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Genome-wide association study for age at puberty in young Nelore bulls

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Abstract

Selection for bulls that would reach puberty early reduces the generation interval and increases fertility and herd productivity. Despite its economic importance, there are few QTL associated with age at puberty described in the literature. In this study, a weighted single-step genome-wide association study was performed to detect genomic regions and putative candidate genes related to age at puberty in young Nelore bulls. Several protein-coding genes related to spermatogenesis functions were identified within the genomic regions that explain more than 0.5% of the additive genetic variance for age at puberty in Nelore bulls, such as *ADAM11, BRCA1, CSNK2A, CREBBP, MEIOC*, *NDRG2, NECTIN3, PARP2*, *PARP9*, *PRSS21, RAD51C, RNASE4, SLX4, SPA17, TEX14, TIMP2* and *TRIP13* gene. Enrichment analysis by DAVID also revealed several GO terms related to spermatogenesis such as DNA replication (GO:0006260), male meiosis I (GO:0007141), double-strand break repair (GO:0006302), base excision repair (GO:0006284), apoptotic process (GO:0006915), cell–cell adhesion (GO: 0098609) and focal adhesion (GO:0005925). The heritability for age at puberty shows that this trait can be improved based on traditional EBV selection. Adding genomic information to the system helps to elucidate genes and molecular mechanisms controlling the sexual precocity and could help to predict sexual precocity in Nelore bulls with greater accuracy at younger age, which would speed up the breeding programme for this breed.

KEYWORDS
beef cattle, *Bos indicus*, GWAS, sexual precocity

1 | INTRODUCTION

Age at puberty is important for cattle performance because it determines the beginning of animal's reproductive life. Selection for fertile bulls that would reach puberty early reduces the generation interval and potentially increases fertility and herd productivity (Fortes et al., 2012; Siddiqui et al., 2008). Although heifer pregnancy is a direct indicator of age at puberty in bulls, scrotal circumference is the most recorded reproductive trait in breeding programmes for beef cattle and is widely used as a main indicator of precocity and fertility. Puberty in bulls has been defined by scrotal circumference thresholds which are inexpensive and easy to measure, are highly heritable and have been correlated to sperm concentration, motility and morphology (Corbet et al., 2011; Siddiqui et al., 2008), age at puberty in bulls and their female relatives (Kluska et al., 2018), and heifer pregnancy (Terakado et al., 2015). Puberty in young bulls could also be characterized by the production of the first ejaculate with at least 50×10^6 sperm with 10% progressive motility (Wolf et al., 1965).

Significant phenotypic differences have been reported for age at puberty among cattle breeds, where Zebu (*Bos indicus*) cattle breeds, such as Nelore, have slower testicular development and tend to reach puberty later than taurine (*Bos taurus*) breeds (Casas, Lunstra, Cundiff, & Ford, 2007; Chase et al., 2001; Lunstra & Cundiff, 2003). Ramírez López et al. (2015), in a study to determine the stage of sexual maturity in Nelore bulls, identified that 84.5% of the bulls that were 19–23 months old were sexually mature. Freneau, Vale Filho, Marques, and Maria (2006) identified that some young Nelore bulls were mature when they were around 15 months old.

Despite its economic importance, there are few QTL associated with age at puberty described in the literature. Fortes et al. (2012), performing a genome-wide association study (GWAS), detected the *CA8, CHD7, CSF2RA, FAM110B, IMPAD1, NSMAF, PCMTD1, PENK, RLBP1, RP1, SDR16C, SNTG1, TOX* and *XKR4* genes on BTA14 playing significant roles on age at puberty in Brahman cattle. The *IGF-1* (Lirón et al., 2012), *MIR551B*, *MECOM* (Fernández et al., 2015), *ISL1, PELO, FST* and *SPZ1* genes (Fernández et al., 2015) have been associated with timing of puberty in Angus bulls. Polymorphisms in thyroglobulin (TG) and follicle-stimulating hormone receptor (*FSHR*) genes have been associated with age at puberty in Guzerat (Fernández et al., 2017) and Nelore (Milazzotto et al., 2008) bulls, respectively.

In this work, we performed a genome-wide association study to detect genomic regions and putative candidate genes related to age at puberty in Nelore bulls. The elucidation of genes and molecular mechanisms controlling this trait should provide a better understanding of the genetic regulation of reproductive performance and could promote earlier prediction of puberty in young bulls that would speed up genetic breeding programmes.

2 | MATERIALS AND METHODS

2.1 | Phenotype and pedigree information

Animal Care and Use Committee approval was not obtained for this study because the genotypic data were from an existing database provided by ANCP (National Association of Breeders and Researchers), Ribeirão Preto, São Paulo, Brazil. We did not collect any new samples for this study. The phenotypic data were collected by Animal Reproduction Laboratory, Faculty of Veterinary Medicine and Animal Science, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil. The methodology of animal management and biological material collection was approved by the Animal Ethics and experimentation Committee of the Federal University of Mato Grosso do Sul, under protocol no 511/2013.

