UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

GENOME SCAN FOR HOMOZYGOSITY ISLANDS AND INBREEDING EFFECT ON REPRODUCTIVE TRAITS IN NELORE BEEF CATTLE

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TÍTULO DA TESE: GENOME SCAN FOR HOMOZYGOSITY ISLANDS AND INBREEDING EFFECT ON REPRODUCTIVE TRAITS IN NELORE BEEF CATTLE

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DADOS CURRICULARES DO AUTOR

Ana Cristina Herrera Rios – nascida no dia 30 de dezembro de 1982 em Tarso, Estado de Antioquia - Colômbia. Filha de Jorge Luís Herrera Morales e Edilma Rios Loaiza. Iniciou seus estudos de formação profissional em fevereiro de 2000 no "Politécnico Marco Fidel Suarez", obtendo o titulo de "Tecnóloga en sistemas de la computación" no ano de 2003. Em 2008, obteve o título de Zootecnista pela "Universidad de Antioquia". Durante a graduação, fez parte do grupo de pesquisa "Genética, Mejoramiento y Modelacion Animal - GAMMA" da mesma universidade, sob orientação do Prof. Dr. Mario Fernando Cerón Muñoz. No período de 2008 a 2014 atuou como professora da "Facultad de Ciencias Agrarias" da "Universidad de Antioquia" nos cursos de "Ingeniería Agropecuária", "Medicina Veterinária" e "Zootecnia". Em março de 2010, ingressou no Programa da Pós-Graduação em "Biologia" da mesma universidade, obtendo o título de mestre em Bilogia em agosto de 2012. Neste mesmo ano, atuou como professora na "Corporación Universitaria La Sallista" no curso de "Zootecnia" e, no ano de 2013, na "Universidad de Córdoba" no curso de mestrado em "Ciencias Veterinarias del Trópico". De 2012 a 2014 participou do desenvolvimento de projetos de pesquisa e extensão da Faculdade de Ciências Agrárias da Universidad de Antioquia. Em março de 2015, ingressou no curso de doutorado do programa de Pós-graduação em Genética e Melhoramento Animal da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho", campus de Jaboticabal, sob orientação da Profa. Dra. Lucia Galvão de Albuquerque. Foi bolsista da CAPES e, posteriormente, bolsista de COLFUTURO-Colômbia e do programa "ENLAZAMUNDOS".

“*Para quem tem fé, não existe sorte, existe Deus. Para quem tem Deus, não existe perda, existe vitória. Para quem crê em Deus, não existe impossível, existem milagres, no momento certo e no tempo certo”.*

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E em memoria da minha avó Maria de Los Angeles Loaiza

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SUMÁRIO

Detecção de ilhas em homozigose e efeito da endogamia sobre características reprodutivas em bovinos nelore

RESUMO – O uso intensivo de biotecnologias reprodutivas tem feito com que se eleve a taxa de nascimento de progênies com maior grau de parentesco (maior taxa de nascimento de meio-irmãos e irmãos completos). Assim, o conhecimento sobre o coeficiente da endogamia média do rebanho torna-se relevante para a eficiência do sistema de produção. Com o advento da genômica, o coeficiente de endogamia (F) pode ser estimado com base na informação de milhares de marcadores do tipo polimorfismos de base única (SNPs), espalhados por todo o genoma. No presente estudo, informações de 3.785 animais da raça Nelore (1,760 machos e 2,025 fêmeas) genotipados para 777.962 SNPs do BovineHD BeadChip (Illumina Inc., San Diego, CA, USA) foram utilizadas com o objetivo de avaliar a taxa de endogamia em rebanhos comerciais da raça Nelore, bem como investigar o seu efeito (depressão endogâmica) sobre a expressão fenotípica de características reprodutivas (idade ao primeiro parto (IPP), ocorrência de prenhez precoce (OPP) e reconcepcão de novilhas (REC)). A estimativa do valor de F, bem como da depressão endogâmica, foi feita utilizando diferentes metodologias: (i) matriz de parentesco genômica com frequências alélicas obtidas da população base (F_G); (ii) matriz de parentesco genômica com frequências alélicas fixadas em 0,5 (F_{GRM}); (iii) com base no excesso de SNPs em homozigose (F_{SNP}); e (iv) corrida de homosigose (F_{ROH}). Os resultados da corrida de homosigose também foram utilizados para identificar os padrões (tamanho e distribuição) dos segmentos ROH na raça Nelore bem como para identificar ilhas de homosigose (segmentos ROH compartilhados por mais de 50% da população). Foram identificados 210.636 segmentos ROH distribuídos nos 29 autossomos e cinco ilhas de homozigose localizadas nos cromossomos 5, 7, 12, 21 e 26, nas quais 43 genes foram identificados. Alguns destes genes (*INHBE, INHBC, STAT6, FGF8* e *DPCD)* foram previamente associados com caracteristicas reproductivas, de crescimento, resposta inmume e adaptabilidade em bovinos. As médias para o coeficiente de endogamia calculado com base nas diferentes abordagens foram: -0,0006 (F_G), 0,4376 (F_{GRM}), 0,5500 (F_{SNP}) e 0,0590 (F_{ROH}). As correlações foram ente baixas F_G-F_{SNP} (-0,28), F_G-F_{GRM} (-0,20), F_G-F_{ROH} (0,21), a moderadas F_{ROH}-F_{SNP} (0,68), F_{ROH}-F_{GRM} (0,72) e fortemente alta para F_{SNP}-F_{GRM} (0,99). O valor médio de F variou de acordo com a metodologia utilizada. O valor extremamente alto do F_{SNP} denota que este método tende a superestimar as taxas de endogamia. Independentemente do método utilizado para obter os valores de F, foi verificado que o aumento de 1% no coeficiente de endogamia médio do rebanho influenciou desfavoravelmente a média das características reprodutivas avaliadas.

Palavras-chave: Autozigose**,** Depressão endogâmica, Gado de corte, corridas de homozigose

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ABSTRACT – The intensive use of reproductive biotechnologies has increased the birth rate of progenies with high degree of relationships (higher birth rate of half- and full-sibs). Thus, the control of herd inbreeding becomes relevant for the efficiency of the production system. With genomics, the inbreeding coefficient can be estimated using thousands of single nucleotide polymorphisms (SNPs), spread throughout the genome. In the present study, information of 3,785 Nelore animals (1,760 males and 2,025 females) genotyped with 777,962 SNP markers of BovineHD BeadChip (Illumina Inc., San Diego, CA, USA) was used with the objective of evaluating the inbreeding rates of Nelore commercial herds, as well as to investigate the effects of inbreeding (inbreeding depression) on the phenotypic expression of reproductive traits (age at first calving (AFC), heifer early pregnancy (EP), and heifer rebreeding (HR)). The inbreeding coefficient (F) and inbreeding depression were estimated based on (i) genomic relationship matrix considering allele frequencies estimated from the base population (F_G); (ii) genomic relationship matrix considering allele frequencies fixed at 0.5 (F_{GRM}); (iii) excess of homozygous SNPs (F_{SNP}); and (iv) runs of homozygosity (F_{ROH}). The runs of homozygosity results were also used to identify the pattern (size and distribution) of ROH segments as well as to identify ROH islands (ROH segments shared by more than 50% of the population). In total, there were identified 210,636 ROH segments and five ROH Islands located on the chromosomes 5, 7, 12, 21 and 26, in which 43 annotated genes were identified. Some of these genes (*INHBE, INHBC, STAT6, FGF8 and DPCD*) were previously associated with reproduction and growth traits, inmume response and adaptability in cattle. The average inbreeding calculated based on different approaches were -0.0006 (F_G), 0.4376 (F_{GRM}), 0.5500 (F_{SNP}) e 0.0590 (F_{ROH}). These correlations ranged from low F_G-F_{SNP} (-0.28), F_G-F_{GRM} (-0.20), F_G-F_{ROH} (0.21), to moderated F_{ROH}-F_{SNP} (0.68), F_{ROH}-F_{GRM} (0.72) and extremely high F_{SNP}-F_{GRM} (0.99). The average population inbreeding coefficient ranged according to the method used. The extremely high value of F_{SNP} indicates that this approach tend to overestimate the inbreeding rates. Independently of the method used to obtain the F values, it was verified that the increase of 1% in the average herd inbreeding unfavorably influenced the mean value of the evaluated reproductive traits.

