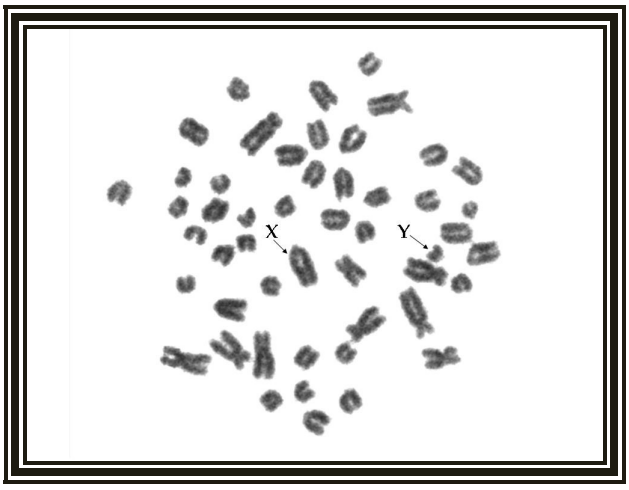
Universidade Federal do Pampa

***Campus* São Gabriel**

Cytogenetic characterization of swamp (*B. bubalis kerebau*) and river (*B. bubalis bubalis*) buffaloes and respective progeny



Tiago Marafiga Degrandi

2010

Universidade Federal do Pampa

Cytogenetic characterization of swamp (*B. bubalis kerebau*) and river (*B. bubalis bubalis*) buffaloes and respective progeny

Tiago Marafiga Degrandi

Artigo apresentado à Comissão de Trabalho de Conclusão do Curso de Ciências Biológicas, Universidade Federal do Pampa — UNIPAMPA, *Campus* São Gabriel, como parte dos requisitos necessários à obtenção do grau de Bacharel em Ciências Biológicas.

Orientador: Prof. Dr. Ricardo José Gunski

Rio Grande do Sul

Junho de 2010

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Aprovado por:

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Presidente, Prof. Dr. Ricardo José Gunski

_____
Prof. Dra. Analía Del Valle Garnero

_____
Prof. Dr. Fabiano Pimentel Torres

São Gabriel, Junho de 2010

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Abstract

The domestic buffaloes are divided in two groups in agreement with your habitat and karyotype number: the “river buffalos” 2n=50 chromosomes and “swamp buffalos” 2n=48 chromosomes. Is reported also the existence of a karyotype intermediate with 2n=49 chromosomes, what evidences hybridization among the river and swamp buffaloes. This work had as objectives to characterize cytogenetically the buffaloes of the breed Murrah, Carabao and type Baio obtaining the patterns of bands C and G. For that they were obtained preparations metaphases through the cultivation of lymphocytes of blood peripheral in 10 ml RPMI 1640 culture medium. For the breed Murrah and type Baio the chromosomal number is of 2n=50 chromosomes, being the first five pairs morphology metacentric and submetacentric and 20 pairs of chromosomes acrocêntricos including the sexual pairs X and Y. For the breed Carabao the observed number was 2n=48 of the first five pairs metacentric and submetacentric and 19 pairs of chromosomes acrocentric. For both groups the sexual chromosomes X and Y are larger and smaller acrocentric, respectively. Of the animals classified as hybrid, with chromosomal number of 2n=49 they presented five pairs of chromosomes meta/ submetacentric and 20 pairs of chromosomes acrocentric including the sexual chromosomes X larger and Y smaller acrocentric; with some such characteristics as: heteromorphic of the pair 1 and absence of the pair's member 24. The pattern of bands G was obtained for identification of the homologous ones. The constituent heterocromatina revealed demarcation centromeric in every complement and the chromosome Y present C-positive. Therefore, the diploid number 2n=49 are enough for identification of hybrid, while in animals 2n=48 and 2n=50 the first pair's morphologic variation serves as marker to classify them as hybrid.

Key-words: Breeds. Genetics. Genome. Reproduction. Selection.

Resumo

Os búfalos domésticos são divididos em dois grupos de acordo com seu habitat e número cariotipico: Os “búfalos de rio” 2n=50 e os “búfalos de pântano” 2n=48. Além disso, é reportado a existência de um cariótipo intermediário 2n=49 cromossomos, que evidencia a existência de hibridização entre os búfalos de rio e de pântano. Este trabalho teve como objetivo caracterizar citogenéticamente búfalos das raças Murrah, Carabao e Tipo Baio; e obter os padrões de bandeamento G e C. Para obtenção de células metafásicas foi utilizado o cultivo de linfócitos de sangue periférico em 10 ml de meio de cultura RPMI 1640. Para a raça Murrah e o Tipo Baio o número cromossômico observado foi 2n=50 cromossomos, destes cinco primeiros pares de morfologia met/submetacêntricos e vinte pares restantes de cromossomos acrocêntricos incluindo os pares sexuais X e Y. Para raça Carabao foi observado 2n=48 cromossomos, cinco primeiros pares de morfologia met/submetacêntricos e dezenove pares de acrocêntricos incluindo X e Y . Para ambos os grupos os cromossomos sexuais X e Y são maior e menor acrocêntricos respectivamente. Dos animais classificados como híbridos com 2n=49 cromossomos apresentando cinco pares de morfologia met/submetacêntricos e vinte pares de acrocêntricos incluindo os cromossomos sexuais X e Y, com algumas características: heteromorfismo do par um, e ausência do homologo no par vinte e quatro. O padrão de bandas G foi utilizado para identificação dos pares homólogos. A heterocromatina constitutiva revelou marcação centromérica in todo complemento e o cromossomo Y apresenta-se C-positivo. Portanto, o número diplóide de 2n = 49 é suficientes para a identificação de híbridos, enquanto nos animais 2n = 48 e 2n = 50 a variação morfológica do primeiro par serve como marcador para classificá-los como híbridos.

