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Genomic study and Medical Subject Headings enrichment analysis of early pregnancy rate and antral follicle numbers in Nelore heifers[1,2]

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ABSTRACT: Zebu animals (*Bos indicus*) are known to take longer to reach puberty compared with taurine animals (*Bos taurus*), limiting the supply of animals for harvest or breeding and impacting profitability. Genomic information can be a helpful tool to better understand complex traits and improve genetic gains. In this study, we performed a genomewide association study (GWAS) to identify genetic variants associated with reproductive traits in Nelore beef cattle. Heifer pregnancy (HP) was recorded for 1,267 genotyped animals distributed in 12 contemporary groups (CG) with an average pregnancy rate of 0.35 (±0.01). Disregarding one of these CG, the number of antral follicles (NF) was also collected for 937 of these animals, with an average of 11.53 (±4.43). The animals were organized in CG: 12 and 11 for HP and NF, respectively. Genes in linkage disequilibrium (LD) with the associated variants can be considered in a functional enrichment analysis to identify biological mechanisms involved in fertility. Medical Subject Headings (MeSH) were detected using the MESHR package, allowing the extraction of broad meanings from the gene lists provided by the GWAS. The estimated heritability for HP was 0.28 ± 0.07 and for NF was 0.49 ± 0.09, with the genomic correlation being −0.21 ± 0.29. The average LD between adjacent markers was 0.23 ± 0.01, and GWAS identified genomic windows that accounted for >1% of total genetic variance on chromosomes 5, 14, and 18 for HP and on chromosomes 2, 8, 11, 14, 15, 16, and 22 for NF. The MeSH enrichment analyses revealed significant ($P < 0.05$) terms associated with HP—"Munc18 Proteins," "Fucose," and "Hemoglobins"—and with NF—"Cathepsin B," "Receptors, Neuropeptide," and "Palmitic Acid." This is the first study in Nelore cattle introducing the concept of MeSH analysis. The genomic analyses contributed to a better understanding of the genetic control of the reproductive traits HP and NF and provide new selection strategies to improve beef production.

Key words: beef cattle, enrichment analysis, genomewide association study, genomics, linkage disequilibrium, Medical Subject Headings terms

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INTRODUCTION

Reproductive traits are economically important in beef cattle production, especially for zebu animals (*Bos indicus*), where heifers often take longer to reach puberty (Abeygunawardena and Dematawewa, 2004; Eler et al., 2014) compared with taurine animals (*Bos taurus*). Reduced heifer fertility impacts the profitability of a given size herd in the production system by decreasing the number of weaned calves and increasing the lifetime costs of herd replacements (Eler et al., 2014).

The inclusion of genomic information in genetic evaluations can be a helpful tool to analyze polygenic traits, such as those for reproduction, shortening the time it takes to obtain reliable breeding value estimates, which can lead to reduced generation intervals and improving genetic gains. It positively affects accuracies by including a more precise measure of genetic similarity between individuals compared with traditional pedigree-based evaluations (Berry et al., 2012; Hayes et al., 2013). Genomic markers such as SNP can be used in genomewide association studies (**GWAS**), a well-established strategy that has identified thousands of markers spread across the cattle genome related to important economic traits (Hu et al., 2016). Genomewide association study relies on linkage disequilibrium (**LD**) between at least 1 marker and the causal mutation or quantitative trait nucleotides responsible for the observed phenotypic variation (Khatkar et al., 2014).

Female fertility can be measured in a number of different ways (Khatkar et al., 2014). Heifer pregnancy (**HP**), defined by the determination of whether or not a heifer exposed to breeding has become pregnant, is a directly measured trait for which data collection is an easy and inexpensive process (Eler et al., 2002). This allows its implementation as a selection criterion in large herds.

Because reproductive technologies, such as ovum pick up and in vitro fertilization (**IVF**), are widely adopted in zebu herds, the numbers of antral follicles (**NF**) has become a relevant trait, due to its association with female performance for ovum pick up and IVF (Baruselli et al., 2015).

A better understanding of genetic factors that affect reproduction could lead to substantial improvement in genetic selection to improve reproductive rates (Kappes et al., 2000). Identification of genomic regions associated with reproductive traits could expand our understanding of reproductive processes and be used to improve reproductive efficiency in cattle, especially in zebu animals.

Medical Subject Headings (**MeSH**) is a collection of comprehensive life sciences terms (Nelson et al., 2004) organized by the U.S. National Library of Medicine (Bethesda, MD); it is the annotation used for PubMed (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3013746/pdf/gkq1237.pdf) documents. These terms are clustered into 16 categories, and the size of MeSh's vocabulary library is approximately twice as large as that of Gene Ontology (Nakazato et al., 2007). They have been recently used in overrepresentation analysis (Morota et al., 2015), permitting the extraction of broad meaning from the gene lists provided by GWAS.

In this study, phenotypic information for HP and NF were collected from Nelore heifers on commercial farms in Brazil. Following whole-genome genotyping, GWAS was performed to identify genomic regions associated with these traits followed by MeSH enrichment analyses of these regions, aiming to increase knowledge about the genetic influence and biological role of genes related to HP. This is the first study in Nelore cattle introducing the concept of MeSH analysis.

MATERIAL AND METHODS

Data Set

The data set consisted of the pregnancy status on 2,283 Nelore heifers exposed to breeding, and around 48% of these (1,099 animals) also had NF measured. The data were from 3 commercial beef cattle farms called *Segredo* (Farm 1), *Engano* (Farm 2) and *CFM* (Farm 3), located in the state of Mato Grosso do Sul in midwestern Brazil and were stored and analyzed by the Animal Breeding and Biotechnology Group of the College of Animal Science and Food Engineering, Pirassununga, São Paulo, Brazil. The animals were about 16 mo old when data was collected, having been raised under similar environmental conditions and receiving salt and mineral supplementation on a pasture-based system, with ad libitum water.

The animals were allocated into 12 contemporary groups (**CG**), formed by the combination of the animal's herd of origin, management group, and year of birth. Records from CG without variability (e.g., all animals had the same pregnancy status) were not considered in the analyses. In addition, animals with an age or weight exceeding 3.5 SD above or below the overall CG mean were excluded.

Traits

Heifer Pregnancy. The diagnosis of pregnancy was performed using ultrasound (Chison 8200VET with 7.5 MHz transducer; Kylumax, Wuxi, China) or transrectal palpation 40 d after AI. Phenotypic records were treated as categorical, assigning the value of 1 (success) to heifers that were diagnosed pregnant and 0 (failure) to those that were not pregnant at the time of diagnosis.

In tropical countries, calving is normally spread from July to November, with heifers entering the October breeding season at between 12 (born in November) and 16 mo old (born in July). So challenging animals around 15 mo old is a strategy to select for sexual precocity that fits in with the routine management of the farms (Eler et al., 2002).

Number of Antral Follicles. Ovarian ultrasound (7.5 MHz transrectal linear transducer, Mindray M5Vet; Mindray, Mahwah, NJ) was performed on those Nelore heifers submitted to fixed-time AI. Visible follicles (≥3 mm of diameter) were counted and recorded on Day 4 of the protocol to establish the total NF recruited in the synchronized follicular wave. This procedure was performed in 2 (*Engano* and *Segredo*) of the 3 farms.

