia.org/home/).

Results

Genetic parameter estimates

The variance components and heritability estimates for PL are presented in Table 1. The heritability for PL was low (0.11), indicating that genetic influences PL are limited, with most of the phenotypic variation (~88%) attributed to environmental effects. Despite this, the presence of genetic variance suggests that selection for improved reproductive performance is possible.

Table 1. Genetic parameters for PL

Trait	σ^2_a	σ^2_{pe}	σ^2_e	h^2
PL	0.14 [0.06–0.22]	0.10 [0.002–0.24]	1.00 [0.97–1.02]	0.11 [0.05–0.16]

σ^2_a: additive genetic variance, σ^2_{pe}: permanent environment variance, σ^2_e: residual variance, h^2: heritability

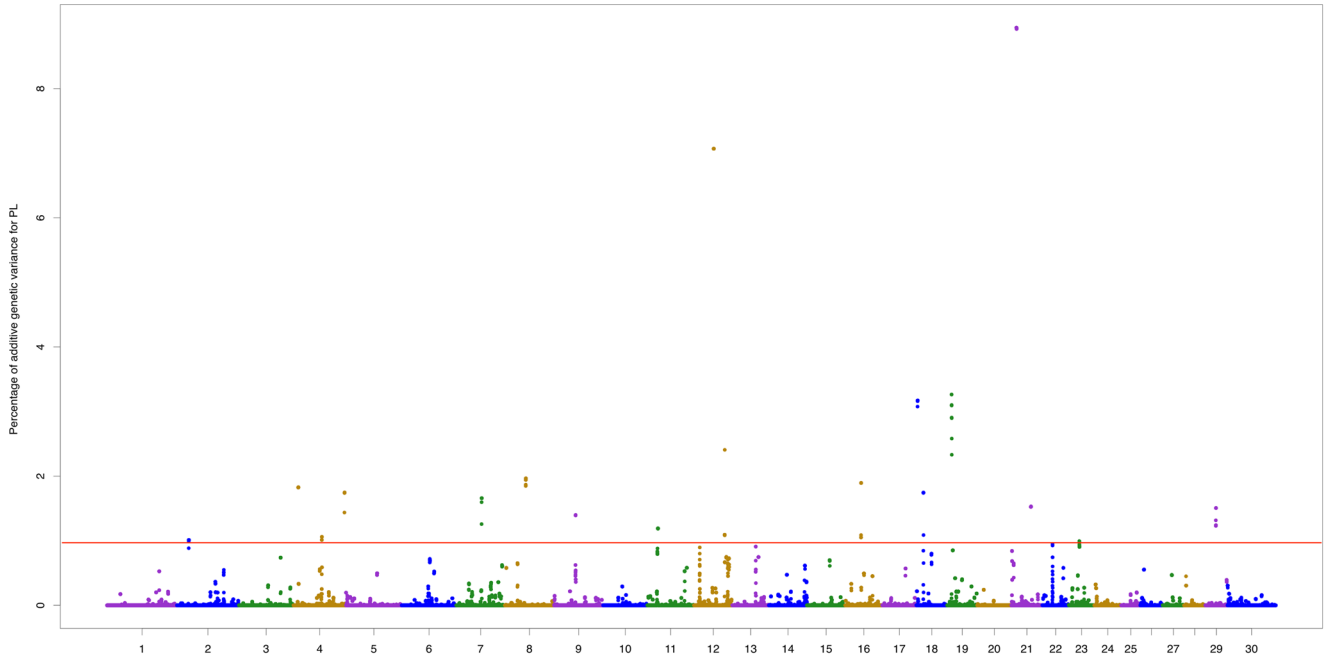


Figure 1. Manhattan plot of the genome-wide association of the studied trait. The X-axis represents the chromosomes, and the Y-axis shows the proportion of the additive genetic variance explained by windows of 10 adjacent SNP. The dots above the red line indicates the genomic regions explaining more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

Genomic regions

Genomic regions and candidate genes were identified based on the % of genetic variance explained by 10 adjacent SNP across the entire bovine genome (Figure 1). The SNPs windows regions which accounted for more than 1% of the genetic additive variance were used to search for candidate genes, which were described in Table 2. A total of 17 candidate regions and 100 genes for PL were identified. The regions were in BTA (*Bos taurus* chromosome) 4, 7, 8, 9, 11, 12, 16, 18, 19, 21, 22, and 29, showing a high number of genes associated with embryonic development and implementation, fertilization, G protein-coupled receptors, embryonic brain development, olfactory receptor activity, and calcium signaling. The genomic window contributing the largest proportion of additive genetic variance was located on chromosome 16 at the 12 Mb position, accounting for 8.68% of the total additive genetic variance.

