CYTOGENETIC ANALYSIS OF THE Y CHROMOSOME OF NATIVE BRAZILIAN BOVINE BREEDS: PRELIMINARY DATA

ANÁLISIS DEL CROMOSOMA Y DE RAZAS BOVINAS NATURALIZADAS BRASILEÑAS: DATOS PRELIMINARES

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ADDITIONAL KEYWORDS

Animal conservation. Animal genetic resources. *Bos taurus taurus. Bos taurus indicus.*

PALABRAS CLAVE ADICIONALES

Conservación animal. Recursos genéticos animales. *Bos taurus taurus. Bos taurus indicus*.

SUMMARY

The present work is a preliminary cytogenetic study carried out on 29 males of the Brazilian breeds Curraleiro, Crioulo Lageano, Junqueira and Pantaneiro, available at Embrapa Genetic Resources & Biotechnology, Brasília, DF and Promissão farm, Poconé, MT. The objective was to investigate their Y chromosome morphology, whether submetacentric or acrocentric, considered as markers of the taurine and zebuine subspecies, respectively. The results showed that of the six Curraleiro animals analyzed, four had an acrocentric and two had a submetacentric Y chromosome, indicating contamination by zebu breeds. All Pantaneiro bulls analyzed had exclusively zebuine (acrocentric) Y chromosome. On the other hand, the Crioulo Lageano and Junqueira bulls had submetacentric Y chromosomes, indicating their taurine origin.

RESUMEN

El objetivo de este trabajo fue realizar un estudio preliminar del cariotipo de bovinos de razas brasileñas: Curraleiro, Crioulo Lageano y Junqueira disponibles en la granja Parque perteneciente a Embrapa Recursos Genéticos y Biotecnología, Brasília, DF y de los Pantaneiros ubicados en el norte del Pantanal, pertenecientes a la finca Promissão, Poconé, MT. Fueron analizados los cariotipos de 29 machos a través de su tipo morfológico de cromosoma Y, submetacéntrico o acrocéntrico, tipos estos considerados como marcadores para las subespecies taurinas y cebuínas, respectivamente. El resultado mostró que, dentro de los seis sementales Curraleiros evaluados, cuatro presentaron Y de cebú (acrocéntrico) y dos de taurino (submetacéntrico), sugiriendo contaminación racial por Cebú. Sin embargo, todos los Pantaneiros analizados presentaron exclusivamente cromosoma Y de Cebú (acrocéntrico), mientras que los bovinos Crioulo Lageano y Junqueira presentaron sólo Y submetacéntrico, confirmando su origen taurino.

INTRODUCTION

According to Epstein and Mason (1984), all domesticated cattle was originated from *Bos primigenius*, extinct in Poland in 1627.

In Brazil, the native bovine breeds descend from the Iberian animals which spread all over the country, through random crosses and by interaction with the environment. As in South America there were no animals of the bovine species at the time of discovery, during the long colonization period the cattle needed to produce

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milk and meat had to be brought from the Iberian Peninsula. It was in the 16th century that bovines first appeared in South America, coming from Portugal and Spain (Athanassof, 1946a).

The breeds introduced by the colonizers became adapted to the new environment, forming the large herds named *Crioulo* (Creole), which differentiated into several varieties (Britto, 1995). Thus, it is difficult to precisely state which breed(s) originated the different native breeds. In some cases, the information about the original populations was lost over time.

According to Mariante *et al*. (1999), in general these breeds have extremely valuable characteristics, such as adaptation to inhospitable conditions, including parasites and infectious diseases, draught and poor quantity and quality of food. Preservation of the native breeds has also a historical value that is the *genetic memory* of the animals which helped colonizing the country. Genetic traces of all these breeds are still left through their crossbreeds. The intervention of man in their reproduction and selection processes did not cause any modification of their potential, besides those granted by nature.

According to Egito *et al*. (2002), the search for more productive breeds led, as of the late 19th and the early 20th centuries, to the import of breeds considered exotic, which, although highly productive, had been selected in regions with a temperate climate. These breeds caused, through absorbing crosses, the replacement and erosion of the local breeds. Although these local breeds presented lower production levels, they stood out for their complete adaptation to the tropics, where they had undergone long-time natural selection. It is therefore necessary to preserve such animal genetic resources, in order to prevent their loss. Genetic diversity within domesticated species is reflected by the variety of types and breeds that exist and by the variation within each one of them, and the loss of a single type or breed compromises the access to its genes and unique genetic combinations, which may be useful to agriculture in the future.