Genotypic Data

Hair samples of 1,267 young heifers (about 16 mo old) from 2,283 available animals were collected for genomic DNA extraction and subsequent genotyping analysis using the GeneSeek GGP *Bos indicus* HD array (Neogen, Lincoln, NE) with 74,677 SNP, specially developed for *B. indicus* cattle. All 1,267 animals had phenotypic records for HP and, of those, 938 also had NF records.

Quality control procedures were performed using PREGSF90 version 1.10 software (Misztal et al., 2014) to reduce spurious associations and, consequently, increase the accuracy of the genomic analyses. The quality control parameters used herein to delete loci were minor allele frequency < 2% and SNP call rate < 95% and when the observed percentage of heterozygous markers differed from expected (Hardy–Weinberg equilibrium) by >15%. Samples with call rate < 90% were also excluded. Genotypes from loci on the Y chromosome, the mitochondria, and markers not assigned to chromosomes were eliminated from the data. After editing, a total of 64,753 SNP and 1,255 genotyped heifers remained in the data set. The frequency of animals among farms and their phenotypic information are summarized in Table 1.

Variance Components Estimation

Genetic and residual variances or covariances for HP and NF were estimated in a bivariate model using the single-step methodology proposed by Legarra et al. (2014) under a Bayesian approach. The statistical model used was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}, \qquad [1]$$

Table 1. Average values (SD) for age, weight, heifer pregnancy (HP) rate and number of antral follicles (NF) of the genotyped (after quality control) Nelore heifers by farm

Item	Farm 1	Farm 2	Farm 3
No. of animals	579	299	377
Age of animals, mo.	16.1 (1.20)	14.1 (0.87)	17.2 (0.52)
Weight of animals, kg	283.2 (28.92)	260.6 (19.20)	276.5 (16.73)
HP	0.33	0.35	0.36
NF	12.07	10.99	–

in which **y** is the vector of the dependent variable (observed phenotypes) for genotyped and nongenotyped animals; **b** is the vector of fixed effects, including the CG and linear covariate for heifer age at pregnancy diagnosis; **a** is the vector of random additive genetic effects; **X** and **Z** are incidence matrices relating **b** and **a** with the dependent variable **y**; and **e** is a vector of random residual errors. Variance components for HP were estimated using a threshold model, which related the observed trait on a categorical scale to an underlying continuous normal scale, whereas NF was modeled as a continuous variable.

The covariance matrix of **a** and **e** assumed

$$\text{var}\begin{bmatrix}\mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{H}\sigma_a^2 & 0\\ 0 & \mathbf{I}\sigma_e^2\end{bmatrix},$$

in which σ_a^2 and σ_e^2 are the variance components of the additive genetic and residual effects, respectively; **I** is an identity matrix of order equal to the number of animals with phenotypes; and **H** is the relationship matrix that combines information from the genotyped and nongenotyped animals considering pedigree information as in Aguilar et al. (2010), with its inverse calculated as

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix}0 & 0\\ 0 & \left(0.95\mathbf{G} + 0.05\mathbf{A}_{22}\right)^{-1} - \mathbf{A}_{22}^{-1}\end{bmatrix},$$

in which **A** is the additive relationship matrix (pedigree), $\mathbf{A_{22}}^{-1}$ is the inverse of the conventional relationship matrix including just the genotyped animals, and **G** is the genomic relationship matrix estimated according to (VanRaden, 2008):

$$\mathbf{G} = \mathbf{MM}'/[2\textstyle\sum p_i(1 - p_i)],$$

in which **M** is a transformed incidence matrix of marker alleles whose elements in the *i*th column are $0 - 2p_i$, $1 - 2p_i$, and $2 - 2p_i$ for genotypes AA, AB, and

BB, respectively; **M′** is the transpose of **M**; and p_i is the frequency of allele B in the *i*th marker.

A total of 2,200,000 chains were generated for this variance component analysis using THRGIBBS1F90 (Misztal et al., 2014), discarding the first 200,000 iterations (burn-in). The convergence of Markov chain Monte Carlo (**MCMC**) chains was verified by Geweke's convergence test and visual inspection of trace plots using the *boa* (Bayesian output analysis) package (Smith, 2007) in R software (R Development Core Team, 2013). The genetic and residual variance estimates were used as prior values in the subsequent Bayesian analysis.

Genomewide Association Study

The LD between markers was estimated using the square of the correlation (r^2; Hill and Robertson, 1968), calculated as

$$r^2 = (AB \times ab - Ab \times aB)^2/(A \times a \times B \times b),$$

in which *A*, *a*, *B*, and *b* are the frequencies of each allele in the studied population. The analyses were conducted with PLINK version 1.9 (Chang et al., 2015).

The associations between SNP markers and the phenotypic information were performed using BayesB methodology, which simultaneously analyzes all SNP data and assumes a different genetic variance for each SNP locus with scaled inverse χ^2 prior distributions and that a fraction $(1 - \pi)$ of the markers have nonzero effects (Meuwissen et al., 2001; Habier et al., 2011). The traits were analyzed separately using GenSel software (Garrick and Fernando, 2013), and the posterior distribution of marker effects was predicted under the statistical model

$$\mathbf{y} = \mathbf{Xb} + \sum_{i=1}^{k} x_{ji}\beta_i\delta_i + e_j, \qquad [2]$$

in which **y**, **X**, and **b** were defined in model [1]; *k* is the number of SNP; $\mathbf{x}_{ji}$ is the column vector representing the SNP covariate at locus *i* for animal *j*, coded as the number of the minor allele frequency allele; β_i is the substitution effect for locus *i*, assuming $\beta_i|\pi$, $\sigma_i^2 \sim \delta_i N(0, \sigma_i^2)$ when $\delta_i = 1$ and $\beta_i = (1 - \delta_i)N(0, \sigma_{\beta i}^2 = 0)$ when $\delta_i = 0$; δ_i is an indicator variable; and $e_j|\sigma_e^2 \sim N(0, \sigma_e^2)$. The prior for δ_i was

$$(\delta_i \mid \pi)\begin{cases} 1; \text{probability}(1-\pi) \\ 0; \text{probability}(\pi) \end{cases},$$

in which π was assumed to be 0.999, which results in about 0.1% of the SNP fitted in the model at each iteration. The marker genetic prior for $\left(\sigma_i^2 \mid \nu_\beta, S_\beta^2\right) \sim \nu_\beta S_\beta^2 X_{\nu_\beta}^{-2}$ χ^2 and the random residual prior $\left(\sigma_e^2 \mid \nu_e, S_e^2\right) \sim \nu_e S_e^2 X_{\nu_e}^{-2}$ with $\nu_\beta = 4$ and $\nu_e = 10$ df, and scale parameters estimated as $\left(\nu_\beta S_\beta^2 + \beta_i\right)/\left(\nu_\beta + 1\right)$ and $\left(\nu_e S_e^2 + \mathbf{e}'\mathbf{e}\right)/\left(\nu_e + n\right)$, respectively, in which n is the number of individuals (Cheng et al., 2015).

A total of 90,000 iterations was considered, where the first 2,000 were discarded (burn-in) and the following 88,000 were used to predict the posterior mean effect of each SNP marker. Rather than using effects of individual markers, the proportion of variance explained by nonoverlapping 1-Mb genomic windows comprising all the SNP effects in that region were used for inference in the genomewide association. This proportion was sampled by

$$q_w = \tilde{\sigma}_{gw}^2 / \tilde{\sigma}_g^2,$$

in which, at every 100th post-burn-in MCMC iteration, $\tilde{\sigma}_{gw}^2$ is the sampled genetic variance explained by SNP inside the genomic window *w* and $\tilde{\sigma}_g^2$ is the sampled total genetic variance. For more details on sampling $\tilde{\sigma}_{gw}^2$ and $\tilde{\sigma}_g^2$, see Fernando and Garrick (2013). Genomic windows that explain at least 1% of total genetic variance based on their posterior means were considered as important regions associated with the traits, with higher chances of being associated with QTL regions (Peters et al., 2012).