Keywords: Autozygosity**,** Beef cattle, Inbreeding depression, *Runs of homozygosity*.

CHAPTER 1 – GENERAL CONSIDERATIONS

1.1 INTRODUCTION

The intensive use of reproductive biotechnologies, such as artificial insemination, in vitro fertilization and embryo transfer, leads to an increase of progenies with close relationships (higher birth rate of half and full-sibs) (VANRADEN et al., 1992; HAYES, 2007; VARGAS and GAMBOA, 2008). In this scenario, it is important to control the inbreeding since it leads to an increase in homozygosity reducing the population genetic variability and often affects the animal survival and fertility (inbreeding depression), causing a significative decrease in the reproductive efficiency of herds.

The inbreeding coefficient (F) and its effect on economically important traits are usually computed using pedigree information. However, a common practice in beef cattle production systems is the use of multiple-sire mating system. As a consequence, the additive relationship matrix based on pedigree becomes less informative, leading to biased estimates of inbreeding. A possible more precise way to assess pairwise relatedness and inbreeding coefficient could be derived with genomic information.

Advances in sequencing and genotyping technologies have allowed to incorporate the information of thousands of single nucleotide polymorphism (SNP) in genetic studies. In genomic era, the relationship between individuals can be evaluated, for example, based on the genomic relationship matrix (G). The G is obtained considering the observed proportion of chromosomal segments shared by individuals, allowing to differentiate the relationship between full-sibs as well as the identification of unknown relationships (i.e. kinship between offspring of multiple sires), and permits to correct errors in genealogy (LEUTENEGGER et al, 2003; VANRADEN, 2008).

The genomic F could be also obtained by calculating the proportion of homozygous SNPs in each individual and/or by runs of homozigosity (ROH) (CURIK et al., 2014; PRYCE et al., 2014). The ROH methodology has been considered as the best alternative to estimate autozygosity (KELLER et al., 2011), being widely

used to investigate population inbreeding (DE CARA et al, 2013; PRYCE et al., 2014). In addition, ROH methodology allows investigating the distribution of homozygosity across the genome and to map genomic regions with high homozygosity, the so-called ROH islands. The identification of ROH islands could unravel genes associated with economically relevant phenotypes under selection in the population (MASTRANGELO et al., 2016).

1.2 OBJECTIVES

I) To estimate the population inbreeding coefficient (F) computed based on different genomic approaches.

II) To use genomic F values to evaluate inbreeding depression on reproductive traits in Nelore Cattle.

III) To identify runs of homozygosity islands in Nelore beef cattle.

1.3 LITERATURE REVIEW

1.3.1 Aspects of Nelore cattle

Historical records state that after being introduced in Brazil from the Indian subcontinent, Indian zebu catlle known as Ongole was crossed with local Bos taurus cattle (creole dams derived from Iberian cattle) looking for a fast increase in the population size (SANTIAGO, 1986), and then backcrossed to the original breeds to recover indicine adaptive and productive traits. Thereafter, repeated crosses with Bos indicus (Ongole) males were used as a breeding strategy to recover pure indicine breed in Brazil with the name of Nelore (DANI et al., 2008). Nowadays, Brazil has the world's second largest commercial cattle herd, with more than 218 million heads (IBGE, 2017), being that almost 70% of the national herd is composed of Nelore breed. The adaptability and high performance in tropical conditions are the main reasons for the prevalence of this breed in Brazil (ALBUQUERQUE et al, 2017).

1.3.2 Inbreeding and relationship coefficients

Inbreeding results from mating individuals with one or more common ancestors (WRIGHT, 1922). The inbreeding coefficient (F) represents the probability of two alleles of a given locus are identical by descent (MALÉCOT, 1948). The F value of an individual is one-half the relationship between its parents and the coefficient of relationship represents the expected percentage of genes in common between two individuals.

The relationship coefficient between two individuals connected in the pedigree by lines of descent from common ancestors is obtained by summing the coefficients computed for every line by which they are connected (WRIGHT, 1922), and can be represented as follows: $R_{XY} = \sum \left(\frac{1}{2}\right)^{n+n'} (1 + F_{CA}) / \sqrt{1 + F_X}\sqrt{1 + F_Y}$, where: R_{XY} is the relationship coefficient between the individuals X and Y; and n and n' are the numbers of generations from X and Y, respectively, to CA (common ancestor) in

question. The inbreeding coefficient of an animal Z (offspring of X and Y) is obtained by: $F_Z = \sum \left(\frac{1}{2}\right)^{n+n'+1} (1 + F_{CA})$, in which F_Z is the inbreeding coefficient of the individual Z.

1.3.3 Genomic inbreeding coefficient

In genomic era, the relationship between individuals has being measured based on genomic relationship matrix (G). In this case, the genomic inbreeding coefficient (F) is obtained by subtracting the value 1 from the diagonal of the G matrix. Thus, the F value of an animal Z is equal to $G_{ZZ} - 1$ (VANRADEN, 2008).

The genomic F value could be also obtained by estimating the proportion of homozygous SNPs in each individual (KELLER et al., 2011) and by the runs of homozygosity (ROH) approach (MCQUILLAN et al., 2008; CURIK et al., 2014; PRYCE et al., 2014). The computation of F values based on the diagonal of matrix G or based on the proportion of SNPs in homozygosity across the genome does not separate loci IBD (Identical by descent) from loci IBS (Identical by state). In this sense, the ROH methodology has been considered as the alternative that better reflects the autozigose (KELLER et al., 2011). Basically, ROH segments are classified as short (associated with ancient common ancestor) or long (associated with recent common ancestor) (DE CARA et al., 2013; FERENCAKOVIC et al., 2013; PRYCE et al., 2014).

1.3.4 Inbreeding depression estimates in cattle

The intensive use of reproductive biotechnologies allows a rapidly spreading of the superior genetic material, contributing to increase the genetic progress. On the other hand, it tends to increase the inbreeding rates. Therefore, inbreeding is often associated with a reduction in the average phenotypic value of one or more traits, the so-called inbreeding depression (MIGLIOR and BURNSIDE, 1995).

Dickerson (1963) used the term inbreeding depression as a reference to livestock productive losses caused by inbreeding. Effects of inbreeding depression

on various economically important traits have being reported in cattle. Burrow (1998), for example, reported that every 1% in increase of inbreeding resulted in a decrease of 0.06 kg in birth weight, 0.44 kg in weight at weaning and 0.69 kg in yearling weight in beef cattle. Schenkel et al. (2002), working with zebu cattle (Gir, Guzerá, Indubrasil, Nelore and Tabapuã), found that for every 10% in increase of individual inbreeding coefficient caused a reduction of 1.7% and 2.1% in pre and post- weaning weight gain, respectively. For every 1% of increase in the inbreeding coefficient, Santana Junior et al. (2010) observed a reduction of 0.85 kg in weight, 1.6 cm in scrotal circumference at 18 months and 8% in heifer probability of pregnancy at 14 months of age in Nelore; Falcão et al. (2001) reported an increase of 1.4 days in the calving interval in Brow Swiss cattle; and Fioretti et al. (2002) found an increase of 0.623 days in age at first insemination and 0.763 days in age at first calving.

Comparing the Inbreeding depression estimated using F values from pedigree-based analysis (F_{PED}) and from the diagonal of the genomic relationship (F_G), Pryce et al. (2014) found that every 1% in increase of inbreeding resulted in a decrease of 21 and 28 liters/lactation respectively for F_{PED} and F_G in Holstein. For Jersey cattle, the authors reported a reduction of 12 and 27 liters/lactation, respectively. The autors considered, what the effect of F_{PED} would reduce the estimates of inbreeding depression. Estimates based on F_G should have a lower sampling error than those based on F_{PED} because it is not affected by incomplete pedigrees and it uses observed rather than expected IBD. The difference in inbreeding depression estimated based on F_G and F_{PED} information implies that estimates using pedigree information may be underestimated.