Enrichment analysis

A total of 100 genes were identified within the 17 genomic regions that explained more than 1% of the additive genetic variance for PL. The functional enrichment analysis revealed 4 GO biological processes, one GO cellular component, 3 GO molecular functions, and 2 KEGG pathways significantly enriched (Table 3, Figure 2, and Figure 3).

Discussion

In our study, pregnancy diagnosis was conducted 60 d after the conclusion of a 90 to 100-d breeding season. Since most cows conceived during the first 2 mo of the breeding season and pregnancy diagnosis occurred 2 mo after its end, the majority of cows were beyond the third month of gestation at the time of pregnancy determination. Consequently, the observed rate of PL of approximately 4.06% aligns with reported rates of fetal loss beyond the third month of gestation in both dairy and beef cattle. For example, in dairy cattle, 1–3% of abortions are reported to occur between days 60 and 90 of gestation (Wiltbank et al., 2016; Albaaj et al., 2023), with an additional 4% estimated to occur between day 90 and term (Santos et al., 2004). Regarding late-term abortions, data from 24.8 million lactations indicate an average abortion rate of 1.2% between days 152 and 251 of gestation in dairy cattle (Neupane et al., 2023). In beef cattle, a meta-analysis by Reese et al. (2020) indicates that 5.8% of pregnancies are lost between days 60 and 100 of gestation. Interestingly, this analysis, which included 40 studies and 30,500 cows, found no differences in PL between *Bos indicus* (5.0%) and *Bos taurus* (5.9%) animals during this late embryonic/early fetal stage. The same meta-analysis, however, indicated that rates of early embryonic mortality, defined as losses occurring between days 32 and 60 or 100, were

Table 2. Genomic regions associated with PL in Brahman Cattle

BTA[1]	Region (bp)	Candidate genes	% Variance explained by SNP windows
4	10278587–10857725	*LOC112446472, SAMD9, VPS50, MIR653, CALCR, MIR489, HEPACAM2, LOC112446530, LOC101906646*	1.77
4	116744972–117014413	*DPP6, PAXIP1, HTR5A, TET3, LOC112446459*	1.78
7	58847118–59327130	*JAKMIP2, C7H5orf46, LOC112447575, SPINK5, SCGB3A2, SPINK1, TRNAC-GCA, TRNAA-AGC, SCGB3A2, LOC100139048*	1.61
7	102968416–103280544	*LOC132345803, LOC787122, SLC25A4*	1.21
8	47624717–48347706	*CEMIP2, C8H9orf85, LOC112447917, ABHD17B, LOC101904574, C8H9orf57*	1.90
9	39917723–40291086	*METTL24, WASF1, GPR6, CDC40*	1.36
11	21740751–22050528	*TMEM178A, THUMPD2*	1.15
12	47213609–47680597	*PIBF1, DIS3, BORA, TRNAS-GGA, MZT1, ENSBTAG00070004440*	6.86
12	71701903–72099398	*LOC100337053, LOC100337076, LOC100848914*	3.31
16	30188773–30611296	*CDC42BPA, AHCTF1, SCCPDH, LOC112441825, LOC112441897, KIF28, LOC781254*	1.84
18	2845267–3275526	*TMEM231, CHST6, TRNAH-GUG, LOC788609, LOC132342775, KARS1, TRNAY-GUA, LOC132342776, ADAT1, TERF2IP, GABAR-APL2, DUXB*	3.08
18	16032434–16243229	*LOC533093, PHKB*	1.69
19	8940285–9174523	*LOC514828, OR4D1B, OR4D1, OR4D2E, MKS1, OR4D2B, OR4D2, OR4D33P, LOC788751, OR4D2G, OR4D2D, OR4D2F, LOC112442599*	3.18
21	11481048–12022835	*LOC101902083, LOC107131592, LOC101902189, LOC112443230, LOC101902131*	8.68
21	46271654–46862117	*LOC107131617, MBIP, LOC101907654, LOC104975406, LOC104975406, NKX2-1, NKX2-8*	1.48
22	22270675–22293335	*LOC540991, LOC614928*	1.06
29	23391568–23842977	*NELL1, LOC112444868, LOC132344181, LOC614928*	1.46

[1]BTA: *Bos taurus* chromosome

Table 3. Gene Ontology terms and pathways revealed by functional enrichment analyses ($P < 0.05$)

