Genomics 114 (2022) 110395

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Integrating genome-wide association study and pathway analysis reveals physiological aspects affecting heifer early calving defined at different ages in Nelore cattle

Lucio F.M. Mota [a,*], Alejandro B. Carvajal [b], Priscila A. Bernardes [c], Marcos E. Buzanskas [d], Fernando Baldi [a,e], Raysildo B. Lôbo [e], Danísio P. Munari [b,f,*]

[a] Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias (FCAV) – Departamento de Zootecnia, Via de Acesso Prof. Paulo Donato Castelane, Jaboticabal, SP, 14884-900, Brazil.
[b] Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias (FCAV) – Departamento de Ciências Exatas, Via de Acesso Prof. Paulo Donato Castelane, Jaboticabal, SP, 14884-900, Brazil.
[c] Universidade Federal de Santa Catarina, Departamento de Zootecnia e Desenvolvimento Rural, Rodovia Admar Gonzaga 1346, Florianópolis, SC, 88034-000, Brazil.
[d] Universidade Federal da Paraíba, Departamento de Zootecnia, Rodovia PB 079 - Km 12, Areia, PB, 58397-000, Brazil,
[e] National Association of Breeders and Researchers, Rua João Godoy 463, Ribeirão Preto, SP 14020-230, Brazil.
[f] National Council for Science and Technological Development (CNPq), Brasilia -, DF, 71605-001, Brazil.

ARTICLE INFO

Keywords:
Biological pathway
Early puberty
Hypothalamus
Metabolic aspects
Multi-trait GWAS
Pituitary gland

ABSTRACT

Heifer early calving (HC) plays a key role in beef cattle herds' economic sustainability and profitability by reducing production costs and generation intervals. However, the genetic basis of HC in Nelore heifers at different ages remains to be well understood. In this study, we aimed to perform a multi-trait weighted single-step genome-wide association (MT w-ssGWAS) to uncover the genetic mechanism involved in HC at 24 (HC24), 26 (HC26), 28 (HC28), and 30 (HC30) months of age in Nelore heifers. The MT w-ssGWAS pointed out four shared windows regions for HC24, HC26, HC28, and HC30 on BTA 5, 6, 14, and 16, explaining a larger proportion of genetic variation from 9.2% for HC30 to 10.6% for HC28. The shared regions harbored candidate genes related with the major gatekeeper for early puberty onset by controlling metabolic aspects related to homeostasis, reproductive, and growth *(IGF1, PARPBP, PMCH, GNRHR, LYN, TMEM68, PLAG1, CHCHD7, KISS1, GOLT1A,* and *PPP1R15B*). The MT w-ssGWAS and pathway analysis highlighted differences in physiological processes that support complex interactions between the gonadotropic axes, growth aspects, and sexual precocity in Nelore heifers, providing useful information for genetic improvement and management strategies.

1. Introduction

Sexual precocity in heifers is a key trait to ensure profitability and sustainability of the beef cattle production system by increasing the number of heifers exposed to the breeding season and weaned calves in cow's lifetime production and the replacement rate of non-precocious heifers by precocious heifers [1,2]. In recent years, greater attention has been directed towards the sexual precocity in Nelore cattle aiming to reduce production costs and generation intervals, leading to more genetic gains rate by generation interval [3]. In this context, Nelore breeding programs in Brazil have included traits related to heifer early pregnancy as selection criteria [4,5]. These Nelore breeding programs have emphasized the selection of sexual precocity, exposing heifers to reproduction between 12 and 17 months or between 19 and 21 months to determine the heifer's early calving (HC) in 24 26, 28, and 30 months [5–7]. On the other hand, the age at which the heifers are exposed to the breeding season of heifers is an important decision for beef cattle farmers.

Attainment of sexual precocity is a complex process whereby a heifer became able to becomes pregnant early and exhibits a complex association with aspects related to genetic, nutritional, and environmental factors, as well as its interactions [8,9]. Establishing a successful early pregnancy in Nelore heifers is considered an intricated process regulated by a polygenic effect involving physiological coordination between growth, neuroendocrine and metabolic mechanisms that culminate in ovulation followed by a regular estrous cycle [10,11]. The main aspect

* Corresponding authors.
E-mail addresses: flaviommota.zoo@gmail.com (L.F.M. Mota), danisio.munari@unesp.br (D.P. Munari).

https://doi.org/10.1016/j.ygeno.2022.110395
Received 18 October 2021; Received in revised form 23 May 2022; Accepted 1 June 2022
Available online 4 June 2022

regarding the early sexual precocity in Nelore heifers has been elucidated by the main biological aspects that regulate the phenotypic variation of early puberty in heifers through genome-wide association studies (GWAS) [9,12,13].

The GWAS approach has found links between early pregnancy and genomic regions, elucidating the major physiological mechanisms in precocious heifers that are activated vs inactivated in heifers with the highest genetic potential to attain early puberty [9,12,13]. In this context, a better understanding of the genetic architecture underlying sexual precocity can be useful in defining priors' assumption about the trait in genomic selection [14] and identifying candidate genes for marker-assisted or gene-based selection. Furthermore, a better knowledge of genomics and biological mechanisms related to sexual precocity obtained from GWAS can aid design efficient genomic selection schemes to improve heifer sexual precocity.

Most of the results from GWAS detected different regions of the genome involved in the phenotypic variation of the traits related to sexual precocity in beef cattle and the potential candidate genes involved in the early puberty onset [12,13,15–17]. Furthermore, no studies have exploited the genomic determinants of sexual precocity throughout different ages. In this context, combining GWAS results and biological enrichment of candidate genes associated with sexual precocity at a different age aid the development of more efficient strategies, aiming to optimize genomic selection for sexual precocity. In addition, the knowledge of the main biological mechanisms targeting genes able to control the transition from pre- to post-puberty in heifers provides a better understanding of biological signals that lead to differences in sexual precocity in heifers [12,13,17] provides the opportunity to develop new strategies that achieve better production without compromising the genetic potential for sexual precocity. Thus, this study was carried out to search genomic regions through multi-trait weighted genome-wide association (MT w-ssGWAS) analysis for Nelore heifer early calving (HC) evaluated at 24, 26, 28, and 30 months in commercial breeding programs to uncover the major biological mechanism to improve the biological knowledge underlying the phenotypic variability for sexual precocity.

2. Materials and methods

2.1. Data source

The phenotypic and genomic information used to evaluate the sexual precocity in Nelore heifers was provided by the Associação Nacional de Criadores e Pesquisadores (National Association of Breeders and Researchers - ANCP, Ribeirão Preto - SP, Brazil; https://www.ancp.org.br/), which conducts the Brazilian Nelore breeding program. The data set includes records from 66,496 heifers with phenotypic information for heifer early calving (HC), from those 8652 heifers were genotyped, belonging to Nelore herds widely distributed in the Southeast and Midwest regions of Brazil.

2.2. Phenotypic information

HC was evaluated at 24 (HC24), 26 (HC26), 28 (HC28), and 30 (HC30) months of age and defined attributing the value of 2 (success) for heifers calving until 24, 26, 28, and 30 months of age respectively, and 1 (failure) otherwise. In addition, Nelore heifers aged between 13 and 17 months were exposed to sexual precocity performance tests in their weaning year, at a breeding season for 90 days, to determine sexual precocity, to attain the heifer early calving at 24 and 26 months. On the other hand, herds exposed Nelore heifers aged between 18 and 21 months to reproduction for 60 days in an anticipated breeding season in February and March, aiming to identify the sexually precocious animals with age at first calving at 28 and 30 months. Heifers were generally raised on pasture with adequate nutritional management (received protein and mineral supplementation) without feed restrictions.

The contemporary groups (CGs) were composed of the herd, year and season of birth, and the management group from birth to year In the study, the CGs ($n = 1497$) were considered as a random effect in the model due to some of the CG showed the same response category (1 or 2), i.e., without variability [18]. The number of CG with heifers classified as precocious was 536 for HC24, 765 for HC26, 912 for HC28, and 1072 for HC30. The descriptive statistics are shown in Table 1.

2.3. Genomic information

A total of 8652 animals were genotyped with the low-density panel (CLARIFIDE® Nelore 2.0) containing over 22,000 markers. The animals genotyped with lower density were imputed to the Illumina BovineHD BeadChip (770 k Illumina Inc., San Diego, CA, USA) using FImpute v.2.2 [19], considering the pedigree information as well as the parentage testing option. In the imputation, the reference population came from 945 sires genotyped with Illumina BovineHD BeadChip assay from the main Nelore lineages (i.e., Karvadi, Golias, Godhavari, Taj Mahal, Akasamu, and Nagpur), which exhibit a high genetic connectedness with the lower-density genotyped animals and the accuracy of imputation was higher than 0.98 [20]. The quality control (QC) of genomic information was performed by removing autosomal markers with a minor allele frequency (MAF) lower than 0.05, a significant deviation from Hardy–Weinberg equilibrium ($P \leq 10^{-5}$), and a call rate of markers and samples lower than 0.90. After quality control, a total of 8545 animals and 454,236 SNP markers have remained for the GWAS analyses.

2.4. Genome-wide association study

The genome-wide association study (GWAS) for heifer early calving (HC) was performed considering a threshold animal model applying a multi-trait weighted single-step genomic BLUP as follows:

$$\boldsymbol{l} = \boldsymbol{X\beta} + \boldsymbol{Za} + \boldsymbol{e}$$

where $\boldsymbol{l}$ is the matrix associated with underlying liabilities for HC at different ages; $\boldsymbol{\beta}$ is the vector of random effect of CG; $\boldsymbol{a}$ is the additive effect of animal and $\boldsymbol{e}$ is the residual effect. The $\boldsymbol{X}$ and $\boldsymbol{Z}$ are the incidence matrices related to random effects of CG and animals. In the model an underlying distribution was considered as follows: $f(HC|\, l_i) = \prod_{i=1}^{n_i} 1(l_i < t_i)1(HC = 1) + 1(l_i > t_i)1(HC = 2)$, where HC is the binary trait (1 or 2) at 24, 26, 28, or 30 months of age, l_i represents the underlying liability for the binary observation i, t_i is the threshold that defines the binary response for the y scale and n_i is the number of information for each trait.

The genetic and residual effects were assumed to be normally distributed (N) as $l \mid \beta, a, R \sim N(X\beta + Za + R)$, in which a is the genetic variance considering the H matrix and R is the residual variance. The vector of random effects for CG (β) was assumed as $\beta \sim p(\beta)$. The additive genetic effect was assumed as $a \mid H, A \sim MVN(0, H \otimes a)$ considering the H matrix and a the genetic variance. Moreover, the a was assumed to follow an inverted Wishart distribution $IW(v_a, v_a S_a)$ combining the pedigree and genomic information, respectively. The residual effect was assumed as an inverse Wishart distribution $R \mid v_e \sim IW(v_e, v_e S_e)$. In the ssGBLUP model, the combined pedigree-genomic relationship matrix (H) was used and its inverse (H^{-1}) was calculated as follows [21]: $H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$ where A_{22}^{-1} represents the subset of the inverse of the pedigree relationship matrix of the genotyped animals, and G^{-1} represents the inverse of the genomic relationship matrix according to VanRaden [22].

In the MT w-ssGWAS the G matrix was constructed as follows: $G = ZDZ'q$ where Z is the SNP matrix assuming 0, 1, and 2 for genotypes AA, AB, and BB; D is a diagonal weight matrix for each SNP marker and q is a weighting factor given as $\sum_{j=1}^{m} 2p_j(1 - p_j)$ where p_j is the second allele

Table 1
Descriptive statistics and genetic parameters for heifer early calving (HC) at 24 (HC24), 26 (HC26), 28 (HC28), and 30 (HC30) months of age, genetic (σ_a^2) and phenotypic (σ_p^2) variance and heritability estimates (h^2) using an animal threshold model through single-step GBLUP.