Palavras-Chave. Raças. Genética. Genoma. Reprodução. Seleção.

Sumário

1. Introdução Geral

Os búfalos vêm ganhando destaque na pecuária brasileira, sendo estes uma importante alternativa para obtenção de proteína animal de qualidade. Características como produtividade, adaptabilidade, fertilidade, são frequentemente associadas a estes animais somando-se a taxas de natalidade superior a 80% e longevidade de 15 anos. Tais fatos remetem ao crescimento populacional de 1,806% observado entre os anos de 1996 e 2005 [16].

No Brasil os búfalos foram introduzidos inicialmente na Ilha de Marajó estado do Pará por volta de 1895 e atualmente encontran-se em todos estados. Segundo estimativas da Associação Brasileira de Criadores de Búfalos (ABCB) o contingente atual de bubalinos no país ultrapassa três milhões de cabeças, representados por quatro raças aqui introduzidas: Murrah, Mediterrâneo, Jafarabadi, Carabao. Além disso está presente no país um quinto grupo genético denominado Tipo Baio, não reconhecido como raça pela ABCB [9].

Os búfalos estão classificados na ordem Artiodactyla , sub-família Bovidae, tribo Bovini, gênero *Bubalus*, que compreende quatro espécies: *Bubalus depressicornis* - Anoa da Planície, *Bubalus quarlesi* - Anoa da Montanha, *Bubalus mindorensis* - Búfalo-anão-de-Mindoro, *Bubalus bubalis* - Búfalo Asiático [1].

Os *Bubalus bubalis* também conhecidos como “water buffaloes”, são divididos em dois grupos de acordo com seu habitat e características citogenéticas: Os “búfalos de rio” *Bubalus bubalis bubalis* apresentam número

cromossômico 2n=50, e os “búfalos de pântano” 2n=48 cromossomos. Além destes é reportado um terceiro grupo de búfalos híbridos entre os grupos de búfalos de rio e de pântano, que é identificado por apresentar 2n=49 cromossomos. Os cromossomos sexuais para espécie do gênero *Bubalus* são de morfologia acrocêntrica sendo o X maior e Y menor do complemento [2,3,4,5].

Muitos estudos têm sido publicados com caracterização citogenética e a identificação de búfalos híbridos (2n=49 cromossomos). Segundo Harisah et al., 1989 estes animais podem apresentar a formação de gametas desbalanceados (n=24 e n=25) sendo viáveis reprodutivamente e capazes de gerar indivíduos férteis [14].

A identificação destes animais parece um tanto fácil, pois o número diplóide 2n=49 já se trata de uma variação na espécie. Porém a identificação destes animais nas gerações F2, F3 e F4 requer maior atenção, pois, números diplóides 2n=50 e 2n=48

podem estar presentes na população e estariam de acordo com o número diplóide descrito para os búfalos de rio e de pântano respectivamente [7].

De uma forma geral a análise citogenética é indicada quando estão presentes na população disfunções tais como: distúrbios na eficiência reprodutiva, na diferenciação sexual, abortos repetitivos, mortalidade neonatal de indivíduos fenotipicamente normais e malformações morfológicas

congênitas, que possam estar relacionados com alterações cromossômicas morfológicas ou numéricas.

Para programas de conservação e melhoramento genético a identificação de alterações cromossômicas é primordial, pois é necessário excluir os animais portadores das etapas reprodutivas do programa. Caso contrário estaria se propagando animais que podem não representar a genética da espécie e/ou consequentemente reduzir as taxas de fertilidade como no caso de animais híbridos.

Com isso este trabalho teve como objetivo caracterizar citogeneticamente búfalos das raças Carabao, Murrah e Tipo Baio cadastrados aos programas de conservação e melhoramento genético da EMBRAPA por meio de técnicas de coloração convencionais e diferenciais como bandeamento G e C.

2. Artigo publicado na revista Veterinária da Universidad Nacional del nordeste Volume 21-2010-Suplemento 1

Cytogenetic characterization of swamp (B.bubalis kerebao) and river (B. bubalis bubalis) buffaloes and respective progeny

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2.1 Introduction

The buffaloes are classified in the order Artiodactyla**,** sub-family Bovidae, tribe Bovini, genus *Bubalus*, which holds four species: *Bubalus depressicornis* – Plain's Anoa, *Bubalus quarlesi* – Hill's Anoa, *Bubalus mindorensis* – dwarf buffalo from Mindoro e *Bubalus bubalis* – Asiatic Buffalo [1].

The Asiatic buffaloes, also known as water buffaloes, are divided in two groups according to their habitat and cytogenetic characteristics: the "river buffaloes"- *B. bubalis bubalis*, which have 2n=50,XX/XY chromosomes, and the "swamp buffaloes"- *B. bubalis kerebau,* which presents 2n=48,XX/XY chromosomes. In addition, the existence of an intermediary karyotype with 2n=49 chromosomes reveals hybridization among river and swamp buffaloes. The sexual chromosomes of the species of this genus have acrocentric morphology, being the X chromosome the biggest and the Y chromosome the smallest of the complement [2,3,4].

