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## **Naturalized Breeds in Brazil: Reports on the Origin and Genetic Diversity of the Pantaneiro Sheep**

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### **Abstract**

Brazil has several breeds of sheep, including animals that developed from breeds brought by settlers soon after their discovery. Over the years, these animals were under the process of natural selection, resulting in breeds that are considered naturalized. The Pantaneiro sheep shows rusticity and ability of adaptation to tropical climate regions and tolerance or resistance to disease and parasites. Molecular tools have marked the discovery of the origin and domestication processes of a wide variety of species, using both nuclear and mitochondrial molecular markers. These tools have aided in the understanding of evolutionary relationships, taxonomies, and demographics of various species and provided support to identify the most important areas for conservation programs, in addition to assisting in the analysis of genetic diversity in domestic, wildlife and endangered species. Researches using these tools show the importance of exploiting the potential of the genetic diversity found in locally adapted livestock. So far, a few studies were performed to observe that Pantaneiro sheep served as maternal basis for the origin of other breeds reared. Moreover, it is possible to suggest an European origin for the sheep populations studied; therefore, more studies using more markers are needed, so that it is possible to prove their origin.

**Keywords:** Pantaneiro sheep, genetic management, phylogeny, mtDNA, nuclear DNA

## 1. Introduction

Brazil has several breeds of sheep, including animals that developed from breeds brought by settlers soon after the discovery. Over the years, these animals were under the process of natural selection of local environmental and climatic conditions, resulting in breeds that are considered naturalized, locally adapted or native [1].

Thus, the characterization of the diversity of naturalized breeds, the genetic relationship between them, as well as knowledge of their origins in other breeds are the first step to obtain subsidies for breeding programs, management, and conservation of naturalized Brazilian sheep [2].

Molecular tools and recent technologies have marked the discovery of the source and domestication processes of species, both using molecular markers such as mitochondrial DNA (mtDNA). These tools have aided the understanding of evolutionary relationships, taxonomies, demographics of various species and provided support to identify the most important areas for conservation programs, and assist in the analysis of genetic diversity in domestic animals, wildlife, and endangered species [3, 4].

The mtDNA shows the haplotype diversity within species; therefore, it becomes a useful tool to establish phylogenetic relationships between the species [5]. On the other hand, the haploid inheritance of markers of the Y chromosome makes them to be extremely sensitive for detecting genetic history, the process of domestication, the relationship between population and the male abundance in gene flow [6].

The Pantaneiro sheep showed greater allelic richness when compared with other populations in a study with other six breeds in the state of Mato Grosso do Sul, Brazil [7]. Considering that the introduction of sheep in South America has occurred concomitant with the process of colonization and the effective population size is in process of formation, high levels of diversity observed in the different studied populations can be related to the fact of selective pressure coupled with miscegenation have implicated in the introgression of genes in populations.

Analyzing the haplotypes found in mtDNA, it was observed that the Pantaneiro sheep was distributed in seven haplotypes and grouped with all other locally adapted breeds that were analyzed [8].

A study performed with Creole breed demonstrated that these animals have a different haplotype compared to the animals of Pantaneiro breed, as there was no grouping in the same network [9]. This suggests a difference between these groups, so more research would be needed to see if it is enough that the Pantaneiro sheep can be recognized as a distinct breed [10]. However, it was also observed that several haplotypes for the Creole breed were close to haplotype formed by Pantaneiro breed which could indicate that, although these animals do not share the same haplotype, they belong to the same haplogroup. The regions where these animals currently found previously belonged to Paraguay, so it is possible that Pantaneiro breed has been influenced by Paraguayan herds. Analysis of their mitochondrial genome can redeem this history showing their genetic diversity from the Creole breed. Thus, the existence of significant differences in the ND5 gene of mtDNA between Pantaneiro and Creole breeds

could indicate that differentiation has occurred between the two breeds, but further research using other markers would be needed to prove the differentiation between Pantaneiro and Creole.