Trait	HC %*	Genotyped		σ_a^2	σ_p^2	h^2	HPD	
		Success	Failure				Low	Upper
HC24	7.15%	1296	3893	0.77 (0.134)	1.77 (0.135)	0.43 (0.041)	0.25	0.50
HC26	13.61%	1630	3559	0.50 (0.067)	1.51 (0.068)	0.33 (0.031)	0.28	0.38
HC28	17.34%	1824	3365	0.47 (0.056)	1.50 (0.057)	0.31 (0.027)	0.27	0.36
HC30	20.80%	2004	3185	0.44 (0.048)	1.51 (0.049)	0.29 (0.025)	0.26	0.35

* Percentage of success for heifer early calving (HC) at 24, 26, 28, and 30 months of age from the total of phenotypic information (66,496 heifers). h^2 - heritability computed as $h^2 = \sigma_a^2/\sigma_p^2$, which the $\sigma_p^2 = \sigma_a^2 + \sigma_h^2 + \sigma_e^2$ where σ_h^2 is the herd variance and σ_e^2 refers to the residual variance; HPD – 95% of the highest posterior density interval.

frequency of the *j-th* SNP marker. The SNP marker effect and weights for MT w-ssGWAS were estimated considering two iterations (first iteration $D = \boldsymbol{I}$ and second D is the weight estimate obtained in step 6) as previously proposed by Wang et al. [23]:

1. In the first step $D = \boldsymbol{I}$ and second step $D = w$ (step 6); 2. Calculate the G matrix ($G = ZDZ'q$); 3. Estimation of the genomic breeding value (GEBV) for animals using the ssGBLUP; 4. Estimation of the SNP marker effect ($\widehat{u}$) based on the GEBV ($\widehat{a}$) of animals from the equation $\widehat{u} = DZ'[ZDZ']^{-1}\widehat{a}$; 5. Estimation of the SNP marker weight (D) to be used in the second step as follows: $D = \widehat{u}^2 2p_j\left(1 - p_j\right)$ where $\widehat{u}^2$ is the allele substitution effect of each SNP marker; 6. The SNP marker weight (D) is normalized to keep the total genetic variance constant. The percentage of genetic variance explained by the SNP markers ($\sigma_{\widehat{u}}^2$) was estimated as described: $\sigma_{\widehat{u}}^2 = \frac{Var(Z_j\widehat{u}_j)}{\sigma_a^2}$ x 100%, where σ_a^2 is the genetic variance; Z_j is the vector of the *j-th* SNP marker of animal and $\widehat{u}_j$ is the SNP effect of the *j-th* markers.

2.5. Model inference and comparison

Samples of the posterior distributions of the genetic parameters were obtained by Bayesian inference using the Gibbs sampling algorithm implemented in the THRGIBBS1F90 program for HC at different ages [24]. The Bayesian analysis consisted of a single chain of 500,000 iterations, considering a burn-in of 50,000 iterations with samples stored at every 100 iterations. The analysis converged through visual inspection using the Bayesian Output Analysis [25], and for the Geweke test [26], the convergence was attained for the evaluated traits with a *p*-value higher than 0.25.

2.6. Statistical test for marker effect

The statistical test for SNP markers was performed by standardizing the SNP effects from the MT w-ssGWAS [27]. In this way, the SNP marker effect (u_k) in each HC evaluated at different ages was estimated using a linear transformation of GEBV ($\widehat{a}$) as $u_k = z'G^{-1}\widehat{a}$ and the prediction error variances of SNP effect estimates as $\sigma_{u_k}^2 = Z'G^{-1}Z\sigma_a^2 - Z'G^{-1}C^{aa}G^{-1}Z$. In this framework the C^{aa} is the portion of the inverse of the mixed model equations associated with the model [27]. Then, the statistical test was performed by the standardization of the SNP effects [27] as follows: $z_k = \frac{u_k}{sd(u_k)}$, where z_k is the statistical test for SNP marker effects in each HC evaluated at different ages. The *p-values* for the SNP effects were computed as $p - value = 2(1 - \phi(|z_k|))$, where $\phi(|z_k|)$ is the cumulative function of the normal distribution for the z-score.

2.7. Gene mapping of significant SNP for M-T WssGWAS

The SNP markers explaining more than 1% of the genetic additive variance for HC at 24, 26, 28, and 30 months of age were deemed significant and grouped at the same chromosome. These grouped SNP markers were used to identify genes located in these regions. After selecting the windows deemed as significant, a search for overlapping windows was defined by the group of significant SNP markers with at least 0.4 Mb to be deemed a shared region. Finally, the linkage disequilibrium (LD) analysis was performed in overlapping regions by computing the r-square (r^2) [28] values for pairwise SNP marker using the Gaston R package [29].

The genes were identified using the NCBI BioSystems database for cattle, using the *Bos taurus* ARS-UCD 1.2 assembly. Functional classification for biological mechanisms and pathways (Gene Ontology - GO) associated with the candidate genes set were identified using the clusterProfiler R Package [30], separately for each heifer early calving (HC 24, 26, 28, and 30), considering as background the bovine database from OrgDb (http://bioconductor.org/packages/release/BiocViews.html#__OrgDb). The enrichment analysis of the given gene set was assessed using Fisher's exact test [31] and considered significant when *p-value* < 0.05. Interactions between protein-coding genes were predicted using the STRING database with default settings, according to Szklarczyk et al. [32].

3. Results and discussion

3.1. Descriptive and genetic parameters

The observed proportion of heifers for HC24 (7.2%) and HC26 (13.6%) was low against HC26 (17.3%) and HC30 (20.8%; Table 1). This lower proportion of Nellore heifers reaching puberty at an early age (HC24 and HC26) may affect the current target of selection from different Nelore breeding programs that have emphasized selection for HC30 that increase its proportion on herds. The inclusion of sexual precocity traits in the selection index has improved the reproductive performance of heifers. The heritability estimates for sexual precocity in heifers changed according to criteria used to define the precocity ranging from high (0.43 ± 0.04 for HC24) to moderate (0.29 ± 0.02 for HC30; Table 1) and are in agreement with previous estimations ranging from 0.28 [33] to 0.37 [6,7]. Based on the results for genetic parameter estimates, HC24 led to a rapid response due to a higher heritability estimate and reduction of the generation interval. There is evidence that HC24 does not negatively impact traits related to reproductive performance, growth, carcass and feed efficiency indicators in Nelore cattle under a tropical production system [5]. In this context, considering sexual precocity as a selection criteria in breeding programs showed that selection for HC combined with improved management practices led to a reduction in the phenotypic means of age at first, calving from nearly 38 to less than 28 months, with a genetic trend of almost −2 days/year [34]. Thus, Nelore breeding programs exposing heifers to mating between 11 and 13 months to identify and select precocious females for HC24, can achieve considerable reductions on phenotypic and genetic trends for age at first calving. In addition, heifers evaluated in sexual precocity tests at early ages (HC24) exposing heifers to mating in a common breeding season in the herd, reduced the costs of an additional breeding season to identify females with HC between 28 and 30 months. However, increased attention has to be directed towards improving

management strategies, mainly the nutritional management of heifers to reach 60% - 65% of their mature body weight before the beginning of the breeding season [8,35]. Consequently, HC24 in these herds has increased from 10% to more than 60%, reducing the number of unproductive females in the herds and promoting the economic sustainability of beef cattle herds through better use of resources.

3.2. SNP variance for sexual precocity

The MT w-ssGWAS pointed out a total of 9, 9, 12 and 11 SNP-windows explaining more than 1% of the genetic variance for HC24 (BTA 2, 3, 4, 5, 6, 9, 14, 16 and 22; Fig. 1 - A), HC26 (1, 4, 5, 6, 11, 14, 16, 22 and 28; Fig. 1 - B), HC28 (1, 2, 4, 5, 6, 7, 11, 13, 14, 16, 22 and 28; Fig. 1 - C) and HC30 (2, 5, 6, 7, 8, 11, 13, 14, 16, 18 and 24; Fig. 1 - D), respectively. These regions explain more than 1% of additive genetic variance were significantly associated with HC at different ages considering a significant level of $-log_{10}(p - value) > 5$ (Supplementary Fig. S1). Some of these genomic regions were shared among the sexual precocity at different ages (Fig. 1 E) and can explain part of the observed genetic correlations among HC at 24, 26, 28, and 30 months (Fig. 1 F). The genetic correlation for HC at different ages decreased when increasing the distance between ages, i.e., HC24 and HC30 ($r = 0.60$), and showed values higher than 0.70 in the adjacent ages (Fig. 1 F). The results of the genetic correlation led us to speculate that expressing HC at different ages has an impact on the outcome of selection strategies for sexual precocity in Nelore cattle. It is of note that the genetic correlation lower than 0.80 leads to differences in the ranking of animals for HC according to the age are expressed. Based on genetic correlation estimation, HC at 24 and 26 months showed more similarity assessed by hierarchical clustering and indicated a clear difference compared with HC at 28 and 30 months (Fig. 1 F).

A higher number of shared windows was observed for HC24, HC26, HC28, and HC30 (4 SNP windows), followed by HC 28 and HC 30 (3 SNP windows) and HC24, HC26, and HC28 (2 SNP windows) which could be explained by the observed genetic correlation coefficients (Fig. 1 F). These results indicated that sexual precocity at different ages has in part the same biological basis, and specific regions may be age-dependent aspects affecting the expression of the mechanisms involved in the transition process heifers from pre- to post-pubertal. Indeed, higher specific genomic regions were observed affecting the HC24 and HC30 (3 SNP windows, Fig. 1 E).

3.3. Specific regions affecting sexual precocity at different months

Specific genomic regions affecting HC in each age evaluated were identified on BTA 2, 3, and 9, for HC24, BTA 1 (139.65–139.87 Mb) for HC26, BTA 1 (127.15–128.55 Mb) for HC28, and BTA 8, 18 and 24 for HC30, implying in different physiology mechanisms leading to sexual precocity in a specific age. The specific variants identified affecting HC24 explained 4.9% of additive genetic variance (Table 2), surround the major genes *MAGI3* on BTA3 (29.65–29.78 Mb) and *MYO7B* on BTA2 (4.94–5.16 Mb) associated with growth aspects, and *MAP3K2* (*MEK/ERK Kinase 2*; BTA2 4.94–5.16 Mb) and *GRIK2* (BTA9 48.03–48.91 Mb) as part of GnRH signaling cascades in the pituitary cells. The gene *MYO7B* is related to muscular formation in different breeds [36] and in ducks showed associations with muscle growth and lipid deposition [37]. The *MAP3K2* gene is related to MAPK pathway activation, which plays an important role in growth and development and insulin resistance [38]. Tosca et al. [38] observed that insulin resistance in cattle affects oocyte maturation. Such findings support the hypothesis that metabolic substrates (insulin and lipid) link nutrition and reproduction, leading to different growth rates, allowing heifers to reach puberty at an early age to become pregnant at 16 months [39]. On the other hand, the genes *MAP3K2* and *GRIK2* are directly associated with GnRH secretion. The *MAP3k2* shows a key role in MEKK, ERK, and JNK signaling cascade, mediating the secretion of LH and FSH hormone enabling regulation of physiological reproductive functions during the onset of puberty [40]. The gene *GRIK2* acts as an excitatory neurotransmitter in the central nervous system by opening ion-channel and has been related to reproduction functions by its effect on gonadotropin-releasing (GnRH) secretion control [41]. In this context, enrichment analysis indicated specific biological processes related to lipid storage (GO:0019915 and GO:0010883) and gonadotropin secretion (GO:0032276) by the action of the major gene set (*HILPDA*, *LEP,* and *KISS1*) were associated with HC at 24 months (Table 3). Indeed, these genes and biological processes with a specific effect highlighted the striking effect linking body energy aspects and early puberty onset by the action of genes affecting the expression of metabolic hormones and neuropeptides, which regulate the levels and release of GnRH hormone [42,43].

