Genome -wide association study for Anti-Müllerian hormone in Nellore cattle

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Summary

Among the economically relevant traits for the beef cattle industry, those linked to reproduction are more important than any other traits. Anti-Müllerian hormone (AMH) has been related as a biomarker to predict the number of health antral follicles for commercial beef cattle. However, information on genomic associated to that hormone is very rare in *Bos indicus*, the relevant breed group in tropical areas. Therefore, the primary objective of this study was to implement a genome-wide association study (GWAS) to identify genomic regions associated to AMH concentration in Nellore cattle, the most important breed in the Brazilian beef industry and investigating the genetic mechanisms underlie this trait. Data from 1,375 females belonging to four farms located in midwestern states of Brazil and genotypes from 917 females of those animals were used. The GWAS analysis was performed with BLUPF90 software using the postGSf90 program. The nine windows significant identified were located on chromosomes 1, 5, 8, 10, 11 and 17 that in total explained 15.86% of the additive genetic variance, considering windows of 150 adjacent SNPs. The identified genes are involved to regulation of ovarian follicle development, ovulation, luteal function, maternal recognition of pregnancy, implantation, maintenance of gestation, ovarian infections and on pituitary function, ubiquitin protein and spermatogenesis associated with fertility and an important signaling pathway increasing the understanding of biological pathways. These results provide pertinent information as the first step to help elucidate which genes affect this trait and further improve the hypothesis of AMH it has been used as a biomarker to predict fertility and utility in future experiments and breeding programs.

Keywords: anti-müllerian hormone, cattle, marker, ovulation, precocity,

Introduction

Reproductive traits are the most important indicators of beef industry profitability and economic value, especially to females (Boligon, et al., 2011). However, the majority of the reproductive traits presents problems as low heritability estimates, measure only on females and late in life (Costa et al., 2015; Terakado et al., 2015; Buzanskas et al., 2017) that infer directly on genetic improvement. In this way, to overcome these problems, indicators of the reproductive efficiency as anti-müllerian hormone (AMH) have been reported since is an important candidate as a marker of female reproductive capacity (Visser & Themmen, 2014; Mossa et al., 2017), play a role in regulating follicle selection (Poole et al., 2016), indirectly reflects the total number of morphologically healthy ovarian follicles in ovaries, and hence,

the ovarian reserve in ruminants (Monniaux et al., 2012) suggesting that AMH could be used as a model to fit the physiological mechanisms.

The GWAS method has been used frequently in livestock studies (Wang et al., 2013; Kadarmideen, 2014; Sharma et al., 2015), and can be a powerful tool to detect genetic variations to interest traits, however, for Zebu cattle (*Bos indicus*) none studies involved genomic analyses for AMH was founded in literature.

The primary objective of this study was to perform a GWAS approach to identify candidate genes and biological functions associated with AMH as a factor that contributes to the high variation in fertility in Nellore heifers.

Material and methods

Data

Phenotypic data from 1,375 females (Nellore cattle) born between 2013 and 2014 in four farms located in midwestern states of Brazil were used. Blood samples of those females were collected around fourteen months of age to obtain AMH circulating concentration (ng/ml). Plasma AMH was perfomed at IgAc (Institute Genese of Scientific Analyses, Sao Paulo, Brazil) and evaluates using enzyme-linked immunosorbent assay (ELISA) kit (Ansh Labs, Webster, TX, USA). The genotype data used were from 944 females, genotyped using the high-density Illumina Bovine HD Assay (Illumina, Inc., San Diego, CA, USA), which contains 777,962 SNPs. Quality control of the genotype data was performed at preGSf90 program process (Misztal et al., 2012). The following criteria were used for the exclusion of SNPs: minor allele frequency (MAF) less than 0.05%, call rate less than 90%, monomorphic SNPs, p-value for Hardy-Weinberg equilibrium less than 10^{-5} and duplicate samples were excluded. After quality control 917 animals and 467,209 SNPs remained for analysis. All phenotypes were collected by veterinarians involved in graduate programs of animal reproduction of the College of Veterinary Medicine and Animal Science of the University of Sao Paulo, located in Sao Paulo, Brazil.

Analysis

The dependent variable used in the analyze was the phenotype observed for AMH. To estimate the SNP effects, the genomic best linear unbiased prediction (GBLUP) (VanRaden et al., 2008) analysis was performed. The model was applied:

$$y = 1\mu + Zg + e \quad (1)$$

where y is the vector of the phenotypes for genotyped animals; 1 is vector of ones; μ is the overall mean; g is the vector of random breeding values; Z is the design matrix to allocating the breeding values; and e is a vector of random residual effect. Also, the contemporary groups (CG) formed by year of birth and farm of origin as fixed effect and the linear effect of age of animal at recording as a covariate were assumed.

The GWAS analysis was performed in BLUPF90 software using the postGSf90 program (Misztal et al., 2012). Results are expressed as the proportion of genetic variance explained considering windows of 150 adjacent SNPs after three interactions. The DAVID tool v6.8 (https://david.ncifcrf.gov/) was used to identify the biological function of the candidates genes.

Results and discussion

Nine SNPs windows that explained more than 1% of the genetic additive variance located on chromosomes 1, 5, 8, 10, 11 and 17 (Figure 1) were used to search for candidate genes. As reported by Irano et al. (2016), the sexual precocity indicators are controlled by a large number of genes, also observed in this study. The window located in chromosome (Chr) 8 at 75.9-76.4 Mb is responsible for the large significant SNP effect, explaining the proportion of genetic additive variance in 4.05%.