Term	*P*-value[1]	Genes
Gene Ontology Biological Process		
GO:0006396~RNA processing	0.007	*DIS3, ADAT1, THUMPD2*
GO:0007186~G protein-coupled receptor signaling pathway	0.010	*OR4D2, OR4D1, OR4D2G, OR4D2B, GPR6, OR4D2F, OR4D2E, LOC514828, OR4D2D, OR4D1B*
GO:0140021~mitochondrial ADP transmembrane transport	0.024	*LOC787122, SLC25A4*
GO:1990544~mitochondrial ATP transmembrane transport	0.024	*LOC787122, SLC25A4*
GO:1990403~embryonic brain development	0.028	*MKS1, CDC40*
Gene Ontology Cellular Component		
GO:0036038~MKS complex	0.025	*MKS1, TMEM231*
Gene Ontology Molecular Function		
GO:0004984~olfactory receptor activity	0.003	*OR4D2, OR4D1, OR4D2G, OR4D2B, OR4D2F, OR4D2E, LOC514828, OR4D2D, OR4D1B*
GO:0004930~G protein-coupled receptor activity	0.01	*OR4D2, OR4D1, OR4D2G, OR4D2B, OR4D2F, OR4D2E, LOC514828, OR4D2D, OR4D1B*
GO:0005471~ATP:ADP antiporter activity	0.021	*LOC787122, SLC25A4*
KEGG pathway		
bta04740: Olfactory transduction	0.006	*OR4D2, OR4D1, OR4D2G, OR4D2B, OR4D2F, OR4D2E, LOC514828, OR4D2D, OR4D1B*
bta04020: Calcium signaling pathway	0.028	*LOC787122, PHKB, HTR5A, SLC25A4*

[1]*P*-value adjusted for multiple comparison test.

greater in *Bos indicus* compared to *Bos taurus*. The authors noted, however, that most studies included in the metanalysis performing pregnancy diagnosis on day 100 were conducted in *Bos indicus* cows, whereas most studies performing pregnancy on day 60 were conducted in *Bos taurus* cows, which may have biased the results. Additional research is needed to determine whether a subspecies effect exists.

There are several risk factors that influence PL rates in cattle. For instance, a recent large-scale study conducted in Brazil with *Bos indicus* Nelore cattle, including 40,104 timed AI records