A significant region on BTA1 with a specific effect on HC26 surround the genes *IGSF5* and *B3GALT5* with unknown biological mechanisms affecting reproduction aspects in female (Table 2). Although specific biological processes related to adipose tissue development (GO:0060612), regulation of lipid transport (GO:0032368), and response to growth hormone (GO:0060416), by the action of genes *LEP*, *GHRL*, *REN,* and *LYN* were pointed out to affect the HC26 (Table 3). The association of these biological mechanisms occurs mainly because of a direct effect on growth aspects affecting the growth rate, which is related to muscle and fat deposition rate [35]. Thus, the relationship of a gene involved in growth, adipose tissue, and lipid transport with HC26 occurs by the fact that cycling heifers need to reach a specific body condition score, which is determined by the ideal fat deposition, i.e., delay in early puberty occurs until the heifers' reach 60% to 65% of the mature body weight and exhibit an ideal ratio of fat and muscle deposition [8,35].

The candidate genes (*GRK7, RNF7, RASA2, ZBTB38, PXYLP1, SPSB4, TRIM42,* and *CLSTN2*) surround the BT1 with a specific effect on HC28 play a key role on body size, lipid, and glucose metabolism (Table 2). The gene *ZBTB38* significantly affects body measurements traits, mainly for cattle's body structure [43] and eye muscle area [44]. This effect could be associated with delayed puberty by increasing the body structure, leading to delays in early pregnancy by increasing the weight at maturity. In this context, heifers with high body weight at maturity reach the age at first calving at older ages due to its unfavorable genetic correlation ($r = 0.52$) [44]. Thus, increasing the mature body weight delays the onset of heifer puberty until an adequate body condition score is associated with their mature body weight [35]. The gene *CLSTN2* is related to lipid metabolism, principally influencing the increase of adipocytes in subcutaneous fat [45] and rib-eye area [46].

Furthermore, the *CLSTN2* expression is related to metabolic disorders such as glucose and insulin [45]. These physiological changes in insulin and glucose blood levels affect reproduction because the glucose and insulin levels represent a key biological link between metabolic factors and the endocrine axis [47] to attain sexual precocity [39]. Samadi et al. [39] observed that Brahman heifers with improved metabolic homeostasis, mainly greater insulin and glucose levels, could affect the development and oocyte quality leading to first ovulation and puberty at an early age. The biological ontologies with a specific effect on HC28 are related to the cellular response to peptide hormone stimulus (GO:0071375), glucose metabolic process (GO:0006006), and insulin-like growth factor receptor signaling pathway (GO:0048009; Table 3). Physiological changes influence the amounts of metabolic substrates peptide hormone signals which directly affect the age at first, calving by its effect on oocyte quality and development of both oocyte and embryo [48].

Three specific SNP regions were deemed as significant for HC30, explained 5.5% of additive genetic variance, and mapped on BTA 8 (*LPL, SLC18A1, ATP6V1B2,* and *LZTS1*), BTA18 (*CDH5*), and BTA24 (Table 2). The major genes *LPL* and *ATP6V1B2* are associated with response to insulin (GO:0032868; Table 3). The gene *LPL* synthesizes fatty acids [49], playing an essential role in the lipid metabolic pathways

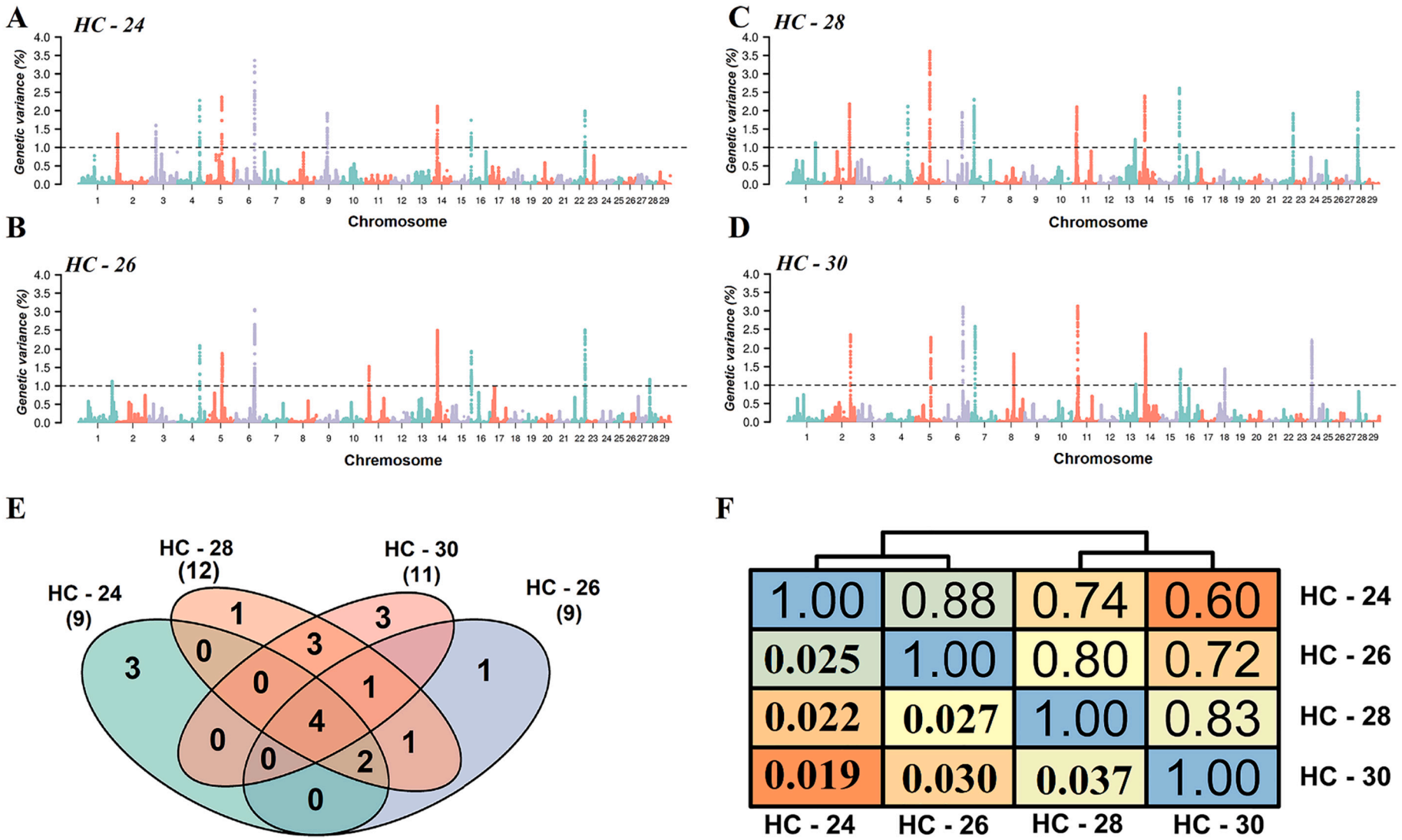

Fig. 1. Manhattan plots of the percentage of the additive genetic variance explained by SNP makers for heifers early calving at 24 (HC24 A), 26 (HC26 B), 28 (HC28 C), and 30 (HC24 D) months. Venn diagram of the number of SNP regions shared by heifer early calving at different months (E) and genetic correlation with its respective standard deviation of estimates bellow the diagonal (F) for heifer early calving at 24 (HC - 24), 26 (HC - 26), 28 (HC - 28), and 30 (HC - 30) months.

Table 2
Specific regions for heifers early calving defined at 24, 26, 28, and 30 months explaining more than 1% of the additive genetic variance (σ_a^2) in Nelore cattle heifers.

BTA	Windows[1]	Size (Mb)	N. SNP[2]	σ_a^2 (%)	Genes
HC 24					
2	4,942,729–5,158,856	0.22	65	1.4	MYO7B, IWS1, PROC, MAP3K2
3	29,651,535–29,787,508	0.14	36	1.6	PHTF1, MAGI3
9	48,038,420–48,918,750	0.28	82	1.9	GRIK2
HC 26					
1	139,653,452–139,875,117	0.22	36	1.1	IGSF5, B3GALT5
HC 28					
1	127,154,516–128,553,466	1.09	79	1.1	GRK7, RNF7, RASA2, ZBTB38, PXYLP1, SPSB4, TRIM42, CLSTN2
HC 30					
8	67,914,870–68,164,937	0.65	66	1.9	LPL, SLC18A1, ATP6V1B2, LZTS1
18	33,761,475–34,135,077	0.37	74	1.4	CDH5
24	15,041,984–15,763,710	0.72	80	2.2	–

[1] Represent the regions of the SNP markers explaining more than 1% of the genetic variance.
[2] Number of SNP markers explaining more than 1% of the additive genetic variance.

Table 3
Gene ontology enrichment analysis for biological processes of the genes identified for heifer early calving (HC) at 24, 26, 28, and 30 months of age, with specific effect across the sexual precocity trait.

ID	Description	p-value	q-value	Gene
Heifer early calving at 24 months				
GO:0019915	lipid storage	0.015	0.047	HILPDA, LEP
GO:0032276	regulation of gonadotropin secretion	0.001	0.027	KISS1, LEP
GO:0010883	regulation of lipid storage	0.007	0.042	HILPDA, LEP
Heifer early calving at 26 months				
GO:0060612	adipose tissue development	0.007	0.036	LEP, GHRL
GO:0032368	regulation of lipid transport	0.007	0.036	LEP, GHRL, REN
GO:0060416	response to growth hormone	0.006	0.035	LYN, GHRL
Heifer early calving at 28 months				
GO:0071375	cellular response to peptide hormone stimulus	0.001	0.011	SOGA1, GNRHR, INSR, LEP, LYN, GHRL, ATP6V1B1, SRC, SLA2
GO:0006006	glucose metabolic process	0.001	0.032	SOGA1, IGF1, INSR, LEP, GHRL, SRC
GO:0048009	insulin-like growth factor receptor signaling pathway	0.001	0.009	GHRH, IGF1, IGFBP2, IGFBP5
Heifer early calving at 30 months				
GO:0044042	glucan metabolic process	0.021	0.041	IGF1, INSR
GO:0045995	regulation of embryonic development	0.007	0.038	IGF1, INSR, TGIF2
GO:0061458	reproductive system development	0.010	0.039	INSR, PLAG1, ETNK2, BIRC6, REN
GO:0032868	response to insulin	0.001	0.033	INSR, LPL, LYN, ATP6V1B2, SLA2

associated with adipose tissue mass [50]. In addition, the *LPL* gene is involved in the hydrolysis of circulating triglycerides and low-density lipoprotein associated with growth and carcass quality traits [51,52]. The gene *ATP6V1B2* has been related to energy homeostasis by its effect on catabolism and anabolism, providing the generation of precursor metabolites and energy [53] and being involved in regulating insulin secretion [54]. The ATP6V1B2 gene was associated with feed efficiency traits in beef cattle [55] and uniformity of yearling weight in Nelore cattle [56]. Enrichment analysis identified biological processes related to glucan metabolic process (GO:0044042) and response to insulin (GO:0032868) are related to energy homeostasis stimulated by anabolic and catabolic ratio metabolism with actions on the regulation of embryonic development (GO:0045995) and reproductive system development (GO:0061458; Table 3). The association of energy homeostasis in HC30 occurs by regulating insulin and glucose, representing the major energy source required for ovarian function and LH secretion [57,58]. Samadi et al. [39] in Brahman heifers raised under adequate nutrition led to greater glucose and insulin levels and then reduced the age at first calving (AFC) by improved metabolic homeostasis.

3.4. Shared regions surround genes for HC at different ages

3.4.1. HC at 26 and 28 months and 28 and 30 months

The significant SNP-window shared between HC at 26 and 28 mapped on BTA28 explained 1.2% and 2.5% of the additive genetic variance respectively and surrounds the gene *RYR2* and *ZP4* (Table 4) and showed a moderate LD in average ($r^2 = 0.40$) between the significant SNP markers (Supplementary Fig. S2). The gene *RYR2* mediates the release of Ca^{2+} from intracellular stores and increases cytosolic Ca^{2+} in response to many different extracellular stimuli, including hormones and neurotransmitters [59]. In addition, the gene *RYR2* plays a key role in amplifying the signal generated by voltage-gated calcium channels, which might improve the LH release meditated by pituitary GnRH because it is a calcium-dependent process [60]. On the other hand, the gene *ZP4* subunits of zona pellucida (ZP) glycoprotein with functions during fertilization and preimplantation development [61]. The expression of *ZP* glycoprotein is required for optimal oocyte growth and fertilization and early embryo migration to the oviduct [61].