Gene Search and Functional Enrichment

The important regions identified by the GWAS were extended on either side (±500 kb) for the functional enrichment analyses and gene search. Annotated genes in these regions were retrieved from the Ensembl Genes 87 database using Biomart software (Aken et al., 2016).

Medical Subject Headings terms were detected using the MESHR package (Tsuyuzaki et al., 2015) for enrichment analyses considering the genes that were associated with HP (127) and NF (91). Medical subject headings identification associated with each Entrez Gene (Maglott et al., 2011)Identifications were obtained from the MESH.BTA.EG.DB annotation package (Tsuyuzaki et al., 2015), assuming a universe of all annotated genes with a unique corresponding Entrez Gene identifications (17,093). The R package uses a hypergeometric test to assess the significance of the enrichment as described by Morota et al. (2015). The terms with a *P*-value < 0.05 were considered significant, applying the Benjamini and Hochberg (1995) procedure to account for multiple testing.

RESULTS AND DISCUSSION

The genomic estimates of heritability on the liability scale by the single-step method were 0.28 (SD 0.07) for HP and 0.49 (SD 0.09) for NF. The

estimated genomic correlation was −0.21 (SD 0.29). This high SD makes the confidence interval straddle 0, suggesting weak or no genetic association between the traits (Table 2). The highest posterior density (0.025–0.975%) interval estimated for the genomic correlation between HP and NF was remarkably wide, varying between −0.781 and 0.329. As the proposed convergence criteria were well met, the width of this interval may be attributed to the limited number of observations included in the present study.

Table 2. Posterior mean, median, SD, and highest posterior density interval 95% (HPD 95%) of heritability (h^2) and genomic correlation for early pregnancy (HP) and number of antral follicles (NF) of the Nelore heifers

Parameter	Mean	Median	SD	HPD 95%
h^2_{HP}	0.28	0.28	0.07	0.15 to 0.43
h^2_{NF}	0.49	0.49	0.09	0.32 to 0.68
$r_{HP \times NF}$[1]	−0.21	−0.20	0.29	−0.78 to 0.33

[1]$r_{HP \times NF}$ = the genomic correlation between heifer pregnancy and number of antral follicles.

Genomewide Association Study

The average LD between adjacent markers was 0.23 ± 0.01, ranging from 0.20 on chromosome 27 to 0.25 on chromosome 5 (Fig. 1), and the overall LD on the same chromosome varied from 0.09 on chromosome 27 to 0.13 on chromosome 6, with an average of 0.11 (SD 0.01; Supplemental Fig. S1 [see the online version of the article at http://journalofanimalscience.org]). Espigolan et al. (2013) reported an r^2 averaging 0.17 for overall SNP in a population of 795 Nelore bulls with approximately 447,000 markers. This observed difference is expected because LD is inversely proportional to the distance between markers, with denser genotypes generally having high overall LD. Biegelmeyer et al. (2016) found average $r2$ of 0.21 ± 0.27 and 0.16 ± 0.20 in 391 Hereford and 2,019 Braford animals, respectively, with approximately 41,000 SNP. As noted by those authors, LD can be influenced by several factors and is, therefore, population specific.

A total of 2,673 genomic windows were constructed for 30 chromosomes (29 autosomes and the X chromosome) including, on average, 25.5 SNP distributed between flanking SNP that were 934 kb apart. The results identified significant regions on chromosomes 5, 14, and 18 for HP and 2, 8, 11, 14, 15, 16, and 22 for NF that each explained >1% of the total genetic variance (Fig. 2 and 3). These genomic regions were used to locate candidate genes and are presented on Tables 3 and 4 for HP and NF, respectively. Other genomic regions on chromosomes 1, 2, 3, 5, 14, 24, 29, and X demonstrated an important influence on HP (Table 5) and genomic regions on chromosomes 2, 12, 15, 19, 24, 25, and 29 demonstrated an important influence on NF (Table 6), explaining >0.5% of the total additive genetic variance. Nonetheless, these regions were not considered in the further analyses.

Six and 7 genomic windows explained at least 1% each of the additive genetic variance for HP and NF, respectively. The cumulative genetic variance explained by these windows was 12.73% for HP and 20.12% for NF. The highest proportion of genetic variance explained by a window for HP was 3.90% in a window located at 73 Mb in chromosome 5, whereas for NF, the most relevant window explained 5.56% of the genetic variance; this window is located at 15 Mb in chromosome 22. Half of the genetic variance was explained by 237 windows for HP and 116 windows for NF, whereas to achieve 90% of explained variance, it would be a similar function of 1,704 windows for HP and 2,673 windows for NF.

Medical Subject Headings Enrichment Analyses

The MeSH enrichment analyses revealed 74 terms related to HP (Supplemental Table S1; see the online version of the article at http://journalofanimalscience.org) and 48 terms related to NF (Supplemental Table S2; see the online version of the article at http://journalofanimalscience.org). After the Benjamini and Hochberg correction, the terms that were significantly ($P < 0.05$) associated with HP were "Munc-18 Proteins," "Fucose," and "Hemoglobins" and the terms that were significantly ($P < 0.05$) associated with NF were "Cathepsin B," "Receptors-Neuropeptide," and "Palmitic Acid."

The mammalian homolog of unc-18 (munc-18) has an important association with hormone secretion in the pituitary cells in mice. Weiss et al. (2007) argued that estradiol affects the production of munc-18, with a central role in regulation of LH secretion. Korteweg et al. (2005) concluded that munc-18 is involved in peptide hormone secretion from the anterior pituitary.

"Fucose" is a hexose deoxy sugar naturally found on mammalian cell surfaces and has been detected in the zona pellucida and on the spermatozoal surface. Romero-Aguirregomezcorta et al. (2015) observed increased levels of spermatozoon–zona pellucida binding and penetration when fucose was added to IVF medium, indicating a key role in fertilization. Studies in the bovine (Lefebvre et al., 1997; Suarez et al., 1998; Tanghe et al., 2004) confirmed that fucose mediates a specific interaction between sperm and oviductal epithelium, thus playing an important role prior in fertilization.