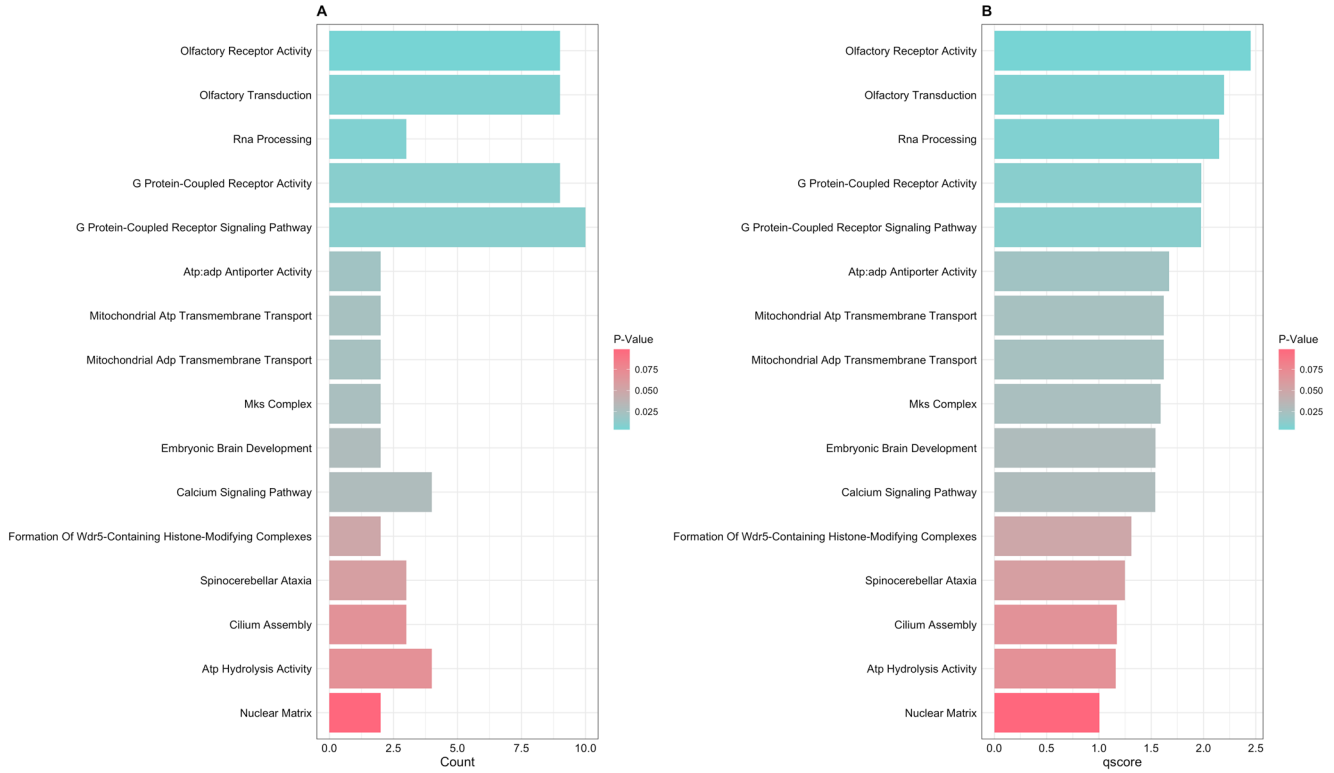


Figure 2. Functional enrichment analysis of genes associated with PL. (A) A bar plot showing the number of genes annotated to each enriched term, colored by *P*-value. (B) The corresponding enrichment scores (q-scores) for the same terms, reflecting the significance of over-representation.

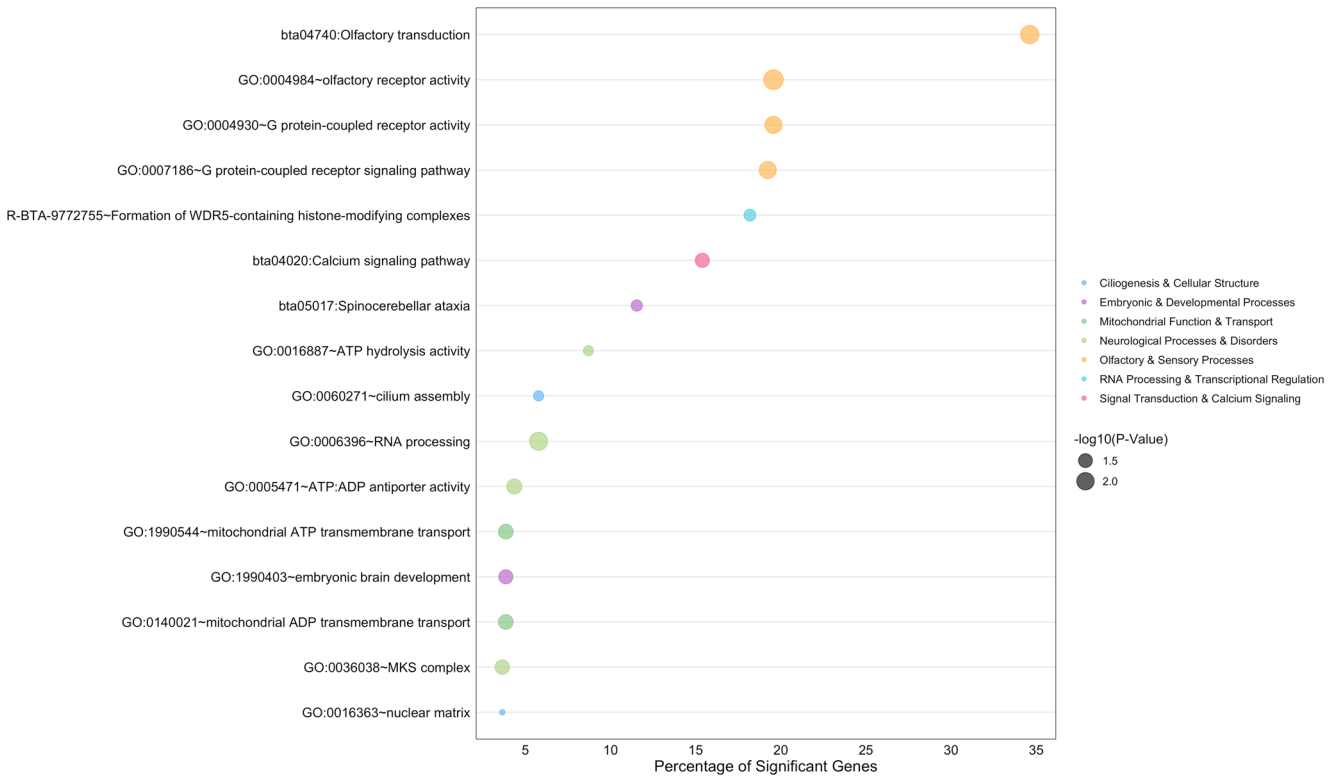


Figure 3. Functional terms and pathways significantly enriched with genes associated with PL. Multiple annotation databases were analyzed, including GO, KEGG, Medical Subject Headings, OMIN, Reactome, and OMIA. The y-axis displays the names of gene sets associated with PL. The size of the dots represents the significance of enrichment (– log10 *P*-Value, Fisher's exact test), and the x-axis represent the percentage of significant genes in each functional term.

