

Cytogenetic identification of Khuzestani water Buffalo

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Article Info	Abstract
<p>Article history:</p> <p>Received: 27 June 2017 Accepted: 21 February 2018 Available online: 15 December 2018</p> <p>Key words:</p> <p>Cytogenetic Karyotyping Khuzestan River buffalo</p>	<p>The domestic water buffalo (<i>Bubalus bubalis</i>) is an economically important livestock species in many Asian and Mediterranean countries. Two types of water buffalos are river and swamp buffaloes. Genetically, the swamp buffalo has 48 chromosomes ($2n = 48$) and the river buffalo has 50 chromosomes ($2n = 50$). Khuzestan province is one of the important centers of buffalo farming in Iran. Since there was no cytogenetic information about Khuzestani buffalo breed, the present study was performed to provide karyotype and cytogenetic study in Khuzestan buffaloes. In this study, blood samples of 40 buffaloes were collected randomly from jugular vein via heparinized syringes. For metaphase smear, blood samples were cultured in RPMI 1640 medium containing fetal bovine serum and phytohemagglutination and then transferred to water bath for 69 hr. After stopping cell division by colcemide and using fixed solution, from each sample six slides were stained with Giemsa staining. Based on the results, number of chromosomes of the Khuzestani buffaloes was 50 ($2n = 50$). Among the 25 pairs of chromosomes, the first 5 pairs of autosomal chromosomes were sub-metacentric, 19 pairs of autosomal ones were acrocentric and the sex chromosomes (X and Y) were acrocentric. Furthermore, X and Y chromosomes were the largest and smallest acrocentric ones, respectively. Therefore, the buffaloes of Khuzestan province are considered as a river type.</p> <p style="text-align: right;">© 2018 Urmia University. All rights reserved.</p>

شناسایی سیتوژنتیک گاومیش آبی خوزستانی

چکیده

گاومیش آبی اهلی (*Bubalus bubalis*) گونه دامی مهمی از نظر اقتصادی در بسیاری از کشورهای آسیایی و مدیترانه ای می باشد. دو نوع گاومیش آبی، گاومیش رودخانه ای و گاومیش باتلاقی وجود دارد. گاومیش باتلاقی از نظر ژنتیکی دارای ۴۸ کروموزوم ($2n = 48$) و گاومیش رودخانه ای دارای ۵۰ کروموزوم ($2n = 50$) می باشد. استان خوزستان یکی از مراکز مهم پرورش گاومیش در ایران است. با توجه به این که اطلاعات سیتوژنتیک پیرامون نژاد گاومیش خوزستانی وجود نداشت، این تحقیق در راستای تهیه کاریوتایپ و انجام مطالعه سیتوژنتیک در گاومیش های خوزستانی انجام پذیرفت. در این مطالعه، از ۴۰ راس گاومیش به طور تصادفی از ورید وداج توسط سرنگ های هپارینه خون گیری به عمل آمد. برای تهیه گسترش های متافازی، نمونه های خون در محیط RPMI 1640 حاوی سرم جنین گاوی و فیتوهاگلوتینین کشت داده شدند و سپس برای ۶۹ ساعت به حمام آب انتقال داده شدند. پس از توقف تقسیم سلولی توسط کلسمید و افزودن محلول ثیوتی، از هر نمونه شش لام با رنگ گیمسارنگ آمیزی گردید. بر اساس نتایج، تعداد کروموزوم های گاومیش های خوزستانی ۵۰ عدد ($2n = 50$) بود. از میان ۲۵ جفت کروموزوم، ۵ جفت نخست کروموزوم های اتوزومی، ساب متاساتریک، ۱۹ جفت کروموزوم های اتوزومی آکروساتریک و کروموزوم های جنسی (X و Y) آکروساتریک بودند. همچنین، کروموزوم های X و Y به ترتیب بزرگ ترین و کوچکترین کروموزوم های آکروساتریک بودند. بنابراین، گاومیش های استان خوزستان از نوع رودخانه ای محسوب می شوند.