A total of three specific SNP windows were shared between HC at 28 and 30 months, explaining from 1% to 2.6% of genetic variance (Fig. 1 C and D) and harbor candidate genes on BTA2 (*SMARCAL1*, *IGFBP2,* and *IGFBP5*), BTA7 (*INSR*), and BTA13 (*GHRH, MYL9, NNAT, NDRG3, TGIF2, RAB5IF, SLA2,* and *SAMHD1*). The major genes region on BTA 2 (*IGFBP2* and *IGFBP5;* 104.30–104.96 Mb) and BTA7 (*INSR;* 15.99–16.41) exhibits a striking effect in metabolic pathways whereby control the insulin (GO:0008286; Table 5) and glucose levels that might help explain the variability in the sexual precocity [62]. In addition, the *IGFBP* gene family has been pointed out as a key factor to control follicle growth by sensitivity to gonadotropins [62,63], and the *INSR* gene is implicated in the regulation of the reproductive aspect due to its actions in the hypothalamic-pituitary-gonadal (HPG) axis and ovaries [64].

Genes mapped on BTA13 (64.89–66.62 Mb), particularly the gene set (*GHRH, MYL9,* and *NNAT*), highlighted the complex physiological transition from the prepubertal phase (accelerated growth) to puberty (sexual maturation) through regulation of growth hormone secretion [65]. The gene *GHRH* affects the age at puberty by its direct function on putative modulatory role in the HPG axis [66], and its action is related to the growth and body condition such as average daily gain, fat thickness, and meat content of carcass [67]. Thus, the gene *GHRH* affects the age at puberty by its direct function on putative modulatory role in the HPG axis, required as an energy homeostasis factor that in many tissues is critical for the maintenance of their metabolic actions by a direct effect

Table 4
Common regions for heifers early calving defined at 24, 26, 28, and 30 months explaining more than 1% of genetic variance (%) in Nelore cattle heifers.

BTA	Windows[1]	N SNP	Size (Mb)	LD[2]	Genetic variance (%)				Genes
					HC24	HC26	HC28	HC30	
5	65,278,733–66,955,623	135	1.67	0.41	2.37	1.88	3.62	2.28	ARL1, ASCL1, CHPT1, DRAM1, GNPTAB, IGF1, MYBPC1, NUP37, PAH, PARPBP, PMCH, SPIC, SYCP3, WASHC3
6	82,880,362–83,641,329	100	0.42	0.52	3.37	3.05	1.95	3.1	CENPC, STAP1, UBA6, GNRHR, TMPRSS11D, TMPRSS11A
14	22,136,921–23,959,114	200	1.82	0.47	2.12	2.49	2.4	2.37	SOX17, RP1, XKR4, TMEM68, TGS1, LYN, RPS20, RF01277, RF00003, MOS, PLAG1, CHCHD7, SDR16C5, SDR16C6, PENK, RF00026, IMPAD1
16	1,003,557–2,246,563	198	1.24	0.38	1.74	1.94	2.61	1.43	ATP2B4, BTG2, CHIT1, ETNK2, FMOD, GOLT1A, KISS1, LAX1, OPTC, PLEKHA6, PRELP, REN, SNRPE, SOX13, ZC3H11A, RF00026, PPP1R15B, PIK3C2B, RF00334
4	91,827,295–93,055,139	105	1.24	0.31	2.28	2.09	2.12	–	GCC1, ARF5, FSCN3, PAX4, SND1, LRRC4, MIR129–1, LEP, RBM28, PRRT4, LOC537848, IMPDH1, HILPDA, FAM71F2, FAM71F1, CALU, OPN1SW, CCDC136, FLNC, MIR2422, ATP6V1F, ATP6V1FNB, KCP, IRF5, TNPO3, MIR1843
22	53,492,772–54,500,345	165	0.98	0.33	1.99	2.5	1.92	–	ATP2B2, SLC6A20, SACM1L, LIMD1, LARS2, TMEM158, CDCP1, CLEC3B, EXOSC7, ZDHHC3, TMEM42, GHRL, SEC13
11	13,380,573–16,086,590	214	2.71	0.30	–	1.03	2.1	3.13	PAIP2B, NAGK, TEX261, ANKRD53, ATP6V1B1, VAX2, CD207, CLEC4F, FIGLA, ADD2, TGFA, FAM136A, XDH, SRD5A2, MEMO1, DPY30, SPAST, SLC30A6, NLRC4, YIPF4, BIRC6, TTC27, LTBP1, RASGRP3
28	10,063,004–10,660,998	90	0.60	0.40	–	1.18	2.5	–	RYR2, RF00402, ZP4
2	104,302,088–104,963,531	100	0.66	0.44	–	–	2.18	2.35	MARCH4, SMARCAL1, RPL37A, IGFBP2, IGFBP5, TNP1
7	15,996,443–16,410,917	85	0.41	0.37	–	–	2.3	2.57	INSR, PEX11G, TEX45, ZNF358, MCOLN1, PNPLA6
13	64,894,313–66,622,751	158	1.73	0.35	–		1.22	1.02	NFS1, ROMO1, RBM39, PHF20, RF00001, SCAND1, CNBD2, EPB41L1, AAR2, DLGAP4, MYL9, TGIF2, RAB5IF, SLA2, NDRG3, DSN1, SOGA1, TLDC2, SAMHD1, RBL1, MROH8, RPN2, RF00428, GHRH, MANBAL, SRC, BLCAP, NNAT, CTNNBL1

[1] Represent the regions of the SNP markers explaining more than 1% of the additive genetic variance. N SNP – number of SNP within the Windows regions.
[2] Represents the average of linkage disequilibrium (r^2) for the windows region (Supplementary Fig. S2 and S3).

Table 5
Gene ontology enrichment analysis for biological processes of the genes identified for heifer early calving (HC) at 24, 26, 28, and 30 months of age, with biological processes shared across the traits (significance tests are shown in Supplementary Table S1 and S2).

ID	Description	Gene associated with the GO			
		HC 24	HC 26	HC28	HC 30
GO:0070977	bone maturation	IGF1, LEP	IGF1, LEP	IGF1, LEP	–
GO:0032274	gonadotropin secretion	KISS1, LEP	KISS1, LEP	KISS1, LEP	–
GO:0046879	hormone secretion	KISS1, LEP, LYN, REN	KISS1, LEP, LYN, GHRL, REN	GHRH, KISS1, LEP, LYN, NNAT, GHRL, REN	–
GO:0009914	hormone transport	KISS1, LEP, LYN, REN	KISS1, LEP, LYN, GHRL, REN	GHRH, KISS1, LEP, LYN, NNAT, GHRL, REN	–
GO:0032275	luteinizing hormone secretion	KISS1, LEP	KISS1, LEP	KISS1, LEP	–
GO:0022602	ovulation cycle process	KISS1, LEP	KISS1, LEP	KISS1, LEP, SRC	–
GO:0060123	regulation of growth hormone secretion	GHRH, KISS1, GHRL	KISS1, GHRL	GHRH, KISS1, GHRL	–
GO:0002791	regulation of peptide secretion	IGF1, KISS1, LEP, LYN, GHRL	IGF1, KISS1, LEP, LYN, GHRL	GHRH, IGF1, KISS1, LEP, LYN, NNAT, GHRL, SRC	–
GO:0008286	insulin receptor signaling pathway	–	–	SOGA1, INSR, LEP, ATP6V1B1, SRC, SLA2	INSR, ATP6V1B2, SLA2
GO:2000241	regulation of reproductive process	–	–	IGF1, INSR, MOS, ZP4, SRC	IGF1, INSR, MOS
GO:0043434	response to peptide hormone	GNRHR, LEP, LYN, BTG2, ATP6V1F	–	–	GNRHR, INSR, LPL, LYN, ATP6V1B2, BTG2, SLA2
GO:0060986	endocrine hormone secretion	KISS1, LEP, REN	KISS1, LEP, GHRL, REN	KISS1, LEP, GHRL, REN	KISS1, REN
GO:0046323	glucose import	IGF1, LEP	IGF1, LEP	IGF1, INSR, LEP	IGF1, INSR
GO:0044060	regulation of endocrine process	KISS1, LEP, REN	KISS1, LEP, GHRL, REN	KISS1, LEP, GHRL, REN	KISS1, REN

on GH receptor (GHR), or indirectly by the mediation of *IGF-1* [68].

The gene *MYL9* on BTA13 regulates muscle contraction by controlling ATPase activity [69], and its effect on sexual precocity in heifers could be associated with a change in muscle metabolism contributing to a reduction in basal energy metabolism [69]. This can be a potential mechanism for heifers raised under an extensive pasture production system aiming to reduce muscle metabolism's energy expenditure and provide free metabolite concentrations for reproduction [9]. Supporting this hypothesis, Cônsolo et al. [70] observed that selecting for a high genetic potential for sexual precocity can affect fat and muscle metabolism. The *NNAT* gene plays a significant role in body metabolism, insulin secretion, adipogenesis differentiation process, and food intake

[71].

3.4.2. HC at 24, 26, and 28 months and HC at 26, 28 and 30

Two common SNP-window genomic regions were identified with a strong effect on sexual precocity evaluated between 24, 26, and 28 months of age (Fig. 1 E) on BTA4 (91.82–93.05 Mb) and BTA22 (65.95–66.58 Mb), explaining a substantial amount of genetic variance, from 2.1% to 2.3% and 1.9% to 2.5%, respectively (Fig. 1 A-C). The region on BTA4 harbors the gene set (*ATP6V1FNB, IRF5*, *FSCN3, PAX4, SND1, HILPDA, LRRC4,* and *LEP*) that regulate the peptide signal-regulating lipid metabolism and energy expenditure and region on BTA22 harbors the genes set (*GHRL*, *TNPO3,* and *SEC13*) with effects on hypothalamic signals and insulin and glucose metabolism (Table 4). The genes *LEP* and *GRHL* are associated with energy pathways for the metabolic regulation of age at puberty [72]. The gene *LEP* acts as an adipose tissue hormone that regulates the feed intake and body energy homeostasis, whereby provides a link between nutritional status and functions on the HPG axis representing the gatekeeper signals to heifer attain the early puberty and development reproductive function [11,73]. The gene *GRHL* has been pointed out as an important regulator of body weight by growth hormone secretion stimulation, increasing the blood glucose levels free fatty acid, and reducing the glucose transport in skeletal muscle [74].

The *HILPDA* on BTA4 was associated with the regulatory signal responsible for adjusting the storage of triglycerides and the intracellular availability of fatty acids [75]. Thus, these genes lead to differences in metabolic hormone and glucose blood level integrating the metabolic signals with the HPG axis in the control of puberty onset. Samadi et al. [39] in Brahman heifers observed that this intricate pathway positively influences reducing the age at puberty.

One SNP window on BTA 11 (13.58–16.08 Mb) was considered shared by HC 26, 28, and 30, although this region shows a large size (2.71 Mb), a medium LD on average ($r^2 = 0.30$) was observed (Supplementary Fig. S2). This region on BTA 11 surrounds the major genes associated with growth factors *LTBP1* and *TGFA* with a key role in the *TGF-β* signaling associated with ovarian function, immunoregulation of pregnancy, and embryo implantation [76]. In addition, these genes affect the sexual precocity through direct effects on ovarian cells and gonadotropins secretion by its association with the *EGFR* gene. The gene *FIGLA* is a germ cell-specific transcription factor and was associated with oogenesis, suggesting a role in follicle growth [77] and playing an important aspect on multiple oocyte-specific genes [61].

