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Research Article

Association between reproductive traits and four microsatellites in Brangus-Ibagé cattle

João F.C. de Oliveira[1,2], Jairo P. Neves1, Eric A. Almeida[2], Clara Sabina Steigleder[2], José Carlos F. Moraes[3], Paulo Bayard D. Gonçalves[1] and Tania A. Weimer[2,4]

[1]*Universidade Federal de Santa Maria, Departamento de Clínica de Grandes Animais, Laboratório de Biotecnologia e Reprodução Animal, Santa Maria, RS, Brazil.*
[2]*Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil.*
[3]*Embrapa Pecuária Sul, Bagé, RS, Brazil.*
[4]*Universidade Luterana do Brasil, Departamento de Biologia e Hospital Veterinário, Canoas, RS, Brazil.*

Abstract

The aim of the present study was to verify associations between reproductive efficiency and four microsatellite markers located in synteny with genes involved in the regulation of reproductive mechanisms. A sample of 107 females from a Brangus Ibagé population (5/8 Aberdeen Angus x 3/8 Nelore) was characterized for ETH225 (D9S1) and MM12E6 (D9S20) microsatellites, mapped on chromosome 9, and HEL5 (D21S15) and AFZ1 (D21S37) on chromosome 21. Associations between the genetic markers and reproductive efficiency were determined by one-way analysis of variance using calving interval (CI), live weight at calving (LWC), live weight at first calving (LW1C) and live weight at second calving (LW2C) as dependent variables. The genotypes were classified according to allele size into homozygous for long alleles, homozygous for short alleles and heterozygous. A longer CI was observed for individuals homozygous for long alleles at the HEL5 locus compared with the others ($p = 0.022$). For the AFZ1 locus, an inverse correlation between allele size and calving interval was observed ($p = 0.022$), suggesting that homozygosity for long alleles at this microsatellite could be advantageous. Analysis of the combined effect of favorable genotypes at HEL5 and AFZ1 indicated that animals with unfavorable genotypes (homozygous for long alleles at HEL5 and homozygous for short alleles at AFZ1) presented a significantly longer CI ($p = 0.003$) when compared to the other genotypes. The ETH225 and MM12E6 systems did not present any association with CI. None of the systems studied showed any significant association with LWC, LW1C or LW2C.

Key words: bovine, IGF-I receptor, estrogen receptor, reproductive efficiency.

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Introduction

Microsatellite markers are particularly useful due to their wide variability, random distribution throughout the genome, Mendelian inheritance (Tautz 1993) and possible influence on gene regulation (Schroth *et al.* 1992; Comings 1998). Microsatellites tend to be concentrated in transcription initiation regions, with the virtual absence of these sequences at intergene positions and in pseudogenes. Sequences rich in alternating purines and pyrimidines, (CA)n, such as microsatellites, are able to form Z-DNA under physiological conditions (Comings 1998). These facts suggest the potential role of microsatellites in gene regulation. Based on these considerations, the association between a microsatellite and a given polygenic phenotype would be not dependant on a specific allele but on a repeat size threshold.

In a study on a Brangus Ibagé cattle population involving individuals with different degrees of fertility, Oliveira *et al.* (2002b) observed differences in the post-partum follicular development. The mean diameter of the larger follicles and the mean number of follicles were larger in individuals with greater reproductive efficiency, suggesting that mechanisms related to post-partum follicular development were important in the classification of individuals when mean calving interval was used as a criterion. Studies conducted on various species have demonstrated that estrogen plays an important role on follicular development, including follicle stimulation and maturation (Goldenberg *et al.* 1972) and the increased expression of

Send correspondence to João Francisco Coelho de Oliveira. Universidade Federal de Santa Maria, Departamento de Clínica de Grandes Animais, Laboratório de Biotecnologia e Reprodução Animal, 97105-900 Santa Maria, RS, Brazil. E-mail: joaofco@biorep.ufsm.br.

follicle-stimulating hormone and luteinizing hormone receptors by granulosa cells (Richards *et al.* 1976; Richards *et al.* 1979). In cattle, the dominant follicle presents higher estrogen levels than follicles entering in atresia (Evans and Fortune 1997). Evidence also indicates that the insulin-like growth factor (IGF) system consisting of IGF-I and -II, IGF receptors and IGF-binding proteins (IGFBP) is important for the modulation of the effect of nutrition on post-partum anestrus (Monget and Martin 1997; Constant *et al.* 2000). Thus, estrogen and IGF-I receptors can be considered good candidates for the study of the possible association between molecular markers and reproductive efficiency.