Bjelland et al. (2013) used SNP information to calculate genomic F values in Holstein based on different approaches: 1) the percentage of homozygous SNPs (F_{PH}); 2) the Runs of homozygosity (F_{ROH}), 3) the genomic relationship matrix (F_{GRM}). These authors reported mean values of 60.5 ± 1.1%, 3.8 ± 2.1% and 20.8 ± 2.3% for F_{PH}, F_{ROH} and F_{GRM}, respectively, and an increase in the mean of the trait days open equal to 1.76, 1.72 and 1.06 days when F_{PH}, F_{ROH} and F_{GRM} increased 1%, respectively. Still according to the authors, the conception rate reduced 0.82% and 0.53% when F_{ROH} and F_{GRM} increased 1%, respectively, while F_{PH} had no significant effect for this trait. For calving ease, measured as 5-point scale, where 1 is ease

calving, the increasing of 1 % of F_{PH}, F_{ROH} and F_{GRM} resulted in an increase of 0.09, 0.03 and 0.04 in the expression of the trait, respectively, indicating that cows with higher inbreeding coefficient present more difficult at calving.

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CHAPTER 2 – GENOME-BASED ASSESSMENT OF INBREEDING DEPRESSION ON REPRODUCTIVE TRAITS IN NELORE CATTLE

ABSTRACT

The availability of genomic information has allowed using thousands of single nucleotide polymorphisms (SNPs) spread over the genome to estimate the inbreeding coefficient (F). The aim of this study was to estimate F, using different genomic approaches, and to estimate the effects of inbreeding on age at first calving (AFC), heifer early pregnancy (EP) and heifer rebreeding (HR) traits in Nelore Cattle. In total, 3,785 animals (1,760 males and 2,025 females) were genotyped using the Illumina BeadChip with 777,962 SNPs. The genomic F values were computed using four approaches: 1) excess of homozygous SNPs (F_{SNP}); 2) runs of homozygosity (ROH); 3) genomic relationship matrix (G matrix) with allele frequencies calculated from the base population (F_G); and 4) G matrix with allele frequencies fixed at 0.5 (F_{GRM}). For inbreeding depression estimates, the F values were used as covariable (linear effect) in single-trait animal models that included fixed effects of contemporary group and random animal and residual effects. The average inbreeding coefficients were 0.055 (F_{ROH}), -0.0006 (F_G), 0.4376 (F_{GRM}), and 0.5560 (F_{SNP}). The correlations among these different F values were: -0.20 (F_G-F_{GRM}), -0.28 (F_G-F_{SNP}), 0.21 (F_G-F_{ROH}), 0.68 (F_{ROH}-F_{SNP}), 0.72 (F_{ROH}-F_{GRM}) and 0.99 (F_{SNP}-F_{GRM}). Inbreeding depression on reproductive traits age at first calving (AFC), heifer early pregnancy (EP), and heifer rebreeding (REC) was verified for increases in each measure of inbreeding coefficient in this Nelore population. All the traits presented significant linear effects.

2.1 INTRODUCTION

Inbreeding leads to an increase in homozygosity reducing the population genetic variability and it is often associated to losses in animal survival, production and fertility, which is known as inbreeding depression (FALCONER, 1989; CHARLESWORTH et al., 2009). The inbreeding coefficient (F) is interpreted as the probability of two alleles are identical by descent (IBD) (WRIGHT, 1922). Traditionally, the F value is computed using pedigree information. However, the pedigree quality is a critical point to obtaining unbiased inbreeding estimates (MUCHA and WINDIG 2009; LUTAAYA et al., 1999). In Brazil, in large herds of beef cattle, the use of multiple-sire mating system is a common reproductive practice, affecting genetic predictions and parameter estimations obtained from pedigree-based analysis.

With the availability of high-density SNP panels, the level of inbreeding may be assessed based on the genomic relationship matrix (VANRADEN, 2008; VANRADEN et al., 2011) or by calculating the proportion of homozygous SNPs (KELLER et al., 2011). In both approaches, however, it is not possible to distinguish alleles IBD from those identical by state (IBS). An alternative to estimate the population autozygosity is the runs of homozygosity (ROH) methodology (MCQUILLAN et al., 2008). A ROH has been defined as an uninterrupted segment of homozygous genotypes resulted from identical haplotypes inherited from related parents (PURFIELD et al., 2017). So, ROH has been widely used as a genomic predictor of population inbreeding, in which the ROH length indicates the number of generations in which inbreeding events have probably occurred (BJELLAND et al., 2013; CURIK et al., 2014; PRYCE et al., 2014; SILIÓ et al., 2013; KIM et al., 2013; FERENČAKOVIĆ et al., 2017). As a general rule, shorter ROH is associated to remote common ancestor and longer ROH to recent common ancestor. Nevertheless, according to Marras et al. (2016), it is important to keep in mind that the existence of a long ROH segment in an individual does not necessarily imply that the region was inherited from a common ancestor without recombination.

Inbreeding depression computed through pedigree-based analysis have been well documented. Currentlly, several researches with livestock animals have been

conducted in order to quantify inbreeding, as well as its effects on traits of interest, using genomic information (MAXIMINI et al., 2011; BJELLAND et al., 2013; PRYCE et al., 2014; SILIÓ et al.,2013; SAURA et al., 2015; FERENCAKOVIC et al., 2017). As stated by Keller et al (2011), a F value derived from pedigree represents an expectation of the genome autozygosity, but it is known that there is variation around this expectation due to the recombination process. In this context, the use of genomic information could increase accuracies of F prediction by improving genetic relationship estimations. According to Keller et al. (2011), genomic methods can, potentially, inform the real percentage of the genome that is autozygous since they provide a direct measure of homozygosity. Only a few studies have used genomic information to estimate inbreeding in Nelore population (ZAVAREZ et al., 2015; MAMANI et al., 2018). The aim of this study was to compare the inbreeding coefficient (F) using different genomic approaches, and, in addition, to estimate the inbreeding effects on reproductive traits of a commercial Nelore population.

2.2 MATERIAL AND METHODS

2.2.1 Genotypic and phenotypic data

A total of 3,785 genotyped Nelore animals (1,760 males and 2,025 females) from three commercial breeding programs (DeltaGen, Paint and Cia do Melhoramento) was used to estimate four different genomic inbreeding coefficients (F_G, F_{GRM}, F_{SNP} and F_{ROH}). The genotypes were generated with the Illumina® BovineHD BeadChip containing 777,962 SNP. Only SNPs located on autosomes were considered in the analyses. For F_G and F_{GRM}, the genotype quality control consisted of the exclusion of SNPs with MAF ≤ 0.01, HWE p-value ≤ 10^{-5}, and call rate ≤ 0.98. Samples with call rate < 0.90 were also removed, resulting in a final dataset with 3,687 animals and 551,821 markers. For F_{ROH} and F_{SNP}, the exclusion criteria were only SNP call rate ≤ 0.98, and sample call rate ≤ 0.90, keeping 3,720 animals with 715,076 markers.

The genotyped females also presented phenotypic information for age at first calving (AFC), heifer early pregnancy (EP) and heifer rebreeding (HR). Age at first calving was defined as the difference (in days) between the date of the first calving and the cow birth date. Early pregnancy is a binary trait defined by attributing the value 2 (success) for the cows with a calving information until 30 months of age and the value 1 (failure) for the cows that presented their first calving after 30 months of age. Heifer rebreeding is also a binary trait in which was attributed the value 2 (success) or 1 (failure) for the cows that had or not a second calving information.

For all traits, contemporary groups (CG) were defined as: year of birth, herd (at birth, weaning, and yearling), and management group (at birth, weaning and yearling). For AFC, observations outside the interval between 3.5 standard deviations below and above the mean of the CG were excluded. For HR and EP, there were excluded CGs in which all animals showed the same response category (1 or 2). The summary of the dataset after data editing is presented in Table 1.