The phenotypic and pedigree information were collected by ANCP breeding programme from 18 Nelore herds located in midwest and southeast regions of Brazil. The animals were pasture-reared in low-throughput production systems. Pedigree information was available on 202,717 animals.

The age at puberty was assessed in 4,235 young Nelore bulls by andrological clinical evaluation and semen collection through electroejaculation in young bulls with scrotal circumference higher than 19 cm. Based on the progressive motility $\geq$10% and a total spermatic concentration $\geq 50 \times 10^6$ spermatozoa in the ejaculate, animals were classified as pubertal or non-pubertal (Wolf et al., 1965).

The andrological clinical evaluation was performed each 90 days, when the ultrasound evaluation was performed to monitor changes in the testicular parenchyma from weaning (7 months) to 22 months of age. Longitudinal–lateral plan images were obtained in four evaluations interspersed by 90 days with a linear probe of 7.5 mHz (Costa-e-Silva et al., 2017). The pixel density was read in a software, and the curve was analysed separately. By the results of these evaluations, the animals could be classified as early maturing (puberty $\leq$14 months), typical maturing (puberty between 14 and 17 months) or traditional (puberty >17 months). The age at puberty was considered as linear, evaluated by the age that each animal reaches puberty, measured in months.

2.2 | Genotyping and quality control

A total of 18,746 Nelore animals were genotyped using the Clarifide® Nelore 2.0 (Zoetis). The genotype quality control (QC) excluded SNPs that were monomorphic, had minor allele frequency (MAF) <5%, call rate lower than 90%, with unknown genomic position, and mapped on sex

chromosomes according to the UMD_3.1 bovine genome assembly. Samples with a call rate lower than 90% were also excluded. After quality control, genotypes on 17,230 SNPs were available for 18,746 young Nelore bulls.

2.3 | Weighted single-step genome-wide association study (WssGWAS)

Variance components for sexual maturity in Nelore bulls were estimated by the average information restricted maximum likelihood method using the AIREMLF90 software (Misztal et al., 2002). Genomic information was not used to estimate variance components. The single-trait model included contemporary group as fixed effect (farm and year of birth, management group at weaning and yearling), random animal genetic effect and the residual effect. In matrix notation, the model can be described as:

$$y = Xb + Wa + e$$

where **y** is the vector of phenotypic records; $\boldsymbol{b}$ is the vector of fixed effect of contemporary groups; $\boldsymbol{a}$ is the vector of additive direct genetic effects; and $\boldsymbol{X}$ and $\boldsymbol{W}$ are the incidence matrices for the effects contained in $\boldsymbol{b}$ and $\boldsymbol{a}$, respectively; $\boldsymbol{e}$ is the residual.

The same animal model was used to estimate the genomic breeding values (GEBV) by the single-step genomic BLUP (ssGBLUP) approach (Aguilar et al., 2010), which combines pedigree and genomic relationships into a realized relationship matrix (**H**). Therefore, the difference between the regular BLUP and ssGBLUP is that the inverse of the pedigree relationship matrix ($\mathbf{A}^{-1}$) is replaced by $\mathbf{H}^{-1}$, which is represented as follows:

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

where $\mathbf{G}^{-1}$ is the inverse of the genomic relationship matrix and $\mathbf{A}_{22}{}^{-1}$ is the inverse pedigree relationship matrix for genotyped animals. The **G** matrix was constructed as described by Van Raden (2008):

$$G = \frac{ZDZ'}{2\sum p_i(1-p_i)'}$$

where **Z** is a matrix of genotypes centred by twice the current allele frequencies of each SNP (p); i is the ith locus; **D** is a diagonal matrix of weights for SNP, which is an identity matrix for the regular ssGBLUP. The **G** was blended with 5% of $\mathbf{A}_{22}$ to avoid singularity problems.

After GEBV were estimated by ssGBLUP, they were back solved to obtain SNP effects as described by Wang et al. (2012):

$$\hat{u} = \lambda DZ' G^{-1} \hat{a}_g$$

where $\hat{\boldsymbol{a}}_g$ is GEBV for genotyped animals; $\boldsymbol{\lambda}$ is the ratio of SNP to additive genetic variances $\left(\frac{\sigma_u^2}{\sigma_a^2} = \frac{1}{\sum_{i=1}^{M} 2p_i(1-p_i)}\right)$. The weight for each SNP was calculated based on SNP effects as follows (Wang et al., 2012):

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

where $\boldsymbol{d}_i$ is the weight for the i-th SNP.