Research shows the importance of exploiting the potential of the genetic diversity found in locally adapted livestock. Innate genes with adaptive values linked to tropical climatic regions compared to breeds created/improved in temperate environments could be highly useful in view of the climate changes predicted for the coming years. The exotic breeds, although considered high performance, can reduced their productivity by not easily conform to the conditions of creation and management taxes in Brazil, combined yet to climatic weather (tropical). The introgression of genes between these populations can produce animals whose general average production and rusticity exceed the means of the parents.

A few studies demonstrated that Pantaneiro breed served as maternal basis for the formation of other breeds reared in the region [8, 11]. Moreover, it is possible to suggest an European origin for the sheep populations studied; therefore, more studies using more markers are needed so that it is possible to prove their origin.

It is assumed therefore that the establishment of conservation programs using molecular tools is crucial to provide information regarding the genetic diversity patterns of locally adapted groups, and allow the same to be used for the production system, adding adaptation features, and rusticity [12].

## 2. Brazilian naturalized sheep

Domestic sheep are belonging to the Bovidae family, *Ovis* genus and *Ovis aries* species. The sheep were the first animals to be domesticated, and it is believed that its origin is descended from the Muflon (*Ovis musimon*) and Urial (*Ovis orientalis*) breeds, since the Urial breed may have contributed to the formation of all European sheep breeds [13].

Brazilian sheep breeds, as well as several species of domestic animals in the country were introduced by Portuguese colonization. Over the years, they have been subjected to natural selection because of their adaptation and survival to the local environmental and climatic conditions, resulting in breeds that are now considered locally adapted or local, creole and native or naturalized [1, 2].

Brazilian naturalized sheep are small and specialized in intensive meat or milk production system, so far they have not undergone to selection process and genetic breeding [2]. These breeds were known for their rusticity and ability of adaptation to tropical and subtropical climate regions, allowing them to hold important attributes of genetic resources. These animals still have tolerance or resistance to disease and parasites, as well as adaptation to availability of food resources and water [14].

However, the search for more productive breeds from the ending of the nineteenth century and the beginning of the twentieth century led to import exotic breeds from temperate climatic

region, which did not have the adaptive traits of local breeds. This way, it occurred indiscriminate crossings, which was determinant to result in rapid replacement and erosion of naturalized animals, endangering their existence [15].

In order to prevent the extinction of naturalized breeds and their genetic heritage, in 1983, the National Research Center for Genetic Resources and Biotechnology (Cenargen) of the Brazilian Agricultural Research Corporation (Embrapa) decided to include animal genetic resource conservation research in its Genetic Resources Conservation program (Brazilian Animal Genetic Resources Conservation program) [16]. Since then, in addition to Cenargen, several Embrapa centers, universities, private companies, and farmers have maintained the conservation of animals, through natural selection and semen, embryos, and oocytes storage in germplasm banks [17].

### 3. Pantaneiro sheep

The Brazilian Pantanal, considered the largest wetland sedimentary plain of the world, is located in the states of Mato Grosso and Mato Grosso do Sul and part of the northern Paraguay and eastern Bolivia. This biome, due to its intrinsic characteristics, made it difficult the exchange for other regions of the country at the time of colonization. So, the first animal's populations developed for breeds and the sheep of Pantanal region is an example of adaptability maintained throughout the years [18].



**Figure 1.** Pantaneiro sheep.

With the expansion of sheep breeding in the state of Mato Grosso do Sul, the genetic group of locally adapted sheep, known as Pantaneiro Sheep (**Figure 1**), can be advantageous to increase the production chain, due to its adaptation to the environmental conditions of the region and by using its rustic genetic material in conservation and breeding programs [7].

In 2005, researchers at the University Anhanguera (UNIDERP), Federal University of Grande Dourados (UFGD), the Brazilian Agricultural Research Corporation (EMBRAPA), and the Federal University of Mato Grosso do Sul (UFMS) initiated studies with the Pantaneiro genetic sheep group in order to identify, preserve, record, and ensure the development of animals and their desirable traits obtained by natural selection. The groups were consisted of animals obtained from farms of Pantanal (Midwest of Brazil), which had similar phenotypic traits but distinct genotypic patterns presented by exotic breeds created in Brazil [7, 18, 19].

