SNP Markers Influence On Breeding Soundness Of Nellore (*Bos indicus*) Bulls[1]

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Introduction

The Brazilian cattle herd has around 190 million head, 80% of them with participation of zebu (*Bos indicus*) breeds, mainly Nellore. The yearly need of replacement bulls is around 450,000 (Ferraz & Felicio, 2010), being close to 400,000 of Nellore breed. Among selection criteria for replacement bulls, breeding soundness is essential, mainly because near to 95% of the Brazilian beef cows are naturally mate. Between 20 and 40% of bulls from an unselected population may have reduced fertility and few are completely sterile (Kastelic & Thundathil, 2008). Manuals and rules to evaluate breeding soundness are broadly described in literature (Henry & Neves, 1998; Kennedy et al., 2002) and are being used in the Brazilian beef industry. DNA techniques are starting to be used in beef cattle genetic evaluation, to recognize uncertain paternity in multi-sire pastures (Van Eenennaam et al, 2007), as it is happening in several production traits, can be very useful to speed up genetic gain in breeding programs. This research was realized to verify the association of DNA SNP markers with ten different traits related to breeding soundness of Nellore young bulls, raised in tropical area of Brazil, close to latitude 20º.

Material and methods

Data

Breeding soundness exams of 1.470 young Nellore bulls, with ages between 18.3 and 30.9 months, reared in three different farms, located in geographical coordinates 20º37'S, 50º13"W; 20º22"S, 49º57"W and 20º28"S, 55º54"W, carried out from 2001 to 2006 and genotyped for 119 genetic markers were used to estimate the effect of those markers on ten traits: traits: scrotum circumference at the physical exam (SC), gross motility (GM), progressive sperm motility (MOT), sperm vigor (VIG), acrossomal defects (ACRO), major sperm defects (MAD), minor sperm defects (MID), total sperm defects (TD) and breeding soundness classification (CLAS). Breeding soundness evaluations were performed by the Animal Reproduction team of Federal University of Viçosa, following the standards established by Blom (1973), Henry & Neves (1998). Data bank maintenance and statistical analysis were performed at Animal Breeding and Biotechnology Group from the College of Animal Science and Feed Engineering, University of Sao Paulo, Brazil.

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Genotyping

DNA samples were obtained from blood or hair follicles. Genotypes came from DNA mass scpectrometry (Sequenom iPlexTM Mass Spec), carried out in laboratories, located in USA and licensed by IGENITY® (Duluth, Georgia), a Merial Ltd subdivision, the company that owns the exploration licenses rights on markers analyzed. A total of 119 markers were studied in this research, related to many metabolic routes. Markers were coded to protect property rights and confidentiality agreements.

Statistical analyses.

Allele and genotypic frequencies for T945M and UCP1 were obtained using PROC FREQ of SAS (2004). Associations between SNPs and traits were studied with using mixed model methodology, in a sire model, using PROC MIXED of SAS (2004), using the model:

$$Y_{ijk} = \mu + CG_i + \beta_1\left(I_{ijk} - \bar{I}\right) + \beta_2\left(M_{ijk} - \overline{M}\right) + S_j + e_{ijk}$$

where Y_{ij} the phenotypic value of an animal, μ is the general mean of the trait, CG_i is the fixed effect contemporary group, β_1 is the coefficient for the covariate age at exam, β_2 is the regression coefficient for a given genetic marker, S_j is the coefficient associate to random effect of sire, and e_{ij} is the random effect of the residual. Allele substitution effect was estimated as suggested by Falconer (1981), using β2. F-statistic was considered significant for allelic substitution effect if the nominal P-value was lower than 0.05 (*) or 0.01 (**). As frequencies of alleles were very unequal, the effects were, also, considered "potential" for 0.05≤P≤0.10 (††) or 0.10≤P≤0.25 (†), respectively.

Results and discussion

The descriptive statistics of the traits analyzed are presented in Table 1.

Table 1. Descriptive statistics for breeding soundness traits of Nellore young bulls.