The present study analyzed the possible association between genetic markers and reproductive efficiency through four microsatellite loci located on the same chromosome as the estrogen receptor and IGF-IR genes.

Materials and Methods

Four microsatellites [ETH225 (D9S1), MM12E6 (D9S20), HEL5 (D21S15) and AFZ1 (D21S37)] were investigated. The first two are located on BTA9 chromosome, the same of the estrogen receptor gene (ESR1), ETH225 at 8 cM, MM12 at about 77 cM, and ESR1 at 9 cM. The other two are located on the BTA21 chromosome which contains the IGF-IR gene, HEL5 at 13 cM, AFZ1 at 75 cM and the IGF1-R locus at 0 cM from AFZ1. (Barendse *et al.* 1997; http://www.ncbi.nlm.nih.gov; http://www. marc.usda.gov; http://www.inra.fr).

The association with reproductive efficiency was tested in a sample of 107 individuals obtained from a Brangus Ibagé herd originating from Embrapa, Pecuária Sul, Bagé, RS, Brazil, previously classified according to the average of their calving interval records, (Oliveira, 2002a). The following parameters were used: calving interval, live weight at calving (LWC), live weight at first calving (LW1C) and live weight at second calving (LW2C). This population has been formed since 1945, its genetic proportion being fixed at 3/8 Nelore x 5/8 Aberdeen Angus. The herd was solely selected according to male adult weight and female size (Oliveira *et al.* 1998), not suffering any selection based on reproductive efficiency. All individuals of the sample possessed reproductive records for at least three calving and had always been managed on a native field.

Genomic DNA was extracted from blood collected by puncture of the jugular vein using an anticoagulant according to the method of Plante *et al.* (1992). Microsatellite fragments were amplified by the polymerase chain reaction (PCR) according to Stone *et al.* (1996) using adjacent primers and annealing temperature specific for each fragment (Table 1). The amplified fragments were separated by vertical non-denaturing polyacrylamide gel electrophoresis with 30 cm of height colored with Ethidium Bromide (Lahiri *et al.* 1997). The final characterization of each allele was performed using pBR322 plasmid digested with restriction enzymes as molecular weight markers. The size of each fragment was determined on digital photographs using the Kodak Digital Science 1D Analysis Software.

Linkage disequilibrium between loci considering individual alleles was tested by the maximum likelihood ratio using the Arlequin program (Schneider *et al.* 2000). Associations analyses between genotype classes of genetic markers and reproductive performance were determined by one-way analysis of variance, considering the genotype classes of the alleles as independent variables and reproductive records measurements as dependant variables. Calving interval data were normalized using a natural logarithm, and LWC data were analyzed without transformation since they showed a normal distribution. The genotypes were grouped according to allele size into the following classes: homozygous for short alleles, homozygous for long alleles and heterozygous.

Results

The number of individuals, the observed alleles, and the frequencies of genotypes grouped in classes according to the allele size of the four systems are shown in Table 2. There was a slight difference in the number of individuals studied per system due to amplification problems of some samples. Total heterozygosity was similar for the four systems, varying from 0.79 to 0.90. Markers on chromosome 21 (AFZ1 and HEL5) were in linkage disequilibrium ($p = 0.04$) but ETH225 and MM12 seem to segregate independently, as expected based on their chromosome distance.

For the HEL5 locus (Table 3 and Figure 1), homozygous for long alleles showed a trend towards a longer calv-

Table 1 - Investigated loci with their chromosome location, primer sequences and annealing temperature.