#### **4. Features of Pantaneiro sheep**

The Pantaneiro sheep is a separate genetic group of sheep breeds, and it presents allelic combination approaching to wool breeds from South Brazil and woolless breeds from Northeast Brazil [20]. As the Pantaneiro sheep are living in the Pantanal region for many years without suffering any kind of artificial selection or genetic breeding, it was concluded that these animals are locally adapted to the region [19]. Evidence of this adaptability can be determined through the wool distribution in the body of the animals, since they show little or no wool in the legs, belly, and neck, once these body parts are in the most contact with water, when there is need for locomotion in local filled with water and dense vegetation [18].

In a biometric analysis of 20 Pantaneiro sheep, lower morphometric data were observed, but similarity exists between native animals of the Pantanal and other sheep breeds created in Brazil. However, the heart girth and rump width measurements were higher in Pantaneiro sheep than the data in the literature for crossing between Ile de France and Ideal breeds. Therefore, the Pantaneiro animals show good potential for genetic breeding sheep breeding [21].

For assessing the morphometric measurements, a study with four lambs obtained the following averages for the characteristics studied: body weight (46.3 kg), body (69.6 cm), and croup length (19, 1 cm), chest circumference (82.7 cm), chest (18.1 cm), and croup width (11.7 cm). The mean values for these characteristics were smaller when compared to the values observed in wool Creole sheep, but they were higher when confronted with three woolless breeds (Santa Inês, Morada Nova, and Brazilian Somalis) [22].

An analysis of morphometric characterization, using 338 naturalized sheep from Mato Grosso do Sul and performed 20 morphometric measurements, concluded that the females of this genetic group have relatively large heads (32.4 cm), and this feature along with the length of the skull (24.2 cm) are larger in females than in males [23].

The Pantaneiro sheep have good productive potential in relation to meat and carcass quality traits, and also for the production of wool, widely used in handicrafts [19]. The lean physique of these animals owing to their nonrequirement for the high-calorie consumption results in low subcutaneous fat accumulation, which is characterized by their rusticity [24].

These animals exhibit average birth weight between 2.5 and 3.5 kg and about 200 to 350 g in average daily gain weight. Still, it was found that the weight data support the production of

lambs, providing slaughtering when the animals are between 4 and 8 months, weighing between 30 and 40 kg, generating high carcass yield, up to 50%, and in addition males and females have similar productive performance [19].

These sheep also features highlighted in relation to the reproductive performance when compared to other breeds of sheep created in Brazil. With reproductive activity during the entire year, females do not show reproductive seasonality, and they can produced more than 1.5 lamb per year, unlike the females of other breeds [19, 25, 26].

The male sheep also have reproductive seasonality, but only when there is little variation of photoperiod, as in the tropics. Moreover, the variation of breed and environmental factors are also crucial to the reproductive performance of animals [19].

As Pantaneiro sheep are not considered a breed, studies of molecular, genetic, and phenotypic characterization are needed in order to prove the differences between these animals and the other breeds. More studies are required to demonstrate the body, production and reproduction characteristics of these animals [27].

## 5. Genetic diversity

Through indiscriminate crossbreeding with exotic breeds, mainly imported from Africa and Europe, it was put at risk the existence and preservation of naturalized breeds that are now important genetic heritage [28]. These animals have characteristics which may be considered useful both from the productive point of view and adaptive such as tolerance or resistance to diseases and parasites and adaptive wide variations related to the availability and quality of food and water. Therefore, the animals best adapted and/or more resistant survived and reproduced to the present day. Thus, the naturalized breeds are a result of the process of natural selection over the years.

Research aimed at the conservation and breeding of naturalized breeds that are important for the selection of animals for the purpose of controlling intersections, avoiding inbreeding, and indiscriminate crossbreeding that thus purebreds are preserved. Therefore, it is necessary to seek a production system that makes evident its potential, so that they are recognized by the creators and that they perceive the possibility of the use of local breeds for higher returns [29].