Table 1 Descriptive statistics of the Nelore reproductive traits used in this study.

Continuous phenotypes				
Trait	**N**	**Mean ± SD**	**Min**	**Max**
Age at first calving (days)	1868	1,056 ± 118	960	1399
Categorical phenotypes				
Traits	**N**	**N_Class**	**(%)**	
Heifer early pregnancy	1651	490	30	
Heifer rebreeding	1482	981	66	

N: Number of observations; SD: standard deviation; Min: minimum value; Max: maximum value; N_Class: number of animals with phenotype category 2 (success); %: percentage of success;

2.2.3 Estimates of inbreeding coefficients

The genomic-based inbreeding coefficient (F) was computed based on four approaches: 1) excess of homozygous SNPs (F_{SNP}); 2) runs of homozygosity (F_{ROH});

3) genomic relationship matrix (G) considering allele frequencies obtained from the base population (F_G); and 4) G matrix considering allele frequencies fixed at 0.5 (F_{GRM}).

The F_{SNP} of individual *i* was estimated as : $F_{SNP} = (OH_i – EH)/(n – EH)$, where F_{SNP} is the inbreeding coefficient of the animal *i*; n is the number of SNPs; OH_i is the observed homozygosity of the animal *i*; and EH is the expected homozygosity for all individuals, being calculated as: $\sum_{j=1}^{n}[1 - 2p_j\,(1 - p_j)]$, where p_j is the minor allele frequency of j^{th} SNP (KELLER et al., 2011).

The F_{ROH} of each animal was calculated as (KELLER et al., 2011; BJELLAND et al. 2013):

$$F_{ROHi} = \frac{\Sigma_t\ length(ROH_t)}{length_{total}}$$

where, ROH_t is the t^{th} ROH in genome of individual i and $length_{total}$ is 2,612,820 kb, which represents the total length of the bovine genome (ZIMIN et al., 2009). The ROH segments were detected using the PLINK software version 1.07 (PURCELL et al., 2007), considering a sliding window with 50 consecutive SNPs, 1 heterozygous per window, 5 missing calls and a minimum proportion of homozygous windows in which each SNP occurs of 0.05 (CURIK et al., 2014). The minimum length of a ROH was set to be 1,000 kb; the required minimum SNP density considered was 1 SNP per 50 kb; and the maximum gap between two consecutive SNPs was set to be 500 kb (KARIMI, 2013).

The genomic relationship matrix (G) was built according to VanRaden, (2008).

$$\mathrm{G} = \frac{\mathrm{ZZ}'}{2\sum_{\mathrm{i}=1}^{\mathrm{m}}\mathrm{p_j}\left(1 - \mathrm{p_j}\right)}$$

where, Z is the genotype matrix that contains the 0-2p values for homozygotes, 1-2p for heterozygotes, and 2-2p for opposite homozygotes and p_j is the allele frequency at locus j^{th}. The F_G of the animal *i* was computed as G_{ii}-1. To compute the F_{GRM}, it was considered a G matrix built assuming $p_j = 0.5$ (VANRADEN et al., 2011).

2.2.4 Inbreeding depression analysis

The *nlme* R package (PINHEIRO et al., 2018) was used to test the significance of the linear and quadratic effects of each genomic-based inbreeding coefficient. The model, for each trait, included fixed effect of CG, defined as above, and the linear and quadract effects of each genomic F coefficient (F_{ROH}, F_{SNP}, F_G, and F_{GRM}) as covariable. For age at first calving (continuous variable) a linear model was assumed and the F-test ($p \leq 0.05$) was used. For heifer early pregnancy and heifer rebreeding traits (categorical variables) generalized linear models were assumed and the Chi-squared test ($p \leq 0.05$) was applied. In this study, only genomic-based inbreeding linear effects were significative. So, only the linear F effect was considered for inbreeding depression computations for all traits.

The general single-trait (linear or threshold) model used to estimate inbreeding depression was:

$$\begin{pmatrix} y \\ or \\ \eta \end{pmatrix} = X\beta + Z\alpha + \varepsilon$$

where y is the vector of observations for AFC and η is the linear predictor for HR or EP; β is the vector of fixed effects (CG and the linear effect of inbreeding coefficient (F) as covariable); α is the animal additive genetic effect, assuming $\alpha \sim N(0, \mathbf{A}\sigma_\alpha^2)$; ε is the residual effect, assuming $\varepsilon \sim (0, \mathbf{I}\sigma_\varepsilon^2)$; and **X** and **Z** are incidence matrices for fixed and random effects, respectively. In the threshold model it was assumed an underlying scale (liability) with normal distribution that could be represented as (SORENSEN and GIANOLA, 2002):

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

where ***U*** is the vector of the underlying scale of order r; θ=(β, α) is the vector of the location parameters of order s with β corresponding to the set of fixed effects and α corresponding to random effect; ***W*** is a known incidence matrix of order r $_X$ s; ***I*** is an identity matrix of order r $_X$ r; and $\sigma_e^2 = 1$.

The linear animal model was implemented by restricted maximum likelihood using the AIREMLF90 program and the Bayesian threshold animal model with a probit link function using the THRGIBBS1F90 program (MISZTAL et al., 2002). For the Bayesian model a total of 500,000 GIBBS chains were generated considering a burn-in period of 100,000 iterations and a 10 cycle thinning interval. The analysis convergence was verified on 40000 samples through graphical and visual inspection (trace plots) and based on the criteria proposed by (SMITH et al., 1997).

2.4. RESULTS AND DISCUSSION

The mean value of F_{SNP} and F_{GRM} were considerably higher than F_G and F_{ROH} (Table 2). These results could indicate that both F_{SNP} and F_{GRM} approaches tend to overestimate the inbreeding values. Bjelland et al. (2013) and Pryce et al. (2014), by using simply the percent of homozygosity, found genomic inbreeding ranging from 60% to 72%, on average, in dairy cattle. These values are comparable to our results for F_{SNP} since F_{SNP} is, basically, an estimate of genomic inbreeding based on excess SNP homozygosity (KELLER et al., 2011).

Table 2. Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of inbreeding coefficient (F) measured with different approaches in a commercial Nelore beef cattle.

F value	Mean	SD	Min	Max
F_G	-0.0006	0.029	-0.190	0.690
F_{GRM}	0.4376	0.013	0.080	0.620
F_{SNP}	0.5560	0.011	0.193	0.700
F_{ROH}	0.0590	0.016	0.0016	0.260

F_G and F_{GRM} – F computed based on genomic relationship matrix with allele frequencies calculated from the base population and with allele frequencies fixed at 0.5, respectively; F_{SNP} – F computed based on excess of homozygosity and F_{ROH} – F computed based on ROH.

As in Bjelland et al. (2013), who used different genomic approaches to estimate inbreeding coefficient in Holstein cattle, we do not present F values computed based on pedigree information. In our case, approximately, 40 % of the animals had unknown sire information due to the use of multiple sires mating. However, most of the estimates of F in Zebu cattle were obtained from pedigree information. Thus, comparisons with previous literature reports for Zebu breeds were done with pedigree-based inbreeding coefficients.

Using pedigree-based analysis, Faria et al. (2002) and (2009), Brito et al. (2013) and Pereira et al. (2016) reported F values means ranging from 0.98% to 2.36% for Brazilian Zebu cattle. Compared to our results, these values are more similar to F_G and F_{ROH} than to F_{SNP} and F_{GRM}, reinforcing the indication that F_{SNP} and F_{GRM} tend to overestimate F values. The statistics Min and Max (Table 2), however, show that in addition to negative values, F_G also reached high (overestimated) F values. On the other hand, the descriptive statistics for F_{ROH} (Table 2) seems to be more similar to pedigree-based inbreeding estimates reported in the literature (FARIA et al., 2002; BRITO et al., 2013; PEREIRA et al., 2016).