across 6 herds (Da Silva et al., 2024), reported an average PL of 5.8% between days 30 and 140 of gestation. The authors noted that parity, herd, body condition score (BCS), and sire significantly affected PL rates. For example, higher rates of embryonic mortality were observed in primiparous cows (7.11%) compared to heifers (5.03%) or multiparous cows (4.26%), and PL rates ranged from 3.37% to 6.89% across the herds evaluated (Da Silva et al., 2024). Lower BCS at the time of breeding was associated with increased PL, whereas BCS gain following AI was linked to reduced embryonic mortality. Additionally, PL varied widely among sires, ranging from 0% to 40% (Da Silva et al., 2024). Additionally, other important variables are known to affect reproductive efficiency, such as individual cow data on nutritional status at the time of breeding, records of disease incidence, the sire used for breeding, influence of AI technician, estrus expression at time of breeding, and the postpartum interval between the calving and breeding seasons (Cushman et al., 2013).

Genetic parameter estimates

Reproductive traits typically have low heritability and respond slowly to genetic selection, which limits the rate of genetic gain. In our study, the heritability estimates for PL was 0.114, which is in the range reported in dairy cattle (0.015 to 0.489). In a study investigating the genetic factors associated with PL in Holstein heifers diagnosed as pregnant 39 ± 3 d after breeding, Sigdel et al. (2021) reported heritability estimates ranging from 0.015 (0.009–0.042) for nulliparous, 0.084 (0.033–0.169) for primiparous, and 0.182 (0.130–0.365) for multiparous cows. Ask-Gullstrand et al. (2023) explored the feasibility of using pregnancy-associated glycoproteins in milk as a tool for breeding selection for pregnancy maintenance and assessed the genetic variation in PL traits in Swedish Red and Swedish Holstein cows. The authors evaluated 3 distinct PL phenotypes across different stages of gestation: total pregnancy loss (TPL), which included losses occurring from 28 d post-AI until calving; fetal loss (FL), defined as PL occurring between 42 d post-AI and calving; and embryonic loss (EL), which refers to losses occurring between 28- and 41-d post-AI. The heritabilities ranged from 0.03 for TPL and 0.02 for FL and EL. Similarly, Sigdel et al. (2022) investigated fetal loss in Holstein cattle in different parity classes using linear and threshold models and observed h^2 estimates ranging between 0.1 to 0.2 for nulliparous heifers, 0.1 to 0.08 in primiparous cows and 0.03 to 0.18 in multiparous cows.

Lastly, Bamber et al. (2009) estimated heritabilities and genetic variances for anovulation (delayed return to cyclicity after calving, h^2=0.160–0.192) and PL occurring between the first and second pregnancy diagnoses after AI. Using a sire–maternal grandsire threshold model, the authors stated that embryo survival has a greater influence on PL than the cow's ability to maintain pregnancy, as the breeding value variance for embryo effects (h^2_d=0.489) was 3 times higher than for maternal effects (h^2_m=0.166). Their findings suggest that selection targeting key reproductive traits, such as prolonged postpartum anovulation and PL, could significantly enhance reproductive efficiency in cattle.

Altogether, our findings, along with existing literature, highlight the potential for selection to enhance reproductive efficiency in cattle by targeting key traits such as PL. While reproductive traits generally exhibit low heritability, the estimates observed in our study indicate the presence of genetic variability for PL, suggesting that selection could contribute to long-term genetic progress. However, a deeper understanding of the genetic architecture of PL is essential for improving reproductive traits that impact productivity and efficiency in production systems.

Gene mapping

Pregnancy Losses are a significant challenge in the cattle industry (Ealy and Seekford, 2019), as they prolong the calving interval, directly impacting herd profitability. Identifying genetic variations associated with PL and uncovering candidate genes that influence the ability to maintain pregnancy are crucial to enhancing reproductive efficiency. In this study, we focused on a subset of genes with functional relevance to fertility for discussion.