Studies related to knowledge of adaptive characteristics of different breeds to the environment can sustain production systems in livestock based on adapted breeds, reducing the impact on the environment and receiving better products for consumption.

With recent technological developments, new molecular tools were developed in order to understand the origin and domestication process of domestic species. These tools helped the discovery of evolutionary relationships, taxonomy, and demographics of a wide variety of species, providing important support both in identifying priority areas for conservation programs and in the understanding of genetic diversity in domestic and wild species threatened with extinction [3].

## 6. Microsatellite markers

Microsatellites are the most widely used marker for the study of genetic diversity and population structure of domestic animals [30]. The abundance of this marker along the genome, its high degree of polymorphism and codominance are the main features that make it an important tool for genomic analysis.

Through the evaluation of eight microsatellite loci in five breeds of unrelated sheep (Romney, Border Leicester, Suffolk, Awassi, Australian, and New Zealand Merino) was found highly significant differences in allele frequencies between individuals, indicating that the genotyping using microsatellite can be a useful tool for examining the evolutionary relationships between the breeds [31]. Studies using microsatellite markers to characterize genotypic and assessments of genetic diversity of sheep described in Spanish breeds, determined the genetic relationship between Swiss breeds. In these studies, microsatellites were efficient to evaluate genetic diversity and demonstration of the genetic diversity between the animals involved [32, 33].

Several studies have shown the use of microsatellite markers in genetic diversity studies of native animals of Brazil. In a study using 27 microsatellite markers to analyze the genetic variability of native breeds of goats in Brazil, the result showed that all microsatellites were polymorphic and showed a high capacity for genetic characterization of these breeds [34]. In another study, microsatellite markers for 18 loci in studies of genetic diversity of sheep naturalizes and exotic in Brazil were used (Santa Inês, Bergamácia Wide Tail, Morada Nova and Somali) and the results showed the efficiency of these markers in the characterization of these breeds because all breeds differed significantly, although they presented low genetic variability [2].

A study with 717 animals was determined the variability of 20 microsatellites in 14 Portuguese sheep breeds. Analysis of these results allowed us to assess the degree of structure of the Portuguese population of sheep and estimate parameters of genetic diversity in each of the breeds [35].

Thus, microsatellites have proven marker of excellence for characterization of new naturalized breeds [36], as well as to genetic variability of population studies [37, 38]. A recent study identified an approach to facilitate the merger of microsatellite data for cross-country comparison of genetic resources when samples are evaluated in different laboratories. This approach can facilitate the merger and analysis of microsatellite data for cross-country comparison and extend the utility of previously collected molecular markers. In addition, this analysis can be used in new and existing conservation programs [39].

Recent research analyzes genetic diversity and population structure among varieties of sheep. Therefore, nuclear microsatellite markers and regions of mitochondrial DNA are used [40].

## 7. Phylogeny and population structure

Despite the sheep breeds are considered adapted to Brazil and these animals were brought to the country by settlers soon after the discovery, few studies have been conducted in order to discover the origin of these animals.

Knowledge of the population structure combined with information about genetic changes can influence future management actions and can be used to develop strategies for using a breed in a particular ecosystem as a model for genetic improvement programs [41]. Conservation programs using molecular tools are crucial for the providing information about the genetic diversity of locally adapted groups, thus allowing them to be included in production systems for integration of adaptation and rusticity features [12].

Recent molecular tools and technologies have marked the discovery of the origin and domestication processes of a wide variety of species, using either nuclear or mitochondrial molecular markers. These tools have aided in the understanding of evolutionary relationships, taxonomy, and demography of several species that will provide support to identify the most important areas for conservation programs, in addition to the analysis of genetic diversity in domestic, wildlife, and endangered species [3, 4].

Two studies [42, 43] demonstrated the existence of at least two major haplogroups in *O. aries* from the control region (D-loop) of mitochondrial DNA (mtDNA) sequencing: one of European origin and another, probably of Asian origin. These results can also be interpreted as two independent domestication events that have occurred for domestic species [44]. Furthermore, it was developed a test based on polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) of mitochondrial cytochrome C oxidase I gene (MT-COI 6) with the restriction enzyme *HinfI* (extracted from bacteria *Haemophilus influenzae* Rf) in order to more easily identify these two haplogroups HA (Asian origin) and HB (European origin) [43].