The gonadotropin releasing hormone 1 (GnRH1) and uromodulin like 1 (UMODL1), genes located at chromosome 8 and 1 respectively, are involved in regulation of ovarian follicle development, since stimulates the release of luteinizing and follicle stimulating hormones (Atkins et al., 2014). These results are confirmed by Rico et al. (2009) and Morotti et al. (2015) that obtained a strong correlation between AMH concentration and number of antral follicles (AFC) suggesting the measurement of AMH concentration helps to identify early precocity heifers through follicular and ovulatory responses. Also, it's possible combine these results from those described by Batista et al. (2014) that performed a comparative of follicogenesis and reproductive physiology between plasma AMH concentrations in *Bos indicus* and *Bos Taurus* heifers.

Chemokine CCL19 and CCL21 genes are also linked with stimulating hormone, which response to prostaglandin E which impact on ovulation, luteal function, maternal recognition of pregnancy, implantation, maintenance of gestation, ovarian infections and on pituitary function that play an important role on female reproduction (Weems et al., 2006). These genes also are presented on others important biological pathways.

Other genes can be grouped according the biological function, like those genes involved in the ubiquitin protein regulation system: UBQLN1, UBAP1, UBAP2, UBERR2, UBE2N, HPD, BAG1, DCAF12 and LEO1 which play a role in hormonal control of reproduction associated with early pregnancy, sperm mitochondria, bovine endometrium, uterine receptivity and important signaling pathway (Yang et al., 2016; Zhao et al., 2017). Also, interestingly, during the gametogenesis controlling in the meiosis process (Baarends et al., 1999).

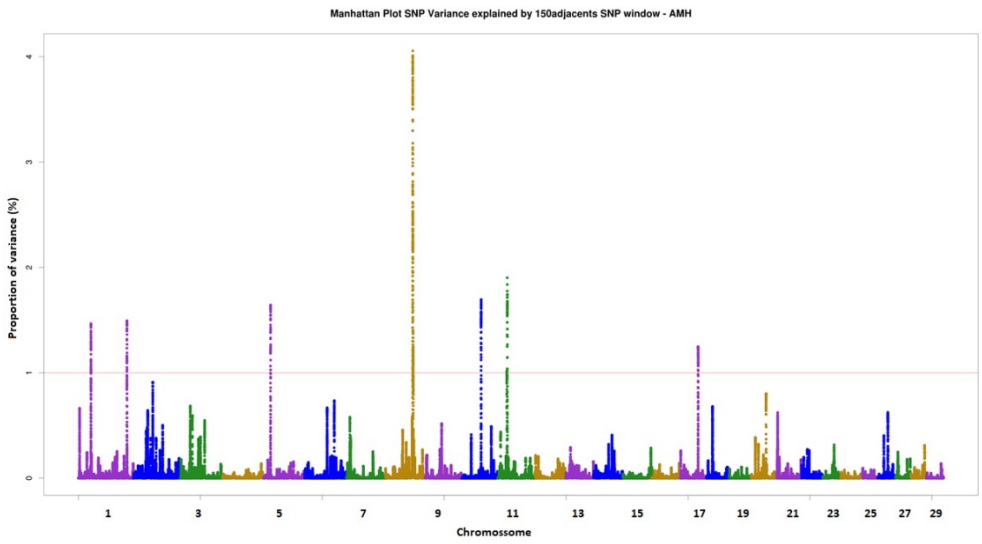

Figure 1. Manhattan plot for Anti-Müllerian hormone (AMH) in Nellore cattle. The X-axis

represents the chromosomes, and the Y-axis shows the proportion of genetic variance explained by windows of 150 adjacent SNPs.

Genes like B4GAL1 and GALT have important function in galactose metabolic process and has been related a variety of clinical manifestations in animals and human, which all galactose metabolites are proposed mechanisms include interference with ovarian apoptosis and gonadotrophin signaling (Liu et al., 2000). In sheep, Campbell et al. (2010) reported the effect on ovarian function, suggesting its potential role in the healthy antral follicle, also maintenance of gestation that could affect cattle as well.

The NFX1 gene located in Chr 8 at 76.8/77.3 Mb response to major histocompatibility complex (MHC) class II expression which provides for organism immune mechanism related to reproduction in cattle, since is relevant to the success of the fertilization that is characterized by the fusion of male and female gametes (Penn et al., 2002).

Besides the females were the focus on this study, some genes associated directly of males reproduction system were found. The genes KDM2 and MNS1 are essential for spermatogenesis process, which has been confirmed by Rajat et al. (2017) since the AMH is secret by Sertoli cells that play a role in spermatogenesis and it's important to sexual differential. The genes DNAI1 and KIF 27 leads at the sperm motility, especially on flagellum movement, that is related to fertility, also important to the success of embryo fertilization (Saacke et al., 2008). Considering these genes furthered gamete selection and cells differentiation can be possible confirm their assignment to variability and genetic selection.

The results have demonstrated that AMH could be applicable as an endocrine marker for ovarian status and fertility capacity for heifers. Also, support and refine further studies to better understanding and improve a model to select young cattle as oocytes donors, embryo production or for breeding season management more accurately accelerating the genetic gain and decreasing generation intervals, consequently reaching the genetic improvement. In addition, more investigation must be performed using a greater number of animals and genomic information to validate the AMH as a biomarker for beef cattle.

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