According to Legarra (2016), negative F_G values represent animals that are more heterozygous than the population average. Fixing allele frequencies at 0.50 (F_{GRM}) avoids inbreeding negative values by, essentially, making F_{GRM} a measure of homozygosity with distribution similar to traditional pedigree-based inbreeding (BJELLAND et al., 2013). In fact, VanRaden et al. (2011) observed that F_{GRM} was more correlated with inbreeding derived from pedigree information in dairy cattle than F_G. According to these authors, since the true markers frequencies in the base population are unkown, it would be better, instead of estimating these frequencies, simply use allele frequencies of 0.50 when computing G matrix.

F_{GRM} and F_{SNP} were extremely correlated to each other (0.99), indicating that these approaches are equivalent in terms of identifying the most and the least-inbred animals. However, F_{GRM} was more correlated with F_{ROH} (0.72) than F_{SNP} (0.68). In turn, F_G presented the lowest correlation with F_{ROH} (0.21) and negative and low correlation with both F_{SNP} (-0.28) and F_{GRM} (-0.20). These results suggest that F_{GRM} may be a better approach than F_G to compute inbreeding, which corroborates with VanRaden et al. (2011) and Bjelland et al. (2013).

Descriptive statistics for F_{ROH} (Table 2) are more similar to previous reports of pedigree-based inbreeding estimates than any other genomic approaches. Moreover, F_{ROH} has been determined as the genomic method that provides F values more correlated with true inbreeding (KELLER et al., 2011; FORUTAN et al., 2018). Thus, F_{ROH} might be the most appropriate genomic method to estimate inbreeding.

All genomic inbreeding coefficients presented significant linear effects on age at first calving (AFC) and on heifer early pregnancy (EP; Table 3). On average, the increase of 1% in any inbreeding coefficient causes an increase in AFC varing from 3.33 to 6.13 days depending on the method. These values are similar to previous pedigree-based reports for various breeds of cattle, e.g. 4.2 days in Alentejana cattle breed (CAROLINO AND GAMA, 2008); 6.2 days in Guzerat dairy population (PANETTO et al., 2010); 0.763 days in Piedmontese breed (FIORETTI et al., 2002); and of 1.7 days in Nelore cattle (PEREIRA et al., 2016).

Table 3. Linear regression coefficient (b_1) and standard error (SE) on the different measures of inbreeding coefficient (F) for reproductive traits in Nelore beef cattle.

Trait	**F value**	**b_1**	**SE**
Age at first calving (days)	F_G	3.67	0.18
	F_{GRM}	5.19	0.18
	F_{SNP}	6.13	1.23
	F_{ROH}	3.33	1.50
Heifer early pregnancy (score)	F_G	-0.12	0.08
	F_{GRM}	-0.09	0.05
	F_{SNP}	-0.13	0.01
	F_{ROH}	-0.03	0.02
Heifer rebreeding (score)	F_G	-0.014	0.01
	F_{GRM}	-0.025	0.09
	F_{SNP}	-0.012	0.01
	F_{ROH}	-0.016	0.02

For EP, there were observed decreases ranging from 3.00 to 13.00% per every 1% increase in genomic inbreeding. Using pedigree information, Santana Junior et al. (2010) found an average decreasing of 10.8% in the mean of EP of Nelore

animals, i.e. representing a decrease of population heifer pregnancy probability. Burrow (1998) also observed a reduction in the pregnancy rate of female reproductive traits in tropical beef cattle breeds caused by increases in herd inbreeding rates.

For HR, in which only the linear effect of F_G significantly affect this trait, was detected decreases of 1.4% per every 1% increase in genomic inbreeding. In a population of Guernsey using pedigree-based analysis, Hermas et al. (1986) revealed an average decreasing of 4.3% in the mean of conception rate for heifers.

In general, small inbreeding depression was observed in this Nelore population independent of the genomic approach used. It is worth mentioning, however, that the continuous monitoring of inbreeding on reproductive traits is recommended to avoid declining of such traits.

2.5 CONCLUSIONS

For using genomic matrix to estimating inbreeding coefficients, the allele frequency should be fixed in 0.5 instead of using population frequencies. Runs of homozygosity (F_{ROH}) seems to be the most appropriate genomic methodology to compute inbreeding. All traits, but heiffer reabreeding, were negatively affected by the linear effect of inbreeding, independent of the method.

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CHAPTER 3 - GENOME SCAN FOR HOMOZYGOSITY ISLANDS IN NELORE BEEF CATTLE

ABSTRACT

The aim of this study was to characterize the genomic homozygosity in Nelore beef cattle in addition to identify runs of homozygosity (ROH) islands that could be involved in selection events associated with economically important traits in Nelore. The dataset used in this study was composed of 3,558 Nelore animals (2,030 females and 1,528 males) genotyped with 777,962 SNP markers of BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). The PLINK program was used to identify the ROH segments, considering the following parameters: i) sliding window of 50 SNPs; ii) 2 heterozygous per window; iii) the minimum length of ROH was set to 1,000 kb; iv) the required minimum SNP density was one SNP per 50 kb; v) the maximum gap between two consecutive SNPs was 500 kb; vi) 5 missing calls were allowed and a proportion of homozygous windows in which each SNP occurs of 0.05 were required in a ROH. The ROH islands represented the ROH segments shared by more than 50% of the population individuals. There were identified 210,636 ROH segments, the findings obtained in this study suggest that on average 7.2% of the genome of this population is autozygous. The average ROH length was 2.97 ± 4.21 Mb, ranging from 1.0 to 98.45 Mb. Five ROH islands were identified on chromosomes 5, 7, 12, 21 and 26, in which 43 annotated genes were identified. Some of these genes *INHBE, INHBC, STAT6, FGF8, DPCD* are involved in sexual development and maturity. Regions found in X chromosome in analysis performed only on females presented a total of 144 genes, of this total, 78 were identified as LOCs that were divided into protein-coding genes, pseudogenes, unknown non-coding RNAs, and proteins uncharacterized in cattle. Runs of homozygosity results evidenced the presence of ROH islands in genomic regions with potential genes affecting reproduction and growth traits, immune response and environmental adaptation in Nelore cattle.

3.1 INTRODUCTION

Runs of homozygosity (ROH) are contiguous homozygous chromosomal segments (MCQUILLAN et al., 2008) generated from identical haplotypes inherited from a common ancestor. Long ROH are related to recent ancestors and short ROH are associated to ancient ancestors (SÖLKNER et al., 2014). The identification of ROH allows a better understanding of the genomic patterns of autozygosity across the genome, relating the ROH length to the number of generations since the common ancestor (HOWRIGAN et al., 2011). In addition, ROH islands could be used to identify candidate genes associated with relevant phenotypes (MASTRANGELO et al., 2016). ROH islands are homozygous genomic regions shared by more than 50 % of the individuals. There is an expectation that these homozygous regions are close or within quantitative trait loci that have been subjected to selection events (GROSSMAN et al., 2010; PEMBERTON et al., 2012). Thus, the ROH islands mapping of a population under selection permits to identify functional genetic variants associated to economically important traits (MARRAS et al., 2014).

In populations under selection, inbreeding is an inevitable consequence since only a subset of individuals is used for breeding (MARRAS et al., 2014). Both natural and artificial selection in livestock may leave a variety of imprints on the genome, which is characterized by reduced genetic diversity and high frequence of ROH segments (PURFIELD et al., 2017). Therefore, ROH islands are likely to be signatures of selection that potentially contain functional genetic variants associated with the expression of various phenotypes, which have been targeted by natural and/or artificial selection (PURFIELD et al., 2017). Mastrangelo et al. (2017), for example, reported the presence of ROH islands harboring candidate genes associated with adaptability, milk production and immune responses in Valle del Belice sheep. According to Zavarez et al., (2015), were found islands in chromosomes 4, 7, and 12 of cattle Nelore involved in resistance to infectious diseases and fertility. Another study, showing noticeable peaks on chromosome 7, 12, and 21 present hotspots of autozygosity (> 40%) putatively hovering selective pressure and contributing with studies based on ROH analysis in Nelore cattle (MAMANI et al., 2018). Hence, the researches of autozygosity (characterizing and

comprehending) could improve the mechanisms involved in the maintaining genetic variability and response to selection in commercial breeding programs of Nelore cattle in Brazil.