The *CDC40* gene, located on BTA9, encodes a protein integral to the pre-mRNA splicing process and is implicated in cell cycle regulation. Dysfunctions in *CDC40* can lead to abnormal gene expression and potential disease-related consequences. In a study aiming to identify gene expression signatures differentiating cattle exposed to *Mycobacterium avium subspecies paratuberculosis* (MAP), Malvisi et al. (2020) identified *CDC40* as part of a potential biomarker set for developing a new diagnostic test for early detection of paratuberculosis. *Mycobacterium avium subspecies paratuberculosis* infection causes Johne's disease, which negatively impacts reproduction in cattle. Infected cows have been associated with lower conception rates and increased days open. Griss et al. (2024) reported a prolonged calving interval by around 30 d and a 1.5 to 3 times higher likelihood of culling per lactation in MAP-positive animals. Additionally, chronically infected cows may experience irregular estrous cycles due to systemic inflammation and malnutrition (Griss et al., 2024). While MAP infection is not typically associated with PL, Whittington and Windsor (2009) suggested that the chronic inflammatory response and altered immune function in infected cows may interfere with embryo implantation and fetal development, leading to early embryonic death, increased PL, and sporadic abortion.

Located on BTA12, the *DIS3* gene is associated with RNA metabolism, microtubule production, and growth and development (Robinson et al., 2015). In a study on Swiss Simmental cattle, Häfliger et al. (2021) performed a reverse genetic screening to identify recessive deleterious variants that may impact fertility and embryonic survival. The authors detected 11 genomic regions with significant homozygosity depletion (deficit of homozygous genotypes), indicating the presence of potentially lethal recessive alleles. Furthermore, the authors performed a whole-genome sequencing of haplotype carriers and identified a loss-of-function mutation in *DIS3* (SH5-related DIS3:p.Ile678fs) (Häfliger et al., 2021). Given the *DIS3* gene's role in RNA metabolism, its disruption likely leads to severe developmental consequences. Moreover, the absence of homozygous individuals carrying the mutation among sequenced animals in their study strongly suggested embryonic lethality. Additionally, *DIS3* has been linked to male infertility in cattle yak (Zhao et al., 2022) and identified as a candidate gene for litter size in sheep and goats (Tao et al., 2021; Sun et al., 2023). In humans, *DIS3* variants have been associated with premature ovarian insufficiency, resulting in loss or complete absence of

ovarian activity (Kline et al., 2024). Furthermore, studies in mice suggest that *DIS3* plays a crucial role in female fertility, as its disruption induces premature meiotic arrest of maturing oocytes, ultimately leading to embryonic lethality (Wu and Dean, 2023).

The MKS transition zone complex subunit 1 gene (*MKS1*, BTA19) plays a critical role in cilia formation and is strongly associated with Meckel–Gruber syndrome (MKS), a severe autosomal recessive developmental disorder caused by primary cilia dysfunction during early embryogenesis (Chen, 2017). Cilia are essential for cell signaling, fluid movement, and embryonic development, and their dysfunction can result in severe congenital disorders (Jenkins and Beales, 2013). *MKS1* mutations account for approximately 7% of MSK cases (Lin et al., 2022). As a congenital ciliopathy, MKS affects embryonic development, often leading to lethal structural abnormalities. In humans, MKS has been associated with neural tube defects, renal cystic dysplasia, liver fibrosis, and polydactyly (Barisic et al., 2014; Chen, 2017; Hartill et al., 2017; Raj et al., 2020), and these anomalies often result in early embryonic lethality or perinatal death. Although MKS is well-documented in humans, it has also been observed in other species, including sheep and mice. Weatherbee et al. (2009) investigated the role of *MKS1* in ciliogenesis by developing a mouse model that closely mimics human MKS phenotypes. Their study demonstrated that *MKS1* loss-of-function in mice leads to neural tube defects, biliary duct abnormalities, disrupted limb patterning, bone malformations, and kidney defects. Additionally, they reported that *MKS1* mutants exhibited dysregulated Shh signaling, a pathway crucial for neural tube formation, limb development, and skeletal growth. These severe structural abnormalities often result in early embryonic death or perinatal lethality. Furthermore, Stayner et al. (2017) identified kidney and liver abnormalities associated with MKS in Perendale and Coopworth sheep.