Traits	N	AVG	STD	MIN	MAX
Scrotal circumference (SC, cm)	1444	32.52	3.032	22.50	51.60
Gross motility (GRO, score)	1449	1.02	1.062	0.0	5.0
Progressive motility (MOT, %)	1449	61.31	23.241	0.0	90.0
Semen vigor (VIG, score)	1449	2.68	1.010	0.0	5.0
Acrossomal defects (ACRO, %)	1449	2.69	3.185	0.0	31.0
Major sperm defects (MD, %)	1449	15.82	15.521	0.0	98.0
Minor sperm defects (MIN, %)	1449	5.13	5.725	0.0	79.0
Total sperm defects (TOT, %)	1449	20.98	17.981	0.0	100.0
Classification (CLAS, score)	1449	1.96	1.312	1.0	4.0

N = nº of observations; AVG = average; STD = standard deviation; MIN, MAX = minimum or maximum values

From the 119 markers, 100 were not used in the final analysis, due to several reasons, like fixation of one of the alleles, very low marker frequency or a small amount of genotyped animals. Only 19 markers were considered in the final analysis, although allele substitution

effect was estimated for all of the markers were that was possible. Allelic and genotypic frequencies of the most important markers are shown in Table 2.

Table 2. Allelic and genotypic frequencies of genetic markers on a Nellore young bull population

Polymorphisms	N	Allelic frequencies (%)		Genotypic frequencies (%)		
		p	q	Homo 1	Hetero	Homo 2
MARK_56	1409	74.27	25.76	55.71	34.05	7.24
MARK_58	1407	17.80	82.20	2.49	30.63	66.88
MARK_59	1391	11.18	88.82	0.36	11.14	88.5
MARK_69	1410	98.37	1.63	96.88	2.98	0.14
MARK_91	1449	9434	5.66	88.96	10.77	0.28
MARK_97	1109	15.78	84.22	2.16	24.23	70.60
MARK_99	1409	80.02	19.98	63.45	33.14	3.41
MARK_100	1437	89.63	10.37	89.49	0.28	10.23
MARK_101	1439	80.47	19.53	64.63	31.69	3.68
MARK_104	1391	97.74	1.26	97.56	2.37	0.07
MARK_105	1350	87.52	12.48	76.74	21.56	1.70
MARK_107	1435	2.37	97.63	0.07	4.60	95.33
MARK_109	1372	98.87	1.13	97.89	1.97	0.15
MARK_111	1398	94.13	5.87	88.84	10.59	0.57
MARK_112	1387	88.75	11.25	79.16	19.18	1.66
MARK_113	1155	64.98	35.02	44.68	40.61	14.72
MARK_116	1251	80.02	19.98	63.55	32.93	3.52
MARK_118	1348	62.17	37.83	38.58	47.18	14.24
MARK_119	1225	38.16	61.84	13.80	48.73	37.47

Homo 1 = homozygous for allele 1 (f= p); Homo 2 = homozygous for allele 2 (f= q), Hetero = heterozygous

The analysis of genes frequencies indicate that a large amount of polymorphisms, that were described, originally, mainly in *Bos Taurus*, are fixed or have a very low frequency in the sample of *Bos indicus* analyzed. However, an important amount of markers showed polymorphisms that allow estimation allele substitution effects.

The association of those 19 markers with the traits was studied and the results are presented in Table 3. The analysis of that table indicates that markers 58, 59, 97, 100 and 105 have statistically significant effects in several traits linked to breeding soundness, while markers 56, 91, 99, 101, 107, 109, 111, 112, 113 and 119 can be potential markers for those traits in Nellore breed. References, in literature, on genetic markers effect on breeding soundness on zebu cattle are very rare, what makes difficult to compare these results with other findings.

The allele substitution effects were estimated for markers and some can be very important. For example, effect of marker 100 and 59 on SC were3.15 and 1.25% of the mean of the trait, while the effect of markers 105 and 59, on MD were 16.12 and 10.92% of its' mean.

Table 3. List of significant markers (identified by their numbers, ordered by P value) for 10 breeding soundness traits on young Nellore bulls.

	Stastistically significant markers		Potential markers	
Traits	**	*	††	†
Scrotal circumference (SC, cm)	100	59	104, 107	109, 111
Gross motility (GRO, score)		58		113, 100, 91, 112,
Progressive motility (MOT, %)	58			109, 91, 111
Semen vigor (VIG, score)	58		109,110	111, 116, 113, 101,
Acrossomal defects (ACRO, %)	105	97	118, 113, 91	107, 112, 119, 101
Major sperm defects (MD, %)	105		59, 97	99
Minor sperm defects (MIN, %)			109, 116	56, 107, 59
Total sperm defects (TOT, %)	105	59		97
Classification (CLAS, score)		59		56, 58

*P≤ 0.05, **P≤ 0.01, ††0.05≤P≤0.10, † 0.10≤P≤0.25

Conclusion

The use of genetic markers as auxiliary selection criteria can help in selection for breeding soundness of Nellore cattle. Improve the size of population sampled, searching for rare polymorphisms and improving allele frequencies, the inclusion of new markers, related to metabolic routes that are important to semen production will bring better results to such kind of studies.

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