3.4.2.1. HC at 24, 26, 28, and 30 months. The MT w-ssGBLUP targeting the sexual precocity in Nelore heifers contributes to understanding the common genetic background associated with HC variability at different ages (Fig. 1). A total of four SNP-window regions shared between the evaluated sexual precocity traits (HC24; HC26; HC28 and HC30) could explain, in part, the genetic correlation estimates among them (Fig. 1 F). These shared SNP windows located on BTA 5, 6, 14, and 16 explained the highest percentage of additive genetic variance at 9.6% for HC24, 9.4% for HC26, 10.6% for HC28, and 9.2% for HC30 (Table 4) and showed a moderate LD average ($0.30 \leq r^2 \geq 0.52$; Supplementary Fig. S2 and S3). The genes that surround these regions are related to mechanisms that compose the essential gatekeepers for sexual precocity through the integration of metabolic hormone action at the brain such as *IGF1* and *PMCH* (BTA5), *GNRHR* (BTA6), *PLAG1,* and *PENK* (BTA14), and *KISS1* (BTA16). These genes provide an integration of multiple regulatory signals whereby links neuroendocrine and metabolic mechanisms, responsible for the transition from the pre- to post-puberty that culminates in ovulation followed by a regular estrous cycle [72]. DeAtley et al. [11], applying the peptidomics approach in Brangus heifers, observed changes in the hypothalamus and pituitary gland activation before and after puberty that influence the sexual precocity.

The region on BTA5 (65.27–66.95 Mb) and BTA14 (22.14–23.96 Mb) surround genes with an important biological effect whereby links metabolic homeostasis and endocrine axis, which contribute to heifers achieving puberty at an early age [11], as well as explain the variability in the sexual precocity and growth aspects [9,13,17]. The gene *IGF1* on BTA5 and *PLAG1* on BTA14 has been directly associated with QTLs affecting the different reproductive traits such as ovulation rate, age at puberty, gestation length, postpartum anestrous interval, and calving ease [9,78,79], indicating that *IGF1* and *PLAG1* genes are related to the different reproductive process by neuroendocrine regulation gatekeeper pathways controlling GnRH release [58,80]. The *PLAG1* gene shows a pleiotropic function on growth and reproduction aspects [9,81,82]. Hence, the *PLAG1* gene can affect the age at puberty in heifers through a direct effect on *IGF1* and *IGF2* levels and then stimulate GnRH neurons [83]. In addition, the gene *MOS* on BTA14 shows a key function on oocyte maturation by activating the MAP kinase cascade, which represents a crucial cellular energy sensor regulating essential roles in oocyte maturation and fertilization [84,85].

The *PENK* gene maps on BTA14 had been associated with AFC in Nelore cattle [9] and encodes an opioid precursor participating in neuron stimulation during the endocrine transition to puberty in cattle [11]. Thus, the BTA5 and BTA14 regions appear to have a potential association with delayed puberty by metabolic-sensing pathways that mediate the body energy reserves and metabolic status, leading to delays in reproductive precocity in heifers until an adequate body condition concerning their frame size is achieved. Several authors have been highlighted the importance of metabolic aspects whereby the growth and sexual precocity-related traits are associated through the metabolic compounds (glucose and insulin) [8,9,13,39,78,79].

The *PMCH* gene on BTA5 encodes three neuropeptides (*MCH, NEI,* and *NGE*), and its biological actions are associated with energy homeostasis, fat deposition, and feed intake [86,87]. *PMCH* variants encoding these three neuropeptides show a key link between energy balance and reproductive physiology [86]. Nelore heifers are raised under different production systems and exposed to different nutrition levels; such conditions demand a higher efficiency in heifers' energy balance during growth phases until they reach puberty [9]. Angulo-Valenzuela et al. [88] observed a favorable effect of SNP within the PMCH gene region in enhancing fertility in Angus and Brangus heifers under desert environment, suggesting a genetic component associated with balancing their reproductive performance and energy metabolism reducing a negative effect of harsh environmental conditions. The *PARPBP* (Poly[ADP-Ribose] Polymerase 1 binding protein) exhibits an indirect effect on metabolism related to oocyte development through *PARP1* gene regulation [89]. The region on BTA6 (82.88–83.64 Mb) surrounding the major gene *GnRHR* that mediates the action of *GnRH* responsible for controlling the reproduction stimulating the secretion of peptide hormone such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) involved in the timing of puberty in heifers [90]. An adequate secretion of *GnRH* represents an important aspect for heifers to achieve early sexual precocity due to improvements in growth rate and reducing the age at puberty in heifers [90].

The gene set (*KISS1, GOLT1A, REN,* and *PPP1R15B*) on BTA16 (1.00–2.25 Mb) is related to GnRH release, estrogen release regulation, and pregnancy pathways. The gene *KISS1* has been indicated as an important gatekeeper regulating the onset of puberty and reproductive function through its action on GnRH pulse by a complex metabolic control [83]. The *KISS1* gene integrates the key peripheral network signals and central pathways linking the metabolic information to the GnRH neurons driving the puberty onset [91]. Mutations or deletions on the *KISS1* gene reduce circulating levels of gonadotropin hormones (LH and FSH), affecting reproductive functions in humans and mice [92,93]. The gene *PPP1R15B* was identified near the SNP markers significantly associated with sexual precocity-related traits in tropical beef cattle [13], and knockout gene studies showed its effects on growth retardation and early embryonic death [94]. The gene *REN* shows an important effect on the renin-angiotensin-aldosterone system (RAS pathway),

acting as a factor in steroidogenesis regulation and leading to changes in ovarian antral development and ovulation [95].

3.5. Gene network and gene ontology annotation shared by HC

Analysis by STRING revealed interactions between candidate genes with shared and specific effects on HC at different months (Tables 2 and 4) were involved in a single network. The key genes identified (*LEP, INSR, IGF1, IGFBP2, IGFBP5, PLAG1, KISS1, SHOX, GHRH, GHRL, LPL,* and *PMCH*) act as signaling factors that regulate the reproductive functions and growth aspects by a pleiotropic biological effect through metabolic homeostasis involved in physiological processes with important effects in the HPG axis (Fig. 2). These genes are related to important biological processes shared between HC evaluated at different ages, highlighting the effect of the main gatekeepers of puberty onset through regulation of the physiological mechanisms (GO:0008286, GO:0046323) and HPG axis (GO:0032274, GO:0046879, GO:0009914, GO:0032275, GO:0060123, GO:0060986, and GO:0044060; Table 5). Age at puberty represents a process governed by the complex interaction between genetic and nutritional aspects that regulate the sexual precocity [8,9,35,39].

Differences in early attainment of puberty come from the favorable genetic merit for age at first calving and adequate nutrition leading to the precocious or late process of the pubertal activation of the HPG axis, a key event in the onset of puberty is the progressive increase of the neurosecretory activity of GnRH neurons in pre- and post-pubertal hypothalamus in heifers [11]. The main metabolic signals related to the puberty onset comes from the interactions of the main genes' LEP, *IGF1*, *GHRL,* and *KISS1* [96], but the genes set *INSR, IGFBP2, IGFBP5, SHOX, GHRH,* and *PLAG1* show a key role biological aspects for early puberty that involves growth hormone (GH; GO:0046879 and GO:0060123), glucose (GO:0046323) and insulin (GO:0008286; Table 5). Adequate growth rates from pre-weaning and weaning to puberty (14–16 months) are critical for Nelore heifer to attain HC early [35,97]. An increase in growth hormone secretion is related to changes in growth rates of precocious heifers to achieve their weight "threshold," occurring the transition of pre-puberty to puberty with an increase in adipose tissue deposition against with lower genetic potential for early pregnancy. Brunes et al. [97] observed that precocious Nelore heifers showed better growth aspects (i.e., body weight from birth to 450 days of age and average daily gain) with 75% and 62% more subcutaneous fat thickness and rump fat thickness on average compared with non-precocious Nelore heifers. These factors observed in precocious heifers could be the key factor leading to an increase in hormonal action of *LEP*, *IGF1*, as well as regions related to *IGF1* hormone control *PLAG1*, *IGFBP2*, and *IGFBP5*, *PMCH,* and *KISS1* enhancing the metabolic signals for the start of the pubertal event [10,62,72]. Hawken et al. [98] observed that increasing body size is associated with the highest mature body weight and correlated with lower IGF1 blood levels leading to puberty onset in advanced ages. Thus, the GWAS results for HC at different ages showed

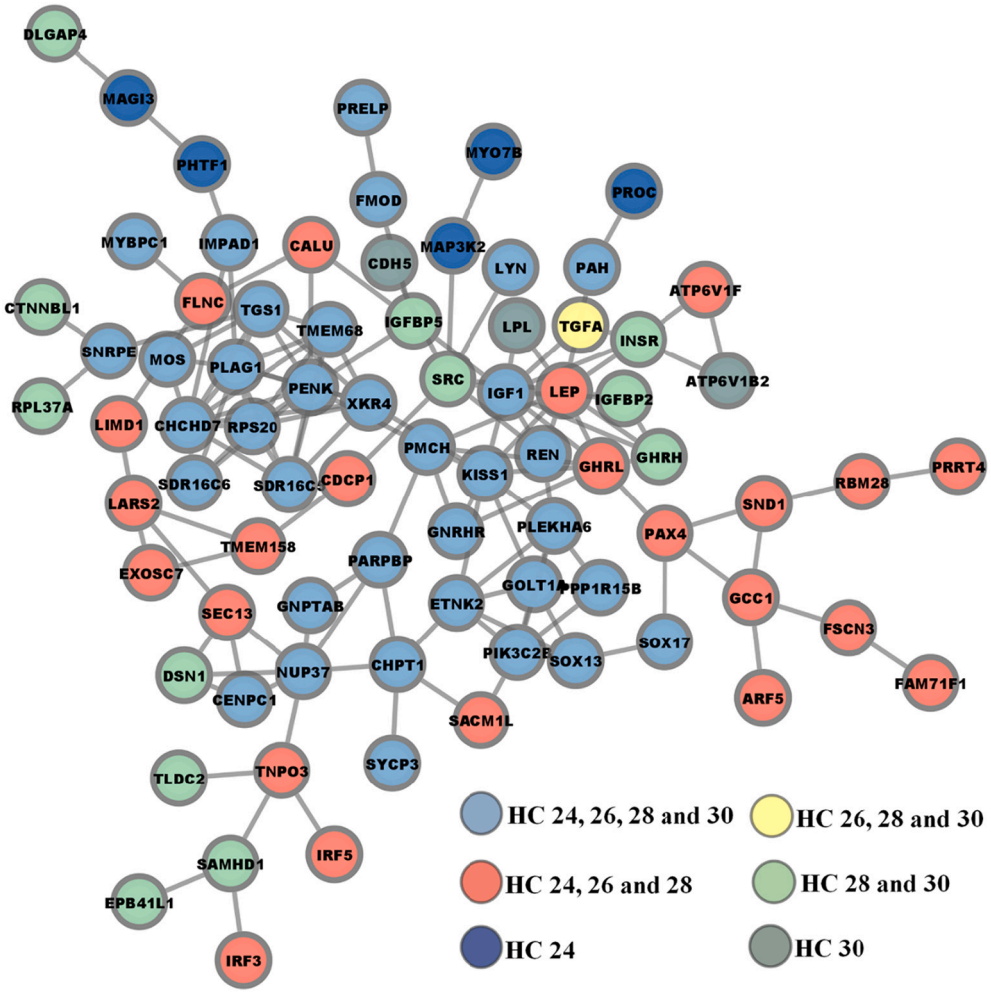

Fig. 2. Network of candidate genes identified within SNP windows regions deemed significantly affecting HC at different ages (24, 26, 28, and 30 months). The gene network was built from known protein-protein interactions (edges) between gene products (nodes) using the string database for *Bos taurus*. The node color represents the shared or specific genes for HC across the 24, 26, 28, and 30 months.

strong evidence of genomic regions affecting physiological events, with changes in circulating metabolic signals, with a key role on reproductive neuroendocrine system maturation to support the onset of puberty in early ages. These results indicate that the functionality of the HPG axis is sensitive to the metabolic status through hormones such as leptin, insulin, Ghrelin (GHRL), and IGF1 are the proposed mediators of this process.