In this context, the present study aimed to characterize the distribution of ROH segments in a commercial Nelore population and to investigate the existence of ROH islands that could harbor candidate genes associated with economically important traits in beef cattle.

3.2 MATERIAL AND METHODS

This study was approved by the ethical committee on the use of animals (CEUA) of the School of Agricultural and Veterinary Sciences (FCAV/UNESP), Jaboticabal – SP, Brazil (protocol number 18.340/16).

3.2.1 Genotypic Data

The dataset used in the present study consisted of genotypic information of 3,558 Nelore animals (2,030 females and 1,528 males), genotyped with the BovineHD BeadChip (Illumina®, Inc., San Diego, CA, USA) containing 777,962 single nucleotide polymorphism (SNP) distributed across the bovine genome. These animals were offspring of 421 sires and 3,425 dams, and were from three commercial breeding programs (DeltaGen, Paint and Cia de Melhoramento). There were considered markers located on autosomes and X chromosome with GenCall score greater than 70% and call rate higher than 95%. Samples with call rate lower than 90% were removed. After genotype quality control, there were 3,492 samples (2,007 females and 1,485 males) and 755,319 SNP markers.

3.2.2 Runs of homozygosity detection

The PLINK software (PURCELL et al., 2007) was used to detect the ROH segments, considering the following parameters: i) sliding window of 50 SNPs; ii) 2 heterozygous per window; iii) the minimum length of ROH was set to 1,000 kb; iv) the

required minimum SNP density was one SNP per 50 kb; v) the maximum gap between two consecutive SNPs was 500 kb; vi) 5 missing calls and a proportion of homozygous windows in which each SNP occurs of 0.05 were allowed in a ROH. (PURCELL et al., 2007; FERENCAKOVIC et al., 2013; KARIMI, 2013). The identification of ROH on the X chromosome was performed considering only the genotypic information of females. The ROH islands represented the ROH segments shared by more than 50% and 70% of the population individuals, considering the autosomes and the X chromosome, respectively.

The X chromosome was used in ROH analysis because shows direct participation in the biological process to the production of gametes, fertility and pregnancy in females. In this sense, the selection for reproductive traits could affect autozygosity levels in X chromosome (PORTO-NETO et al., 2013) caused by the favorable allele fixation in females under as it probably contains regions under relatively strong positive selection (SCHAFFNER, 2004).

The genes located at ROH islands, i.e. ROH segments with frequencies exceeding 50% of the whole population, were mapped using the NCBI Map Viewer tool (www.ncbi.nlm.nih.gov/mapview/) (NCBI, 2015), considering the UMD3.1 bovine assembly. Functional analysis of the mapped genes was performed using the DAVID v6.7 software (HUANG et al, 2009).

3.3 RESULTS

3.3.1 Distribution of runs of homozygosity

The average percentage of chromosome coverage by ROH is shown in Figure 1. The highest average, considering the autosomes, was observed on chromosome 28 (6.97%), followed by chromosome 27 (6.94%) and 25 (6.79%). The chromosome 1 presented the lowest percentage of coverage by ROH with only 2.13%.

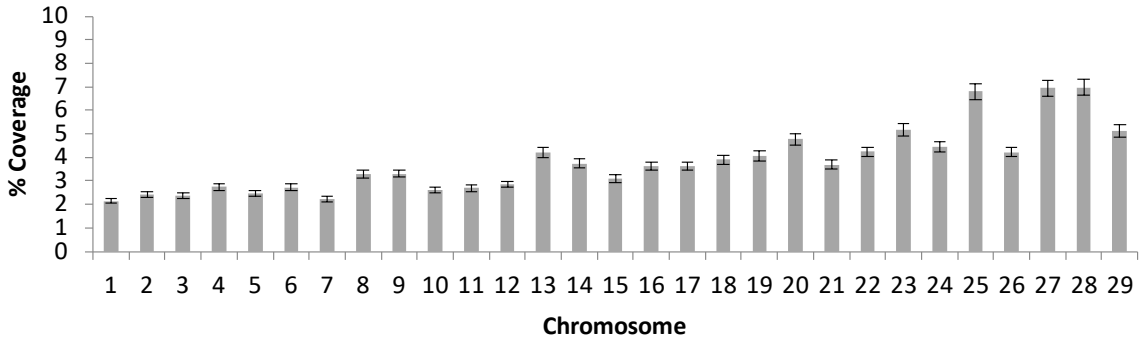

Figure 1. Average percentage of coverage by runs of homozygosity in Nelore cattle.

A total of 210,636 ROH segments was identified across the 3,492 animals. The number of SNPs per window ranged from 50 to 26,810 with an average of 789 SNPs. The mean length of ROH identified per individual was 2.93 ± 4.21 Mb, ranging from 1.0 to 98.45 Mb. On average, 7.2 % of the genome of this Nelore population was covered by ROH. The number of ROH by class of length (Table 1) shows the prevalence of shorter ROH (1 - 4 Mb), which represents 83.08% of all o segments identified, even though the proportion of the genome covered by them was relatively small.

Table 1. Distribution of the runs of homozygosity (ROH) by class of length.

Class	Number of ROH	Percent	ROH length mean	Standard Deviation
$ROH_{1\text{-}4\ Mb}$	175.007	83.08	2.35	0.40
$ROH_{4\text{-}8\ Mb}$	20.899	9.90	5.57	1.12
$ROH_{8\text{-}16\ Mb}$	10.620	5.04	10.95	2.21
$ROH_{>16\ Mb}$	4.110	1.95	25.17	10.04

The ROH islands can be defined as genomic regions with reduced genetic diversity and, consequently, high homozygosity around selected loci that might harbor targets of positive selection (PEMBERTON et al., 2012). The distribution of ROH segments showed the existence of five ROH islands located on five autosome chromosomes (BTA 5, 7, 12, 21, and 26) (Figure 2). These ROH islands harbor 43 genes (Table 2), in which several of them have a prominent importance in feed efficiency, reproduction traits, meat quality, growth, adaptation and immune system

(SAHANA et al., 2013; SANTOS-BIASE et al., 2012; GUTTRIDGE, 2017; DU et al., 2013; HOSSEINI et al., 2015; MARCHESI el al., 2017; KISER et al., 2017) (Table 3).

Gene ontology (GO) and pathway analysis (KEEG) were performed by DAVID tool to obtain a broad functional insight into the set of genes. According to the functional enrichment analysis, performed only set genes found in autosome chromossomes, seven over-represented terms were found for the biological processes: mammary gland epithelial cell proliferation (GO:0033598); spermatogenesis (GO:0007283); apoptotic process (GO:0006915); positive regulation of pathway-restricted SMAD protein phosphorylation (GO:0010862); regulation of MAPK cascade (GO:0043408); cell development (GO:0048468); and SMAD protein signal transduction (GO:0060395). Pathway analysis showed that GLI1 and BTRC genes were involved in Hedgehog signaling pathway (bta04340).