All the analyses were performed using the BLUPF90 software (Misztal et al., 2016). For the weighted single-step GWAS (WssGWAS; Wang et al., 2012), the steps from the construction of **G** up to the calculation of SNP weights were repeated twice, meaning that computed weights were used to update GEBV and SNP effects. After the second round, the genome-wide association was verified based on the percentage of additive genetic variance explained by 10-SNP sliding windows.

The percentage of the additive genetic variance explained by $\boldsymbol{i}^{\text{th}}$ window was calculated as described by Wang et al. (2014):

$$\frac{\text{var}(a_i)}{\sigma_a^2} * 100 = \frac{\text{var}\left(\sum_{j=1}^{n} Z_j \hat{u}_j\right)}{\sigma_a^2} * 100$$

where $\boldsymbol{a_i}$ is genetic value of the $\boldsymbol{i}$-th region consisting of 10-SNP sliding window length physical size, $\boldsymbol{\sigma}_a^2$ is the total genetic variance, z_j is vector of genotype of the $\boldsymbol{j}$-th SNP for all individuals, $\hat{\boldsymbol{u}}_j$ is SNP effect of the $\boldsymbol{j}$-th SNP within the $\boldsymbol{i}$-th region, $\boldsymbol{n}$ is the number of SNP in a window. The genomic regions displaying more than 0.5% of the additive genetic variance for age at puberty in young Nelore bulls were prospected for possible QTL related to this trait.

2.4 | Gene annotation and enrichment analysis

The Ensembl Biomart tool with the Genes 94 database (Haider et al., 2009) was used to identify the gene content of genomic regions displaying more than 0.5% of the additive genetic variance, selecting a 500 Kb window around each significant region (upstream and downstream). The search for relevant ($p < .05$) Gene Ontology (GO) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways was performed with Database for Annotation, Visualization and Integrated Discovery (DAVID) v. 6.8 tool (Huang, Sherman, & Lempicki, 2009a, 2009b) using the UMD3.1 assembly as genome reference.

3 | RESULTS

The sexual maturity analysis revealed 627, 1,200 and 2,408 young bulls classified as early maturing (puberty between