واژه های کلیدی: خوزستان، سیتوژنتیک، کاریوتایپ، گاومیش رودخانه ای

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Introduction

The domestic water buffalo (*Bubalus bubalis*) is an important livestock resource in many countries of Asia, Mediterranean regions and Latin America. The estimated number of buffaloes in the world is more than 199 million scattering in 42 countries. Almost 97.00% of buffaloes are growing in Asia,^{1,2} more than 56.00% of them are in India, about 1.00% are in Europe and others are bred in other parts of the world.³

Generally, there are two types of domesticated buffaloes; the river buffalo (*B. bubalis* subsp. *bubalis*) and the swamp buffalo (*B. bubalis* subsp. *kerabau*). The swamp buffalo has 48 chromosomes, their color is often black and sometimes they have a faint bar on neck. Their body weight in adult females and males is 400 and 500 kg, respectively. The river buffalo has 50 chromosomes. They often have black color, sometimes with white patches on the body. The river buffalo color is occasionally gray, brown and rarely white. Their body weighs in adult males and females are 300 - 700 and 250 - 650 kg, respectively.⁴

Cytogenetic study is used to determine standard karyotype of farm animals which is helpful for recognition of chromosomal abnormalities.⁵ There are some studies performed to determine standard karyotype of river buffaloes in Iran,⁶ Pakistan,⁷ India,⁸ Brazil,⁹ Thailand¹⁰ and Egypt.^{5,11}

There were about 250 thousand heads of buffalo in Iran in 2016. Based on the climate conditions and region, there are three main ecotypes of buffalo in Iran including Azeri ecotype in Ardabil and Western and Eastern Azerbaijan provinces, North ecotype in Guilan and Mazendaran provinces and Khuzestani ecotype in Khuzestan province.¹² There are 90 thousand of buffaloes in Khuzestan province.¹³ In this region, buffaloes are rearing for milk, meat and leather production. Their heights at withers of adult males and females are 148 and 141 cm and their body weights in adult males and females are 650 and 550 kg, respectively.¹⁴ The diet of this kind of buffalo is often green grass, sugarcane waste and sometimes barley grain. Since classification of Khuzestan province buffaloes was unknown regarding cytogenetic and banding, so the aim of the present study was to evaluate the number and banding of buffaloes chromosomes in Khuzestan province.

Materials and Methods

Blood samples were collected from 40 water buffaloes (27 males and 13 females) with different phenotypes from Khuzestan province located in southwest of Iran. Available information such as gender, age, breed and weight was recorded for sampled animals. Blood samples were taken

with heparinized syringe from the jugular vein. Samples were stored in 4 °C until delivering to Narges Genetics Laboratory of Ahvaz for laboratory analyses. To prepare mitosis metaphases, blood samples were cultured in RPMI 1640 medium with fetal bovine serum and phytohemagglutinin and transferred to the ban Marie for 69 hr. After stopping cell division by colcemide (Gibco Laboratory, Grand Island, USA), fixative solution was added. From each sample, six slides were prepared. The G-banding using trypsin and Giemsa (GTG) technique or protocol was carried out for staining.¹⁵ After banding, using an optical microscope (CX31; Olympus co, Tokyo, Japan), by magnification of 400 and 1000, the chromosomes were evaluated. Then, by Leica microscope (DM 2500; Leica Microsystems, Wetzlar, Germany) and Leica image processing software (CW4000 CytoFISH; Leica), the metaphase chromosomes were studied. Also, by standard karyotype of water buffalo, the homologous chromosomes were classified.