The karyotypic divergence among river and swamp buffaloes results from a tandem fusion between telomere-centromere of chromosomes 4p and 9 of the river buffalo, originating the chromosome 1 of the swamp buffalo, reducing the chromosomal number to 2n=48 [3,5]. According to Wurster and Benirschke[6], chromosomal alterations like tandem fusion are a primary mechanism of differentiation in the Bovidae family [6].

In Brazil, the buffaloes were introduced around 1895 and estimations suggest that the contingent is bigger than three million of animals, according to ABCB-Brazilian Association of Buffaloes Breeders (Associação Brasileira de Criadores de Búfalos),

which recognizes four breeds: Murrah, Mediterrâneo, Jafarabadi, Carabao. There is a fifth group named type Baio, which is not recognized as a breed [7,9].

The breed Carabao and the type Baio are object of conservation studies, since both are in advanced state of genetic erosion and threatened to extinction [7,8]. On the other hand, the race Murrah is well dispersed in the country due to its potential for milk production. However, it frequently presents congenital malformations, as effect of the inbreeding, being object of genetic improvement studies [9]. The purpose of this study was to evaluate cytogenetically buffaloes of the breeds Carabao, Murrah and type Baio, using conventional staining and C- and G-banding techniques.

2.2 Material and Methods

In this work, 92 exemplars of Brazilian buffaloes were studied. Out of them, 54 buffaloes of the Carabao breed (37 females and 17 males), 25 type Baio (17 females and 8 males) from the Pará State and 13 of the Murrah breed (6 males-1 with muscle hyperplasia and 7 females-2 with albinism) from the Rio Grande do Sul State. Around 10 mL of peripheral blood was extracted from each exemplar using Vacutainer tubes with heparin.

The peripheral blood was employed to the establishment of lymphocytes cultures for 72 h at 37ºC, following Moorhead[10]: in 10 mL of culture medium RPMI 1640 (GIBCO) enriched with 20% of fetal bovine serum, phytoemagglutinin and penicillin was added to 1mL of blood. The colchicine (0,05%) was added one hour before the end of the culturing. The cells were treated with hypotonic solution (KCl 0,075M) during 20 minutes and the fixation was performed three times with methanol-acetic acid 3:1.

For the analyses and chromosomal counting, the chromosomes were stained with Giemsa and 30 metaphases were observed for each exemplar. Metaphases were photographed and karyotypes were mounted. For the C-banding technique the methodology of Ledesma [11] was followed and the G-banding technique was performed using the protocol of Seabright [12].

2.3 Results and Discussion

The diploid number of 2n=50 chromosomes was observed for buffaloes of the Murrah breed and of type Baio (Figure 1). For the Carabao breed, 2n=48 chromosomes were observed (Figure 2). In three exemplars, the chromosomal number was 2n=49, corresponding to hybrid animals between river and swamp buffaloes (Figure 3). The sexual chromosomes X and Y were identified as the biggest and smallest acrocentric chromosomes of the complement, respectively [2,4,13]. The chromosomal morphology of the three chromosomal groups shares some similarities and characteristics (Table 1).

The chromosomal divergence among river and swamp buffaloes occurs in the first pair of the swamp buffalo (big, metacentric) and the presence of 18 acrocentric pairs. In the hybrid, the karyotype is characterized by a heteromorphic pair and the absence of the homologue chromosome in the pair 24.

According to Harisah, the F2 of hybrids can result in three different chromosomal categories 2n=50, 2n=49 and 2n=48. Therefore, the chromosomal morphology of the first pair can be utilized for the identification of hybrids of the F2 when they present a diploid number according to the species. Such groups are consequence of the formation of unbalanced gametes n=24 and n=25 in F1 hybrids [14].

Furthermore, an heteromorphism of the X chromosome was observed in one female exemplar of the race Murrah (Figure 4), described by Pires in 1998 just for the Mediterrâneo breed, when studying animals from the state of São Paulo. According to Albuquerque, such fact can be originated from the crossing between breeds in order to increase the variability of the Murrah breed. When in a closed group, this breed presents high frequency of congenital malformations, as effect of the consanguineous matting [15,9].

In the C-banding pattern, the presence of constitutive heterochromatin-HC in pericentromeric regions was identified from the first to the fifth pair. In the other pairs of the chromosomal complement, HC was observed in the centromeric region. The sexual X chromosome holds interstitial bands, while the Y chromosome is C-positive. For the studied buffaloes, no differences in the localization of the HC among the three chromosomal groups was observed [15] (Figure 5).

The G-banding pattern was useful for the identification of the pairs in non-hybrids animals with diploid numbers 2n=48 and 2n=50. In hybrid animals, variations in the karyotype formation in animals F1, F2 and F3 were observed in a hybrid offspring (Figure 6).

3. Conclusão

Os resultados deste trabalho comprovam a existência de búfalos híbridos (2n=49 cromossomos) no Brasil. Evidenciam também a formação cariotipica apresentada por estes animais em uma progênie com gerações F1, F2, F3, e F4 apresentado cariótipos 2n=48 e 2n=50 cromossomos, aparentemente normais correspondentes as subespécies *B. bubalis kerebao* e *B. bubalis bubalis* respectivamente, mas que tratam-se de híbridos.

Conclui-se também que variações morfológicas entre os pares cromossômicos podem ser utilizadas para identificação/classificação de híbridos entre búfalos de rio e de pântano.

