Genome association analysis for pregnancy status following parturition in crossbred beef cattle

Estudo de associação genômica de prenhez pós-parto em vacas de corte

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Abstract

The aim of this study was to evaluate genetic diversity of nine molecular markers, six short tandem repeats - STRs (BM4325, BMS3004, ILSTS002, IDVGA51, HEL5, AFZ1) and three single nucleotide polymorphisms (SNPs; Lep*Sau*3A1 A-B, Lep*Sau*3A1 1-2, and FSHR*Alu*1), linked to genes involved in reproductive function and their possible effect on reproductive performance. For this purpose, 81 crossbred beef cows were used in this study. The animals were classified into two groups (fertile and sub-fertile cows) based on their pregnancy status after two breeding seasons. High genetic diversity level was observed highlighted by the polymorphic content information ranging 0.23 to 0.87 and expected heterozygosity from 27 to 89%, with an average of 62%. Alleles *BM4325 103, BMS3004 129, ILSTS002 137, IDVGA51 177, LEPSau3A1 A, LEPSau3A1 1, HEL5 149, AFZ1 119* and *FSHRAlu1 G* presented high frequencies. Two STRs (IDVGA51 and ILSTS002), linked to Leptin and LHβ genes, respectively, were associated to reproductive performance. These data support previous findings suggesting the potential use of IDVGA51 and ILSTS002 STRs for reproductive performance selection.

Keywords: Bovine. Marked-assisted selection. Reproductive performance. Short tandem repeats. Single nucleotide polymorphisms.

Resumo

Foi avaliada a diversidade genética de nove marcadores moleculares, dos quais seis do tipo *short tandem repeats* - STR (BM4325, BMS3004, ILSTS002, IDVGA51, HEL5, AFZ1) e três do tipo *single nucleotide polymorphisms* - SNPs (Lep*Sau*3A1 A-B, Lep*Sau*3A1 1-2 e FSHR*Alu*1), ligados a genes envolvidos na reprodução e seus efeitos na performance reprodutiva. Foram examinadas amostras de sangue de 81 vacas sem raça definida, os animais foram classificados em dois grupos (vacas férteis e subférteis) baseado nas taxas de prenhez de duas estações reprodutivas. Alto nível de diversidade genética foi observado, revelando alto conteúdo de informação polimórfica, variando de 0,23 a 0,87 e heterozigosidade esperada de 27 a 89% com 62% em média. Os alelos mais frequentes foram *BM4325 103*, BMS3004 129*, ILSTS002 137*, IDVGA51 177*, LEPSau3A1 A, LEPSau3A1 1, HEL5 149*, AFZ1 119** e *FSHRAlu1 G.* Os marcadores IDVGA51 e ILSTS002, ligados aos genes da leptina e LHβ, respectivamente, foram associados a performance reprodutiva. Esses dados suportam achados prévios que sugerem o potencial uso desses marcadores na seleção de animais com maior performance reprodutiva.

Palavras-chave: Bovinos. Seleção assistida por marcadores. Performance reprodutiva. Repetições curtas em tandem. Polimorfismos de nucleotídeo único.

Introduction

Reproductive efficiency is a major factor affecting livestock production. Ovarian activity during postpartum period depends on follicle stimulating hormone *(FSH),* luteinizing hormone *(LH)*, insulin like growth factor *(IGF-1)* (BUTLER et al., 2008; THIENGTHAM; PARKINSON; HOLMES, 2008),

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Received: 17/06/2013
Approved: 08/01/2014

leptin *(LEP)* and their receptors (LIEFERS et al., 2003; LIEFERS et al., 2005; PASSOS et al., 2007). At the end of pregnancy, hypothalamic-pituitary axis responds to a negative feedback effect from placental and ovarian steroids by suppressing FSH release and depleting LH stores (YAVAS; WALTON, 2000a). After reestablishment of LH stores in the anterior pituitary, between Days 15 and 30 postpartum (YAVAS; WALTON, 2000a), cow-calf connection increases the postpartum anestrous due to the negative effect on LH release, which affect the final oocyte maturation and ovulation (WILLIAMS et al., 1996; YAVAS; WALTON, 2000b). IGF-1 and leptin are other important mediators of dietary intake and/or energy balance effects on cow's fertility. Increased concentrations of IGF-1 could directly stimulate proliferation or steroidogenic capacity of thecal and/or granulosa cells (DISKIN et al., 2003). Leptin, an adipocyte hormone, regulates energy homeostasis, plays a role in signalizing nutritional status to the central reproductive axis and seems to control LH and FSH release by the pituitary (MOSCHOS; CHAN; MANTZOROS, 2002; ZIEBA; AMSTALDEN; WILLIAMS, 2005).