3.6. Future perspectives for heifer early calving selection

Our results indicated the feasibility of direct selection for anticipating heifer reproductive life through selecting heifers for HC24, but the heritability estimate (0.43), indicated a possible potential combination of genetics and environmental factors that could potentially affect the sexual precocity in Nelore heifers. In addition, under tropical environments, Nelore heifers are raised under heterogeneous production systems that represent an important source for attaining sexual precocity. In this context, breeding programs selecting for HC24 must consider this effect in their selection decisions or reduce the environmental variance to obtain a lower variability across the production system. These differences across environments where heifers are raised could lead to environmentally dependent SNP markers, whereby genomic regions show an important effect in a specific environmental level and not in others, changing its magnitude and direction [7]. The GWAS results indicated a significant list of genes related to body energy homeostasis, metabolic status, and signaling mechanisms as the relevant modifiers of sexual precocity, mainly for HC24. Altogether, there are notable differences in the biological processes for HC expressed at different ages that are useful for predicting the sexual precocity potential in Nelore heifers. Knowledge about these genomic regions might aid in designing efficient selection strategies to improve HC24 in Nelore cattle raised under harsh conditions due to HC at different ages being an energy-dependent process.

4. Conclusions

Heifer early calving (HC) showed a polygenic architecture with a moderate to high heritability according to the threshold age considered, which respond favorably to genomic selection. The genetic correlation obtained for HC defined at different ages suggests that common variation is likely to be explained by mutations in shared genomic regions that surround genes able to control early puberty onset and specific physiological processes associated with sexual precocity for each age Nelore heifers. These results indicated significant SNP markers surrounding genes related to energy homeostasis and a signaling mechanism in the hypothalamus as the main factor to induce early puberty in Nelore cattle. The pleiotropic genomic regions between HC evaluated at 24, 26, 28, and 30 months were located on BTA 5 (65.28–66.95 Mb), 6 (82.88–83.64 Mb), 14 (22.14–23.96 Mb), and 16 (1.00–2.24 Mb) confirmed a group of genes that are important for metabolic homeostasis as gatekeeper messengers for modulating the sexual precocity. In general, the genomic regions identified for HC across different ages are linked by metabolic conditions and genes that regulate reproduction functions through the hypothalamic-pituitary-gonadal axis and endocrine parameters associated with precocity.

Availability of data and materials

The Nellore phenotypic and genotypic data were provided from the National Association of Breeders and Researchers - ANCP (https://www.ancp.org.br/) and the data are available for academic use from the authors by request.

Author statement

DPM, FSBR, and RBL conceived, designed, and supervision the research. LFMM and ABC data curation and formal analysis. LFMM, MEB, PAB, FSBR, and DPM conceptualization, methodology, review & editing. LFMM and ABC original draft. LFMM preparation, creation, and/or presentation of the published work.

Declaration of Competing Interest

The authors declare no competing interest.

Acknowledgments

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil, finance code 001. The authors acknowledge the National Association of Breeders and Researchers - ANCP (Ribeirão Preto, São Paulo, Brazil) for providing the dataset used in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2022.110395.

References

[1] I.B. Formigoni, J.B.S. Ferraz, J.A.I.V. Silva, J.P. Eler, R.C. Brumatti, Valores econômicos para habilidade de permanência e probabilidade de prenhez aos 14 meses em bovinos de corte, Arq. Bras. Med. Vet. e Zootec. 57 (2005) 220–226, https://doi.org/10.1590/s0102-09352005000800013.

[2] C.H. Laske, B.B.M. Teixeira, N.J.L. Dionello, F.F. Cardoso, Breeding objectives and economic values for traits of low input familybased beef cattle production system in the state of Rio Grande do Sul, Rev. Bras. Zootec. 41 (2012) 298–305, https://doi.org/10.1590/S1516-35982012000200010.

[3] S.E. Moorey, F.H. Biase, Beef heifer fertility: importance of management practices and technological advancements, J. Anim. Sci. Biotechnol. 11 (2020) 1–12, https://doi.org/10.1186/s40104-020-00503-9.

[4] J.V. Portes, G.R.O. Menezes, M.D. MacNeil, L.O.C. da Silva, A. Gondo, J. Braccini Neto, Selection indices for Nellore production systems in the Brazilian Cerrado, Livest. Sci. 242 (2020) 1+8, https://doi.org/10.1016/j.livsci.2020.104309.

[5] M. Bonamy, S. Kluska, E. Peripolli, M.V.A. de Lemos, S.T. Amorim, R.J. Vaca, R. B. Lôbo, L.M. de Castro, C.U. de Faria, F. Borba Ferrari, F. Baldi, Genetic association between different criteria to define sexual precocious heifers with growth, carcass, reproductive and feed efficiency indicator traits in Nellore cattle using genomic information, J. Anim. Breed. Genet. 136 (2019) 15–22, https://doi.org/10.1111/jbg.12366.

[6] J.P. Eler, A.B. Bignardi, J.B.S. Ferraz, M.L. Santana, Genetic relationships among traits related to reproduction and growth of Nelore females, Theriogenology. 82 (2014) 708–714, https://doi.org/10.1016/j.theriogenology.2014.06.001.

[7] L.F.M. Mota, G.A. Fernandes Jr., A.C. Herrera, D.C.B. Scalez, R. Espigolan, A.F. B. Magalhães, R. Carvalheiro, F. Baldi, L.G. Albuquerque, Genomic reaction norm models exploiting genotype × environment interaction on sexual precocity indicator traits in Nellore cattle, Anim. Genet. 51 (2020) 210–223, https://doi.org/10.1111/age.12902.

[8] M.V.C. Ferraz, A.V. Pires, M.H. Santos, R.G. Silva, G.B. Oliveira, D.M. Polizel, M. V. Biehl, R. Sartori, G.P. Nogueira, A combination of nutrition and genetics is able to reduce age at puberty in Nelore heifers to below 18 months, Animal 12 (2018) 569–574, https://doi.org/10.1017/S1751731117002464.

[9] L.F.M. Mota, F.B. Lopes, G.A. Fernandes Júnior, G.J.M. Rosa, A.F.B. Magalhães, R. Carvalheiro, L.G. Albuquerque, Genome-wide scan highlights the role of candidate genes on phenotypic plasticity for age at first calving in Nellore heifers, Sci. Rep. 10 (2020) 1–13, https://doi.org/10.1038/s41598-020-63516-4.

[10] S.R. Ojeda, A. Lomniczi, A. Loche, V. Matagne, G. Kaidar, U.S. Sandau, G.A. Dissen, The transcriptional control of female puberty, Brain Res. 1364 (2010) 164–174, https://doi.org/10.1016/j.brainres.2010.09.039.

[11] K.L. DeAtley, M.L. Colgrave, A. Cánovas, G. Wijffels, R.L. Ashley, G.A. Silver, G. Rincon, J.F. Medrano, A. Islas-Trejo, M.R.S. Fortes, A. Reverter, L. Porto-Neto, S. A. Lehnert, M.G. Thomas, Neuropeptidome of the hypothalamus and pituitary gland of Indicine × taurine heifers: evidence of differential neuropeptide processing in the pituitary gland before and after puberty, J. Proteome Res. 17 (2018) 1852–1865, https://doi.org/10.1021/acs.jproteome.7b00875.

[12] M.S. Tahir, L.R. Porto-Neto, C. Gondro, O.B. Shittu, K. Wockner, A.W.L. Tan, H. R. Smith, G.C. Gouveia, J. Kour, M.R.S. Fortes, Meta-analysis of heifer traits identified reproductive pathways in Bos indicus cattle, *Bos Indicus*, Cattle. Genes. 12 (2021) 1–27, https://doi.org/10.3390/genes12050768.

[13] T.P. Melo, M.R.S. Fortes, T. Bresolin, L.F.M. Mota, L.G. Albuquerque, R. Carvalheiro, Multitrait meta-analysis identified genomic regions associated with sexual precocity in tropical beef cattle, J. Anim. Sci. 96 (2018) 4087–4099, https://doi.org/10.1093/jas/sky289.

[14] M. Piles, R. Bergsma, D. Gianola, H. Gilbert, L. Tusell, Feature selection stability and accuracy of prediction models for genomic prediction of residual feed intake in

