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Diversity of microsatellites linked to the *FSH*β gene, their usefulness for individual identification and association with reproductive performance

Diversidade de microssatélites ligados ao gene *FSH*β, sua utilidade para identificação individual e associação com o desempenho reprodutivo

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ABSTRACT

*The genetic diversity of three microsatellites (ILSTS027, MBO22, BM4325) mapped on the bovine chromosome 15 and linked to the follicle-stimulating hormone beta gene (FSHß) was investigated in cows of a Brangus Ibagé herd and the efficiency of these markers for individual identification and parentage control was estimated. Possible associations between each molecular marker and cow reproductive performance were also analyzed. Six alleles were detected in BM4325 and ILSTS027 and 12 in MB022, the most frequent being BM4325*101, BM4325*103, ILSTS027*169 and MB022*229. Polymorphic information content ranged from 0.58 to 0.88 while expected heterozygosity ranged from 65% to 89%, with an average mean value of 77%. Although only three markers were studied, the combined values indicate a high power of exclusion of a false parentage (94%) and of individual identification (3.8 x 10^{-4}). The association analyses based on statistics parameters [MB022 (n=104, CI=545.3±127.0, WFC=349.9±53.4), BM4325 (n=106, CI=542.2±124.9, WFC=350.5±54.4) and ILSTS027 (n =105, CI=543.4 ± 124.5, WFC=350.1±54.5)] indicated no positive association between each microsatellite and weight at first calving. Calving interval (CI) also seemed not to be influenced by the ILSTS027 or MB022 system. However, carriers of at least one BM4325*101 allele presented a CI about 54 days shorter than the other animals (P=0.04; n=106). This marker could be useful for marker-assisted selection, allowing the improvement of reproductive performance, at least in the Brangus Ibagé herd.*

Key words: *microsatellites, bovine genetic diversity, individual identification, reproductive performance, marker-assisted selection.*

RESUMO

*A diversidade genética de três microssatélites (ILSTS027, MBO22, BM4325) mapeados no cromossomo bovino 15 e ligados ao gene do hormônio folículo estimulante, cadeia β (FSHβ) foi investigada em fêmeas de um rebanho bovino Brangus Ibagé. Além de estimar a variabilidade genética do rebanho, avaliou-se a eficiência destes marcadores para a identificação individual e controle de paternidade. Verificaram-se também possíveis associações entre os marcadores e o desempenho reprodutivo. Seis alelos foram detectados em BM4325 e ILSTS027 e 12 foram observados em MB022, os mais freqüentes sendo, BM4325*101, BM4325*103, ILSTS027*169 e MB022*229. O conteúdo de informação polimórfica variou entre 0,58 a 0,88 enquanto a heterozigosidade esperada oscilou entre 65% e 89%, sendo o valor médio de 77%. Embora apenas três marcadores tenham sido investigados, os valores combinados indicam alto poder de exclusão de um falso progenitor (94%) e de identificação individual (3,8 x 10^{-4}). As análises de associação baseadas nos parâmetros estatísticos [MB022 (n=104, CI=545,3±127,0, WFC=349,9±53,4), BM4325(n=106, CI=542,2±124,9, WFC=350,5±54,4) e ILSTS027(n=105, CI=543,4±124,5, WFC=350,1±54,5)] não indicaram associação positiva entre os microssatélites e o peso da vaca ao parto. O intervalo entre partos também não parece ser influenciado pelos marcadores ILSTS027 ou MB022. No entanto, portadores de pelo menos um alelo BM4325*101 apresentaram intervalo entre partos 54 dias mais curto que os demais animais (p=0,04; n=106). Este marcador pode ser útil para seleção assistida por marcadores, permitindo a melhoria do desempenho reprodutivo, pelo menos no rebanho Brangus Ibagé.*

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Palavras-chave: *microssatélites, diversidade genética em bovinos, identificação individual, desempenho reprodutivo, seleção assistida por marcadores.*

INTRODUCTION

Molecular markers can be used to improve animal selection by a methodology known as marker-assisted selection (MAS). This method is particularly important to study traits of difficult measurement and low heritability, and to select young animals for traits that can only be measured after sexual maturity (MONTGOMERY & KINGHORN, 1997). The first step in the development of a MAS program is the detection phase, in which DNA polymorphisms are used as linked or direct markers to detect quantitative trait loci (QTL) segregation in a particular population (DAVIS & DENISE, 1998). Molecular markers can also be useful in parentage and individual identification, in the analysis of breeding control and in the identification of gene introgression for livestock improvement (WEIMER, 2003). However, the genetic variability of the population should be evaluated, before the first MAS phase and before the use of genetic markers for parentage or individual identification, .

Microsatellites are DNA markers abundant and are distributed all over the genome with a high degree of polymorphism, being excellent markers for the use in MAS and in parentage and breeding control.