Some native Brazilian breeds, although named differently and living in distinct regions, present similar phenotypes which raise doubts about their identity as a distinct racial group or native type. It is not known if these populations are genetically similar or not. Even if they belong to the same breed, they may have accumulated different alleles due to genetic drift, as a consequence of geographic isolation and adaptation to different ecological niches (Egito *et al*., 2002).

Curraleiro

According to Athanassof (1946b), this breed is named *Curraleiro* in some States, such as Goiás and Tocantins, and *Pé-Duro* in others, such as Piauí and Maranhão. It is a native bovine breed that has not yet been improved, originated from the bovines introduced in Brazil by the Portuguese and Spaniards during the time of colonization. The Curraleiro breed stems from *Bos taurus ibericus* and, given its European origin, it is assumed to belong to the subspecies *Bos taurus taurus* (Britto, 1995).

Aiming to prevent its extinction, as well as that of other Brazilian breeds, EMBRAPA has developed a project for their preservation, to be carried out by the *Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia* (National Research Center for Genetic Resources and Biotechnology) and by the *Centro de Pesquisa Agropecuária do Meio-Norte* (Agricultural Research Center of the Mid-North) (CPAN). CPAN maintains a preservation unit for the Curraleiro cattle at São João do Piauí, PI, in the semi-arid zone of the Brazilian Northeast, with approximately 300 animals (EMATER).

Tambasco *et al*. (1985) found both types of Y chromosomes (acrocentric and submetacentric) in the Curraleiro breed. The frequency of the acrocentric Y chromosome

was higher than that of the submetacentric Y chromosome. In 1999, Britto and Mello confirmed this finding.

Pantaneiro

Pantaneiro bovine, also named Cuiabano or Tucura, descends from the Spanish breeds introduced in America during the colonization process of the Plata Basin. According to Mazza *et al*. (1994), the literature on the phenotypic characteristics of Pantaneiro bovine, from the 16th to the 18th centuries, is rather scarce. Publications from the 20th century portray these animals as short of stature, with short, brown, dark and red-brown hair. The authors believe that the descriptions of the animals of the Pantanal region, made in the 20th century, are not reliable, because in that time the crosses with their domestic and even zebuine breeds were still occurring. Based on morphological measurements and weight development of Pantaneiro cattle taken the germoplasma bank analysis of the data confirm their reduced body size, with average weight at birth of 24 and 22 kg and, in the adult phase, of 375 and 298 kg, for male and females, respectively.

The remaining populations, few and far between, of Pantaneiro cattle can be at present in the Northern part of the Pantanal, in the state of Mato Grosso, in areas where maximum flooding takes place along the Paraguay, Cuiabá, São Lourenço and Bento Gomes rivers. To the South, the Pantaneiro cattle are practically extinct. In this moment have only two populations from these breeds in the Pantanal region. One in the Northern in Promissão farm, that doing a good work the rescue the breeds in this areas and in the South there are a conservation in situ nucleus conducting by Embrapa Pantanal, that have a lot of research about this breeds in the region.

More than three centuries of native pasture adaptation to flooded Pantanal regions conferred the Pantaneiro bovine with rusticity, high fertility and the ability to survive under conditions of water and food stress conditions.

Crioulo Lageano

These bovines from Southern Brazil (Lages, State of Santa Catarina) are those which keep the greatest resemblance to the type of bovine introduced by the conquerors, therefore of indisputable Iberian origin. This breed has evolved by natural selection over four centuries and almost disappeared as a consequence of indiscriminate crosses. When the Catarinense Highland was colonized, the settlers brought the Franqueiro cattle with them that probably mated with the bovines which already existed there, originating the Crioulo Lageano cattle (Spritze *et al*., 2003).

Currently, the population of these bovines is reduced to about 500 animals, of which more than 80% belong to a single breeder. Research works conducted in the 1980's by *Embrapa Recursos Genéticos e Biotecnologia* and by the Federal University of Santa Catarina, in collaboration with a few private breeders, showed advantages in exploring these bovines for crosses and also as a pure breed, under the raising conditions of the South Brazilian Highland (Spritze *et al*., 2003).

Tambasco *et al*. (1985) studied animals of the Crioulo Lageano breed and found a higher frequency of the submetacentric Y than of the acrocentric Y chromosome, as opposed to the Curraleiro breed.

Junqueira

Formed in the State of Minas Gerais, the Junqueira cattle breed is a product of crosses between Caracu and other Brazilian varieties. It is currently found in the Jequitinhonha River basin. The animals are robust, with a voluminous body with long and spiraled horns (EMATER). Their tail is thick and hairy; their hair is yellow, white- and reddish-brown speckled or patchy (Athanassof, 1946b). They are resistant to parasites and abiotic stresses, like the breed

that has probably originated this one, Caracu, a Brazilian breed that also developed in this region.