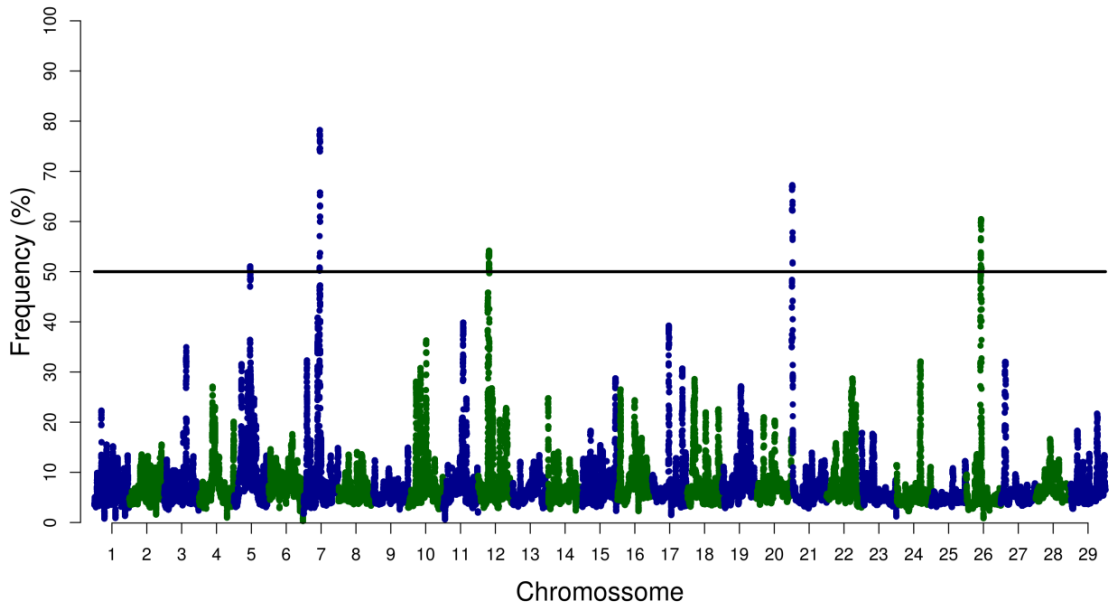

Figure 2. Distribution of ROH segments per autosome. The line represents the threshold used to identify the ROH islands.

Table 2. Chromosome (Chr), position and annotated genes of ROH islands identified in Nelore cattle.

Chr	Physical Position (bp)	Gene Symbol
5	56,286,256 – 56,721,249	*NAB2, INHBE, GLI1, MBD6, LRP1, NEMP1, MARS, STAC3, R3HDM2, ARHGAP9, MYO1A, INHBC, SHMT2, NDUFA4L2, SNORA62, bta-mir-2430, bta-mir-2431, NXPH4*
7	52,048,743 – 52,599,065	*DNAJC18, TMEM173, CXXC5, PAIP2, SLC23A1, SPATA24, PROB1, SIL1, UBE2D3, MZB1, SNORA74*
12	28,768,075 – 29,187,016	*2AR1L, FRY*
21	916,117 – 1,455,150	*5S-rRNA, U6*
26	22,135,723 – 22,535,723	*FGF8, POLL, DPCD, FBXW4, BTRC, NPM3, MGEA5, KCNIP2, STAT6 C10orf76, 5S-rRna*
X	22,832 –1,455,199	*AGTR2,CT83,KLH24,SLC6A14*
X	8,849,180 – 10,144,898	*STAG2, LOC782784, VN2R412P, LOC615454, SH2D1A,TENM1, LOC104976318, LOC100335567, MIR2285G-3, LOC513322, MIR2483, LOC104970464, LOC101902870, LOC101904092, LOC782963, TRNAE-UUC*
X	15,866,834 -17,401,780	*ARHGAP36, LOC100300546, IGSF1, LOC528006, LOC528106, OR13H1, LOC786185, LOC614771, LOC104971281, LOC104971280, STK26, FRMD7, RAP2C, TRNAC-GCA, MBNL3, LOC104971282, HS6ST2, LOC101903011, LOC786718, LOC101902899, LOC783491, USP26, LOC104969930, LOC783325, LOC783362*
X	23,129,547 – 24,197,195	*F9, MCF2, ATP11C, MIR505, LOC100297368, LOC100851236, CXHXorf66, LOC785249, LOC787011, LOC507017, LOC785156, LOC781911*
X	30,603,248- 31,831,300	*FMR1, FMR1NB, LOC100335894, AFF2*
X	140,420,549 -148,820,237	*GYG2,XG, CD99, LOC104970645, ZBED1, LOC101902961, DHRSX, LOC52189, LOC790019, LOC104970636, LOC104970637, TMSB4X, TLR8, TLR7, PRPS2, LOC101903955, LOC101908437, FRMPD4, LOC101904216, TRNAD-GUC, MSL3, LOC104970633, ARHGAP6, USP9Y, ZRSR2Y, LOC100336906, LOC781072, CLCN4, WWC3, LOC100851938, LOC101905082, LOC104970454, LOC783100, UTY, LOC101905564, DDX3Y, LOC783370, LOC104970455, LOC100336498, PRDM7, LOC101908812, LOC101906113, LOC100851174, LOC101905707, LOC101905856, LOC101905937, LOC101905930, LOC783713, LOC101906130, LOC614318, CLDN34, LOC100337198, OFD1Y, SHROOM2, LOC104970638, GPR143, LOC101907958, EIF1AY, EIF2S3Y, TBL1X, LOC104970640, TRNAC-GCA, MIR584-8, ANOS1, LOC104970644, LOC104970643, LOC104970642, LOC101902182, LOC101902122, LOC104976420, LOC100847588, TRNAE-UUC, PNPLA4, LOC101902311, TRNAT-AGU,TRNAG-CCC, LOC104970641, STS, LOC104976421, PUDP, LOC10084762, TRNAV-CAC, NLGN4X, LOC783541*

In the X chromosome, there were found six ROH islands which harbor 144 genes (Table 2). Of this total, 78 were identified as LOCs that were divided into protein-coding genes, pseudogenes, unknown non-coding RNAs, and proteins

uncharacterized in cattle. LOC101904216, TRNAD-GUC, MSL3, LOC104970633 and ARHGAP6 genes found this study have been associated with reproductive traits (TEIXEIRA et al., 2017).

Table 3. Traits associated with genes found on ROH islands in Nelore Cattle.

Trait	Chr	Genes	Autor
Feed efficiency	5	*NAB2*	Boyle et al., 2009
	26	*C10orf76, STAT6, FGF8, DPCD*	Sahana et al., 2013 Tizioto et al., 2015; Moore et al., 2008
Reproduction	5	*INHBE, INHBC, ARHGAP9*	Bhardwaj et al., 2012;
	7	*PAIP2, PROB1, SPATA24, SIL1, UBE2D3*	Orozco-Lucero et al., 2014; Zheng et al., 2007; Brancorsini et al., 2008; Liu et al., 2009, Viana et al., 2016; Ghanem et al., 2007
	21	*U6*	Nascimento et al., 2016
	26	*MGEA5, STAT6, FGF8, DPCD*	Assidi et al., 2010; Santos-biase et al., 2012; Peñagaricano et al., 2012; Fortes et al., 2010; Fortes et a al., 2011
Meat quality	5	*GLI1, LRP1, R3HDM2, MYO1A, SHMT2, NDUFA4L2, ARHGAP9*	Liu et al., 2014; Tizioto et al., 2013; Tremblay et al., 2007; Schiff et al., 2015; Tello et al., 2011; Nowak et al., 2011
	7	*SLC23A1, CXXC5*	Wyke et al., 2004; Guttridge, 2017; Du et al ., 2013
	12	*FRY*	Guillemin et al., 2011
	26	*FBXW4, NPM3, KCNIP2, STAT6, BTRC, FGF8, DPCD*	Schlemi, 1997;Tizioto et al ., 2013; Kee, 2008; Cassar-malek and Picard, 2016; Bernard et al., 2007; Wu et al., 2010
Growth	5	*STAC3, NXPH4, ARHGAP9*	Hosseini et al., 2015; Sun et al., 2016; Ge et al., 2014
	26	*STAT6, FGF8*	Texeira et al., 2005; Maffei et al.,1995; Rincon et al., 2009
Adaptation	5	*SHMT2*	Pamok et al., 2009; Panieri and Santoro, 2016; Mujahid et al., 2006; Marchesi el al., 2017.
	26	*STAT6*	Ostrand-Rosenberg et al., 2004; Hou et al., 1994; Quelle et al., 1995;
Immune system	7	*TMEM173, SLC23A1, MZB1*	Zheng et al., 2014; Rosenbaum et al., 2014; Kiser et al., 2017; Ganz and Nemeth, 2015.