The "Hemoglobins" term was also significant, suggesting a possible association with reproduction. Hemoglobins are the oxygen-carrying proteins of erythrocytes, and vascularity within the ovary has been asso-

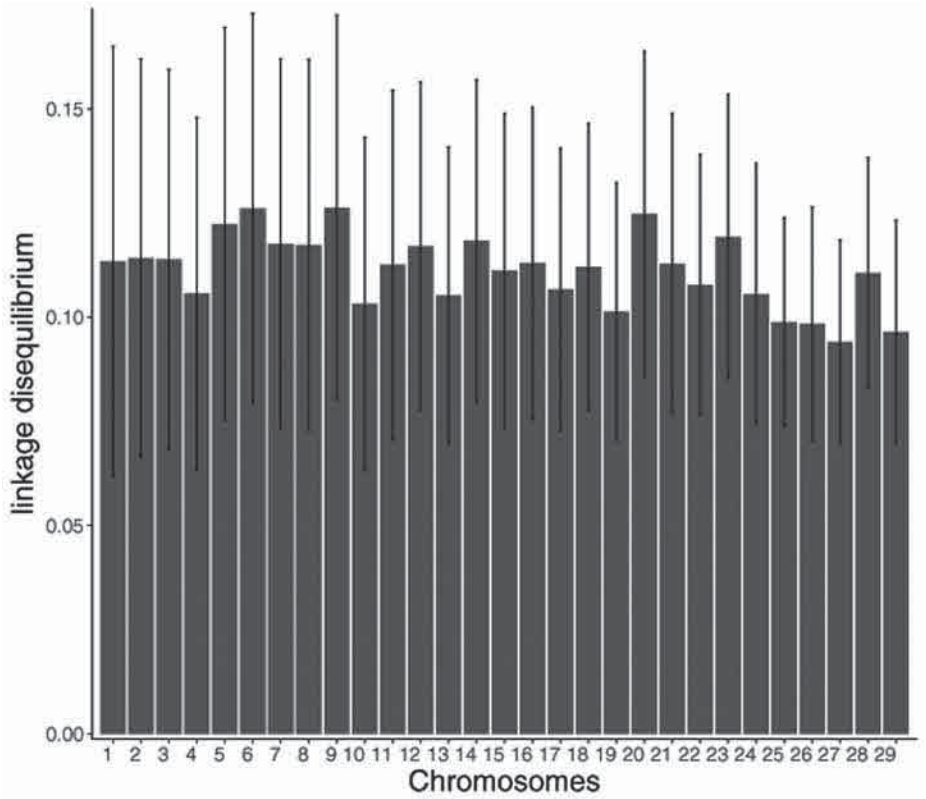

Figure 1. Mean values (±SD) of linkage disequilibrium (r^2) of adjacent markers by chromosomes in the studied population of Nelore heifers.

ciated with oocyte health (Van Blerkom, 2000). Brown et al. (2014) concluded that hemoglobin is expressed by the follicular cells of the ovary, suggesting an influence on endocrine regulation through induction of a hypoxic stage that interferes with the LH surge.

"Cathepsin B," a lysosomal cysteine proteinase, was negatively correlated with the quality of bovine oocytes and embryos (Balboula et al., 2013). According to Balboula et al. (2013), heat stress, a common condition in tropical countries, could lead to an increase in the expression of cathepsin B, with consequent impairment of oocyte development and female fertility.

The "Receptor, Neuropeptide" term refers to cell surface receptors that bind specific neuropeptides initiating intracellular modifications that can change the comportment of cells. Many of these neuropeptides are hormones when outside of the nervous system. The neuropeptide kisspeptin has been positively associated with GnRH and gonadotropin secretion (Amstalden et al., 2014), and the function of kisspeptin neurons may be influenced by postweaning weight gain in heifers. Redmond et al. (2011) related this neuropeptide to the onset of puberty in lambs through its role in activation of the hypothalamic–adenohypophyseal axis.

Palmitic acid is one of the most common SFA present in the follicular fluids of cattle, sheep, and pigs (McEvoy et al., 2000). Mu et al. (2001) associated the plasma concentration of SFA with granulosa cell apoptosis in humans, which may influence reproductive disorders. In cattle, a high concentration of palmitic acid in follicular fluid has negative effects on oocyte maturation, fertilization, and cleavage rate (Leroy et al., 2005). Marei et al. (2010) found that dietary supplementation with fatty acids may influence oocyte maturation and early embryonic development. Zeron et al. (2001) reported high follicular fluid concentrations of SFA in high temperatures (summer), which is associated with negative impacts on fertility (García-Ispierto et al., 2007).

Genes Associated with Heifer Pregnancy

Chromosome 5. Twenty-four genes in 3 genomic windows on chromosome 5 had large effects on HP. A study by Kappes et al. (2000) found a significant peak on this chromosome (approximately 40 cM) for ovulation rate and suggested that this chromosome may harbor important genes for this trait in cattle. The results of Khatkar et al. (2014) found highest meta-GWAS scores on chromosome 1, 5, 13, and 16 for fertility traits.

The genes *TOM1* and *HO-1* are associated with pregnancy in humans (Dumesic et al., 2015). Assidi et al. (2011) noted that *TOM1* downregulates the transcription factor AP1, which is involved in the LH pathway in preovulatory bovine granulosa cells. They also confirmed that *TOM1* is a negative biomarker of oocyte competence. *Heme-oxygenase 1* (*HO-1*) is an

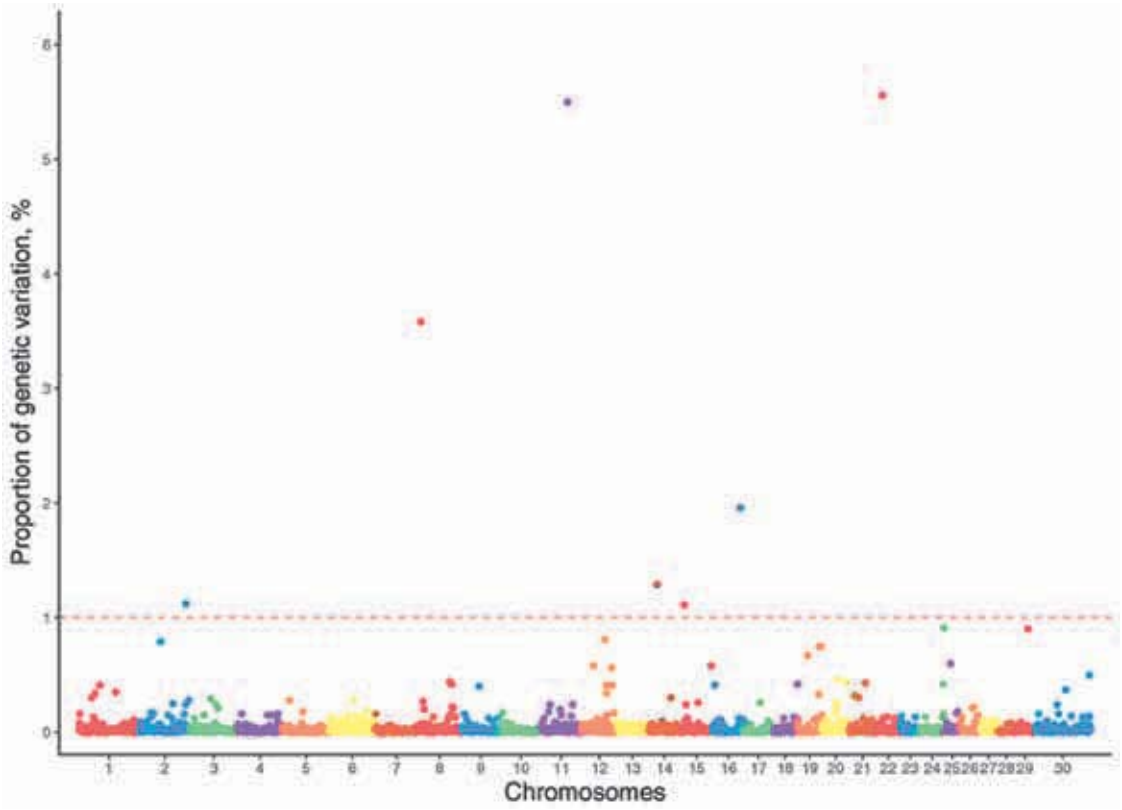

Figure 2. Manhattan plot of the genomewide association study for pregnancy in Nellore heifers. The dashed red line represents the threshold of the proportion of the explained genetic variance.