Quanto as técnicas de bandeamento, pode-se concluir que a técnica de bandeamento C não fornece suporte para identificar búfalos híbridos. Porém, a técnica de bandeamento G serve como marcador para classificação de búfalos híbridos, pois não é possível observar homologia no padrão de bandas entre os pares cromossômicos.

Sendo assim, as variações morfológicas entre os pares cromossômicos, e a técnica de bandeamento G, constituem uma importante ferramenta para análise cariotípica, tanto para os programas de melhoramento quanto de conservação dos recursos genéticos, identificando alterações cromossômicas como no caso de búfalos híbridos.

4. Figuras e Tabelas

4.1 Tabela 1

TABLE 1

Number and morphology chromosome for buffaloes of the breeds: Murrah, Carabao, type Baio and hybrid.

Species	Breed	Karyotype number	Meta/Submetacentric	Acrocentric
B. Bubalis bubalis	Murrah and Type Baio	2n=50	1° ao 5° pair	6° ao 25° pair X largest e Y smaller
Hybrid	Type Baio x Carabao	2n=49	1° ao 5° pair *	6° ao 25° pair ** X largest e Y smaller
B. Bubalis kerebau	Carabao	2n=48	1° ao 5° pair	6° ao 24° pair X largest e Y smaller

*Pair 1 heteromorphic; * * Absence in the pair 24.

4.2 Figura 1

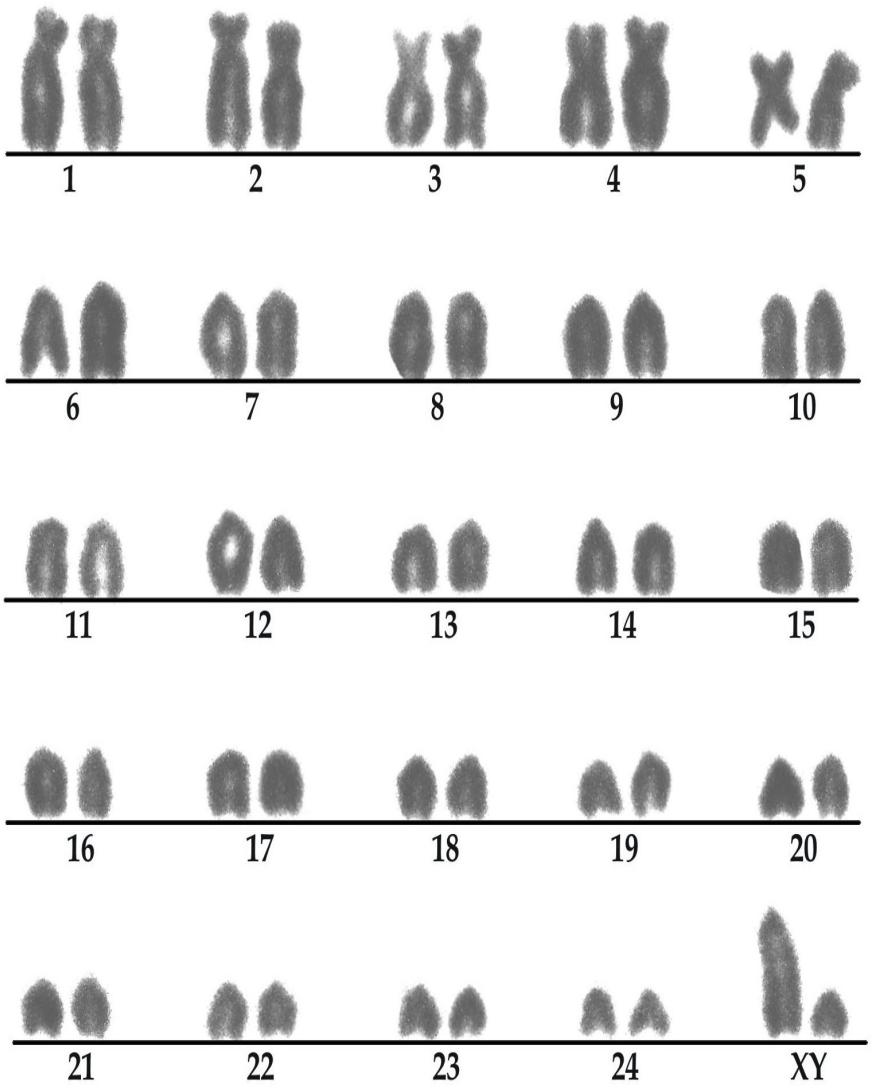

FIGURE 1-Conventionally stained karyotype of male river buffalo 2n=50.