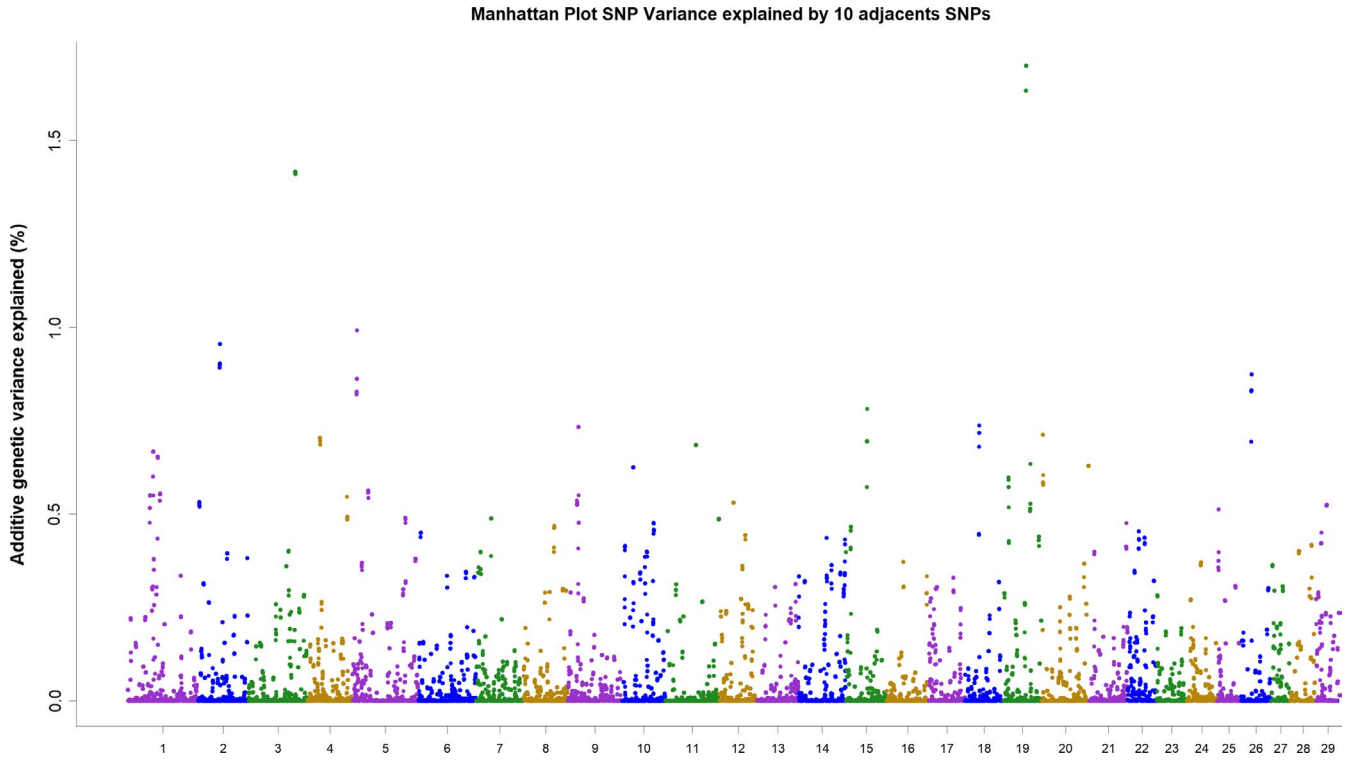

FIGURE 1 Proportion of additive genetic variance explained by windows of 10 adjacent SNP for age at puberty in young Nelore bulls

9 and ≥14 months), typical maturing (puberty between 14 and ≥17 months) and traditional (puberty >17 months), respectively. The estimated heritability for age at puberty in Nelore bulls was 0.3457 ± 0.059. The additive genetic direct variance was 4.23, whereas the residual variance was 8.01.

A total of 26 windows were identified each explained more than 0.5% of the additive genetic variance for sexual maturity in young Nelore bulls (Figure 1, Table 1), which explained a total of 18.95% of additive genetic variance for the trait.

A total of 480 protein-coding genes were identified within the genomic regions that explain more than 0.5% of the additive genetic variance for age at puberty in Nelore bulls. Among then, several genes have been previously identified as related to spermatogenesis functions such as *ADAM11, PARP2*, *PARP9, NECTIN3, SPA17, PRSS21, TRIP13, NDRG2, RNASE4, TEX14, MEIOC, SLX4, RAD51C, BRCA1, CSNK2A, CREBBP* and *TIMP2* gene.

The functional enrichment analysis by DAVID software revealed 10 GO biological processes, four GO cellular components, seven GO molecular functions and two KEGG pathways significantly over-represented (Table 2). We highlighted the following terms related to spermatogenesis such as DNA replication (GO:0006260), male meiosis I (GO:0007141), double-strand break repair (GO:0,006,302), base excision repair (GO:0006284), cell–cell adhesion (GO: 0098609) and focal adhesion (GO:0005925).

4 | DISCUSSION

The heritability estimated for puberty in Nelore bulls was moderate (0.35 ± 0.06), indicating that genetic progress for this trait is feasible, and so, it would respond favourably to direct selection. Based on the heritability estimated, the puberty in Nelore bulls could be improved based on traditional estimated breeding value (EBV) selection. However, genotyping costs has decreased dramatically in recent years and nowadays it costs almost the same as measuring phenotypes related to puberty in bulls, which requests ultrasound evaluation and semen analysis. In addition, genotyping provides accurate and reliable results contributing to improve our understanding of the complexity of biological mechanisms related to puberty in young bulls and could help to predict sexual precocity in Nelore bulls with greater accuracy at younger age, which would speed up the breeding programme for this breed. Eventually, the genes identified as related to puberty in bulls could be used as molecular markers or to develop an SNP genotyping array for reproductive traits.

Regarding to genes identified by wssGWAS, we highlighted some genes that could be related to early puberty in Nelore bulls, according their functions. The function of those genes in other mammals such as rodents and humans was discussed only if their functions in cattle were not previously described. However, we carefully verified whether these genes are orthologous among mammals, which are generally assumed to retain a similar function to that of the ancestral

TABLE 1 Genomic windows of 10 adjacent SNPs that explain more than 0.5% of the additive genetic variance (Var) for age at puberty in young Nelore bulls

Chromosome	Start position (bp)	End position (bp)	Var
25	1,990,059	2,773,521	0.51247
29	28,192,104	29,327,349	0.52499
12	31,933,210	33,215,852	0.53099
2	2,814,353	4,339,368	0.53283
9	12,252,672	12,946,867	0.53618
4	11,1863,574	11,262,6,112	0.54631
1	55,832,662	57,660,748	0.55065
1	85,067,382	86,351,590	0.55540
5	32,481,519	33,430,648	0.56312
19	10,085,159	10,609,583	0.59781
10	22,278,002	26,325,867	0.62519
20	70,415,256	71,296,022	0.62949
19	53,831,725	54,815,158	0.63426
1	77,442,921	78,724,790	0.65387
1	66,550,779	68,066,718	0.66807
11	66,118,270	66,789,317	0.68508
4	30,109,820	31,403,232	0.70410
20	1,576,537	1,961,868	0.71243
9	14,838,816	15,312,685	0.73410
18	26,282,010	27,121,621	0.73729
15	40,459,840	41,947,856	0.78142
26	16,753,151	17,577,730	0.87407
2	65,069,037	66,524,648	0.95542
5	7,201,534	7,862,085	0.99193
3	95,662,104	96,724,963	1.41717
19	44,261,882	45,332,964	1.70062

gene that they evolved from and to share other key properties (Gabaldón & Koonin, 2013).