Genetic markers or polymorphisms, inside or linked to IGF-1 and Leptin could influence their expression by modifying DNA conformation and, therefore, could affect the postpartum anestrus interval. Thus, genetic markers can improve animal selection by a methodology named marker-assisted selection (MAS) (DAVIS; DENISE, 1998; DEKKERS, 2004). Previous studies described associations among reproductive performance (ALMEIDA et al., 2003; DUARTE; MORAES; WEIMER, 2005; OLIVEIRA et al., 2005; WEIMER et al., 2007), STRs (short tandem repeats) and SNPs (single nucleotide polymorphisms) within leptin, IGF-1, FSH and LH receptors genes, however, not related to crossbred herds. Studies searching for associations among bovine superovulatory response, embryo production and the FSHR gene are described (AGUIAR, 2008; CORY et al., 2013). Polymorphisms found in FSHR gene are associated with reproductive characteristics in humans, such as FSH concentrations in women (MARCA et al., 2013) and testicular volume in men (GRIGOROVA et al., 2013). In Holstein cattle, the FSHR has been investigated as a superovulatory marker (YANG et al., 2010).

Based on genetic and reproductive interactions, the objective of this study was to evaluate and describe genetic diversity of nine molecular markers (six short tandem repeats - STR, and three single nucleotide polymorphisms -SNP) in crossbred beef cows, which represents the major bovine commercial target, and its association with pregnancy status following parturition of beef cows.

Material and Methods

Animals

Crossbred cows (n = 81), resulting from the crossing of Braford bulls with Hereford, Aberdeen Angus, Normand, Limousin, Brown Swiss, or Brangus (5/8 Aberdeen Angus X 3/8 Nelore), multiparous, age from four to six years old and a body condition score of 3 (MORAES; JAUME; SOUZA, 2007) at Days 60 to 81 postpartum, were selected for the experiments. Therefore, animals younger than four years (negative nutritional balance) were not included, and mature animals with body condition around 3 were included in order to have a 50% chance of pregnancy. All animals were managed exclusively on native grass pasture, mainly composed of *Paspalum* sp. and *Axonopus* sp. Native grass pasture yields 1,500 to 3,000 kilograms of dry matter per hectare in this region.

Climate and management characteristics

According to the Köppen's classification (PEEL; FINLAYSON; MCMAHON, 2007), the climate of this region is subtropical with consistent rainfall throughout the year. Average annual rainfall is 1350 mm, ranging from 1080 to 1620 mm. Average annual temperature is 17.6ºC, with January the hottest month (24ºC), and June the coldest (12.5ºC). The soils of the region are predominantly represented by Fluventic

Eutrochrepts fine clay type (EMBRAPA, 1999), with average levels of organic matter exceeding 3% on the horizon surface. Levels of phosphorus are low and those of calcium are high (MACEDO, 1984). The annual breeding season lasts from mid-November to mid-January. Cows that remain open have an extra breeding season from early April to mid-May. Both breeding seasons were performed with fertile bulls. Animals were classified into two groups (fertile and sub-fertile) based on their pregnancy status after the two breeding seasons.

Animal groups and samples collection

Blood samples were collected from the tail vein or artery in EDTA following the Brazilian Principles of Veterinarian Medical Ethics (CFMV, Código de Ética Profissional do Médico Veterinário, 2001) and the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, International Guiding Principles for Biomedical Research Involving Animals, 1985), and stored at -20°C until processed. Forty-two out of 81 cows were classified as fertile. The fertile group produced a calf in the previous reproductive season and became pregnant during the first breeding season, with a calving interval shorter than 365 days. The remaining 39 cows were classified as sub-fertile because they did not calf in the previous reproductive season and remained open after two breeding seasons. The fertile group was submitted to pregnancy diagnostic twice during the spring and winter to confirm pregnancy status before sample collection, while sub-fertile group was examined once during the spring.