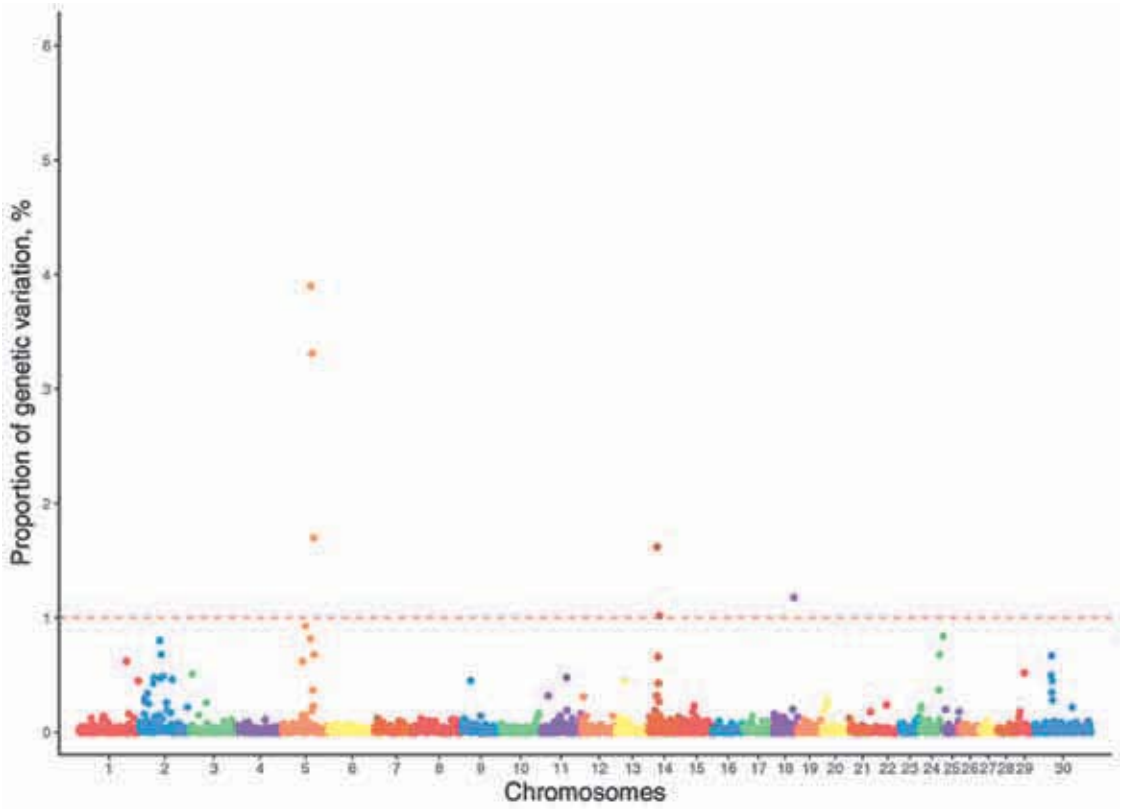

Figure 3. Manhattan plot of the genomewide association study for the number of antral follicles in Nellore heifers. The dashed red line represents the threshold of the proportion of the explained genetic variance.

Table 3. Genes harbored in genomic windows that explained more than 1% of the additive genetic variance for early pregnancy in Nelore heifers

CHR[1]	Position (UMD 3.1 bovine assembly[2])	Genes	Var,[3] %	No. of SNP[4]
5	72,521,518–74,466,890	*APOL6*, *HMGXB4*, *HMOX1*, *ISX*, *LARGE1*, *MB*, *MCM5*, *RASD2*, *RBFOX2*, *SNORA76*, and *TOM1*	3.90	22
	76,522,303–78,484,850	*BICD1*, *DNM1L*, *FGD4*, *KIAA1551*, *PKP2*, *SYT10*, and *YARS2*	3.31	27
	80,633,605–82,455,158	*CCDC91*, *COX7ALP1*, *ERGIC2*, *FAR2*, *KLHL42*, and *PTHLH*	1.70	22
14	22,507,464–24,484,847	*ATP6V1H*, *FAM150A*, *LYPLA1*, *MRPL15*, *NPBWR1*, *OPRK1*, *PCMTD1*, *RB1CC1*, *RGS20*, *RP1*, *SOX17*, *ST18*, *TCEA1*, *U2*, *U6*, and *XKR4*	1.62	29
18	54,518,279–56,456,772	*ALDH16A1*, *BAX*, *BCAT2*, *BOSTAUV1R403*, *BOSTAUV1R404*, *C19orf68*, *C5AR1*, *C5AR2*, *CA11*, *CABP5*, *CCDC114*, *CCDC155*, *CCDC9*, *CD37*, *CRX*, *DBP*, *DHDH*, *DHX34*, *DKKL1*, *ELSPBP1*, *EMP3*, *FAM38E*, *FCGRT*, *FGF21*, *FLT3LG*, *FTL*, *FUT1*, *FUT2*, *GLTSCR1*, *GLTSCR2*, *GRIN2D*, *GRWD1*, *GYS1*, *HRC*, *HSD17B14*, *IZUMO1*, *KCNA7*, *KCNJ14*, *KDELR1*, *KPTN*, *LHB*, *LIG1*, *LIN7B*, *LMTK3*, *MAMSTR*, *MEIS3*, *NAPA*, *NOSIP*, *NPAS1*, *NTF4*, *NTN5*, *NUCB1*, *PIH1D1*, *PLEKHA4*, *PPFIA3*, *PPP1R15A*, *PRRG2*, *PTH2*, *RASIP1*, *RCN3*, *RPL18*, *RPS11*, *RUVBL2*, *SAE1*, *SEC1*, *SEPW1*, *SLC17A7*, *SLC6A16*, *SLC8A2*, *SNRNP70*, *SPACA4*, *SPHK2*, *SULT2A1*, *SULT2B1*, *SYNGR4*, *TEAD2*, *TMEM143*, *TMEM160*, *TRPM4*, *TULP2*, *ZC3H4*, *ZNF114*, and *ZNF541*	1.18	27
14	28,561,608–30,475,350	*GGH*, *NKAIN3*, *TTPA*, and *YTHDF3*	1.02	23

[1]CHR = Bovine autosome.

[2] Zimin et al., 2009.

[3]Var = genetic variance explained by the window.

[4]No. of SNP is the number of SNP in the window.

oxidative stress-related gene, and high levels of expression in cumulus cells is associated with reduced oocyte fertilization in humans (Bergandi et al., 2014). These results support the hypothesis that this genomic region (72–74 Mb) is involved with HP outcomes.

In a study by Hawken et al. (2012), chromosome 5 harbored the most important markers associated with reproductive traits, such as age at puberty, in Tropical Composite cattle. The most significant SNP was located at around 96 Mb, which is close to the regions reported in this study (72–83 Mb). Other studies (Schulman et al., 2008; Allan et al., 2009; Kim et al., 2009; Sahana et al., 2010; Luna-Nevarez et al., 2011) also related this chromosome to reproductive traits. *Insulin-like growth factor 1* (*IGF1*) is located at 66 Mb and is frequently associated with reproductive traits in cattle (Fortes et al., 2013). The average LD of adjacent markers between the locations of the middle of this gene (66,564,289 bp) and the middle of the closest genomic window reported in this study (73,494,204 bp) was 0.34 (95% confidence interval 0.30–0.39), suggesting a possible linkage between these markers.