Seminal plasma is a complex secretion of sugars, lipids, enzymes, prostaglandins, proteins, inorganic ions, organic salts and several other components produced by the testes, epididymis and accessory sex glands of the male which influences several biological processes related to sperm maturation, sperm membrane stabilization and capacitation beyond interact with the oviduct and oocyte (Druart et al., 2013).

Stable intercellular bridges are a distinct feature of spermatogenesis in mammalian germ cells which have been recognized as the unique means of intercellular communication and their loss causes spermatogenesis disruption (Greenbaum, Iwamori, Buchold, & Matzuk, 2011). The *TEX14* (testis-expressed gene 14), a testis-expressed gene and germ cell-specific component, encodes a protein necessary for intercellular bridges in germ cells converting midbodies into stable intercellular bridges, which are crucial for successful spermatogenesis. The loss of germ cell intercellular bridges causes sterility through spermatogenesis disruption (Greenbaum, Iwamori, Agno, & Matzuk, 2009; Kim et al., 2015).

The *PRSS21* (serine protease 21) gene, as well as *TEX14*, also participates in the male gamete generation (GO:0048232) biological process, which encodes a cell-surface anchored serine protease known as testisin, which is abundantly expressed by premeiotic testicular germ cells and sperm (Scarman et al., 2001). Deficient *PRSS21*-mouse displays sperm with several defects that occur during epididymal transit, such as heterogeneity in sperm form and angulated flagella, reduced numbers of motile sperm, and abnormal sperm volume, suggesting that testisin is a proteolytic factor that is responsible for epididymal sperm cell maturation, spermatozoa-fertilizing ability (Netzel-Arnett et al., 2009) and contributes to the zona pellucida-binding complex in stallion spermatozoa, which could be involved in the proteolytic cascade that arranges the sperm surface for interaction with the oocyte (Swegen et al., 2019).

The *NDRG2* (NDRG family member 2) gene regulates testicular development and spermatogenesis in rats, which is involved in cell differentiation, physiological and pathological apoptosis of germ cells (Hou et al., 2009). The *SPA17* (sperm autoantigenic protein 17) gene encodes a conserved highly antigenic protein found in acrosome and fibrous sheath of the sperm flagellum, which has been implicated in cell–cell adhesion functions, sperm maturation, capacitation, acrosome reaction and binding of sperm to the zona pellucida of the oocyte (Chiriva-Internati et al., 2009; Grizzi et al., 2003).

The *ADAM11* gene encodes a member of a disintegrin and metalloprotease (ADAM) protein family, which play important roles in several biological processes including spermatogenesis and sperm functions affecting the maturation of sperm and influencing their adhesion and migration in the uterus (Edwards, Handsley, & Pennington, 2008).

Spermiogenesis includes several processes such as transcriptional silencing, chromatin condensation and extensive morphological changes as spermatids transform into mature sperm. The *RNASE4* (ribonuclease A family member 4) gene encodes a protein that has been found in bull seminal vesicle (Westfalewicz et al., 2017) and it has been related to fluid sperm maturation in cattle (Selvaraju et al., 2018). The TIMP metallopeptidase inhibitor 2 (*TIMP2*) is a member of the TIMP gene family that encodes inhibitors of the matrix metalloproteinases and has been associated with semen quality and fertility in bulls (Kasimanickam et al., 2012; McCauley, Zhang, Bellin, & Ax, 2001).

The *PARP2, PARP9* and *PARP14* genes are members of poly (ADP-ribose) polymerase (PARP) family which plays a crucial role in maintaining the genomic integrity of several cell types acting in DNA repair, maintenance of genomic