Loci	Chromosome	Primers	Annealing temperature (°C)
HEL5	21	5'-GCAGGATCACTTGTTAGGGA-3' 5'-AGACGTTAGTGTACATTAAC-3'	60
AFZ1	21	5'-TTGGACGACAAAACTCACGG-3' 5'-GTGGCTGGACTGGTCTGGTT-3'	50
ETH225	9	5'-GATCACCTTGCCACTATTTCCT-3' 5'-ACATGACAGCCAGCTGCTACT-3'	60
MM12	9	5'-CAAGACAGGTGTTTCAATCT-3' 5'-ATCGACTCTGGGGATGATGT-3'	50

Table 2 - Number of animals, allele sizes and genotype frequencies of the individuals classified as homozygous for short (H. Short), homozygous for long (H. Long) and heterozygous.

Locus	Total alleles	N	Allele size (bp)		Genotype frequency		
			Short	Long	H. short	H. long	Heterozygous
HEL5	12	101	147-157	159-169	0.27	0.49	0.24
AFZ1	9	103	115-121	123-129	0.38	0.16	0.46
ETH225	11	105	139-149	151-159	0.27	0.49	0.24
MM12	9	106	117-127	129-133	0.25	0.20	0.55

Table 3 - Results of association analyses between calving interval and microsatellite genotype classes homozygous for short (H. Short), homozygous for long (H. Long) and heterozygous.

Locus	Calving interval (means ± SE)						
	n	H. short	n	H. long	n	Heterozygous	p
HEL5	27	522.23 ± 27.1^{a}	50	569.16 ± 17.90^{b}	24	509.06 ± 20.28^{ab}	0.072
AFZ1	39	574.17 ± 22.5^{a}	17	489.47 ± 18.60^{b}	47	540.43 ± 17.96^{ab}	0.076
ETH225	40	536.71 ± 17.7	15	563.68 ± 38.88	50	543.57 ± 18.69	0.853
MM12	27	542.23 ± 23.9	21	577.25 ± 32.33	58	532.35 ± 15.57	0.407

a, b, c Values with different superscript in the same row differ significantly ($p < 0.10$).

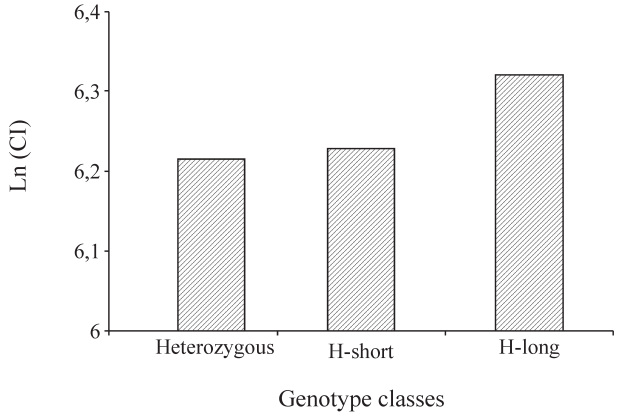

Figure 1 - Natural logarithm of mean calving interval (CI) obtained from different genotype classes of the HEL5 locus grouped into homozygous for short alleles(H-short), homozygous for long alleles (H-log) and heterozygous. When homozygous for short alleles and heterozygous were grouped, the difference was significant (p = 0.022).

ing interval compared with the other individuals (p = 0.072). Additional analysis grouping homozygotes for short alleles with heterozygotes and contrasting them with homozygotes for long alleles revealed a significant association (p = 0.022), suggesting an effect of long alleles on the prolonged calving interval.

