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Prospecting polymorphisms in the *PPP3CA* and *FABP4* genes and their association with early pregnancy probability in Nellore heifers

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ABSTRACT

Early pregnancy probability (P16) in heifers is a trait of high economic value for beef cattle production. Early pregnancy is defined based on the conception and calving of a heifer given that the animal had entered the breeding season at about 16 months of age and is considered a strong predictor of puberty in Nellore cattle. The aim of this study was to identify polymorphisms in the protein phosphatase 3 – catalytic subunit, alpha isozyme (*PPP3CA)* and fatty acid-binding proteins 4 (*FABP4)* genes, which have been associated with P16. The exon regions of the candidate genes were sequenced in 380 heifers. Two polymorphisms were detected in *PPP3CA* and 13 in *FABP4*. A deletion (rs134413439) was identified in the *FABP4* gene in all samples, which could be a variation that occurs across breeds since the reference genome is from a Hereford cow. Two SNPs in the *FABP4* gene were associated with P16 after Bonferroni correction. However, none of the haplotypes exerted a significant effect ($P > 0.05$). This study showed that the *PPP3CA* and *FABP4* genes are polymorphic in Nellore cattle. Furthermore, the *FABP4* gene was found to be associated with early pregnancy in heifers, reinforcing the contribution of lipid metabolism to reproduction.

1. Introduction

Reducing the age at first conception and first calving promotes improvement of reproductive efficiency and has a major impact on the efficiency of beef cattle production systems (Beretta et al., 2001; Fortes et al., 2010; Teixeira et al., 2002). Furthermore, this reduction positively influences genetic progress by decreasing the generation interval and permitting to increase the intensity of selection (Van Melis et al., 2010). Early pregnancy probability (P16) is considered a good indicator of age at puberty and might be used as a selection criterion. However, since this trait is expressed late and only in females, it has been little used in breeding programs not only in Brazil but also in the rest of the world (MacNeil et al., 2006).

In an attempt to identify the association between known polymorphisms in genes related to adipose tissue and P16 in Nellore heifers, Dias et al. (2015) used data from 1689 precocious (calvings up to 18 months) and non-precocious heifers genotyped with the High-Density Bovine SNP BeadChip (Illumina, Inc). Fifty-seven candidate genes and 443 SNPs were analyzed. The authors found a significant effect of haplotypes located in the protein phosphatase 3 – catalytic subunit, alpha isozyme (*PPP3CA*) and fatty acid-binding proteins 4 (*FABP4*) genes on P16 and concluded that these genes exert an influence on this trait.

Among many factors, the influence of adipose tissue on P16 is due to the fact that this tissue is one of the main sites of sex steroid metabolism (Kershaw and Flier, 2004). Taniguchi et al. (2008) found the *PPP3CA* gene to be differentially expressed and it is down-regulated during the adipogenesis. Furthermore, Martin et al. (2007) observed that blockade of this gene caused infertility in male rats.

The *FABP4* gene is a member of the FABP family which are fatty acid-binding proteins. The gene is located on bovine chromosome 14. Studies have reported an association of this gene with variations in cholesterol percentage (Hoashi et al., 2008). Michal et al. (2006) demonstrated a key role of FABP4 in the absorption, transport and metabolism of fatty acids. Free fatty acids that are transported by FABP4 can be activated and oxidized to produce acetyl-CoA and NADH. Part of this acetyl-CoA is used for the biosynthesis of cholesterol which, in turn, is the precursor of steroid hormones (Berg et al., 2002). Steroids are

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involved in the differentiation, growth and physiology of reproductive organs (Havlíková et al., 2006; Pearce and Jordan, 2004).

The objective of the present study was to identify polymorphisms in the exon regions of the *PPP3CA* and *FABP4* genes and to evaluate their possible association with P16 in heifers.

2. Material and methods

2.1. Animals

The data and samples used in the present study were collected from 2036 Nellore heifers born in the years 2007 and 2008 and exposed to bulls in the anticipated breeding seasons of 2009 and 2010 on farms belonging to the company Agropecuária Jacarezinho Ltda., State of Bahia, Brazil. Data regarding pregnancy (success or failure) were obtained, as well as information about the pedigree, register and management of these animals. In addition, hair follicles were collected from the tail for DNA extraction.

On these farms, the animals were exposed to bulls during two breeding seasons. An anticipated breeding season lasting 60 days occurs between February and April to identify animals that reach puberty early. All heifers aging 16–18 months were exposed, regardless of weight and body condition. The mating system for heifers is a controlled breeding, with the ratio of 1:50 bull per heifers. Approximately, 60 days after the end of the anticipated breeding season, heifers were submitted to rectal palpation for the evaluation of pregnancy. Heifers that did not conceive in the anticipated breeding season are used in the cow breeding season of the subsequent year when they are about two years of age.