Follicule stimulating hormone (FSH) is a pituitary gonadotropin consisting of an α–subunit common to all gonadotropins, noncovalently associated with a specific β–subunit that determines the FSH activities. This hormone stimulates follicular development in the ovary, prepares follicles for ovulation and luteinization, induces the LH receptor in granulose cells so that they become responsive to LH, stimulates the production of the steroid hormone, progesterone, stimulates aromatase which converts testosterone to estrogen, and stimulates the production of plasminogen activators (WARD et al., 1991). FSHβ is a candidate gene for the evaluation of associations between genetic markers and cow productive performance that could be useful in MAS, due to its involvement in all of these steps of reproductive development.

This investigation analyzes the genetic diversity concerning three microsatellites located on the bovine chromosome 15 close to the FSHβ (mapped in a relative position of 59.9cM) gene: (BM4325, MB022, both in the same relative position as FSHβ, 59.9cM and ILSTS027, at 66.3 cM; http://www.marc. usda.gov/genome). In addition to the evaluation of the genetic variability of the herd, we estimated the efficiency of these markers for individual identification and parentage control and the possible associations between each of these molecular markers and cow reproductive performance.

MATERIAL AND METHODS

Samples were obtained from females of a Brangus Ibagé herd. This is a composite beef cattle (5/8 Aberdeen Angus x 3/8 Nelore) resulting from the crossing between Aberdeen Angus cows (***B. primigenius taurus***) and Nelore bulls (***B. primigenius indicus***) performed by the Brazilian Agricultural Research Corporation (EMBRAPA Pecuária Sul, Bagé, RS, Brazil). The selection program began in 1945, emphasizing body weight measurements at birth, at weaning adjusted to 205 days and at 18 months of age, without any special criterion on selection for fertility. All the animals have been managed exclusively on native pasture in an extensive livestock system (OLIVEIRA et al. 1998), with the mating season extending from November 15 to February 15. Calving interval (CI) data were obtained for females of the experimental herd as described by OLIVEIRA et al. (2002). As an indicator of cow fertility the weight at first calving (WFC) was computed as a predictor of the growth potential of the heifers. The breeding procedure includes single sire mating in small paddocks in groups of about 40 females, for paternity identification. Among a total of 287 cows, samples were obtained of 106 animals from which there were information about at least three CI. Blood sample was obtained from the jugular vein using ACD (citric acid, citrate, dextrose) as anticoagulant (ALMEIDA et al., 2003)

Genomic DNA was extracted from blood samples by the method of PLANTE et al. (1992). Microsatellites were amplified by the polymerase chain reaction (PCR) as described by STONE et al. (1996), but using primers and annealing temperatures specific for each fragment (Table 1). PCR products were analyzed by vertical electrophoresis in 7% non-denaturing polyacrylamide gels by the method of LAHIRI et al. (1997) and detected with ethidium bromide fluorescence with standard UV lightbox.

The expected heterozygosity (h), the content of polymorphic information (PIC), the probability of parentage exclusion (PE) and the probability of individual identity (PI) were estimated according to:

Table 1 - Primers sequences and annealing temperatures used to analyze the microsatellites in the present study

Microsatellites	Primers sequences	AT (°C)	References
MB022	CTTGGGATATAGACTTAGTGGCATG GCACAAGTCACAGTTTCTAAGGCTA	60	CRAWFORD et al. (1995)
BM4325	AGAGTCAGACAGGACTGAGCG CTGTAACTTGCAAATGTCTCGG	54	KAPPES et al. (1997)
ILSTS0027	GTGTGTGTTGGTTAAGACTGG GAATCATAGACCTGACTTCC	58	KEMP et al. (1995)

AT: annealing temperature; Mg Cl_2 concentration: 1.5mM, for all microsatellites

$h = \{[1-(\Sigma x_i^2)]\ 2n\} / 2n-1$, where x_i is the frequency of each i allele and n is the number of individuals; NEI (1978)

$PIC = 1- (\Sigma x_i^2) - (\Sigma\ 2\ x_i^2 x_j^2)$, where x_i and x_j are the frequencies of i an j alleles; BOTSTEIN et al. (1980)

$PE = a_1-2a_2+a_3+3(a_2a_3-a_5)-2(a_2^2-a_4)$, where $a_n = \Sigma\ x_i^n$ and are the moments of the xi gene frequencies with $a_1 = 1$; CHAKRAVARTY & LI (1983)

$PI = (\pi\ (\Sigma\ Gi_k^2)$, where π is the product and G_{ik} is the probability of each i genotype class in each k locus; VAN ZEVEREN et al. (1989).

The associations between the genetic markers and the productive parameters (calving interval, CI; weight at first calving, WFC) were verified by analysis of variance (one-way ANOVA) or by the t-test, using the SPSS (1997), according to the model:

$$Y_{ij} = \mu + A_i + e_{ij}$$

where, Y_{ij} is the CI or WFC phenotype of the jth individual; μ is the effect of the population mean; A_i is the effect of the ith genotype class; and e_{ij} is the random error component. Since CI did not present normal distribution, it was corrected by natural logarithm transformation before the statistical analysis.

RESULTS

The allele frequencies and the diversity parameters (PIC and h) for the three polymorphisms investigated and in table 2. The sample size presented small variations among systems due to amplification problems in some samples. Six alleles were detected in BM4325 and ILSTS027 and 12 were observed in MB022, the most frequent being *BM4325*101*, *BM4325*103*, *ILSTS027*169* and *MB022*229*. Polymorphic information content ranged from 0.58 to 0.88, while expected heterozygosity ranged from 65% to 89%, with an average mean value of 77%.