pigs using machine learning, Front. Genet. 12 (2021), https://doi.org/10.3389/fgene.2021.611506.
[15] N. Irano, G.M.F. de Camargo, R.B. Costa, A.P.N. Terakado, A.F.B. Magalhães, R.M. D.O. Silva, M.M. Dias, A.B. Bignardi, F. Baldi, R. Carvalheiro, H.N. de Oliveira, L. G. de Albuquerque, Genome-wide association study for indicator traits of sexual precocity in Nellore cattle, PLoS One 11 (2016) 1–14, https://doi.org/10.1371/journal.pone.0159502.
[16] M.E. Buzanskas, A. Grossi, R.V. Ventura, F.S. Schenkel, T.C.S. Chud, N.B. Stafuzza, L.D. Rola, S.L.C. Meirelles, F.B. Mokry, M.A. Mudadu, R.H. Higa, M.V.G.B. da Silva, M.M. de Alencar, L.C. Regitano, D.P. Munari, Candidate genes for male and female reproductive traits in Canchim beef cattle, J. Anim. Sci. Biotechnol. 8 (2017) 67, https://doi.org/10.1186/s40104-017-0199-8.
[17] R.R. Mota, S.E.F. Guimarães, M.R.S. Fortes, B. Hayes, F.F. Silva, L.L. Verardo, M. J. Kelly, C.F. de Campos, J.D. Guimarães, R.R. Wenceslau, J.M. Penitente-Filho, J. F. Garcia, S. Moore, Genome-wide association study and annotating candidate gene networks affecting age at first calving in Nellore cattle, J. Anim. Breed. Genet. 134 (2017) 484–492, https://doi.org/10.1111/jbg.12299.
[18] J. Ødegård, T.H.E. Meuwissen, B. Heringstad, P. Madsen, A simple algorithm to estimate genetic variance in an animal threshold model using Bayesian inference, Genet. Sel. Evol. 42 (2010) 29, https://doi.org/10.1186/1297-9686-42-29.
[19] M. Sargolzaei, J.P. Chesnais, F.S. Schenkel, A new approach for efficient genotype imputation using information from relatives, BMC Genomics 15 (2014) 1–12, https://doi.org/10.1186/1471-2164-15-478.
[20] F. Brito Lopes, C.U. Magnabosco, T.L. Passafaro, L.C. Brunes, M.F.O. Costa, E. C. Eifert, M.G. Narciso, G.J.M. Rosa, R.B. Lobo, F. Baldi, Improving genomic prediction accuracy for meat tenderness in Nellore cattle using artificial neural networks, J. Anim. Breed. Genet. 137 (2020) 438–448, https://doi.org/10.1111/jbg.12468.
[21] I. Aguilar, I. Misztal, D.L. Johnson, A. Legarra, S. Tsuruta, T.J. Lawlor, A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score, J. Dairy Sci. 93 (2010) 743–752, https://doi.org/10.3168/jds.2009-2730.
[22] P.M. VanRaden, Efficient methods to compute genomic predictions, J. Dairy Sci. 91 (2008) 4414–4423, https://doi.org/10.3168/jds.2007-0980.
[23] H. Wang, I. Misztal, I. Aguilar, A. Legarra, R.L. Fernando, Z. Vitezica, R. Okimoto, T. Wing, R. Hawken, W.M. Muir, Genome-wide association mapping including phenotypes from relatives without genotypes in a single-step (ssGWAS) for 6-week body weight in broiler chickens, Front. Genet. 5 (2014) 134, https://doi.org/10.3389/fgene.2014.00134.
[24] I. Misztal, S. Tsuruta, D. Lourenco, I. Aguilar, A. Legarra, Z. Vitezica, Manual for BLUPF90 Family of Programs, Univ. Georg, Athens, USA, 2018. http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all7.pdf.
[25] B.J. Smith, boa: an R package for MCMC output convergence assessment and posterior inference, J. Stat. Softw. 21 (2007) 1–37, https://doi.org/10.18637/jss.v021.i11.
[26] J. Geweke, Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments, in: J.M. Bernado, J.O. Berger, A.P. Smith, A.F. M. Dawid (Eds.), Bayesian Stat, 4th ed., Clarendon Press, Oxford, UK, 1992, pp. 169–193.
[27] J.L. Gualdrón Duarte, R.J. Cantet, R.O. Bates, C.W. Ernst, N.E. Raney, J.P. Steibel, Rapid screening for phenotype-genotype associations by linear transformations of genomic evaluations, BMC Bioinformatics 15 (2014) 1–11, https://doi.org/10.1186/1471-2105-15-246.
[28] W.G. Hill, A. Robertson, Linkage disequilibrium in finite populations, Theor. Appl. Genet. 38 (1968) 226–231, https://doi.org/10.1007/BF01245622.
[29] H. Perdry, L. Dandine-Roulland, Gaston — Genetic Data Handling (QC, GRM, LD, PCA) & Linear Mixed Models, R Packag. Version 1.5.5 83, 2018, pp. 1–29, https://doi.org/10.1159/000488519.
[30] G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS 16 (2012) 284–287, https://doi.org/10.1089/omi.2011.0118.
[31] E.I. Boyle, S. Weng, J. Gollub, H. Jin, D. Botstein, J.M. Cherry, G. Sherlock, GO:: TermFinder–open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes, Bioinformatics 20 (2004) 3710–3715, https://doi.org/10.1093/bioinformatics/bth456.
[32] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K.P. Tsafou, M. Kuhn, P. Bork, L.J. Jensen, C. Von Mering, STRING v10: protein-protein interaction networks, integrated over the tree of life, Nucleic Acids Res. 43 (2015) D447–D452, https://doi.org/10.1093/nar/gku1003.
[33] G.A. Oliveira Júnior, B.C. Perez, J.B. Cole, M.H.A. Santana, J. Silveira, G. Mazzoni, R.V. Ventura, M.L.S. Júnior, H.N. Kadarmideen, D.J. Garrick, J.B.S. Ferraz, Genomic study and medical subject headings enrichment analysis of early pregnancy rate and antral follicle numbers in Nelore heifers, J. Anim. Sci. 95 (2017) 4796–4812, https://doi.org/10.2527/jas2017.1752.
[34] G.A. Fernandes Júnior, D.A. Silva, L.F.M. Mota, T.P. de Melo, L.F.S. Fonseca, S. Silva, R. Carvalheiro, L.G. De Albuquerque, Sustainable intensification of beef production in the tropics: the role of genetically improving sexual precocity of heifers, Animals. 12 (2022) 1–25, https://doi.org/10.3390/ani12020174.
[35] A.A. Miszura, M.V.C. Ferraz, R.C. Cardoso, D.M. Polizel, G.B. Oliveira, J.P. R. Barroso, L.G.M. Gobato, G.P. Nogueira, J.S. Biava, E.M. Ferreira, A.V. Pires, Implications of growth rates and compensatory growth on puberty attainment in Nellore heifers, Domest. Anim. Endocrinol. 74 (2021), 106526, https://doi.org/10.1016/j.domaniend.2020.106526.
[36] J.L. Doyle, D.P. Berry, R.F. Veerkamp, T.R. Carthy, R.D. Evans, S.W. Walsh, D. C. Purfield, Genomic regions associated with muscularity in beef cattle differ in five contrasting cattle breeds, Genet. Sel. Evol. 52 (2020) 1–18, https://doi.org/10.1186/s12711-020-0523-1.
[37] L. Wang, X. Li, J. Ma, Y. Zhang, H. Zhang, Integrating genome and transcriptome profiling for elucidating the mechanism of muscle growth and lipid deposition in Pekin ducks, Sci. Rep. 7 (2017) 3837, https://doi.org/10.1038/s41598-017-04178-7.
[38] L. Tosca, C. Ramé, C. Chabrolle, S. Tesseraud, J. Dupont, Metformin decreases IGF1-induced cell proliferation and protein synthesis through AMP-activated protein kinase in cultured bovine granulosa cells, Reproduction 139 (2010) 409–418, https://doi.org/10.1530/REP-09-0351.
[39] F. Samadi, D. Blache, G.B. Martin, M.J. D'Occhio, Nutrition, metabolic profiles and puberty in Brahman (Bos indicus) beef heifers, Anim. Reprod. Sci. 146 (2014) 134–142, https://doi.org/10.1016/j.anireprosci.2014.03.004.
[40] S. Kraus, Z. Naor, R. Seger, Intracellular Signaling pathways mediated by the gonadotropin-releasing hormone (GnRH) receptor, Arch. Med. Res. 32 (2001) 499–509, https://doi.org/10.1016/S0188-4409(01)00331-9.
[41] K.J. Iremonger, S. Constantin, X. Liu, A.E. Herbison, Glutamate regulation of GnRH neuron excitability, Brain Res. 1364 (2010) 35–43, https://doi.org/10.1016/j.brainres.2010.08.071.
[42] M.J. D'Occhio, P.S. Baruselli, G. Campanile, Influence of nutrition, body condition, and metabolic status on reproduction in female beef cattle: a review, Theriogenology. 125 (2019) 277–284, https://doi.org/10.1016/j.theriogenology.2018.11.010.
[43] J. Roa, E. Aguilar, C. Dieguez, L. Pinilla, M. Tena-Sempere, New frontiers in kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function, Front. Neuroendocrinol. 29 (2008) 48–69, https://doi.org/10.1016/j.yfrne.2007.07.002.
[44] V.R.N. Gaviolli, M.E. Buzanskas, V.A.R. Cruz, R.P. Savegnago, D.P. Munari, A. R. Freitas, M.M. Alencar, Genetic associations between weight at maturity and maturation rate with ages and weights at first and second calving in Canchim beef cattle, J. Appl. Genet. 53 (2012) 331–335, https://doi.org/10.1007/s13353-012-0100-6.
[45] S. Ugi, S. Maeda, Y. Kawamura, M.A. Kobayashi, M. Imamura, T. Yoshizaki, K. Morino, O. Sekine, H. Yamamoto, T. Tani, M. Rokushima, A. Kashiwagi, H. Maegawa, CCDC3 is specifically upregulated in omental adipose tissue in subjects with abdominal obesity, Obesity 22 (2014) 1070–1077, https://doi.org/10.1002/oby.20645.
[46] M.H.A.A. Santana, R.V. Ventura, Y.T. Utsunomiya, H.H.R.R. Neves, P.A. Alexandre, G.A. Oliveira Junior, R.C. Gomes, M.N. Bonin, L.L. Coutinho, J.F. Garcia, S.L. Silva, H. Fukumasu, P.R. Leme, J.B.S.S. Ferraz, A genomewide association mapping study using ultrasound-scanned information identifies potential genomic regions and candidate genes affecting carcass traits in Nellore cattle, J. Anim. Breed. Genet. 132 (2015) 420–427, https://doi.org/10.1111/jbg.12167.
[47] M. Amstalden, G.L. Williams, Neuroendocrine control of estrus and ovulation, in: Bov. Reprod, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2014, pp. 203–218, https://doi.org/10.1002/9781118833971.ch23.
[48] P.C. Garnsworthy, K.D. Sinclair, R. Webb, Integration of physiological mechanisms that influence fertility in dairy cows, Animal 2 (2008) 1144–1152, https://doi.org/10.1017/S1751731108002358.
[49] H.J. Lee, Y.J. Chung, S. Jang, D.W. Seo, H.K. Lee, D. Yoon, D. Lim, S.H. Lee, Genome-wide identification of major genes and genomic prediction using high-density and text-mined gene-based SNP panels in Hanwoo (Korean cattle), PLoS One 15 (2020) 1–18, https://doi.org/10.1371/journal.pone.0241848.
[50] F. Xu, Z. Gao, J. Zhang, C.A. Rivera, J. Yin, J. Weng, J. Ye, Lack of SIRT1 (mammalian sirtuin 1) activity leads to liver steatosis in the SIRT1+/− mice: a role of lipid mobilization and inflammation, Endocrinology 151 (2010) 2504–2514, https://doi.org/10.1210/en.2009-1013.
[51] X.P. Wang, Z.M. Luoreng, F. Li, J.R. Wang, N. Li, S.H. Li, Genetic polymorphisms of lipoprotein lipase gene and their associations with growth traits in Xiangxi cattle, Mol. Biol. Rep. 39 (2012) 10331–10338, https://doi.org/10.1007/s11033-012-1910-7.
[52] D. Oh, B. La, Y. Lee, Y. Byun, J. Lee, G. Yeo, J. Yeo, Identification of novel single nucleotide polymorphisms (SNPs) of the lipoprotein lipase (LPL) gene associated with fatty acid composition in Korean cattle, Mol. Biol. Rep. 40 (2013) 3155–3163, https://doi.org/10.1007/s11033-012-2389-y.
[53] C.-S. Zhang, B. Jiang, M. Li, M. Zhu, Y. Peng, Y.-L. Zhang, Y.-Q. Wu, T.Y. Li, Y. Liang, Z. Lu, G. Lian, Q. Liu, H. Guo, Z. Yin, Z. Ye, J. Han, J.-W. Wu, H. Yin, S.-Y. Lin, S.-C. Lin, The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and mTORC1, acting as a switch between catabolism and anabolism, Cell Metab. 20 (2014) 526–540, https://doi.org/10.1016/j.cmet.2014.06.014.
[54] G.-H. Sun-Wada, T. Toyomura, Y. Murata, A. Yamamoto, M. Futai, Y. Wada, The a 3 isoform of V-ATPase regulates insulin secretion from pancreatic β-cells, J. Cell Sci. 119 (2006) 4531–4540, https://doi.org/10.1242/jcs.03234.
[55] M.K. Abo-Ismail, M.J. Kelly, E.J. Squires, K.C. Swanson, S. Bauck, S.P. Miller, Identification of single nucleotide polymorphisms in genes involved in digestive and metabolic processes associated with feed efficiency and performance traits in beef cattle, J. Anim. Sci. 91 (2013) 2512–2529, https://doi.org/10.2527/jas2012-5756.
[56] L.H.S. Iung, H.A. Mulder, R. Neves, R. Carvalheiro, Genomic regions underlying uniformity of yearling weight in Nellore cattle evaluated under different response variables, BMC Genomics 19 (2018) 1–13, https://doi.org/10.1186/s12864-018-5003-4.
[57] R. Sartori, M.M. Guardieiro, R.S. Surjus, L.F. Melo, A.B. Prata, M. Ishiguro, M. R. Bastos, A.B. Nascimento, Metabolic hormones and reproductive function in