At the AFZ1 locus, homozygous for long alleles presented shorter calving interval, homozygous for short alleles showed longer calving interval while heterozygotes had intermediate values (p = 0.065; Table 3). Analysis of variance considering only homozygous individuals revealed an inverse correlation between allele size and calving interval (p = 0.022), suggesting that homozygosity for

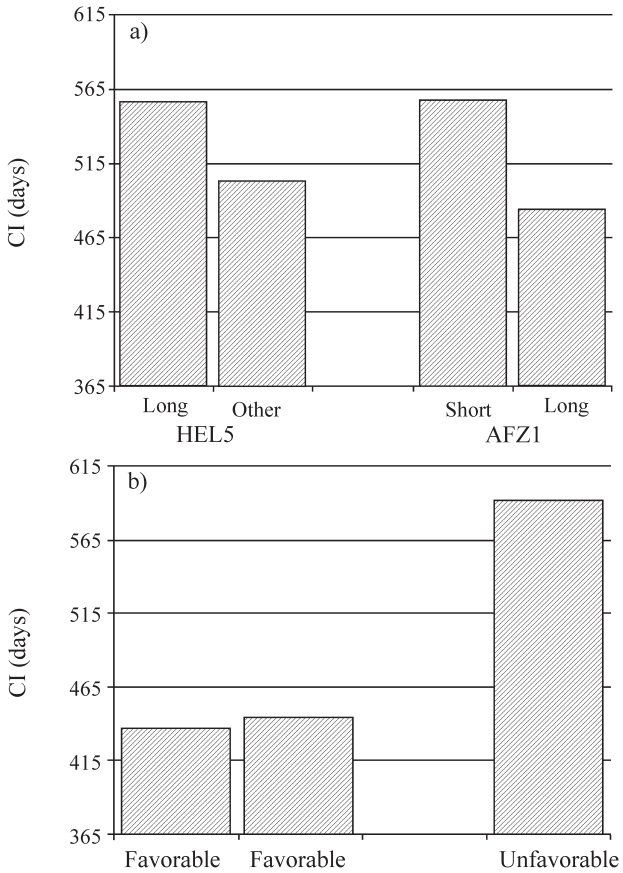

Figure 2 - Mean calving interval (CI; untransformed data) obtained from the different genotype classes of the HEL5 and AFZ1 microsatellites assessed independently (A). Panel B shows favorable (lower CI) and unfavorable (higher CI) genotypes based on the combination of results obtained from both loci.

long alleles at this microsatellite could be advantageous. Based on these results, we investigated the combined effect of these two microsatellites on calving interval. For this purpose, the genotypes were classified into favorable (individuals with at least a short allele at HEL5 and homozygous for long alleles at the AFZ1 locus) and unfavorable genotypes (individuals homozygous for long alleles at HEL5 and homozygous for short alleles at AFZ1). Statistical analysis comparing favorable and unfavorable genotypes with the other categories demonstrated highly significant associations ($p = 0.004$ and $p = 0.003$, respectively; Figure 2).

With respect to LWC (Table 4), homozygous for long alleles at AFZ1 showed consistently a slightly higher LWC than the other individuals but the differences were not statistically significant. There were also no significant associations between LWC and HEL5, ETH225 and MM12E5 genotypes.

Discussion

The present study investigated possible associations between reproductive performance and loci selected based on the role of estrogen and IGF-I in the control of the regulation of folliculogenesis and the reinitiation of postpartum cyclicity in beef cattle.

The results indicated associations of AFZ1 and HEL5 markers mapped in synteny with the gene encoding IGF-IR on reproductive performance. The IGF system, consisting of IGF-I and -II, IGF-IR, and IGFBP, has been shown to be an important component of the mechanisms that coordinate ovarian follicular function in different species, including cattle (Spicer and Encternkamp 1995). The alterations in concentration and expression pattern of the IGF system components and their marked interference with growth and follicular dominance mechanisms may suggest a relevant influence of this system on regulation of the cyclicity reinitiation in cows after calving. The IGF-IR mRNA had been detected in theca interstitial and granulosa cells, with its levels increasing during the development of dominant follicles, suggesting its role in follicular development.