4.3 Figura 2

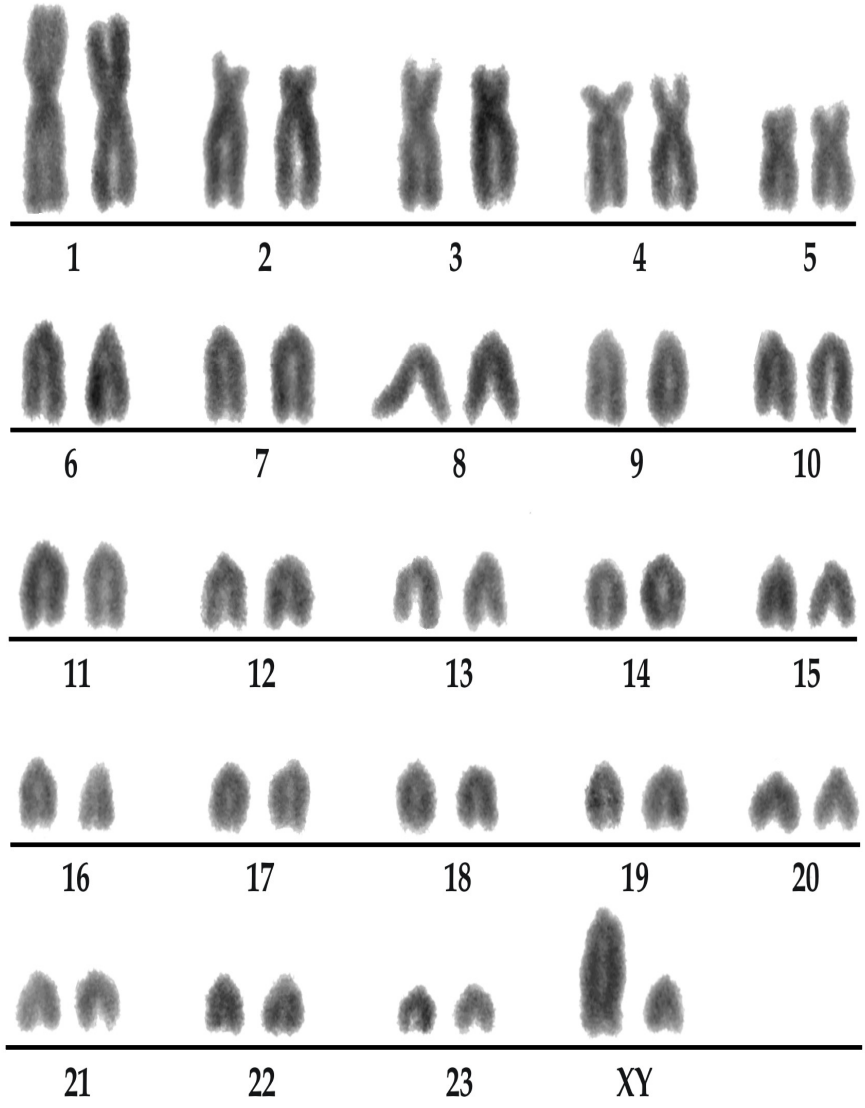

FIGURE 2- Conventionally stained karyotype of male swamp buffalo 2n=48.

4.4 Figura 3

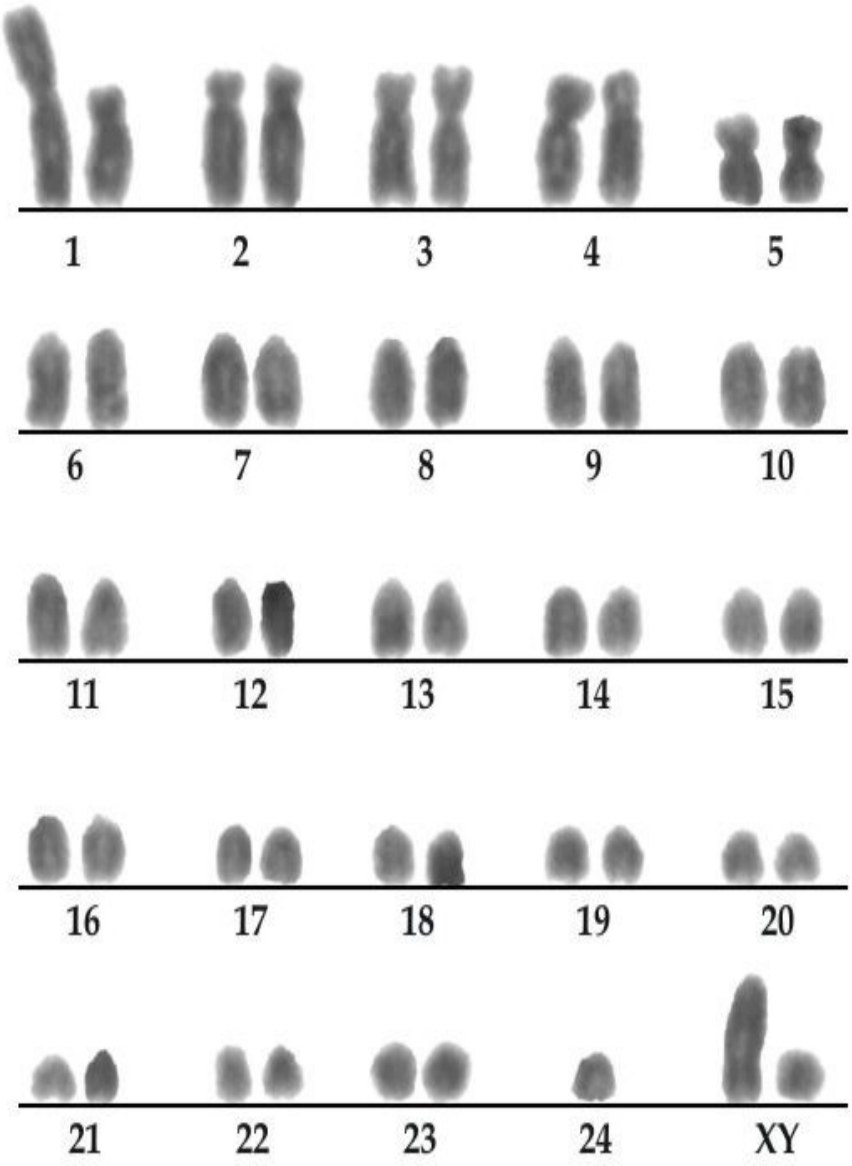

FIGURE 3- Conventionally stained karyotype of male swamp buffalo 2n=49.