Polymorphisms detection

DNA was extracted from peripheral blood mononuclear cells (PBMCs) as described by Miller, Dykes and Polesky (1988). STRs were amplified by the polymerase chain reaction (PCR) as previously described (KEMP; BREZINSKY; TEALE, 1992; BISHOP et al., 1994; HOUDE et al., 1994; JORGENSEN; KONFORTOV; MILLER, 1996; STONE; KAPPES; BEATTIE, 1996; KAPPES et al., 1997; POMP et al., 1997) and SNPs according to Houde et al. (1994); Stone, Kappes and Beattie (1996) and Marson et al. (2005). STR amplification products and SNP amplicons (submitted to *Sau*3AI or *Alu*I endonucleases cleavages) were analyzed by vertical electrophoresis in non-denaturing polyacrylamide gel (LAHIRI; ZHANG; NURNBERGER JR., 1997). The STR ILSTS002 presented some amplification issues and only 40, instead of 42, of the fertility cows were analyzed for this marker.

Statistical analyses

Genotype and allele frequencies were determined by direct counting. Expected heterozygosity (H) and polymorphism information content (PIC) were estimated according to Nei (1978) and Botstein et al. (1980). Association analyses between alleles or genotypes and pregnancy state were performed using the Fisher exact test comparing all alleles or genotypes randomly; when a positive association was detected, the significant class was compared with the others by a Chi-Square test. When the associations involved more than one allele, they were grouped in classes of short and long alleles as suggested by Comings (1998). This approach was used for IDVGA51 (short alleles: 173-177 bp; long alleles: 179-183 bp) and ILSTS002 (short: 127-137 bp; long: 139-143 bp) markers. As the animals had been managed exclusively on extensive livestock system, no individual information was available for statistical analyses. All statistical analyses were made using SPSS software (v. 10.0.5, 1999).

Results

Allele frequencies for STR and SNP markers are presented in Table 1. The number of alleles in STR markers varied from three in BMS3004 to thirteen in HEL5, and the most frequent alleles were: *BM4325 103, BMS3004 129, ILSTS002 135* and *ILSTS002 137, IDVGA-51 177, HEL5 149* and *AFZ1 119*. The most

frequent SNPs were: LEP *Sau*3A1 A, LEP *Sau*3A1 1, and FSHR Alu1 G alleles. Polymorphic content information ranged from 0.23 to 0.87 while expected heterozygosity from 27 to 89%, with an average of 62%.

Significant association was verified between *IDVGA51* and ILSTS002 and reproductive status. Cows carrying at least one *IDVGA51 181* allele (P = 0.05) were more frequent in the sub-fertile group, while cows carrying the alleles *IDVGA51 173 (P = 0.01)* and *IDVGA51 177 (P = 0.02)* were more frequently found in the fertile group (Table 2). Based on these results, alleles were grouped in classes according to the size. Short alleles (173-177 bp) were carried in a significantly higher frequency *(P = 0.04)* by the fertile cows.

For ILSTS002 STR (Table 2), only one association was verified. Cows with at least one ILSTS002 137 allele were about 20% more frequent in the sub-fertile cows group *(P = 0.03)*. No significant associations were verified between the fertility groups and genotypes or allele classes for BM4325, BMS3004, HEL5, AFZ1, LEP*Sau*3A1 and FSHr*Alu*1 markers.

Discussion

The genetic diversity of markers analyzed in this herd is one of highest so far detected within cattle populations in southern Brazil (Table 3). These data suggests that the management applied to this herd is not affecting the genetic variability expected for crossbred herds.

Associations between reproductive performance and molecular markers have been described (Table 4). Comparing the present study with previous data (Table 4), only two associations were verified, the IDVGA51 and ILSTS002 STRs. Almeida et al. (2003) analyzed lifetime calving intervals of beef cows and

Table 1 - Allele frequencies of nine molecular markers (six STRs and three SNPs) in a beef cattle herd