TABLE 2 KEGG pathways and Gene Ontology terms revealed by DAVID analyses

Term	*N*	P-value	Genes
Biological process			
GO:0090501–RNA phosphodiester bond hydrolysis	4	1.62E−04	*ANG, RNASE4, ANG2, RNASE6*
GO:0098609–cell–cell adhesion	7	0.004283	*LIMS2, CCDC80, PKD1, ESAM, NECTIN3, CSTA, VMP1*
GO:0000122–negative regulation of transcription from RNA polymerase II promoter	21	0.006085	*HCLS1, CBX4, TP63, CBX2, CNOT1, PDX1, CBX8, TAGLN3, ZNF205, ZNF174, TRIM37, HDAC5, SALL2, VDR, CHD8, HEXIM2, SAP130, HEXIM1, E4F1, ZNF219, HDAC7*
GO:0006260–DNA replication	6	0.016362	*SLX4, GINS3, TBRG1, E4F1, POLQ, BRCA1*
GO:0006915–apoptotic process	12	0.016430	*CSNK2A2, CLPTM1L, DNASE1, EI24, DAD1, BIRC5, RPS6KB1, FAM162A, TRAF7, CUL1, WDR92, C1D*
GO:0006536–glutamate metabolic process	3	0.021010	*GOT2, ALDH18A1, NAGS*
GO:0006302–double-strand break repair	5	0.025903	*PARP9, APLF, DTX3L, MEIOC, TRIP13*
GO:0090502–RNA phosphodiester bond hydrolysis, endonucleolytic	4	0.034230	*RNASE1, ENDOU, BRB*
GO:0006284–base excision repair	4	0.040870	*USP47, POLQ, PARP2, APEX1*
GO:0007141–male meiosis I	3	0.042553	*RAD51C, MEIOC, TRIP13*
Cellular component			
GO:0005737–cytoplasm	100	0.021410	*FLYWCH1, DBF4B, PRR11, PKMYT1, RPS6KB1, PDLIM1, PNP, KLHL7, GTF2E1, NLRC3, CDKN2C, DDX25, MLST8, CASKIN1, ZNF641, SOCS3, RBFOX3, EFTUD2, DTX3L, HCFC1R1, SPA17, ASB8, HEPACAM, PARP14, CDCA7L, CSTA, TPPP2, HSPBAP1, PPY, FBXO40, TPRG1, ASB16, TK1, SLX4, HEXIM2, HEXIM1, NDRG4, DRC7, SKA2, NDRG2, BLNK, DHX8, EEF1A1, PRSS54, OSGEP, LPP, CREBBP, SPDL1, RNPS1, KCTD5, BRCA1, FXR1, HDAC5, NMT1, RNF6, TUBD1, POMP, DHX40, PARP2, HDAC7, GFAP, EZH2, KCNIP1, TMEM235, SENP6, AFMID, CD96, CCDC184, ETAA1, TERT, MEIOC, ARHGAP27, TPPP, C1QL1, ARL4D, MAP3K14, PARVA, C1D, HYLS1, CYTH1, ENDOU, COL2A1, ZNF174, MEFV, PKD1, APEX1, ZNF263, PLEK, HCLS1, BIRC5, FZD2, PKNOX2, TEX14, MEOX1, ZBED5, SP4, SETD6, USP47, TEP1, FEZ1*
GO:0035102–PRC1 complex	3	0.027172	*CBX4, CBX2, CBX8*
GO:0005654–nucleoplasm	46	0.040641	*FLYWCH1, IQCB1, CLUAP1, SEPT4, RAD51C, DBF4B, APLF, SOX2, TP63, CBX2, CHEK1, RPS6KB1, CBX8, RPS2, ZNF174, SENP6, KLHL7, VDR, SLX4, HEXIM2, HEXIM1, E4F1, NDRG2, APEX1, DHX8, ZNF263, ZNF641, GINS3, DTX3L, ARHGAP27, RNPS1, TOX4, TRAP1, RNF6, DUSP3, PARP9, ZBED5, CCDC113, SP4, CFAP20, TUBD1, THOC6, POMP, PARP2, METTL17, KPNA1*
GO:0005925–focal adhesion	15	0.043237	*LIMS2, FLT1, LPP, PDLIM1, FZD2, RPS2, MMP14, SLC9A3R2, ATP6V0C, ACTR3, SENP1, RPS29, PHLDB2, PARVA, ITGA2B*
Molecular function			
GO:0004519–endonuclease activity	8	2.09E−06	*RNASE10, RNASE1, RNASE11, ANG, RNASE4, ANG2, RNASE6, DNASE1L2*
GO:0004540–ribonuclease activity	6	4.96E−05	*RNASE10, RNASE11, ANG, RNASE4, ANG2, RNASE6*
GO:0003676–nucleic acid binding	25	0.001591	*ZNF398, ZNF263, ZNF641, GTF3A, RNASE1, DBF4B, RNASE4, ANG2, RNASE6, ZNF75A, POLR2D, ZNF518A, ZNF205, ZNF174, RNASE10, TNRC6C, RNASE11, ANG, DDX25, SP4, ZNF597, ZNF319, RNASE13, BRB*
GO:0004522–ribonuclease A activity	3	0.001901	*RNASE1, BRB*

(Continues)

TABLE 2 (Continued)