During the step of data preparation, inconsistent data resulting from registration errors were eliminated. The contemporary groups (CG) were formed considering all effects shared by the animals and always maintaining connectedness among groups. For P16, the CG were defined by farm, year and season of birth of the heifers. The CG containing less than ten animals and those without variation in the trait (all females became pregnant or all females were empty) were excluded. After analysis of consistency, 1689 heifers remained and the rate of early pregnancy at 16 months was 30.52%. Of these, 380 heifers were selected for sequencing of the candidate gene regions based on the pregnancy rate of the CG, considering a minimum rate of 30%. Thus, the 380 females selected belonged to 19 CG and the rate of early pregnancy of the 380 heifers was 40.26%.

2.2. Candidate genes

Based on the position of the haplotypes described by Dias et al. (2015), exon regions that flank or a located within haplotypes exhibiting significant effects at the 5% level were delimited. Thus, the four exons of the *FABP4* gene and exons 2 and 3 of the *PPP3CA* gene were used. Fig. 1, adapted from Ensembl (Cunningham et al., 2015), illustrates the haplotypes described by Dias et al. (2015) and the exons selected for sequencing.

Table 1
Region per amplicon containing the exon/intron of the candidate genes.

Gene	Chr	Location		Length (bp)
		Start	End	
PPP3CA	6	24,812,677	24,812,991	314
PPP3CA	6	24,985,650	24,985,860	211
PPP3CA	6	25,060,198	25,060,332	135
FABP4	14	46,833,660	46,833,886	227
FABP4	14	46,834,340	46,834,451	112
FABP4	14	46,835,034	46,835,216	183
FABP4	14	46,837,916	46,838,058	143

Chr: chromosome.

2.3. DNA extraction, customized panel of MiSeq sequencing, and detection of variants

DNA was extracted from the caudal hair follicle by the phenol-chloroform-isoamyl alcohol method (Sambrook et al., 1989). A custom panel was built with the TruSeq Custom Amplicon® Kit (Illumina, Inc) using the DesignStudio software (available at: http://designstudio.illumina.com/) based on the position of the exons. For full coverage of the exon region, part of the introns and intergenic region was used. The size of the amplicon used for customization was 425 bp, ensuring 100% coverage of the delimited region of each exon (Table 1).

An amount of 250 ng DNA was used to generate amplification products covering the regions. Indexed adapters were added to the ends of the DNA amplicons by limited cycle PCR. The DNA libraries obtained were validated in an Agilent Bioanalyzer 2100 (Agilent Technologies, USA). Next, the DNA libraries were sequenced (2 × 250 paired-end) with the Illumina MiSeq® System using the MiSeq Reagent Kit v2 (500 cycles) according to manufacturer instructions. Image analysis and base calling were performed using the MiSeq software.

The sequences in FASTQ format were aligned with the UMD3.1. bovine reference genome through BaseSpace® (available at: https://basespace.illumina.com/) (Illumina, Inc.) using the TruSeq Amplicon tool. The same tool was employed for the detection of variants using the somatic option. Only variants with a minor allele frequency (MAF) > 5% were considered. Gene annotation was performed with Ensembl and only variants with a Phred quality score ≥ 40 were considered.

2.4. Allele and Genotype Frequencies and Hardy-Weinberg Equilibrium

Descriptive statistics were generated with the Plink software (Purcell et al., 2007). Allele and genotype frequencies of the SNPs were calculated as the ratio between the number of a certain allele and the total number of alleles (Falconer and Mackay, 1996). Possible deviations of the observed genotype frequency from the expected frequency were tested by the chi-square test in order to determine whether the locus of interest was in Hardy-Weinberg equilibrium (HWE) in the population sample studied (Falconer and Mackay, 1996).

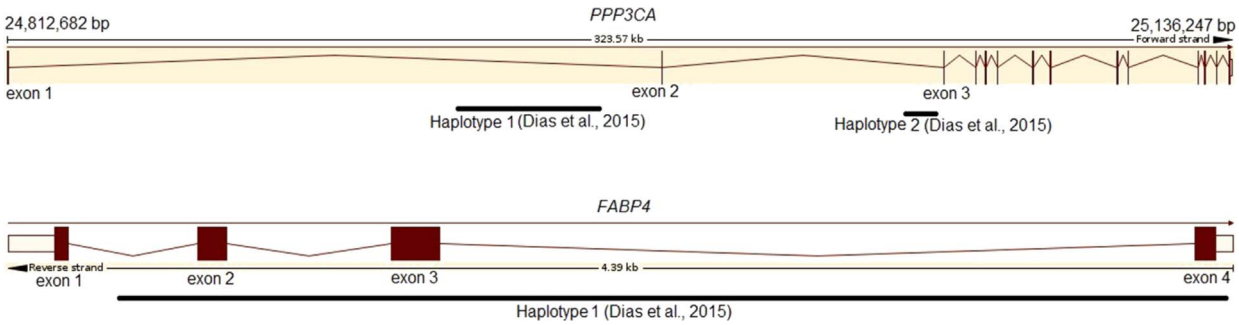

Fig. 1. Schematic diagram, adapted from Ensembl, illustrating the position of haplotypes with significant effects (Dias et al., 2015) and the exons selected for sequencing.