4.5 Figura 4

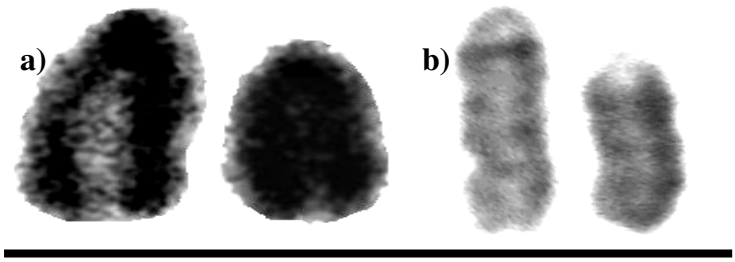

Figure 4: Heteromorphism in the X chromosome pairs. a) Conventional staning. b) G-banding

4.6 Figura 5

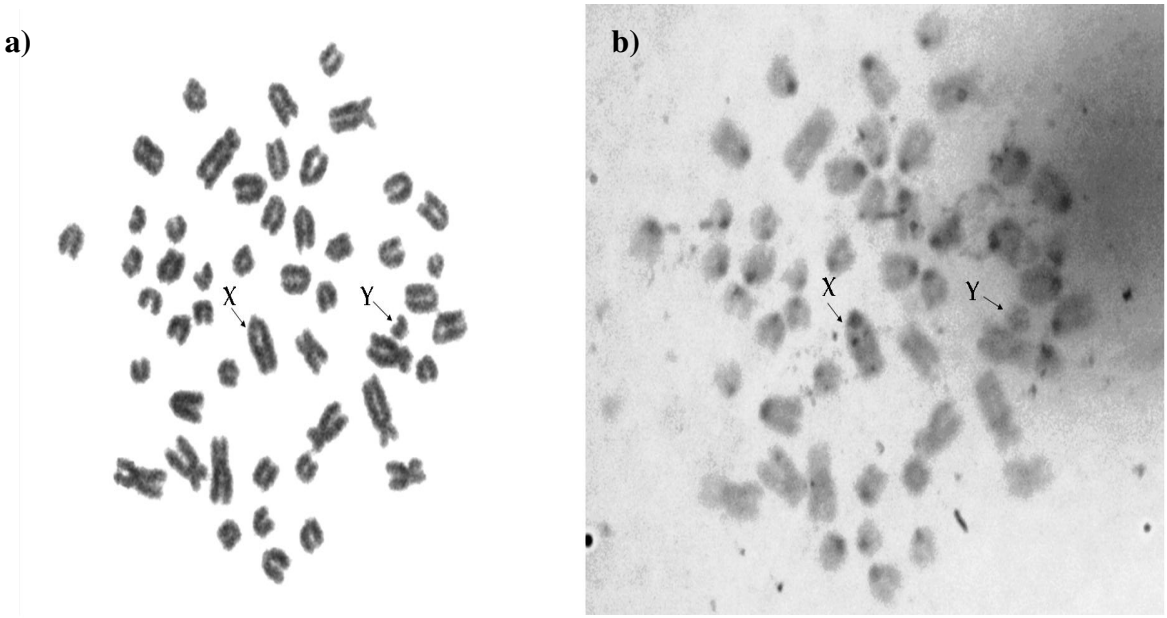

FIGURE 5: Metaphases 2n=50 chromosomes a) Conventional staining; b) Technique C-Banding

4.7 Figura 6

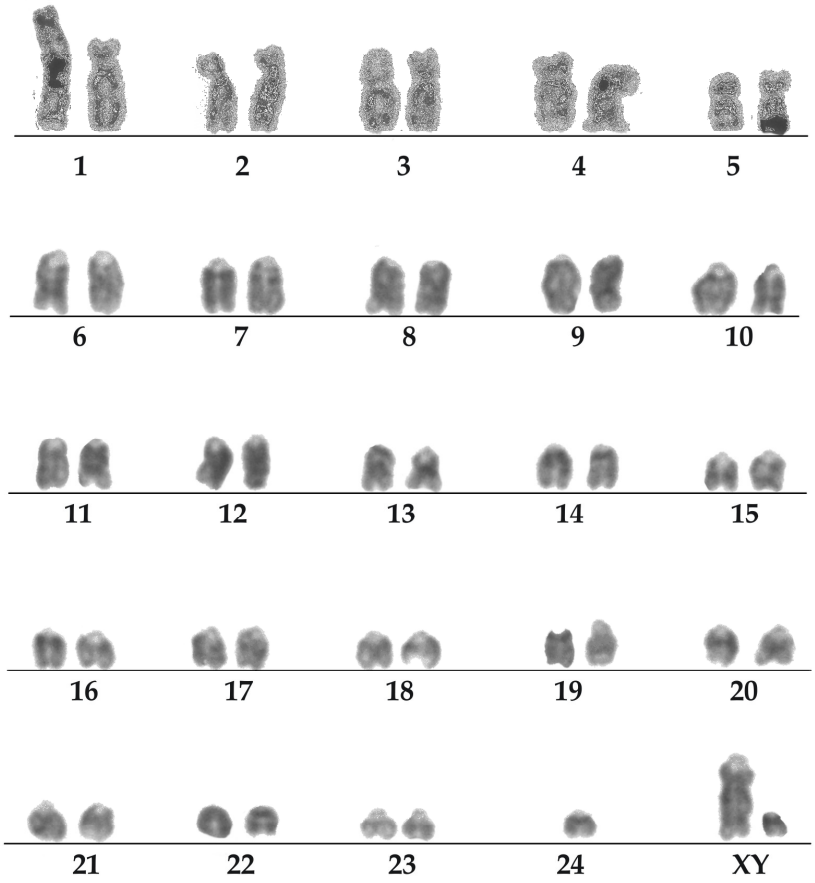

FIGURE 6- Karyotypeof male hybrid buffalo 2n=49 cromossomes with technique G-banding.