3.4 DISCUSSION

ROH segments distribution across genomic regions in Nelore show differences leading to regions with abundant ROH (Figure 1). The genome scan to ROH islands detected regions on BTA 7, 12 and 21 overlapping with regions that have been reported by Karimi (2013). Genomic regions in autozygosity higher than

50% on BTA 7 and 21 show the highest peak, Karimi (2013) has pointed out the same ROH patterns in these regions in tropical breeds (Gyr, Brahman and Nelore cattle). Genomics regions identified underlying or flanking the ROH islands on BTA 7 and 12 have also been reported by Zavarez et al, (2015) and, according to these authors, these are regions affecting resistance to infections and puberty. These regions have been reported to be potentially under selection through the comparison of Bos taurus and indicus breeds (PORTO-NETO et al., 2013) and as locus autozygosity regions in Nelore cattle (MAMANI et al., 2018).

The highest proportion of homozygosity on the autosomes was found on BTA 7 (79.12%), shown in Figure 2. These results are in agreement with (ZAVAREZ et al., 2015), who used a distribution of chromosome-wise autozygosity for ROH > 0.5Mb in a Nelore population and was previously described as a ROH hotspot in three taurine and indicine breed by Sölkner et al., (2014). This region has been implicated in the control of parasitemia in cattle (HANOTTE et al., 2003).

3.4.1. Candidate genes within runs of homozygosity

In this chapter, we do discuss the genomic regions associated with ROH, but focus on some selected genes found on this regions that show associations with several specific traits related to livestock breeding. Many the candidate genes found in the ROH islands are coding protein and they are related to cellular growth, development and regulation. Some of these genes participate of biological process affecting feed efficiency of the bovine. The C10orf76 gene affects feed conversion rate (SAHANA et al., 2013) and NAB2 encodes a protein induced by EGR1 that interacts with several genes associated with residual feed intake (BOYLE et al., 2009; TIZIOTO et al., 2015).

Some these genes are also directly involved in sexual development and maturity such as the PROB1 and U6 genes that were associated with puberty in *Bos indicus* (LIU et al., 2009; NASCIMENTO et al., 2016). *FGF8,* INHBE, INHBC, MGEA5, PAIP2 and UBE2D3 genes affecting foliculogenesis and oocytes maintenance (ASSIDI et al., 2010; BHARDWAJ et al., 2012, OROZCO-LUCERO AND SIRARD, 2014, GHANEM et al., 2007; BURATINI et al., 2005; SANTOS-BIASE

et al., 2012). The INHBE and INHBC were over-represented to positive regulation of pathway-restricted SMAD protein phosphorylation (GO:0010862), SMAD protein signal transduction (GO:0060395) and cell development (GO:0048468). SMAD proteins comprise a family of structurally similar proteins that are the main signal transducers for receptors of the transforming growth factor beta (TGF-B) superfamily, which are critically important for regulating cell development and growth (DERYNCK et al., 1998).

The *SPATA24* gene plays a critical role in male reproduction during spermatogenesis (BRANCORSINI et al., 2008; ZHENG et al., 2007). The protein encoded by SIL1 was found in seminal plasma of Holstein bulls with high fertility, suggesting an influence of SIL1 in male fertility caused by increase of the sperm motility (VIANA et al., 2016). This study, PAIP2 and GLI1 genes were over-represented on spermatogenesis (GO:0007283).

Over-represented GO term also was related with reproductive cells: mammary gland epithelial cell proliferation (GO:0033598). BTRC genes were over-represented to this GO terms. BTRC and GLI1 also were involved in Hedgehog (Hh) signaling pathway, this pathway is involved in the regulation of cell proliferation, differentiation and turnover in a variety of mammalian embryonic and adult tissues including bovine ovarian granulosa and theca cells (FRANCO and YAO, 2012). These results could indicate a positive selection of genes related to reprodutive traits in this Nelore population.

The GLI1 also have been affecting meat quality (LIU et al., 2014). A GLI1 participates of the Hh signaling pathway, members of GLI gene family explained the greatest amount of additive genetic variance for meat tenderness in Nelore steers (TIZIOTO et al., 2013). We found MYO1A gene, belonging to the myosin family that play key regulatory roles in the initiation and development of skeletal muscle and the maintenance of its phenotype. The MYO1A gene was associated with meat quality in chicken (DU et al., 2016). FRY is an encode protein gene involved in actin filament reorganization. Actin filaments were related to meat quality traits in beef cattle (GUILLEMIN et al., 2011).

The *NPM3* was expressed in *Longissimus thoracis* of young Charolais bulls and was correlated to tenderness, juiciness and flavor (CASSAR-MALEK and

PICARD, 2016; BERNARD et al., 2007). Polymosfisms in FGF8 gene have been associated with female reproductive traits, carcass quality, yield grade, growth, feed efficiency and lipid metabolism (MARQUES et al., 2009; MOORE AND MARQUES , 2008). This gene participates in the MAPK signaling pathway, which is one of the major intracellular signaling pathways affecting myogenesis that is relevant to postmortem meat quality (PONSUKSILI et al., 2009; WU et al., 2010). In this study, the INHBE and INHBC were over-represented to regulation of MAPK cascade (GO:0043408), showing that these genes may have a putative effect on meat quality.

Other important gene found was the SLC23A, which belongs to the superfamily of the SLC (Solute-carrier) genes responsible for the transport of glucose and other sugars, bile salts and organic acids (HE et al., 2009). Genes from this superfamily were associated to carcass and meat quality traits in Nellore (FERNANDES JÚNIOR et al., 2016; MAGALHÃES et al., 2016).

The STAC3, NXPH4, ARHGAP9, STAT6 and FGF8 genes have been associated with growth and development processes in bovine (HOSSEINI et al., 2015; SUN et al., 2016; GE et al., 2014; TEXEIRA et al., 2005; MAFFEI et al.,1995; RINCON et al., 2009). *STAT6* also presents an important function on regulatory network of fertility in cattle and was associated with age at first corpus luteum, an indicator trait of early puberty in heifers (FORTES et al., 2010, 2011). The gene *SHMT2* was associated with the metabolism of some amino acids, such as glycine, serine and threonine, indicating there is an intimate connection between cellular metabolism and redox homeostasis related to environment adaptation processes (PAMOK et al., 2009).

The MZB1, TMEM173 and SLC23A1genes are associated with regulation of the immune system. The MZB1, was associated with impairs in humoral immune responses and antibody secretion in plasma cells that naturally undergo ER stress in mice (ROSENBAUM et al., 2014). The gene TMEM173 was over-representated on apoptotic process (GO:0006915) this study, and was related to activation of innate immune response and regulation the expression of stress response and immune response in the serum of heat-stressed and normal Holstein cows (ZHENG et al., 2014). The SLC23A1 gene was previously associated with susceptibility to

Mycobacterium avium ssp. paratuberculosis (Map) tissue infection in two American Holstein populations (KISER et al., 2017).

In this study were found six ROH island on chromosome X. Zavarez et al. (2015), described four genomic regions in autozygosity on chromosome X in Nelore females. We mapped many LOC genes (N=78), in which most are transcribed and untranslated or unannotated in cattle. The higher autozygosity on the X chromosome is expected because approximately, they present a rate of recombination 1/3 smaller than the autosomes (HAMMER et al., 2010).

This results showed that the identification and understand the play a role that the genes of the chromosome X have in economic traits in is a challenging task. The region X:154866834-17401780 identified in the present study was partially overlapped with one region previously reported in genome-wide study by Teixeira et al. (2017) who reported a proportion of variance explained by windows with effects for stayability (0.71%), containg some genes as LOC101904216,TRNAD-GUC,MSL3, LOC104970633 and ARHGAP6 that have been associated with reproductive traits. De Camargo et al. (2015) performed a genome-wide association in the Nelore catlle and found genes located on the chromosome X as indicators of male fertility and correlated with female sexual precocity and reproductive longevity.

3.5 CONCLUSIONS

The ROH distribution obtainable in the present study suggests a higher contribution of remote inbreeding to autozigosity in this Nelore population since there was a high prevalence of short ROH in the genome.

The ROH islands mapping showed various candidate genes associated to growth, reproduction, feed efficiency, meat quality, adaptation and immune system traits.

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