Junqueira is a breed developed in the inland of the State of São Paulo between the 18th and the 19th centuries, with an aptitude for meat. In the past, their long horns were used to manufacture *berrantes*, a kind of musical instrument. Nowadays this breed is critically endangered, with less than a hundred animals left in the whole country (Diniz and Euler, 2005). Pires *et al*. (2004) found only submetacentric Y chromosome in the Junqueira breed.

Until this moment don't have much information in the literature regarding the morphology of the Y chromosome of these animals. For other hands this information is basic and very helpful to use in animal conservation program.

The first chromosome studies in bovines were carried out in 1892 by Bardeleben, who described 2n= 16 chromosomes for the species. Several works performed in the decades of 1910 and 1920 described different chromosome numbers (Schoenfeld, 1902; Hoof, 1919; Masui, 1919; Wodsedalek, 1920), until 1931, when Krallinger described for the first time the correct chromosome number of taurines as 2n= 60, with submetacentric X and Y chromosomes. In 1964, Monnier-Cambon described the Y chromosome of zebuines as acrocentric.

Different shapes of the Y chromosome in the same breed have been described by several authors (Potter *et al.*, 1979; Moraes, 1978; Pinheiro, 1979; Halnan and Watson, 1982; Xin and Lin, 1993; Frisch *et al*., 1997).

In the Brazilian breeds, the Y chromosome has been analyzed by a few local authors. Tambasco *et al*. (1985) described the morphology of the Y in the Caracu, Mocho Nacional, Curraleiro and Crioulo Lageano breeds. These authors observed a dimorphism (acrocentric and submetacentric) of the Y chromosome in these breeds. Britto and Mello (1999) analyzed the Y chromosome of the Curraleiro breed and, like the former authors, found both morphological types. The Junqueira breed showed only a submetacentric Y chromosome, but this study was based on three animals (Pires *et al*., 2004). Issa *et al*. (2006) showed that the Pantaneiro cattle breed also presents the dimorphism, most of the animals have an acrocentric Y.

Giovambattista *et al*. (2000) studied the geographic distribution and the haplotype frequency of the Y chromosomes of *Bos taurus* and *Bos indicus* in Argentine and Bolivian Creole breeds, using cytogenetic and molecular techniques. The taurine haplotype (submetacentric Y chromosome) was found in 100% of the Argentine Creole breeds, whereas in the Bolivian breeds both a submetacentric and an acrocentric Y chromosome were found, the first morphological type being more frequent than the latter.

According to Hanotte *et al*. (2000), 69 African bovine populations from 22 countries were analyzed for a marker locus (INRA124) of the Y chromosome. From the 984 males studied, 693 (70%) and 291 (30%) showed the indicus and taurus allele, respectively.

The present work had the objective of collaborating with Brazilian Agricultural Research Corporation (Embrapa) and Goiás Federal University (UFG) in the characterization process from the native bovine breeds, by means of a cytogenetic study of the Y chromosome, and help the farmers in the animal conservation in Brazil.

MATERIAL AND METHODS

For the analysis of the Y chromosome, we used 12 males (six Curraleiro, two Crioulo Lageano and four Junqueira animals) from *Embrapa Recursos Genéticos e Biotecnologia* in Brasilia. To the Pantaneiro breed we used 17 unrelated males from Promissão farm, Poconé, MT.

Blood was collected from the jugular vein using 4 ml Vacutainer tubes with sodium

heparin for lymphocyte culture. About 3 ml of blood were collected in each tube. Then, the tubes were placed in a styrofoam container with reusable preserving ice (euthetic ice) and recyclable ice (Serono) and shipped by mail. Incubation of the cultures started 28 hours after blood collection, with a relative success. Cell culture was performed using the standard technique with 4ml culture medium, 1 ml fetal calf serum, 0.1 ml phytohemagglutinin and 8 drops of blood, 70-72 hours of incubation, 50 minutes colchicine and 20 minutes hypotonic (KCl 0.075M) treatment. After fixation in 3:1 methanol/acetic acid, the material was dripped on slides, stained with Giemsa and examined under a regular optical microscope. At least 15 metaphases of good quality from each animal were examined and photographed using digital microphotography.

RESULTS AND DISCUSSION

In the present work, all animals studied presented a karyotype with 2n= 60 chromosomes. The autosomes were acrocentric and the X chromosome was submetacentric, its size being approximately the same as that of the pair 1 autosomes.