Chromosome 14. Eighteen genes were associated with both HP and NF in a window (22–24 Mb) on chromosome 14. Hawken et al. (2012) associated multiple clusters of SNP related to reproductive traits in 843 Brahman and 866 Tropical Composites beef heifers, with the most significant markers located between 22 and 25 Mb. A meta-GWAS study (Khatkar et al., 2014) found the highest score on chromosome 14 for sexual maturity in cattle.

These same regions had large effects in the current study for both HP and NF, emphasizing their importance in reproductive traits and suggesting a possible pleiotropic effect. This hypothesis was reinforced by comparing the correlation of the genomewide estimated genomic values of HP and NF with the estimated genomic values when considering only the markers present in referenced genomic window (Cole et al., 2009). The estimates were −0.14 (95% confidence interval −0.21 to −0.08) when considering all SNP and −0.30 (95% confidence interval −0.36 to −0.24) considering only SNP in the referened window. A Fisher r-to-Z transformation test was performed to assess significant differences between the estimates, and the obtained result ($P < 0.01$) supports the pleiotropic effect previously hypothesized for this region.

SOX17 is a well-known gene linked with primordial germ cells, which are the precursors of spermatozoa and oocytes (Irie et al., 2015). Irie et al. (2015) suggested that *SOX17* plays a key role in the maintenance of early human germline cells and also supports human uterine adenogenesis and glandular functions (Guimarães-Young et al., 2016). Mice with a null allele of *SOX17* show decreased expression of the progesterone receptor promoter gene compared with the wild-type, with a consequent impairment of female fertility.

The *RGS20* gene belongs to the regulator of G-coupled protein signaling (RGS) family of proteins.

Table 4. Genes harbored in genomic windows that explained more than 1% of the additive genetic variance for number of antral follicles in Nelore heifers

CHR[1]	Position (UMD 3.1 bovine assembly[2])	Genes	Var,[3] %	No. of SNP[4]
22	14,507,849–16,474,506	*ACKR2*, *ANO10*, *CCDC13*, *CCK*, *HHATL*, *HIGD1A*, *KLHL40*, *LYZL4*, *NKTR*, *SEC22C*, *SNRK*, *SS18L2*, *TCAIM*, *TOPAZ1*, *TRAK1*, *VIPR1*, *ZBTB47*, *ZNF197*, *ZNF445*, and *ULK4*	5.56	25
11	69,520,906–71,472,289	*ALK*, *BRE*, *C11H2orf71*, *CLIP4*, *FAM179A*, *FOSL2*, *LBH*, *PLB1*, *PPP1CB*, *SNORD53_SNORD92*, *SPDYA*, *TRMT61B*, *WDR43*, and *YPEL5*	5.50	27
8	6519,096–8474,290	*ADAM29*, *BLK*, *C8orf74*, *CTSB*, *DEFB134*, *FAM167A*, *FDFT1*, *GATA4*, *GLRA3*, *HPGD*, *MTMR9*, *NEIL2*, *PINX1*, *SOX7*, *TDH*, and *XKR6*	3.58	22
16	70,502,173–72,455,202	*ARL8A*, *ELF3*, *GPR37L1*, *LGR6*, *PPP1R12B*, *PROX*, *PTPN14*, *PTPN7*, *RNPEP*, *RPS6KC1*, *SMYD2*, *SYT2*, and *UBE2T*	1.96	25
14	22,507,464–24,484,847	*ATP6V1H*, *FAM150A*, *LYPLA1*, *MRPL15*, *NPBWR1*, *OPRK1*, *PCMTD1*, *RB1CC1*, *RGS20*, *RP1*, *SOX17*, *ST18*, *TCEA1*, and *XKR4*	1.29	29
2	122,533,476–124,485,696	*COL16A1*, *FABP3*, *HCRTR1*, *LAPTM5*, *MATN1*, *NKAIN1*, *PEF1*, *PUM1*, *SDC3*, *SERINC2*, *SNRNP40*, and *TINAGL1*	1.12	22
15	8507,403–10,464,870	*ARHGAP42* and *CNTN5*	1.11	24

[1]CHR = Bovine autosome.

[2] Zimin et al., 2009.

[3]Var = genetic variance explained by the window.

[4]No. of SNP is the number of SNP in the window.

It is a regulator of G protein α subunits, inducing them into the inactive, guanosine diphosphate (**GDP**)–bound form (The UniProt Consortium, 2015). This gene has an important function in cell division, where it is essential for the first mitotic division of the embryo. Mouse zygotes with a loss of *RGS14* expression were not able to progress to the 2-cell stage (Martin-McCaffrey et al., 2004). Feuerstein et al. (2012) identified *RGS2* as biomarker of oocyte development competence, with significantly ($P < 0.05$) different levels of expression in pregnant and nonpregnant patients.

The *PLAG1* gene, located at 25 Mb of chromosome 14 in cattle, is often associated with reproductive traits in different species, with reported functions related to cell proliferation by regulation of growth factors (Juma et al., 2016). Chen et al. (2007) concluded that the expression of this gene in the hypothalamus and pituitary glands affects egg production. Hensen et al. (2004) identified significant associations with growth rate and fertility reduction in both males and females using *PLAG1* knockout mice. In cattle, variants of this gene have also been linked to growth and fertility traits as well as changes in *IGF1* gene expression (Fortes et al., 2012a,b; Hawken et al., 2012). Although the exact gene location was not reported in our results, the average LD of adjacent markers between the location of the middle of this gene (25,026,431 bp) and the middle of the genomic window (23,496,156 bp) reported in this study was 0.32 (95% confidence interval 0.21–0.44), suggesting possible linkage between these markers.

Chromosome 18. The genomic window (54–56 Mb) on chromosome 18 included 83 different genes. The galactoside *2-α-L-fucosyltransferase* (*FUT2*) gene appears to be stimulated by estrogen, and the correlated expression of fucosylated H-typed-1 carbohydrate epitope in the endometrial epithelia may be involved with embryo implantation (White and Kimber, 1994). The *FUT1* gene was associated with daughter pregnancy rate as well as total number of piglets born (HongYan et al., 2009; Cochran et al., 2013), and Ortega et al. (2016) also found significant associations between this gene and cow conception rate and daughter pregnancy rate in Holstein cattle.

The proapoptotic *Bcl-2 associated X protein* (*BAX*) gene accelerates programmed cell death, and it has been shown that the absence of this gene results in infertility in males (Gaddis et al., 2016). However, in females, Bcl-2 knockout mice exhibit increased oocytes and follicle numbers (Greenfeld et al., 2007), although te Velde and Pearson (2002) found that upregulation of this gene resulted in enhanced follicle damage and premature ovarian failure in mice.

Luteinizing hormone β polypeptide (*LHB*) is known to promote ovulation by stimulating the ovaries to synthesize steroids (The UniProt Consortium, 2015). Luteinizing hormone is secreted by the pituitary gland and plays an important role in gonadal functions. Weiss et al. (1992) associated a homozygous mutation in the *LHB* gene with delayed puberty in humans. Normal pulsatile release of this hormone is crucial for successful induction of LH receptors on the granulosa cells of the developing dominant follicle (Luo et al., 2011). Bender et al. (2014) reported a positive correlation between feed intake and LH concentration, with both high and low values being harmful to embryo development.