Finally, the *THUMPD2* (BTA11) and *TMEM231* (BTA18) genes have been implicated in placental development, pregnancy, and fertility. Kikas et al. (2019) identified *THUMPD2* as a gene potentially associated with placental expression quantitative trait loci (eQTLs). Further investigation is needed to determine how *THUMPD2* contributes to key placental functions, as understanding its role in placental dysfunction could help establish it as a potential genetic marker for PL. *TMEM231* (Transmembrane Protein 231) is a core transition zone protein and a key component of the MKS complex, playing a critical role in ciliary function and Hedgehog signaling regulation (Chih et al., 2011; Garcia-Gonzalo et al., 2011; Sang et al., 2011; Shi et al., 2017), as it helps regulate protein trafficking into the ciliary membrane, ensuring the proper localization of Hedgehog pathway components. Since Hedgehog signaling is essential for embryonic development, cell differentiation, and organ formation (Murdoch and Copp, 2010), disruptions in *TMEM231* function may contribute to developmental abnormalities associated with ciliopathies. Neupane et al. (2017) identified *TMEM231* as a candidate gene influencing uterine capacity, pregnancy, and fertility in crossbred (Angus × Polled Hereford) heifers. Later, Kiser et al. (2025) investigated genes and gene sets associated with low conception rates in Holstein cows and identified *TMEM231* as a candidate gene involved in cellular component assembly.

Enrichment analysis

Functional enrichment analysis revealed a small number of significantly GO terms and pathways, which can be attributed to the diversity of functions performed by these genes, corroborating the complexity of the studied trait. We highlighted the significant GO terms and pathways that could be related to PL, such as G protein-coupled receptor signaling pathway (GO:0007186), G protein-coupled receptor activity (GO:0004930), MKS complex (GO:0036038), embryonic brain development (GO:1990403), olfactory receptor activity (GO:0004984), olfactory transduction (bta04740), and calcium signaling pathway (bta04020).

G protein-coupled receptors comprise the largest family of cell-surface proteins involved in signal transduction that play crucial roles in regulating a wide range of physiological processes including those related to reproduction, from conception to labor, participating in the maintenance of pregnancy. Many reproductive disorders have been attributed to alterations in G protein-coupled receptors, such as ectopic pregnancy (Bianchi et al., 2021), preeclampsia (Vidal et al., 2022), unregulated hormonal levels (Casarini and Simoni, 2021; Radomsky et al., 2024), uterine myometrial contractions (Walker et al., 2022), failures in embryo development (Shimada and Mukhopadhyay, 2017), development of recurrent spontaneous abortion due to dysregulation of immune cells at the maternal-fetal interface (Yang et al., 2024), among others. A total of 10 protein-coding genes were identified related to G protein-coupled receptor signaling pathway (GO:0007186) and G protein-coupled receptor activity (GO:0004930), in which we highlight *OR4D1*. The *OR4D1* gene encodes the olfactory receptor traditionally associated with detecting odorants in the olfactory system. However, some studies indicate that *OR4D1* is also expressed in non-olfactory tissues, including the male reproductive system (Hartmann et al., 2013; Elango and Kekäläinen, 2025). In humans, *OR4D1* has been identified as one of several olfactory receptors present on the sperm cell surface (Flegel et al., 2016). These receptors are believed to participate in the process by which sperm navigate chemical gradients to locate the oocyte (sperm chemotaxis). Specifically, activation of *OR4D1* by certain ligands can trigger intracellular calcium transients, influencing sperm motility and directional movement (Veitinger et al., 2011). These findings suggest that *OR4D1* may play a functional role in reproduction by helping guide sperm to the egg and supporting successful fertilization.

MKS complex (GO:0036038) refers a multi-protein complex located at the ciliary transition zone to regulate the contents of cilia. Cilia are organelles of almost all vertebrate cells with a pivot role in sensing and transducing environmental signals from tissue development in embryonic development to throughout adult life (Huljev Frković et al., 2022). A wide range gene mutations that alter cilia are embryonic lethal or results in congenital disorders (Amack, 2022). Proteins encoded by the *MKS1* and *TMEM231* genes are part of the MKS complex and are potential candidate genes for PL.

Embryonic brain development (GO:1990403) refers to the biological process that results is the progression of the brain from its formation to the mature structure. It is well known that embryonic central nervous system malformations may result in abortion (Leibovitz et al., 2022). Studies have been reported genes that regulates fetal brain differentiation and

development associated with PL in Holstein cows (Sigdel et al., 2021; Suarez et al., 2024).

In the olfactory system, the olfactory receptors are expressed at the cell membrane of sensory neurons present in olfactory epithelium to detect odorants and direct sensory axons toward target sites in the brain (Francia and Lodovichi, 2021) which are essential for survival and reproduction of many species, including cattle (Parker Gaddis et al., 2016; Neupane et al., 2017). Olfactory proteins play a crucial role in fertility due to their close interaction with the hypothalamic-pituitary axis and the regulation of reproductive hormones, which are essential for preparing and maintaining the uterus for pregnancy (Kiser et al., 2019). Copy number variant regions are over-represented in the olfactory receptor genes, which have been associated with fertility traits in beef cattle, such as uterine capacity, cell–cell communication during embryogenesis, chemotaxis, tissue growth, and regeneration (Kadri et al., 2014; McDaneld et al., 2014; Neupane et al., 2017). Olfactory receptor genes have been described in lethal haplotypes in Angus beef cattle (Hoff et al., 2017). Studies in humans also have been described an association between PL and enriched in genes involving in olfactory receptor activity (Bai et al., 2023; Gu et al., 2023; Zhang and Ma, 2024).