As for the Y chromosome, four (66.67%) of the six Curraleiro animals analyzed had an acrocentric Y chromosome **(figure 1)**, and two (33.33%) had a submetacentric Y chromosome **(figure 2)**. Tambasco *et al*. (1985) showed that in the Caracu, Curraleiro and Mocho Nacional breeds the frequency of acrocentric Y chromosomes was higher than that of submetacentric Y's. In turn, in the Crioulo Lageano breed, the result was the opposite. In 1999, Britto and Mello studied the morphology of the Y chromosome of the Curraleiro cattle and found that 68% of the bulls had an acrocentric and 32% a submetacentric Y chromosome.

We found only submetacentric Y chromosomes, both in the Crioulo Lageano and in the Junqueira breeds (**figures 3** and **4**). As the number of animals in our sample was small, we believe that with a larger number of animals we might find both acrocentric and submetacentric Y chromosomes.

All of the 17 animals of Pantaneiro breed analyzed in the present work from the Northern part of the Pantanal, had an acrocentric Y chromosome **(figure 5)**. However, Issa *et al*. (2006) showed that Pantaneiro breed from Nhumirin Farm, Corumbá, MS, South part of the Pantanal, had

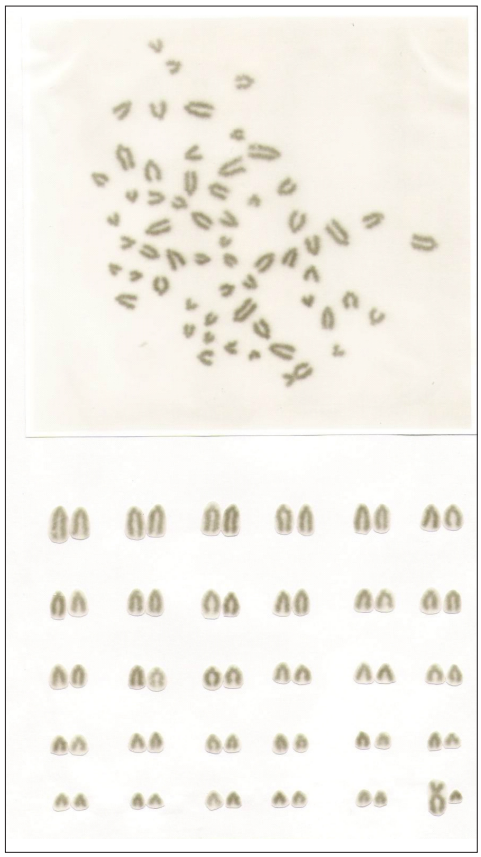

Figure 1. *Karyotype of Curraleiro cattle showing an acrocentric Y chromosome, typical of* Bos taurus indicus*. Chromosome constitution: 2n= 60,XY.* (Cariotipo de ganado Curralleiro mostrando cromosoma Y acrocéntrico, típico de *Bos taurus indicus*, constitución cromosómica: 2n= 60,XY).

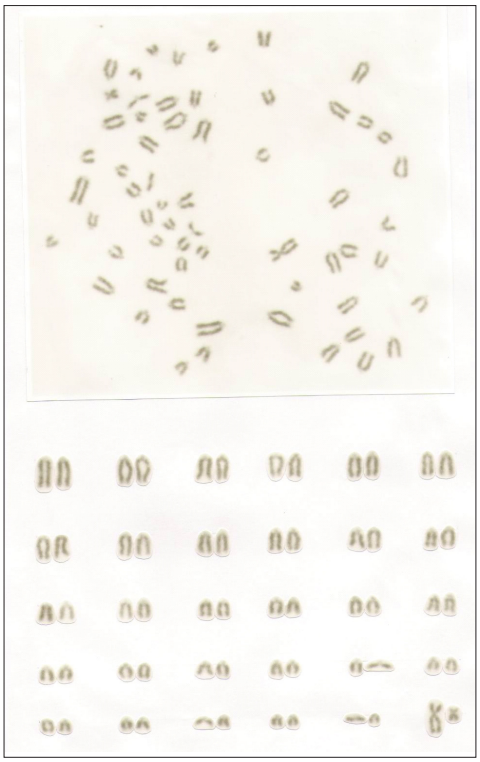

***Figure 2**. Karyotype of Curraleiro cattle showing a submetacentric Y chromosome, typical of* Bos taurus taurus*. Chromosome constitution: 2n= 60,XY.* (Cariotipo de ganado Curraleiro mostrando cromosoma Y sub. metacéntrico, típico de *Bos taurus taurus*, constitución cromosómica: 2n= 60,XY).