Term	*N*	P-value	Genes
GO:0004861–cyclin-dependent protein serine/threonine kinase inhibitor activity	3	0.025351	*HEXIM2, CDKN2C, HEXIM1*
GO:0044212–transcription regulatory region DNA binding	8	0.028551	*SALL2, SOX2, ZNF200, CBX4, TP63, ZNF219, BRCA1, ZNF174*
GO:0046872–metal ion binding	40	0.034709	*GTF3A, APLF, PLEKHM1, YPEL2, SLC6A3, ENDOU, ZNF200, DMRTA2, COL2A1, ZNF75A, TIMP2, ZNF518A, ZNF205, ZNF174, GTF2E1, DNTT, MCCC1, ZNF597, E4F1, APEX1, NTHL1, TERT, ZNF398, ZNF263, ZNF641, OSGEP, GTPBP8, ANG2, PFKM, HDAC5, AMDHD2, ZSCAN10, PPM1E, ZBED5, SP4, ZNF213, ZNF319, ZNF219, GPATCH8, HDAC7*
KEGG pathway			
bta00240:Pyrimidine metabolism	6	0.046193	*POLR1D, POLR2D, ENTPD1, PNP, CANT1, TK1*
bta04520:Adherens junction	5	0.046742	*CSNK2A2, WASF3, SORBS1, CREBBP, NECTIN3*

stability, transcriptional regulation, apoptosis and necrosis (Ame, Spenlehauer, & Murcia, 2004; Celik-Ozenci & Tasatargil, 2013; Said & Khosravi, 2012). Maintaining the integrity of sperm DNA is vital to male fertility and the PARPs have several roles during spermatogenesis, sperm maturation and even in ejaculated sperm through protection against chemically induced damage (Agarwal et al., 2009; Celik-Ozenci & Tasatargil, 2013).

The functional enrichment analysis by DAVID software revealed 10 GO biological processes, four GO cellular components, seven GO molecular functions and two KEGG pathways over-represented ($p < .05$). Spermatogenesis is a complex process related to the development of spermatozoa in the seminiferous tubules of the testis and epididymis, that involves cell proliferation and differentiation during male germ cell development including several processes such as mitosis, meiosis, DNA repair and spermiogenesis (Shaha et al., 2010; Griswold 2015; Gunes, Al-Sadaan, & Agarwal, 2015). We identify several over-represented ($p < .05$) GO terms related to these processes, such as DNA replication (GO:0006260), male meiosis I (GO:0007141), double-strand break repair (GO:0006302), base excision repair (GO:0006284), apoptotic process (GO:0006915), cell–cell adhesion (GO: 0098609) and focal adhesion (GO:0005925).

Spermatogenesis is a highly ordered differentiation process that includes meiosis I and II successive cellular divisions. Male meiosis I (GO:0007141) biological process encompasses the steps by which a cell progresses through the first meiotic division in the male germ cell. The *MEIOC, RAD51C* and *TRIP13* genes were identified as related to male meiosis I biological process. The *MEIOC* (meiosis specific with coiled-coil domain) gene, which has restricted expression towards testis in human, encodes an essential protein to extended meiotic prophase required to complete chromosome dynamics and meiotic recombination processes besides to avoid degradation of transcripts of genes critical for meiotic prophase (Abby et al., 2016; Soh et al., 2017). The *RAD51C* gene encodes a protein that plays important roles in the repair of DNA and homologous recombination in germ line cells, which is required for gene reassortment and proper chromosome segregation at meiosis (Liu, Tarsounas, O'Regan, & West, 2007). *RAD51C* deficiency in mice results in infertility because the spermatocytes undergo a developmental arrest during the meiotic prophase I (Kuznetsov et al., 2007). The *TRIP13* (thyroid hormone receptor interactor 13) encodes a conserved AAA + ATPase that interacts with thyroid hormone receptors and is required for normal execution of multiple aspects of chromosome structure development, meiotic recombination, formation of higher order chromosome structures and repair (Marcet-Ortega et al., 2017; Pacheco et al., 2015; Roig et al., 2010). Mice spermatocytes with *TRIP13* deficiency trigger a recombination-dependent response that arrests spermatocytes in pachynema without incorporation of the testis-specific histone H1t into their chromatin, resulting in cell apoptosis in response to the arrest or defect in sex body formation (Marcet-Ortega et al., 2017).

Several genes were identified as related to DNA replication (GO:0006260) biological process and, among them, we highlighted the *SLX4* and *BRCA1* genes. The *SLX4* (SLX4 structure-specific endonuclease subunit) gene, also known as *BTBD12*, assists in the resolution of DNA secondary structures arises during the DNA repair and recombination processes. The *SLX4* works as a scaffold for several DNA repair activities and has been identified in mouse premeiotic spermatogonia and meiotic spermatocytes with roles in premeiotic primordial germ cell proliferation and meiotic crossover formation maintain genome stability throughout gametogenesis (Holloway et al., 2011). During embryogenesis, loss of

SLX4 results in impaired primordial germ cell proliferation and increased apoptosis, which decreases the spermatogonial pool in the early postnatal testis, that is the primordial germ cell in developing testis is depended of *SLX4* (Holloway et al., 2011).