Table 5. Description of genomic windows that explain more than 0.5% of the total genetic variation of heifer pregnancy in Nelore heifers

CHR[1]	SNP_start[2]	SNP_end[3]	Pos_start,[4] bp	Pos_end,[5] bp	Size,[6] bp	No. of SNP[7]	%Var[8]
5	rs109437025	rs110687761	73,021,518	73,966,890	945,372	22	3.90
5	rs42561706	rs137385583	77,022,303	77,984,850	962,547	27	3.31
5	rs110496647	rs136544553	81,133,605	81,955,158	821,553	22	1.70
14	rs41724652	rs133297141	23,007,464	23,984,847	977,383	29	1.62
18	rs136460244	rs41891085	55,018,279	55,956,772	938,493	27	1.18
14	rs41624840	rs136805030	29,061,608	29,975,350	913,742	23	1.02
5	rs42917128	rs136339681	60,131,115	60,941,344	810,229	23	0.93
24	rs136828522	rs137238317	60,015,982	60,980,620	964,638	43	0.84
5	rs137127461	rs109435449	72,059,606	72,982,750	923,144	28	0.82
2	rs42509691	rs134051905	54,015,771	54,997,281	981,510	23	0.80
24	rs109329309	rs135881583	52,121,696	52,957,990	836,294	23	0.68
5	rs110450288	rs133794376	82,043,465	82,961,509	918,044	26	0.68
2	rs133912634	rs134084039	58,011,152	58,971,614	960,462	25	0.68
X	rs134685381	rs137716652	44,037,579	44,972,292	934,713	21	0.67
14	rs135852767	rs42298467	25,021,594	25,986,431	964,837	22	0.66
5	rs110797637	rs137576699	51,113,065	51,990,170	877,105	25	0.62
1	rs136647907	rs133111309	125,026,356	125,988,072	961,716	24	0.62
29	rs134769207	rs42172278	24,031,236	24,999,881	968,645	24	0.52
3	rs109945234	rs42368646	3017,659	3968,604	950,945	42	0.51
X	rs134673004	rs134676523	43,104,725	43,892,074	787,349	22	0.50

[1]CHR = Bovine autosome.

[2]SNP_start = first SNP of the window.

[3]SNP_end = last SNP of the window.

[4]Pos_start = position of the first SNP.

[5]Pos_end = position of the last SNP.

[6]Size is the size of the window.

[7]No. of SNP is the number of SNP in the window.

[8]%Var = percentage of the additive genetic variance explained by the window.

The genes *SLC17A7*, *SLC6A16*, and *SLC8A2* are part of the solute carrier family. *SLC17A7* is regulated by estrogen and encodes the vesicular glutamate transporter 1 (Vglut1) protein, which is expressed in cells of the hippocampus (Bellocchio et al., 2000) and may be associated with estrogen regulation (Sárvári et al., 2015). *SLC6* specifically transports neurotransmitters (e.g., dopamine and serotonin), AA (e.g., gamma 1-aminobutyric acid [**GABA**]), and osmolytes (e.g., betaine, taurine, and creatine; Höglund et al., 2005). *SLC8A2* mediates the electrogenic exchange of Ca^{2+} against Na+ ions across the cell membrane. Choi et al. (2014) reported that *SLC8A* is expressed in the uterine endometrium of pregnant pigs and concluded that calcium extrusion molecules may be associated with the establishment and maintenance of pregnancy.

The sphingosine kinase (SPHK) gene family is part of the sphingolipid metabolic pathway, which is highly activated in decidua during pregnancy (Mizugishi et al., 2007). The isoform genes *SPHK1* and *SPHK2* participate in the pathway of the bioactive lipid *sphingosine 1-phosphate* (*S1P*) and have been associated with vasoconstriction in human uterine arteries during pregnancy (Hudson et al., 2007). Mizugishi et al. (2007) found that mutant mice for *SPHK1* and *SPHK2* produced infertile females, with reduced production of *S1P*.

Genes Associated with the Number of Antral Follicles

Chromosome 2. The associated genomic window on chromosome 2 (122–124 Mb) harbored 12 genes. The intracellular fatty acid–binding proteins (FBP) are associated with metabolism and transport of long-chain fatty acids that have import roles in oocyte meiotic maturation prior to ovulation (Sanchez-Lazo et al., 2014). The small nuclear ribonucleoproteins have been related to oocyte maturation, fertilization, and early embryogenesis in mouse (Prather et al., 1990).

Parisi and Lin (1999) associated the *Pumilio 1* (*PUM1*) gene in *Drosophila* with embryogenesis, primordial germ cell proliferation, germline stem cell division, and the oogenic process. *PUM1* is also involved in oocyte maturation in *Xenopus* (Nakahata et al., 2001). The presence of anti-Pum antibody, or the overexpression of Pum1, has significant impacts on oocyte maturation (Nakahata et al., 2003). Mak et al.

Table 6. Description of genomic windows that explain more than 0.5% of the total genetic variation of number of antral follicles in Nelore heifers

CHR[1]	SNP_start[2]	SNP_end[3]	Pos_start,[4] bp	Pos_end,[5] bp	Size,[6] bp	No. of SNP[7]	%Var[8]
24	rs133021126	rs109305902	62,008,417	62,594,751	586,334	24	0.91
29	rs42183484	rs135436243	33,000,052	33,914,381	914,329	23	0.90
12	rs109641299	rs134942057	60,015,627	60,994,461	978,834	28	0.81
2	rs42321794	rs134609840	56,028,202	56,979,919	951,717	21	0.79
19	rs109030622	rs135605613	61,000,109	61,990,875	990,766	45	0.75
19	rs136160175	rs41919438	56,008,922	56,945,797	936,875	55	0.75
19	rs137834616	rs137557574	26,010,131	26,962,473	952,342	29	0.67
25	rs134031092	rs134416406	16,006,913	16,986,653	979,740	21	0.60
12	rs110539435	rs109044869	30,015,791	30,992,759	976,968	30	0.58
15	rs137836273	rs110334648	81,018,176	81,864,846	846,670	32	0.58
12	rs137815663	rs109275028	79,019,126	79,976,465	957,339	27	0.56
X	rs133820946	rs135451827	144,008,894	144,988,891	979,997	35	0.50

[1]CHR = Bovine autosome.

[2]SNP_start = first SNP of the window.

[3]SNP_end = last SNP of the window.

[4]Pos_start = position of the first SNP.

[5]Pos_end = position of the last SNP.

[6]Size is the size of the window.

[7]No. of SNP is the number of SNP in the window.

[8]%Var = percentage of the additive genetic variance explained by the window.

(2016) reported that this gene plays a critical role in the establishment of primordial folliculogenesis, meiosis, and female fertility in mice.

Chromosome 8. Sixteen genes were reported in the associated genomic region on chromosome 8 (6–8 Mb). Tenghe et al. (2016) identified associations with endocrine fertility traits, such as luteal activity, in Holstein cows. The protein *GATA4* is part of the erythroid transcription factor (GATA) family of zinc finger transcription factors, with possible involvement in early gonadal development in mice and humans (Bouma et al., 2007; Lourenço et al., 2011), and a mutation in GATA4 can compromise the cycle of anti-Müllerian hormone. Lourenço et al. (2011) also concluded that *GATA4* is related to cases of sex development disorders and congenital heart defects in humans.