Markers	Alleles													H	PIC
SRTs															
BM4325	***97***	***99***	***101***	***103***	***105***	***107***	***109***	***111***							
	0.01	0.03	0.13	0.50	0.25	0.04	0.03	0.01						0.67	0.62
BMS3004	***129***	***132***	***138***												
	0.66	0.29	0.05											0.48	0.40
ILSTS002	***127***	***129***	***131***	***133***	***135***	***137***	***139***	***141***	***143***	***145***					
	0.01	0.01	0.04	0.11	0.27	0.28	0.06	0.18	0.03	0.01				0.80	0.76
IDVGA-51	***173***	***175***	***177***	***179***	***181***	***183***	***185***								
	0.09	0.27	0.30	0.09	0.09	0.14	0.02							0.79	0.76
HEL5	***147***	***149***	***151***	***153***	***155***	***157***	***159***	***161***	***163***	***165***	***167***	***169***	***171***		
	0.06	0.18	0.15	0.10	0.03	0.02	0.02	0.04	0.09	0.12	0.10	0.05	0.03	0.89	0.87
AFZ1	***111***	***113***	***115***	***117***	***119***	***121***	***123***	***125***	***127***	***129***					
	0.01	0.05	0.18	0.06	0.21	0.13	0.16	0.14	0.05	0.01				0.85	0.83
SNPs															
LEP*Sau*3A1 (A/B)[1]	***A***	***B***													
	0.77	0.23												0.34	0.28
LEP*Sau*3A1[1]	***1***	***2***													
	084	0.16													
FSHR*Alu*1	***C***	***G***													
	0.45	0.55												0.49	0.37

[1]LEPSau3A1. Allele B and allele 2 = presence of restriction site.

Table 2 - Association analysis performed between IDVGA51 and ILSTS002 alleles (N = number of alleles) or allele groups (short or long) and fertility status in a beef cattle herd

Comparisons	Fertile cows		Sub-fertile cows		χ^2	P
	N	%	N	%		
IDVGA 173 x	12	14	3	4	5.82	0.01
Others alleles	72	86	75	96		
IDVGA 177 x	32	38	17	22	5.34	0.02
Others alleles	52	62	61	78		
IDVGA 181 x	4	5	10	13	3.67	0.05
Others alleles	80	95	68	87		
IDVGA Long alleles (179-185 bp)	22	26	32	41	4.0	0.04
IDVGA Short alleles (173-177 bp)	62	74	46	59		
ILSTS002 137x	14	17	30	38	4.61	0.03
Others alleles	66	83	48	62		
ILSTS002 135x	26	32	17	22	1.56	0.21
Others alleles	54	68	61	78		
ILSTS002 Long alleles (139-145bp)	23	29	21	27	0.66	0.79
ILSTS002 Short alleles (127-137bp)	57	71	57	73		

Table 3 - Observed heterozygosity in several cattle breeds from southern Brazil

Breed	H	References
Brangus	0.63	Almeida *et al.* (2003); Duarte *et al.* (2005); Oliveira *et al.* (2005); Weimer *et al.* (2007)
Nelore	0.53	Aguiar *et al.* (2008)
Charolais	0.42	Almeida *et al.* (2007)
Aberdeen Angus	0.51	Aguiar *et al*(2008)
Crossbred	0.62	Present paper

Table 4 - Associations previously described between genetic markers and reproductive parameters in beef cattle from Southern Brazil

Markers	Gene	Associations	References
BM4325	*FSHβ*	Carriers of *101* allele presented lower calving interval (CI)	Duarte *et al.* (2005)
ILSTS002	*LHβ*	Carriers of *135* allele presented higher CI	Weimer *et al.*(2007)
BMS3004	*LHβ*	Heterozygous animals presented lower CI	Weimer *et al.* (2007)
IDVGA51	*Leptin*	Carriers of *181* allele presented higher CI	Almeida *et al.* (2003)
HEL5[1]	*IGF-IR*	Homozygous for long alleles presented higher CI	Oliveira *et al.* (2005)
AFZ1[2]	*IGF-IR*	Homozygous for short alleles presented higher CI	Oliveira *et al.* (2005)
Lep *Sau*3A1 (1/2)	*Leptin*	Carriers of *2* allele presented higher CI	Almeida *et al.* (2003)
Lep *Sau*3A1 (A/B)	*Leptin*	Heterozygous animals presented higher weight at first calving	Almeida *et al.*(2003)
FSHR *Alu*1 (G/C)	*FSHR*	Nelore cattle the G allele (0.745) was more frequent than the C allele (0.255)	Marson *et al.*(2005)

[1] Short alleles: 147-157; long alleles: 159-169;
[2] Short alleles: 115-121; long alleles: 123-129.