The *BRCA1* gene encodes a nuclear phosphoprotein that contains multiple functional domains to interact with several molecules (Deng & Brodie, 2000). Although the major function of *BRCA1* is to maintain genome integrity, this gene plays important roles in several biological processes and pathways including spermatogenesis, where *BRCA1* is required for chromatin silencing, crossing-over of homologous chromosomes and DNA-damage repair to maintain the genomic stability (Turner et al., 2004; Xu et al., 2003). Cressman et al. (1999), in a study with *BRCA1*-deficient mice, identified infertility in males due to meiotic failure than occurs during prophase I of meiosis resulting in spermatogonia apparently normal but absence of spermatids and spermatozoa.

DNA repair mechanisms protect the genomic integrity and stability of germline cells that includes DNA mismatch repair, nucleotide excision repair, base excision repair, double-strand break repair and postreplication repair in the male germ line cells (Gunes et al., 2015). In this study, the double-strand break repair (GO:0006302) and base excision repair (GO:0006284) biological processes were over-represented. The DNA double-strand break repair involves the repair by homologous and non-homologous mechanisms to obtain a continuous DNA helix, while the base excision repair (GO:000628) is a highly coordinated mechanism responsible for detecting and removal altered bases by DNA glycosylase enzyme.

The cell–cell adhesion (GO: 0098609) biological process is defined as the attachment of a cell to another cell via adhesion molecules. Byrne, Leahy, McCulloch, Colgrave, and Holland (2012) described the set of proteins present at the mature bull sperm plasma membrane that are related to cell adhesion, which are vital to for ensuring spermatogenesis progression, including maturation, capacitation, sperm–egg interaction and fertilization.

In mammalian seminiferous epithelium of the adult testis and the epididymis, cell–matrix and cell–cell interactions are crucial to cellular events, such as maintenance of cell/tissue homeostasis, spermatogenesis in the testis and sperm maturation in the epididymis (Cyr, 2011; Dubé & Cyr, 2013). Focal adhesion (GO:0005925) is related to a small region on the surface of a cell known as cell–matrix adhesions that anchors the cell to the extracellular matrix and that forms a point of termination of actin filaments that play important roles in essential biological processes such as regulation of gene expression, cell motility, proliferation, differentiation and survival. During spermatogenesis, focal adhesion helps to maintain the dynamic interactions between Sertoli cells and developing spermatids, facilitating the orientation and migration of spermatid across the seminiferous epithelium (Siu & Cheng, 2008; Yan et al., 2007).

The adherens junction (bta04520) KEGG pathway is related to the most common type of intercellular adhesions, important for maintaining tissue architecture and cell polarity, limiting cell movement and proliferation. We highlighted the *CSNK2A, CREBBP* and *NECTIN3* genes related to adherens junction pathway. The *CSNK2A2* (casein kinase 2 alpha 2) gene encodes a subunit of the protein kinase enzyme, a serine/threonine protein kinase that is involved in several cellular processes, including cell growth of germ cells and apoptosis. The *CSNK2A2* is involved in the biogenesis of the acrosome and has been found preferentially expressed in later stages of spermatogenesis (Xu et al., 1999). Disruption of *CSNK2A2* gene leads to male infertility due to globozoospermia and oligospermia (Xu et al., 1999).

The *CREBBP* (CREB-binding protein) gene is related to transcriptional coactivation of different transcription factors and has been associated with azoospermia (Sabetian & Shamsir, 2016). Partial disruption of *CREBBP* gene in mice interferes in germ cell development at the spermatocyte stage (Hummler et al., 1994) because CREB-binding protein is required to produce a critical Sertoli cell-derived factor for germ cell survival (Scobey et al., 2001). The *NECTIN3* (nectin cell adhesion molecule 3) encodes a member of nectin family that works as adhesion molecules at adherens junctions and is essential to the development and positioning of elongated spermatids within the seminiferous tubules (Nishimura & L'Hernault, 2017). *NECTIN3* plays a critical role in spermatid development because they are crucial for the formation and maintenance of Sertoli–spermatid junctions (Inagaki et al., 2006).

5 | CONCLUSION

Age at puberty in young Nelore bulls has shown to have a moderate heritability indicating that this trait would respond favourably to selection. Regarding to the genetic architecture, a total of 26 windows were identified by WssGWAS that explained a total of 18.95% of the additive genetic variance; therefore, this trait follows a polygenic model of inheritance with several genetic variants of small effects. Additionally, the functional enrichment analysis revealed significant GO terms and KEGG pathways which may play relevant roles and help to understand the molecular mechanisms controlling age at puberty in Nelore bulls.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available for exclusive research purpose. Requests to access data should be sent to presidencia@ancp.org.br (Dr. Raysildo B. Lôbo, President of ANCP).

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