Results

Based on the results of present study, the age of studied buffaloes was 5 to 10 years old, the color was black, gray and brown and the weight was 450 to 650 kg. Based on the results, diploid chromosomes of both sexes were 50 ($2n = 50$). This indicates that the studied buffaloes were river type. Chromosome G-banding using trypsin and Giemsa (GTG) analysis showed that buffaloes of Khuzestan province have five pairs of sub-metacentric chromosomes (1, 2, 3, 4 and 5 chromosome) and 20 pairs of chromosomes including sex chromosomes are acrocentric. The length of chromosomes decreased gradually from chromosome number 1 to 25 and there was a little difference in the size of homologous chromosomes.

In karyotyping analysis of male and female river buffaloes, it was found that chromosome 1 was the biggest and chromosomes 2 to 5 were shortened gradually. They had specified p and q arms. Chromosomes 6 to 24 were acrocentric and classified by descending slope. In sex chromosomes, X chromosome was the biggest and Y chromosome was the smallest acrocentric chromosome (Figs. 1 and 2).

By GTG banding, possibility of accurate homologous chromosomes determination was provided. In the present study, it was determined that by GTG banding, most of the centromeres of Khuzestan province buffalo's chromosomes were not stained. Therefore, regions of centromeres were easily detected.

The fundamental numbers (FNs) were 60 in both male and female buffaloes. The 20 arms were belonged to sub-metacentric chromosomes (chromosomes 1, 2, 3, 4 and 5) and 40 arms were belonged to acrocentric chromosomes (6 to 25). Acrocentric chromosomes had only p arms.

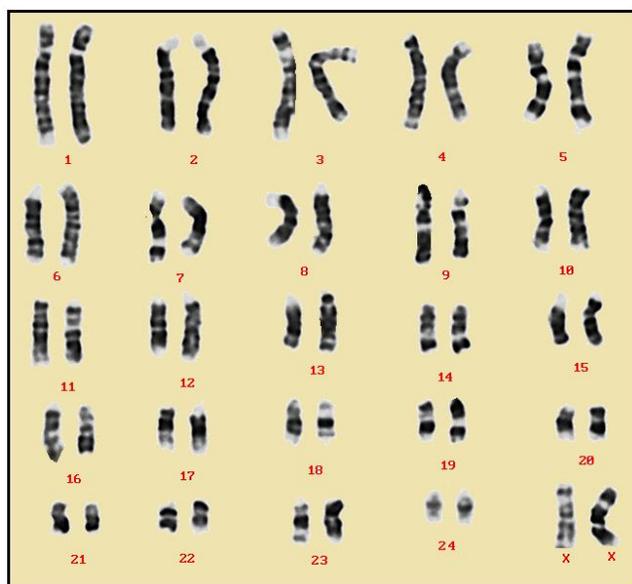


Fig. 1. Karyotype of Khuzestan water buffalo (female, XX, 48) using strips of G-banding using trypsin and Giemsa (GTG).

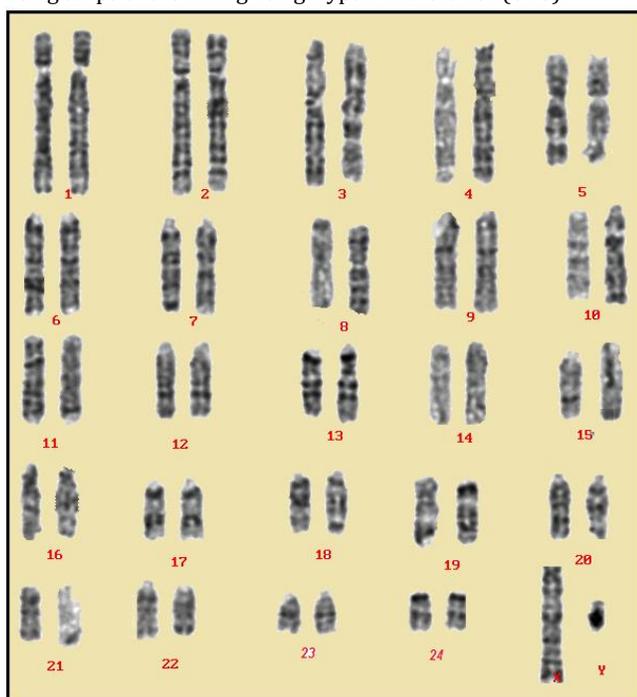


Fig. 2. Karyotype of Khuzestan water buffalo (male, XY, 48) using strips of G-banding using trypsin and Giemsa (GTG).