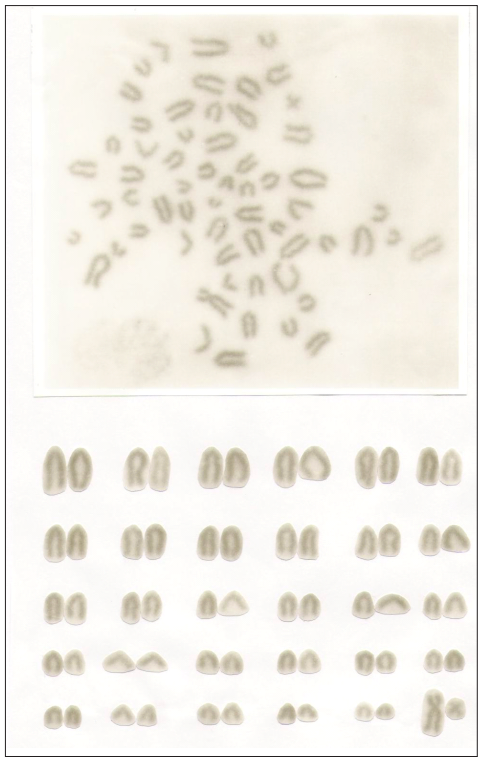

***Figure 3**. Karyotype of Crioulo Lageano cattle showing a submetacentric Y chromosome, typical of* Bos taurus taurus*. Chromosome constitution: 2n= 60,XY.* (Cariotipo de Ganado Crioulo Lageano mostrando cromosoma Y sub. metacéntrico, típico de *Bos taurus taurus*, constitución cromosómica: 2n= 60,XY).

a dimorphism. Of the 12 animals analyzed, nine (75%) had an acrocentric Y, and three (25%) a submetacentric Y chromosome.

Up to this moment, the native Brazilian bovine breeds, which present the different types of Y chromosome, present the same morphologic and productive characteristics. It was observed in the Pantaneiro cattle breed (Abreu *et al*., 2006). The aim of this work is to contribute to management of the animal conservation in the most of farms in Brazil, not to elimination animals because the chromosome morphology. However, this data showing that it is possible to do a selection based on chromosome morphology from Pantaneiro breeds if the farmers have interesting in doing selection to taurine types.

CONCLUSIONS

The Y chromosomes of the Crioulo Lageano and Junqueira breeds are submetacentric, and those of the Curraleiro breed present dimorphism, with a higher frequency of acrocentric and a lower frequency of

***Figure 4**. Karyotype of Junqueira cattle showing a submetacentric Y chromosome, typical of* Bos taurus taurus*. Chromosome constitution: 2n=60,XY.* (Cariotipo de ganado Junqueira mostrando cromosoma Y sub. metacéntrico, típico de *Bos taurus taurus*, constitución cromosómica: 2n= 60, XY).

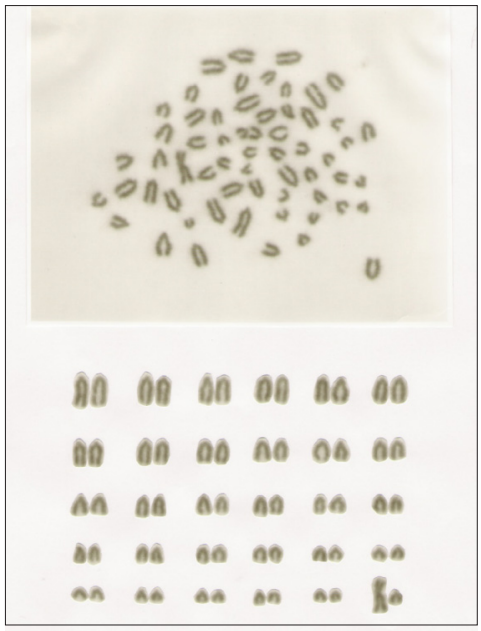

***Figure 5**. Karyotype of Pantaneiro cattle showing an acrocentric Y chromosome, typical of* Bos taurus indicus*.* Chromosome constitution: 2n= 60,XY. (Cariotipo de ganado Pantaneiro mostrando cromosoma Y acrocéntrico, típico de *Bos taurus indicus*, constitución cromosómica: 2n= 60, XY).

submetacentric Y chromosomes. The Pantaneiro breeds from the Northern part of the Pantanal showing only frequency of acrocentric. When the zebuines were introduced in Brazil, in the late 19th and early 20th centuries, the animals brought by the colonizers after the discovery underwent a long process of breed contamination. Thus, the acrocentric Y chromosome found in part of the animals studied was acquired through such random crossings.

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