observed that *IDVGA51 181* allele was associated to poor reproductive performance. In this paper it was have found two new associations previously unreported. Alleles IDVGA51 173 and IDVGA51 177 were positively associated with pregnancy status. When the alleles were grouped according to their size (in base pairs), animals carrying short alleles (173-177 bp) presented better reproductive performance than animals carrying long alleles (Table 2). IDVGA51 is mapped at 87 cM from the beginning of BTA4 chromosome and at 2 cM downstream from the *LEP* gene (KAPPES et al., 1997). Therefore, the possibility of IDVGA51 as an enhancer transcription region was not excluded (a region with sequences that activate transcription and can be located thousands of base pairs upstream or downstream from the controlled gene); thus, influencing gene expression (LI et al., 2004), and the effect being dependent of number of repeats (COMINGS, 1998). According to Passos et al. (2007), cows carrying the IDVGA51 181 allele showed higher expression of the *LEP* gene in the subcutaneous tissue. It is well known that leptin plays a role in the reproductive status by linking reproduction and nutrition (CHEHAB et al., 2002). Leptin expression is regulated by several hormones and proteins, and stimulates LH and FSH release by the pituitary (LIEFERS et al., 2005). Additionally, leptin excess, deficiency, or resistance is associated with abnormal reproductive function (MOSCHOS; CHAN; MANTZOROS, 2002). It is possible, that the differences in reproductive performance between groups carrying long or short alleles could be due to difference in *LEP* gene expression.

Another association detected was a significant higher frequency of *ILSTS002 137* in cows with lower reproductive performance (Table 2). In a previous study, Weimer et al. (2007) reported that animals carrying at least one *ILSTS002 135* allele have on average, longer calving intervals over the reproductive lifetime. Several reasons may explain the difference between the previous findings by Weimer (2007) and the present study. It would be highly unlikely to be due to an error in genotyping, since all genotype analyses were performed using a control sample supplied by Weimer et al. (2007). This is also unrelated to the length of alleles, as there were no significant differences between short (127-137 bp) and long (139-145 bp) alleles. The discrepancy may be the consequence of linkage disequilibrium (LD) between ILTS002 STR and a mutation in the *LHβ* gene, which modify its expression. However, due to the absence of pedigree information, this hypothesis could not be tested. ILSTS002 is mapped at 59.9 cM from the beginning of BTA18 chromosome (KEMP; BREZINSKY; TEALE, 1992) 6 cM apart from *LHβ* gene. It could play a role in gene regulation, even being distant from the *LHβ* gene. According to Li et al. (2004) STRs downstream or upstream from target gene sequences (even located thousands cM from the target gene) could regulate gene expression by altering the primary, secondary or tertiary structure of DNA, by binding to transcription or translation factors, or by affecting RNA editing.

In contrast to previous findings (ALMEIDA et al., 2003; DUARTE; MORAES; WEIMER, 2005; OLIVEIRA et al., 2005; WEIMER et al., 2007), no association was found between reproductive performance and the BMS3004, BM4325, HEL05, AFZ1, and LEP *Sau*3/A1 markers. Therefore, these associations seem to be specific to the previous herds and might not be useful for MAS in other cattle breeds or herds. It is important to note that the studies involving BMS3004, BM4325, HEL05, AFZ1, and LEP *Sau*3/A1 were performed based on multiple calving interval records. Thus, it is possible that the analysis of a single calving interval, as in the present study, does not provide sufficient statistical power to corroborate the previous reports.

Herein, association analyses confirmed previous results showing that two STRs, IDVGA51 and ILSTS002, linked to *LEP* and *LHβ* genes, respectively, are associated to reproductive performance. It is suggested the use of IDVGA51 and ILSTS002 STRs

in the implementation phase of Marker-Assisted Selection to obtain animals with better reproductive performance. Furthermore, it is recommended the use of these markers as a tool to generate and select a better offspring and also reduce the time selecting animals with an optimum post-partum pregnancy capability.

Acknowledgements

This work was supported by Programa de Apoio a Núcleos de Excelência (PRONEX), Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Financiadora de Estudos e Projetos (FINEP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenadoria de Estudos e Projetos (CAPES), EMBRAPA/Pecuária Sul and Universidade Luterana do Brasil. We thank L. Carneiro, N. Benavides for the laboratory support and Dr. Luiz E. Henkes and Dr. Alfredo Antoniazzi for the critical reading of the manuscript.

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