The estimated probabilities for parentage exclusion (PE) and individual identification (PI) using these polymorphisms are in table 3. The most informative marker was MB022 while BM4325 presented the lowest values. Although only three markers were studied, the combined values of PE and PI indicated a high power of exclusion (94%) and of individual identification (3.8×10^{-4}) for these markers.

Table 4 displays the descriptive statistics for CI and WFC for each microsatellite sub-sample. The small differences among them were due to differences in sample size resulting from amplification problems for some DNA samples.

No positive association was verified between each of the microsatellites and weight at first calving. Calving interval also seemed not be influenced by the ILSTS027 or MB022 marker. However, carriers of the *BM4325*101* allele presented a CI about 54 days shorter than the other animals (Table 5).

Table 2 - Allele frequencies and genetic diversity parameters (PIC and h) for MB022, BM4325 and ILSTS027 microsatellites in a beef cattle herd.

MB022; n=104		BM4325; n=106		ILSTS027; n=105	
Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
*201	0.04	*97	0.01	*161	0.03
*203	0.11	*99	0.04	*163	0.06
*205	0.09	*101	0.42	*165	0.12
*207	0.03	*103	0.41	*167	0.24
*209	0.11	*105	0.06	*169	0.32
*211	0.07	*117	0.06	*171	0.22
*215	0.07				
*217	0.01				
*219	0.16				
*221	0.05				
*225	0.08				
*229	0.17				
PIC	0.88		0.58		0.74
h	0.89		0.65		0.77

n: sample size; PIC:polymorphic information content; h: expected heterozygosity;
H (average expected heterozygosity) = 0.77.

Table 3 - Probability of identity (PI) and of parentage exclusion (PE) estimated using three microsatellite loci.

Microsatellites	PI	PE
MB022	0.022	0.78
BM4325	0.191	0.38
ILSTS027	0.090	0.55
Joint probability	3.78×10^{-4}	0.94

DISCUSSION

The high levels of genetic diversity (h and PIC) observed here agree with previous data for the same herd (ALMEIDA et al., 2000, 2003) and suggest that the selective procedure applied did not reduce the high variability expected for crossbreeding.The probabilities of parentage exclusion and individual identification are estimates of great importance in animal selection, mainly to identify genetically superior animals or their progenies. These two estimates are inversely correlated. The higher the value of the probability of exclusion, the greater the chance of excluding a false progenitor and the smaller the probability of identifying two identical individuals at random in the herd. Effective markers for these evaluations are of fundamental importance and the microsatellites herein investigated presented high levels of efficiency and could be very useful for animal identification in this herd.

The approach of candidate gene is very effective to detect genetic markers useful for MAS. In this paper the role of markers linked to the FSHβ candidate gene on productive performance of the herd was evaluated. No QTL linked to FSHβ gene have been so far described, while QTLs affecting reproduction have been verified on bovine chromosomes 5, 7, 18, 19 and 23 (ASHWELL et al., 2004; CRUICKSHANK et al., 2004), and QTL on chromosome 15 has been found to affect tenderness (KEELE et al., 1999).

Table 4 - Descriptive statistics of calving interval (CI) and cow weight at first calving (WFC).

Marker	n	CI	WFC
		Mean ± SD	Mean ± SD
MB022	104	545.3 ± 127.0	349.9 ± 53.4
BM4325	106	542.2 ± 124.9	350.5 ± 54.4
ILSTS027	105	543.4 ± 124.5	350.1 ± 54.5

n = sample size.

Table 5 - CI (and lnCI) in *BM 4325*101* carriers compared with other animals. Data are reported as mean ± standard deviation (sd).

BM 4325	n	Mean±sd (CI)	Mean±sd (lnCI)
101carriers	73	525.3±113.5	6.2±.2
Others	33	579.5±142.1	6.3±.2

t test = 2.1; P=.035 (analysis performed using natural logarithm transformation).

Our results suggest that WFC is not influenced by the microsatellites investigated while CI seems to be influenced by BM4325. The microsatellites analyzed are located at 15q2.4, MBO22, BM4325 in the same relative position as FSHß gene (59.9cM), whereas ILSTS027 is mapped at 66.3cM (http://www.marc.usda.gov/genome). Although it is difficult to evaluate the direct effect of genetic marker variability on gene function, it is possible that microsatellites could influence gene expression (COMINGS, 1998), the effect being dependent on the repeat size. It is possible that the microsatellite size could interfere with gene action, in this case altering FSH function in follicular growth and ovulation. On the other hand, it is possible that BM4325 microsatellite could be in linkage disequilibrium with some FSHβ mutation that may be influencing its biological role. The positive association observed needs to be confirmed in other breeds to determine if it is a general phenomenon or a phenomenon specific for this herd. Even if it is breed specific it could be useful for MAS, allowing the early selection of Brangus Ibagé cows, carriers of the BM4325*101 allele, to improve the reproductive performance of the herd.

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