Balboula et al. (2010) found that inhibition of the *cathepsin B* (*CTSB*) protein positively affected oocyte production, resulting in an increased number of good quality embryos in bovines. They concluded that *CTSB* could be used as a marker for low-quality oocytes, in agreement with Bettegowda et al. (2008), who also reported negative correlations between *CTSB* and oocyte competence. However, Warzych et al. (2016) found favorable associations of *CTSB* in bovine cumulus cells with oocyte quality, suggesting that the relationship of the gene to oocyte quality is more complex that initially thought.

Members of the a disintegrin and metalloprotease domain (**ADAM**) gene family are known to play key roles in follicular development and ovarian organization and functioning (Brown et al., 2005; Shozu et al., 2005; Feng et al., 2016) in humans (Gao et al., 2007; Pyun et al., 2014) and mammals in general (Russell et al., 2015). Members of the ADAM with thrombospondin (ADAMTS) gene family have been extensively studied, and it is associated with follicular development through control of extracellular matrix remodeling (Russell et al., 2015). Brown et al. (2005) reported that early antral follicles were significantly reduced in ovaries of *ADAMST-1* knockout mice. Gene expression has also been observed in germ and somatic cells in mice testis, suggesting association with reproduction (Choi et al., 2004; Han et al., 2009).

Chromosome 11. Eleven genes were reported in the genomic window at chromosome 11 (69–71 Mb). *SPDYA* is a protein-coding gene expressed in pathways related to oocyte meiosis (The UniProt Consortium, 2015). Additionally, it was also associated with oocyte maturation in humans (Barnes et al., 2003) and *Xenopus* (Cheng et al., 2005). Porter et al. (2002) concluded that this class of genes is an essential component of cell proliferation pathways.

FOS-like antigen 2 (*FOSL2*) participates in the regulation of steroidogenesis, which includes the estrogen hormone (Hatzirodos et al., 2014b). This gene encodes components of *activator protein 1* (*AP-1*), which participates in the terminal differentiation of

granulosa cells to luteal cells (Sharma and Richards, 2000). Przygrodzka et al. (2015) suggested that elements from the FOS gene family are activated in porcine corpora lutea with acquired luteolytic sensibility, playing an important role in structural luteolysis in pigs.

The protein *yippee-like 5* (*YPEL5*) is part of the yippee like (YPEL) gene family involved with cell cycle progression and growth, with high expression in oocytes (Hosono et al., 2010; Rajput et al., 2013). Hatzirodos et al. (2014a) reported significant upregulation of this gene in large follicles (>10 mm) compared with small follicles (<5 mm) in taurine cattle.

Chromosome 15. Only 2 genes were present in the associated genomic window on chromosome 15 (8–10 Mb). The protein-coding gene *Rho GTPase activating protein 42* (*ARHGAP42*) influences blood pressure in vascular smooth muscle (Bai et al., 2013). *ARHGAP42* also is involved in biological processes related to the regulation of Rho protein, which is part of a family of proteins involved in regulation and timing of cell division (Yoshizaki et al., 2003). Spencer et al. (2013) associated this gene with age at menarche and age at natural menopause in African American women.

Chromosome 16. The associated genomic window on chromosome 16 (70–72 Mb) harbored 13 genes. La Sala et al. (2015) discussed the association of the *G protein-coupled receptor 37* (*GPR37*) with gonadal differentiation in mice. Mutant animals (GPR37-null) had impaired testis development, affecting postnatal Sertoli cell proliferation and maturation, resulting in significant reduction of sperm count. This gene may have similar functions in females, affecting granulosa cell proliferation and animal fertility.

Although the precise function of *leucine-rich repeat-containing G protein coupled receptor 6* (*LGR6*) is still unknown, other leucine-rich repeat-containing G-protein coupled receptor (LGR) genes have been characterized as part of the insulin gene family, which also includes important reproductive genes including *LH*, *FSH*, and *TSH* (Lu et al., 2005). *LGR7* and *LGR8* are receptors for relaxin hormone, which is primarily produced by the corpus luteum. *LGR4* knockout mice were infertile and exhibited abnormal development of the reproductive tract (Mendive et al., 2006).

Protein phosphatase 1 regulatory subunit 12B (*PPP1R12B*) may be involved with insulin signaling (Pham et al., 2012). This gene is associated with smooth muscle contractibility and regulation of uterine cell hypertrophy in the early stages of gestation (Lartey et al., 2016). Ryu et al. (2016) found that *PPP1R12B* participates in protein phosphorylation, which may influence reproduction in humans.

Protein tyrosine phosphatases (**PTP**) regulate many cellular processes, such as metabolism, cell–cell adhesion, cell migration, cell growth, and the expression of transformation growth factor beta, which is a key regulator of follicle development in mammals (Knight and Glister, 2006; Bastian et al., 2015). van Eekelen et al. (2010) reported that 48 PTP genes are expressed in zebra fish embryos, with the majority of them being maternally derived.

Chromosome 22. Twenty genes were reported in the associated genomic window on chromosome 22 (14–16 Mb). Baillet et al. (2011) showed that *testis and ovary-specific PAZ domain gene 1* (*TOPAZ1*) is expressed in the gonads of sheep and mice, particularly in pregnant females during fetal gonad development. They concluded that the gene has an important function in germ cell meiosis. Supporting these findings, Luangpraseuth-Prosper et al. (2015) found that this gene also plays an important role in the progression of meiosis in oocytes and spermatogenesis in mice.

Lysozyme-like (**LYZL**) genes belong to the class of c-type lysozymes, which are widely distributed in the animal species and which have protective bacteriolytic functions in host defense (Zhang et al., 2005). *LYZL4* is expressed in the epithelium of the human epididymis, and it has also been observed on the surface of human embryonic stem cells (Gu et al., 2011). When mouse spermatozoa were incubated with anti-*LYZL4* antibodies, there was a concomitant loss of fertilizing ability (Sun et al., 2011). Some studies (McReynolds et al., 2014; Kwon et al., 2015) have suggested that this gene could be used as a biomarker for male fertility. The LYZL genes may have similar functions in females, participating in the process of germ cell development

In summary, this study provides evidence of the genomic complexity involved in reproductive traits. Genomic regions on chromosomes 5, 14, and 18 showed important associations (that explained >1% of the total additive genetic variance) with HP, whereas regions on chromosomes 2, 8, 11, 14, 15, 16, and 22 had large associations with NF. Although the same genomic window on chromosome 14 was associated with both traits, their genetic correlation was not relevant, suggesting that the selection for one trait has no interference in the other trait. The MeSH terms "Munc18 Proteins," "Fucose," and "Hemoglobins" were significantly related to HP, and the MeSH terms "Cathepsin B," "Receptors, Neuropeptide," and "Palmitic Acid" were related to NF. Increasing the knowledge about associated genomic regions and genes may be useful for enhancing genomic selection, increasing evaluation accuracy, and making better selection decisions (Boichard et al., 2016).

Conclusions

Genomewide association studies allowed us to identify genomic regions associated with the reproductive traits heifer pregnancy and number of antral follicles. Medical Subject Headings enrichment analyses identified important biological processes that are related to the expression of the phenotypes, providing useful information about the genetic components of these traits. The gene search suggested that some genomic regions harbor important genes related to the traits studied, which could be used in genomic prediction to improve reproductive performance.

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