Discussion

Water buffaloes are classified into swamp and river types. These two types have distinct morphological, cytogenetic (chromosome number; river: $2n = 50$ and swamp: $2n = 48$) and behavioral traits.¹¹ The cytogenetic difference results from the fusion between chromosomes 4 and 9 in a swamp buffaloes. Therefore, all chromosome arms are preserved between both water buffalo types.¹⁶

Cytogenetic study of the Khuzestan province buffaloes revealed that the number of chromosome is 50 including 48 autosome and two sex chromosomes. Female buffaloes had two large acrocentric X chromosomes but male buffaloes had one large acrocentric X chromosome and one small acrocentric Y chromosome.

The results of present study were consistent with studies on water buffaloes by Cribiu *et al.* in Egypt,¹⁷ Iannuzzi in Italy,^{4,18} Yadav *et al.* in India,¹⁹ Ali *et al.* in Pakistan,⁷ Kenthao *et al.* in Thailand,¹⁰ Sali and majeed in Iraq²⁰ and by Yavasoglu *et al.* in Turkey.²¹

Yavasoglu *et al.* have observed that from 25 chromosome pairs of Anatolian buffaloes one pair was sex, five pairs were sub-metacentric and 19 pairs were acrocentric. The result of their study is similar to present study. Ahmad *et al.* have found that Nili-Ravi buffaloes of Pakistan have five pairs of sub-metacentric chromosome and 20 pairs of acrocentric chromosome, similar to present study. Supanum *et al.* have proposed that swamp buffaloes of Thailand have two pairs of metacentric, two pairs of acrocentric, one pair of sub-metacentric, 18 pairs of telocentric and one pair of sex chromosome,²² different from present study.

Based on the results of Ali *et al.* Nili-Ravi buffaloes had five pairs of sub-metacentric, 19 pairs of acrocentric and one pair of acrocentric sex chromosome.²³ Cribiu *et al.* have found that in 25 pairs of Egyptian buffaloes chromosome, five pairs are sub-metacentric and 20 pairs are acrocentric chromosome (sex chromosome included).¹⁷ Their results are similar to present study.

Kenthao *et al.* have found that Mehsani buffaloes of Thailand are river type and have 25 pairs of chromosome.¹⁰ They have found that Mehsani buffaloes have two pairs of metacentric, three pairs of sub-metacentric, 19 pairs of telocentric and one pair of telocentric sex chromosomes. Results of their study are different from present study.

Pournourali *et al.* have found that both Mazani and Azeri buffaloes are river type and have 50 chromosomes.⁶ They have found that from 25 pairs of chromosome, five pairs are sub-metacentric and 20 pairs are telocentric chromosomes. Their result is similar to present study regarding number of chromosomes and sub-metacentric chromosomes but differs in other bandings.

This difference between Khuzestan buffalo and other Iranian buffaloes is probably related to climate because according to climate conditions, Iranian buffaloes consist of three main categories including Azeri, North and Khuzestan ecotypes.¹²

Based on the results of present study, the FNs of Khuzestan river buffaloes were 60 in males and females. Results of the current study were similar to study of Pournourali *et al.*,⁶ Kenthao *et al.*,¹⁰ De Hondt and Ghanam,¹¹ Chandra,²⁴ Bongo and Hilmi and Iannuzzi.^{18,25}

The results showed that the buffaloes rearing in the southwest of Iran are river buffaloes and have 50 chromosomes with five pairs of sub-metacentric, 19 pairs of acrocentric and one pair of sex acrocentric chromosomes.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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