Suggestions that repetitive DNA sequences act on the regulation of gene expression are relatively recent but evidence is accumulating, especially for genes related to some human multifactorial diseases (Krontiris *et al.* 1993; Comings *et al.* 1996). The approach involving the establishment of allele classes for microsatellite loci proposed by Comings (1998), instead of considering the association with each allele, permits the inclusion of a larger number of individuals per genotype, thus increasing the reliability of association tests. Another aspect to be considered is the higher mutation rate observed for microsatellites compared to other sequences, as well as the type of mutation (stepwise) frequently observed in these repetitive DNA regions (Schlötterer, 1998). In view of these considerations, it is reasonable to suppose that genotypes of these systems that only differ in a small number of base pairs exert a similar effect on Quantitative Trait Locus (QTL). The results of the present study, using this approach permitted the detection of highly significant associations, validating the use of this

Table 4 - Results of association analyses between live weight at calving, live weight at first calving and live weight at second calving and the microsatellite genotype classes.

System	Allele class			p
	H. short	Heterozygous	H. long	
Live weight at calving				
AFZ1	384.52 ± 4.59	391.57 ± 4.29	396.26 ± 6.70	0.30
ETH225	387.10 ± 4.61	394.73 ± 4.05	385.55 ± 7.47	0.36
HEL5	387.07 ± 5.60	398.35 ± 5.93	389.42 ± 4.12	0.34
MM12	397.63 ± 5.63	387.07 ± 3.68	388.30 ± 6.36	0.28
Live weight at 1st calving				
AFZ1	340.62 ± 8.56	347.11 ± 7.97	364.00 ± 12.96	0.33
ETH225	343.15 ± 8.69	360.62 ± 7.68	330.64 ± 14.51	0.12
HEL5	348.62 ± 10.94	357.83 ± 11.63	348.64 ± 7.89	0.79
MM12	349.30 ± 10.64	351.11 ± 7.32	348.45 ± 12.37	0.98
Live weight at 2nd calving				
AFZ1	392.82 ± 11.19	400.72 ± 10.17	405.71 ± 16.73	0.78
ETH225	399.73 ± 10.87	398.43 ± 9.82	398.07 ± 18.37	0.99
HEL5	383.15 ± 13.40	421.65 ± 14.25	396.58 ± 9.66	0.14
MM12	402.19 ± 13.19	394.42 ± 9.08	403.85 ± 15.32	0.82

Data are reported as means ± SE.

method and representing a pioneering step towards marker-assisted selection in domestic animals.

According to Moody *et al.* (1996), the HEL5 locus is in linkage disequilibrium with the gene encoding IGF-IR which agrees with the linkage disequilibrium here verified between HEL5 and AZF1 since this last marker is at 0 cM from IGF-IR. These data suggest that these microsatellites may be acting on the IGF-IR regulation or that their different allele classes are linked to alternative forms of this gene, resulting in a positive or negative effect on calving interval.

The estrogen receptor (E2R) gene is mapped on chromosome 9, probably at 9 cM (www.ncbi.nlm.nih.gov), but no reports of microsatellite markers associated with it have been described. Therefore, we chose markers randomly distributed along the chromosome, one close and the other far from it (at 8 cM and 77 cM, respectively). Although estradiol plays a relevant role in the mechanisms of manifestation of follicular dominance and divergence, no association was observed with any of the systems studied.

The use of a more flexible probability level ($p < 0.10$) for association studies involving multifactorial characteristics has been suggested by Haviland *et al.* (1997). In view of the small effect of each locus, a less rigid criterion prevents type 2 statistical errors. In the present study, only associations with a probability of less than 0.05 were considered to be significant, while a probability level of 0.10 was established as a previous criterion to perform the subsequent analyses, grouping of the allele in classes.

The associations observed in the present study indicate the possible utilization of favorable genotypes of the HEL5 and AFZ1 microsatellites in this population to increase reproductive efficiency. However, since the population studied here is a synthetic race, additional studies involving *Bos Taurus* and *Bos indicus* populations are necessary in order to determine whether the associations observed here can be extrapolated as a tool for marker-assisted selection in beef cattle. Additionally, further investigations, using screening approaches such as SSCP or evaluation of previously described polymorphic markers in IGFI-R locus (Moody *et al.*, 1996), are needed to understand possible modulatory effects involved in reproductive efficiency.

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