The study of the mtDNA region, which can be called DNA barcoding, uses partial DNA sequences of the MT-COI 6 gene to identify and designate both new species as described previously, helping to unravel the diversity [45].

A study using PCR-RFLP from MT-COI 6 gene using *HinfI* restriction enzyme was performed to molecularly characterize, over the existing haplogroups, some sheep breeds used in the state of Mato Grosso do Sul [8]. The study with the MT-COI RFLP gene indicated the applicability of this molecular tool to classify most of the animals as belonging to the European haplogroup, highlighting the European origin of the state breeds.

Researchers analyze genetic diversity and population structure among varieties of White, Red, and Black Morada Nova hair sheep from flocks in the northeastern Brazilian semiarid region. In this study, the use of 15 nuclear microsatellite markers and two regions of mitochondrial DNA identified the existence of substantial differences between the Red and White varieties of this sheep and should be used as separate genetic resources and to improve conservation programs [40].

The origin of sheep from some of the breeds in the state of Mato Grosso do Sul, Brazil is important because these are part of the genetic heritage of the state and by knowing their

phylogeny it is possible to improve the management of these breeds, aiming its conservation and the use of the productivity of these animals in our environment.

The Creole sheep has been reared for centuries in the Brazilian states of Rio Grande do Sul and Santa Catarina, where there are two known varieties: Fronteira and Serrana [46]. Considering the geographic distribution of sheep in Brazil and phenotypic similarities between the animals, it is thought that Pantaneiro sheep originated from the Creole sheep, and research has been carried out to determine whether the difference between the groups is sufficient for the Pantaneiro sheep to be recognized as a separate breed [10]. NADH dehydrogenase is one of the main enzymes found in respiratory complexes in mammals. The subunit five (*ND5*) was used to study sheep diversity [47, 46]. The former study determined subspecies of *Ovis ammon* in Mongolia by sequencing this region, and the results suggested the existence of two subspecies (*O. ammon ammon* and *O. ammon darwini*). The genetic differentiation was found between animals of the Creole sheep in the south of Brazil belonging to the varieties Serrana and Fronteira [44].

Genetic polymorphisms in mitochondrial DNA (mtDNA) reveal haplotype diversity within species, and are therefore a useful tool for establishing phylogenetic relationships at the species level [5] The Pantaneiro breed presents a higher genetic variability than other breeds of sheep reared in tropical altitude regions. Therefore, it is important to develop research that aims at their conservation and genetic improvement [7].

In order to assess the variation between a population of Pantaneiro sheep in the state of Mato Grosso do Sul and Creole sheep in the south of Brazil through molecular analysis of the mtDNA *ND5* region, an study revealed that Creole sheep have a different haplotype compared to Pantaneiro sheep, suggesting that differentiation has occurred between these groups; therefore, more research would be necessary that can be recognized Pantaneiro sheep as a distinct breed [9, 10]. Furthermore, several haplotypes in the Creole sheep were close to the one formed by the Pantaneiro breed animals which may indicate that, although these animals do not share the same haplotype, they belong to the same haplogroup. The geographical region where these animals are found today belonged previously to Paraguay, so it is possible that the Pantaneiro breed has been influenced by Paraguayan breeds and the analysis of its mitochondrial genome might confirm this assumption, by showing their genetic diversity from the Creole.

Thus, the significant differences identified for the mtDNA *ND5* gene between Pantaneiro and Creole sheep may indicate that differentiation has occurred in both breeds; however, further research using other markers is required to investigate this further. Additional management measures need to be carried out in this herd to reduce inbreeding and optimize genetic variation.

Other aspects besides the distribution of genetic diversity have to be taken into account when dealing with conservation strategies of species. Historical, cultural, and traditional aspects about the use of particular breeds are relevant issues. Furthermore, the selection practiced by sheep breeders can favoring alleles for which the surrogate neutral markers used in diversity surveys are not necessarily fully representative.

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