cattle, Anim. Reprod. 10 (2013) 199–205. https://pdfs.semanticscholar.org/fbf9/5b3b80a951ae04b55027d69df1599f44af07.pdf (accessed January 19, 2019).
[58] R.M. Garcia-Garcia, Integrative control of energy balance and reproduction in females, ISRN Vet. Sci. 2012 (2012) 1–13, https://doi.org/10.5402/2012/121389.
[59] P.S. McPherson, K.P. Campbell, Characterization of the major brain form of the ryanodine receptor/Ca2+ release channel, J. Biol. Chem. 268 (1993) 19785–19790, https://doi.org/10.1016/S0021-9258(19)36582-2.
[60] S. Sundaresan, J. Weiss, A.C. Bauer-Dantoin, J.L. Jameson, Expression of ryanodine receptors in the pituitary gland: evidence for a role in gonadotropin-releasing hormone Signaling, Endocrinology 138 (1997) 2056–2065, https://doi.org/10.1210/endo.138.5.5153.
[61] S. Pennetier, S. Uzbekova, C. Guyader-Joly, P. Humblot, P. Mermillod, R. Dalbiès-Tran, Genes preferentially expressed in bovine oocytes revealed by subtractive and suppressive hybridization, Biol. Reprod. 73 (2005) 713–720, https://doi.org/10.1095/biolreprod.105.041574.
[62] S. Llewellyn, R. Fitzpatrick, D.A. Kenny, J.J. Murphy, R.J. Scaramuzzi, D. C. Wathes, Effect of negative energy balance on the insulin-like growth factor system in pre-recruitment ovarian follicles of post partum dairy cows, Reproduction. 133 (2007) 627–639, https://doi.org/10.1530/REP-06-0122.
[63] S.E. Echternkamp, A.J. Roberts, D.D. Lunstra, T. Wise, L.J. Spicer, Ovarian follicular development in cattle selected for twin ovulations and births, J. Anim. Sci. 82 (2004) 459, https://doi.org/10.2527/2004.822459x.
[64] D. Laskowski, R. Båge, P. Humblot, G. Andersson, M.A. Sirard, Y. Sjunnesson, Insulin during in vitro oocyte maturation has an impact on development, mitochondria, and cytoskeleton in bovine day 8 blastocysts, Theriogenology. 101 (2017) 15–25, https://doi.org/10.1016/j.theriogenology.2017.06.002.
[65] C.L. Cardoso, A. King, A. Chapwanya, G. Esposito, Ante-Natal and Post-Natal influences on neonatal immunity, growth and puberty of calves—a review, Animals. 11 (2021) 1212, https://doi.org/10.3390/ani11051212.
[66] K.L. Hull, S. Harvey, Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions, Int. J. Endocrinol. 2014 (2014) 1–24, https://doi.org/10.1155/2014/234014.
[67] M.M. Franco, R.C. Antunes, H.D. Silva, L.R. Goulart, Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in landrace pigs, J. Appl. Genet. 46 (2005) 195–200.
[68] A.O. Rini, C. Sumantri, A. Annggraenini, GHRH|HaeIII gene polymorphism in dairy and beef cattle at national livestock breeding centers, Media Peternak. 36 (2013) 185–191, https://doi.org/10.5398/medpet.2013.36.3.185.
[69] B. Picard, M. Gagaoua, Muscle Fiber properties in cattle and their relationships with meat qualities: an overview, J. Agric. Food Chem. 68 (2020) 6021–6039, https://doi.org/10.1021/acs.jafc.0c02086.
[70] N.R.B. Cônsolo, J. da Silva, V.L.M. Buarque, A. Higuera-Padilla, L.C.G.S. Barbosa, A. Zawadzki, L.A. Colnago, A. Saran Netto, D.E. Gerrard, S.L. Silva, Selection for growth and precocity alters muscle metabolism in Nellore cattle, Metabolites. 10 (2020) 1–10, https://doi.org/10.3390/metabo10020058.
[71] J.L. Braun, M.S. Geromella, S.I. Hamstra, V.A. Fajardo, Neuronatin regulates whole-body metabolism: is thermogenesis involved? FASEB BioAdvances. 2 (2020) 579–586, https://doi.org/10.1096/fba.2020-00052.
[72] J. Roa, D. García-Galiano, J.M. Castellano, F. Gaytan, L. Pinilla, M. Tena-Sempere, Metabolic control of puberty onset: new players, new mechanisms, Mol. Cell. Endocrinol. 324 (2010) 87–94, https://doi.org/10.1016/j.mce.2009.12.018.
[73] A.K. Odle, N. Akhter, M.M. Syed, M.L. Allensworth-James, H. Beneš, A.I. M. Castillo, M.C. MacNicol, A.M. MacNicol, G.V. Childs, Leptin regulation of gonadotrope gonadotropin-releasing hormone receptors as a metabolic checkpoint and gateway to reproductive competence, Front. Endocrinol. (Lausanne). 8 (2018) 367, https://doi.org/10.3389/fendo.2017.00367.
[74] J.S. Jennings, A.E. Wertz-Lutz, R.H. Pritchard, A.D. Weaver, D.H. Keisler, K. Bruns, Circulating ghrelin and leptin concentrations and growth hormone secretagogue receptor abundance in liver, muscle, and adipose tissue of beef cattle exhibiting differences in composition of gain, J. Anim. Sci. 89 (2011) 3954–3972, https://doi.org/10.2527/jas.2010-3597.
[75] M.A. de la Rosa Rodriguez, S. Kersten, Regulation of lipid droplet homeostasis by hypoxia inducible lipid droplet associated HILPDA, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids. 1865 (2020), 158738, https://doi.org/10.1016/j.bbalip.2020.158738.
[76] J.L. Juengel, A.H. Bibby, K.L. Reader, S. Lun, L.D. Quirke, L.J. Haydon, K. P. McNatty, The role of transforming growth factor-beta (TGF-beta) during ovarian follicular development in sheep, Reprod. Biol. Endocrinol. 2 (2004) 1–11, https://doi.org/10.1186/1477-7827-2-78.
[77] A. Okunomiya, A. Horie, H. Tani, Y. Sato, S. Takamatsu, J.B. Brown, M. Sugimoto, J. Hamanishi, E. Kondoh, N. Matsumura, M. Mandai, Figla promotes secondary follicle growth in mature mice, Sci. Rep. 11 (2021) 1–10, https://doi.org/10.1038/s41598-021-89052-3.
[78] M.R.S. Fortes, Y. Li, E. Collis, Y. Zhang, R.J. Hawken, The IGF1 pathway genes and their association with age of puberty in cattle, Anim. Genet. 44 (2013) 91–95, https://doi.org/10.1111/j.1365-2052.2012.02367.x.
[79] M.R.S. Fortes, K. Kemper, S. Sasazaki, A. Reverter, J.E. Pryce, W. Barendse, R. Bunch, R. McCulloch, B. Harrison, S. Bolormaa, Y.D. Zhang, R.J. Hawken, M. E. Goddard, S.A. Lehnert, Evidence for pleiotropism and recent selection in the PLAG1 region in Australian beef cattle, Anim. Genet. 44 (2013) 636–647, https://doi.org/10.1111/age.12075.
[80] M.C. Lucy, Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for post-partum nutrition and reproduction, Reprod. Domest. Anim. 43 (2008) 31–39, https://doi.org/10.1111/j.1439-0531.2008.01140.x.
[81] L. Karim, H. Takeda, L. Lin, T. Druet, J.A.C. Arias, D. Baurain, N. Cambisano, S. R. Davis, F. Farnir, B. Grisart, B.L. Harris, M.D. Keehan, M.D. Littlejohn, R. J. Spelman, M. Georges, W. Coppieters, Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature, Nat. Genet. 43 (2011) 405–413, https://doi.org/10.1038/ng.814.
[82] A.G.T. Pereira, Y.T. Utsunomiya, M. Milanesi, R.B.P. Torrecilha, A.S. Carmo, H.H. R. Neves, R. Carvalheiro, P. Ajmone-Marsan, T.S. Sonstegard, J. Sölkner, C. J. Contreras-Castillo, J.F. Garcia, Pleiotropic genes affecting carcass traits in Bos indicus (Nellore) cattle are modulators of growth, PLoS One 11 (2016) 1–13, https://doi.org/10.1371/journal.pone.0158165.
[83] L. Pinilla, E. Aguilar, C. Dieguez, R.P. Millar, M. Tena-Sempere, Kisspeptins and reproduction: physiological roles and regulatory mechanisms, Physiol. Rev. 92 (2012) 1235–1316, https://doi.org/10.1152/physrev.00037.2010.
[84] C.K. Prasad, M. Mahadevan, M.C. MacNicol, A.M. MacNicol, Mos 3′ UTR regulatory differences underlie species-specific temporal patterns of Mos mRNA cytoplasmic polyadenylation and translational recruitment during oocyte maturation, Mol. Reprod. Dev. 75 (2008) 1258–1268, https://doi.org/10.1002/mrd.20877.
[85] H.-Y. Fan, Q.-Y. Sun, Involvement of mitogen-activated protein kinase Cascade during oocyte maturation and fertilization in mammals, Biol. Reprod. 70 (2004) 535–547, https://doi.org/10.1095/biolreprod.103.022830.
[86] J. Naufahu, A.D. Cunliffe, J.F. Murray, The roles of melanin-concentrating hormone in energy balance and reproductive function: are they connected? Reproduction. 146 (2013) R141–R150, https://doi.org/10.1530/REP-12-0385.
[87] Adan Murray, Baker Walker, Nijenhuis Thody, Wilson Yukitake, Melanin-concentrating hormone, Melanocortin receptors and regulation of luteinizing hormone release, J. Neuroendocrinol. 12 (2001) 217–223, https://doi.org/10.1046/j.1365-2826.2000.00440.x.
[88] N.I. Angulo-Valenzuela, M.G. Thomas, D.G. Riley, J.F. Medrano, J.R. Reyna-Granados, C.M. Aguilar-Trejo, P. Luna-Nevárez, A SNP within the PMCH gene as a molecular marker associated with fertility traits in Angus and Brangus beef heifers raised under a desert environment, Trop. Anim. Health Prod. 53 (2021) 1–6, https://doi.org/10.1007/S11250-021-02794-0.
[89] Q. Wei, W. Ding, F. Shi, Roles of poly (ADP-ribose) polymerase (PARP1) cleavage in the ovaries of fetal, neonatal, and adult pigs, Reproduction. 146 (2013) 593–602, https://doi.org/10.1530/REP-13-0174.
[90] S. Madgwick, A.C.O. Evans, A.P. Beard, Treating heifers with GnRH from 4 to 8 weeks of age advanced growth and the age at puberty, Theriogenology. 63 (2005) 2323–2333, https://doi.org/10.1016/j.theriogenology.2004.10.011.
[91] F. Samadi, N.J. Phillips, D. Blache, G.B. Martin, M.J. D'Occhio, Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pastures, Anim. Reprod. Sci. 137 (2013) 137–144, https://doi.org/10.1016/J.ANIREPROSCI.2012.12.012.
[92] N. De Roux, E. Genin, J.C. Carel, F. Matsuda, J.L. Chaussain, E. Milgrom, Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10972–10976, https://doi.org/10.1073/pnas.1834399100.
[93] R. Lapatto, J.C. Pallais, D. Zhang, Y.M. Chan, A. Mahan, F. Cerrato, W. Le Wei, G. E. Hoffman, S.B. Seminara, Kiss1−/− mice exhibit more variable hypogonadism than Gpr54 −/− mice, Endocrinology. 148 (2007) 4927–4936, https://doi.org/10.1210/en.2007-0078.
[94] H.P. Harding, Y. Zhang, D. Scheuner, J.J. Chen, R.J. Kaufman, D. Ron, Ppp1r15 gene knockout reveals an essential role for translation initiation factor 2 alpha (eIF2α) dephosphorylation in mammalian development, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 1832–1837, https://doi.org/10.1073/pnas.0809632106.
[95] R. Ferreira, B. Gasperin, M. Rovani, J. Santos, M. Barreta, R. Bohrer, C. Price, P.B. D. Gonçalves, Angiotensin II signaling promotes follicle growth and dominance in cattle, Endocrinology. 152 (2011) 4957–4965, https://doi.org/10.1210/en.2011-1146.
[96] S.R. Ojeda, V. Prevot, S. Heger, A. Lomniczi, B. Dziedzic, A. Mungenast, Glia-to-neuron signaling and the neuroendocrine control of female puberty, Ann. Med. 35 (2003) 244–255, https://doi.org/10.1080/07853890310005164.
[97] L.C. Brunes, F.S. Baldi, M.F.O. Costa, G. Quintans, G. Banchero, R.B. Lôbo, C. U. Magnabosco, Early growth, backfat thickness and body condition has major effect on early heifer pregnancy in Nellore cattle, An. Acad. Bras. Cienc. 93 (2021) 1–10.
[98] R.J. Hawken, Y.D. Zhang, M.R.S. Fortes, E. Collis, W.C. Barris, N.J. Corbet, P. J. Williams, G. Fordyce, R.G. Holroyd, J.R.W. Walkley, W. Barendse, D.J. Johnston, K.C. Prayaga, B. Tier, A. Reverter, S.A. Lehnert, Genome-wide association studies of female reproduction in tropically adapted beef cattle, J. Anim. Sci. 90 (2012) 1398–1410, https://doi.org/10.2527/jas.2011-4410.