Calcium signaling (bta04020) acts are intracellular second messenger mediating cell signaling and participating in all aspects of female reproduction, including oocyte maturation, fertilization, proper placental development and function, and embryo development (Machaty, 2024; Zhang and Ma, 2024), playing a critical role in infertility, PL and developmental defects (Paudel et al., 2018). Genes from calcium signaling pathway have been associated with PL in dairy cattle (Oliver et al., 2019; Sigdel et al., 2021).

Four protein-coding genes identified by GWAS in genomic regions associated with pregnant loss act in calcium signaling pathway (bta04020), in which we highlight the *HTR5A* gene. The *HTR5A* gene encodes the 5-hydroxytryptamine receptor 5A, a serotonin receptor that belongs to the G protein-coupled receptor family. This receptor is primarily expressed in the central nervous system, where it modulates neurotransmission by inhibiting adenylate cyclase, ultimately affecting intracellular signaling pathways such as cyclic AMP (cAMP) (Blackburn, 2009). These pathways are involved in a wide range of physiological and behavioral processes, including the regulation of stress responses. In a recent study by Littlejohn et al. (2020), HTR5A was found to be hypermethylated in the promoter region of prenatally stressed Brahman bull calves, suggesting reduced gene expression in these animals. Given the critical role of serotonin signaling in neural development and behavioral regulation, downregulation of HTR5A may reflect an epigenetic response to maternal stress that alters neurodevelopmental trajectories in the offspring. Importantly, dysregulated serotonin signaling has been linked to disruptions in hypothalamic-pituitary-gonadal axis function, altered timing of puberty, changes in sexual behavior, and impaired hormone release related to fertility in rodents, fish, and macaques (Bethea et al., 2002; Gaspar et al., 2003; Prasad et al., 2015). Therefore, reduced HTR5A expression in prenatally stressed animals may influence reproductive outcomes indirectly through alterations in neuroendocrine signaling, stress resilience, or behavior relevant to reproductive success (Mueller and Bale, 2008), supporting the concept of a neuroepigenetic pathway through which early-life stress might have long-term effects on reproductive performance (Bale et al., 2010).

Conclusion

Our findings confirm that PL is a low-heritability trait, meaning that environmental factors play a major role in its expression. Nonetheless, we identified meaningful additive genetic variation, indicating that genetic selection could still be leveraged to improve reproductive outcomes.

Several genomic regions explained substantial portions of the genetic variance and harbored candidate genes involved in key biological pathways, including embryonic development, immune function, and cellular signaling, highlighting the multifactorial nature of PL. A potential limitation of this study is the number of genotyped animals (n = 921), which may have limited our ability to detect regions with small effect sizes. Therefore, validation of the identified genomic regions in larger and independent populations is essential. Despite this, our results reveal the complex genetic architecture of PL and provide new insights into the molecular mechanisms underlying PL. By deepening our understanding of the genetic mechanisms involved, our study provides a foundation for developing genomic selection strategies to reduce reproductive failure, increase cow longevity, and improve the overall sustainability and productivity of beef cattle operations.

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Author Contributions

Sabrina Thaise Amorim (Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing—original draft), Nedenia Bonvino Stafuzza (Conceptualization, Investigation, Writing—review & editing), Daniel Cardona Cifuentes (Conceptualization, Writing—review & editing), João G. N. Moraes (Investigation, Writing—review & editing), Barbara Roqueto dos Reis (Investigation, Writing—review & editing), Riley Messmann (Investigation, Visualization, Writing—review & editing), Luis Camaripano (Investigation, Resources, Writing—review & editing), and Fernando Baldi (Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Software, Writing—review & editing)

Conflict of interest statement. The authors declare that they have no competing interests.

Availability of Data and Materials

The data supporting the results of this article are property of the ANCP (Associação Nacional de Criadores e Pesquisadores,

Ribeirão Preto, São Paulo, Brazil) cattle producers, and this information is commercially sensitive, therefore, the data cannot be made available.

Ethics Approval and Consent to Participate

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

Consent for Publication

Not applicable.

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