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6. Anexo 1:

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Cytogenetic characterization of swamp (*B. bubalis kerebau*) and river (*B. bubalis bubalis*) buffaloes and respective progeny*

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Abstract

The domestic buffaloes are divided in two groups in agreement with your habitat and karyotype number: the "river buffalos" 2n=50 chromosomes and "swamp buffalos" 2n=48 chromosomes. In addition, is reported the existence of a karyotype intermediate with 2n=49 chromosomes, that evidency hybridization among the river and swamp buffaloes. The goal of this work is to characterize cytogenetically the buffaloes of the breed Murrah, Carabao and type Baio, obtaining the patterns of bands C and G. Were obtained preparations metaphases through the cultivation of lymphocytes of blood peripheral in 10 ml RPMI 1640 culture medium. For the breed Murrah and type Baio the chromosomal number is of 2n=50 chromosomes, being the first five pairs morphology metacentric and submetacentric and 20 pairs of chromosomes acrocentric including the sexual pairs X and Y. For the breed Carabao the observed number was 2n=48 of the first five pairs metacentric and submetacentric and 19 pairs of chromosomes acrocentric. For both groups the sexual chromosomes X and Y are larger and smaller acrocentric, respectively. Among the animals classified as hybrid, with

chromosomal number of 2n=49, they presented five pairs of chromosomes meta/submetacentric and 20 pairs of chromosomes acrocentric including the sexual chromosomes X larger and Y smaller acrocentric with some characteristics as: heteromorphic of the pair 1 and absence of the pair's member 24. The pattern of bands G was obtained for identification of the homologous ones. The constituent

heterochromatin revealed demarcation centromeric in every complement and the chromosome Y present C-positive. Therefore, the diploid number 2n=49 are enough for identification of hybrid, while in animals 2n=48 and 2n=50 the first pair's morphologic variation serves as marker to classify them as hybrid.

Key Words: breeds, genetics, genome, reproduction, selection.

INTRODUCTION

The buffaloes are classified in the order Artiodactyla, sub-family Bovidae, tribe Bovini, genus *Bubalus*, which holds four species: *Bubalus depressicornis* – Plain's Anoa, *Bubalus quarlesi* – Hill's Anoa, *Bubalus mindorensis* – dwarf buffalo from Mindoro e *Bubalus bubalis* – Asiatic Buffalo [1].

The Asiatic buffaloes, also known as water buffaloes, are divided in two groups according to their habitat and cytogenetic characteristics: the "river buffaloes"- *B. bubalis bubalis*, which have 2n=50,XX/XY chromosomes, and the "swamp buffaloes"- *B. bubalis kerebau*, which presents 2n=48,XX/XY chromosomes. In addition, the existence of an intermediary karyotype with 2n=49 chromosomes reveals hybridization among river and swamp buffaloes. The sexual chromosomes have acrocentric morphology, being the X chromosome the biggest and the Y chromosome the smallest of the complement [2,3,4].

The karyotypic divergence among river and swamp buffaloes results from a tandem fusion between telomere-centromere of chromosomes 4p and 9 of the river buffalo, originating the chromosome 1 of the swamp buffalo, reducing the chromosomal number to 2n=48 [3,5]. According to Wurster and Benirschke[6], chromosomal alterations like tandem fusion are a primary mechanism of differentiation in the Bovidae family [6].

In Brazil, the buffaloes were introduced around 1895 and estimations suggest that the contingent is bigger than three million of animals, according to ABCB-Brazilian Association of Buffaloes Breeders (Associação Brasileira de Criadores de Búfalos), which recognizes four breeds: Murrah, Mediterrâneo, Jafarabadi, Carabao. There is a fifth group named type Baio, which is not recognized as a breed [7,9].

The breed Carabao and the type Baio are object of conservation studies, since both are in advanced state of genetic erosion and threatened to extinction [7,8]. On the other hand, the race Murrah is well dispersed in the country due to its potential for milk production. However, it frequently presents congenital malformations, as effect of the inbreeding, being object of genetic improvement studies [9]. The purpose of this study was to evaluate cytogenetically buffaloes of the breeds Carabao, Murrah and type Baio, using conventional staining, C- and G-banding techniques.

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MATERIAL AND METHODS

In this work, 92 exemplars of Brazilian buffaloes were studied. Out of them, 54 buffaloes of the Carabao breed (37 females and 17 males), 25 type Baio (17 females and 8 males) from the Pará State and 13 of the Murrah breed (6 males-1 with muscle hyperplasia and 7 females-2 with albinism) from the Rio Grande do Sul State. Around 10 mL of peripheral blood was extracted from each exemplar using Vacutainer tubes with heparin. The peripheral blood was employed to the establishment of lymphocytes cultures for 72 h at 37°C, following [10]: in 10 mL of culture medium RPMI 1640 (GIBCO) enriched with 20% of fetal bovine serum, phytoemagglutinin and penicillin was added to 1mL of blood. The colchicine (0,05%) was added one hour before the end of the culturing. The cells were treated with hypotonic solution (KCl 0,075M) during 20 minutes and the fixation was performed three times with methanol-acetic acid 3:1. For the analyses and chromosomal counting, the chromosomes were stained with Giemsa and 30 metaphases were observed for each exemplar. Metaphases were photographed and karyotypes were mounted. For the C-banding technique the methodology of [11] was followed and the G-banding technique was performed using the protocol[12].

RESULTS AND DISCUSSION

The diploid number of 2n=50 chromosomes was observed for buffaloes of the Murrah breed and of type Baio (Figure 1). For the Carabao breed, 2n=48 chromosomes were observed (Figure 2). In three exemplars, the chromosomal number was 2n=49, corresponding to hybrid animals between river and swamp buffaloes (Figure 3). The sexual chromosomes X and Y were identified as the biggest and smallest acrocentric chromosomes of the complement, respectively [2,4,13]. The chromosomal morphology of the three chromosomal groups shares some similarities and characteristics (Table 1).

The chromosomal divergence among river and swamp buffaloes occurs in the first pair of the swamp buffalo (big, meta-centric) and the presence of 18 acrocentric pairs. In the hybrid, the karyotype is characterized by a heteromorphic pair and the absence of the homologue chromosome in the pair 24.

According to Harisah, the F2 of hybrids can result in three different chromosomal categories 2n=50, 2n=49 and 2n=48. Therefore, the chromosomal morphology of the first pair can be utilized for the identification of hybrids of the F2 when they present a diploid number according to the species. Such groups are consequence of the formation of unbalanced gametes n=24 and n=25 in F1 hybrids [14].

Furthermore, an heteromorphism of the X chromosome was observed in one female exemplar of the race Murrah (Figure 4), described by [15] just for the Mediterrâneo breed, when studying animals from the state of São Paulo. According to Albuquerque, such fact can be originated from the crossing between breeds in order to increase the variability of the Murrah breed. When in a closed group, this breed presents high frequency of congenital malformations, as effect of the consanguineous matting [15,9].

In the C-banding pattern, the presence of constitutive heterochromatin-HC in pericentromeric regions was identified from the first to the fifth pair. In the other pairs of the chromosomal complement, HC was observed in the centromeric region. The sexual X chromosome holds interstitial bands, while the Y chromosome is C-positive. For the studied buffaloes, no differences in the localization of the HC among the three chromosomal groups was observed [15] (Figure 5).

The G-banding pattern was useful for the identification of the pairs in non-hybrids animals with diploid numbers 2n=48 and 2n=50. In hybrid animals, variations in the karyotype formation in animals F1, F2 and F3 were observed in a hybrid offspring (Figure 6).

Table 1: Number and morphology cromossomic for buffaloes of the breeds: Murrah, Carabao, type Baio and hybrid.

Species	Breed	Karyotype number	Meta/Submetacentric	Acrocentric
B. Bubalis bubalis	Murrah e Type Baio	2n=50	1° ao 5° pair	6° ao 25° pair X largest e Y smaller
Hybrid	Tipo Baio x kerebao	2n=49	1° ao 5° pair *	6° ao 25° pair ** X largest e Y smaller
B. Bubalis kerebao	kerebao	2n=48	1° ao 5° pair	6° ao 24° pair X largest e Y smaller

*Pair 1 heteromorphic; * * Absence in the pair 24.

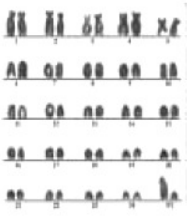

Figure 1 : Karyotype of male type Baio 2n=50.

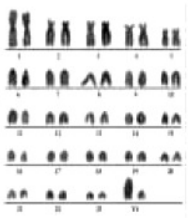

Figure 2: Karyotype of male Carabao breed 2n=48.

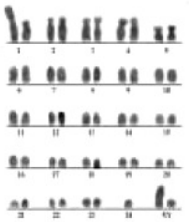

Figure 3 : Karyotyp e of male hybrid 2n=49.

Figure 4 : Heteromorphism in the X chromosome pair for breed Murrah. a) Conventional staning. b) G-banding

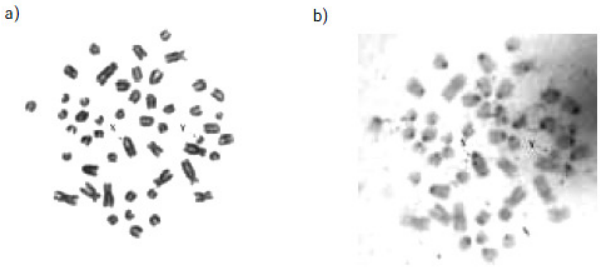

Figure 5: Metaphases 2n=50 chromosomes a) Conventional staining; b) Technique C-Banding

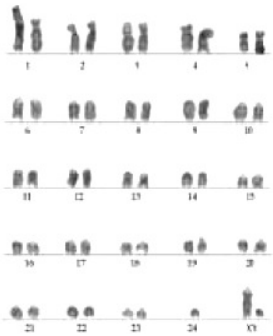

Figure 6: Karyotype of male hybrid buffalo 2n=49 cromossomes with technique G-banding.

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