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İ Ç İ N D E K İ L E R / C O N T E N T S

GTG Banded Karyotype of Anatolian River Buffalo (*Bubalus bubalis*, 2n=50)

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The water buffalo (*Bubalus bubalis)* is one of the most important farm animals of Turkey. There are two types of the water buffalo: river and swamp. While the chromosome number of the river type is 2n=50, that of swamp type is 2n=48. It was reported that the Anatolian water buffalo has 2n=50 chromosomes as being river type but the GTG banded karyotype has not been reported so far. We here report for the first time a GTG banded karyotype of the Anatolian water buffalo.
Keywords: Anatolian water buffalo, karyotype, GTG banding.

Anadolu Mandası *(Bubalus bubalis, 2n=50)* GTG Bantlı Karyotipi

Anadolu Mandası*(Bubalus bubalis)* Türkiye yetiştirilen önemli çiftlik hayvan genetic kaynaklarından biridir.Mandalarda Nehir ve bataklık mandaları olmak üzere iki alt gurup bulunur.Nehir Mandalarının kromozom sayısı 2n=50 iken Bataklık Mandalarının 2n=48 dir .Anadolu mandalarının kromozom sayısı nehir mandası olarak 2n=50 olarak rapor edilmiş olmakla beraber henüz GTG bantlı karyotip çalışması yapılmamıştır.Bu çalışmada Anadolu Mandalarının GTG bantlı karyotipi ilk olarak gösterilmiştir.
Anahtar Kelimeler: Anadolu mandası, , karyotip, GTG bandı

Introduction

Anatolian water buffalo breeds play an important role for the Turkey's native animal genetic resources; nevertheless, so far no comprehensive cytogenetic investigation has been performed on this important economic breed (Soysal et al., 2015). The domesticated water buffaloes are believed to have been derived from the Indian wild Buffalo (*Bubalusarnee*). Riverine and swamp buffaloes were domesticated almost together around 2000 B.C., the former in the indus walley and the latter in the Yangtse valley. Domestic buffalo reached Southern East Europe by 12th century, later taken to the America, Australia and Africa in 20th century. Buffalo is important for local economies in many parts of the world, for production of milk and meat and drought animals. Differences in climate, local geography, cropping systems and size of farms characterize the management in different countries. Riverine breed development has largely centered in Indo-Pakistan subcontinent. According to FAO (2013) data there are about 200 million domesticated Buffalo raised in the 42 countries of which 193.8 million (97%) are in Asia of the five world continents. Total Turkish buffalo population according to the Turkish Statistic institute (www.turkstat.gov.tr) is 121.826 in 2014(Singh and Singh, 2015).

Water buffalo (*Bubalus bubalis*) includes two subspecies known as river and swamp buffaloes, which differ in chromosome number and morphology. The riverine buffalo has 50 chromosomes while swamp Buffalo has 48 chromosomes due to tandem fusion translocation between riverine buffalo chromosomes 4 and 9 (Di Berardino and Iannuzzi, 1981). However, the two subspecies are inter-fertile and produce progeny with 49 chromosomes.

Anatolian river buffalo is not yet suitable for intensive commercial dairy farming without a rapid its genetic improvement, based on information produced from genomic studies (Ali et al., 2010). Confirmation of normal chromosomal morphology is the first step towards implementing strategies to improve genetic merit for production and reproductive traits. Cytogenetic diversity within different livestock species is critical to delineate evolutionary genetic relationship and to provide basis to localize economically important genes on different chromosome in the absence of genetic maps (Iannuzzi et al., 2003).

Materials and Methods

Peripheral blood samples were collected into sodium heparin containing tubes from healthy female and male Anatolian water buffaloes from in a farm in the city of Tekirdag, Turkey. Briefly, 0.5ml of whole blood was cultured in 10 ml of RPMI 1640 (Biological industries, Israel) medium containing fetal bovine serum (15%), L-glutamine (Biological industries, Israel), penicillin streptomycin antibiotics mixture (Biological industries, Israel) and Concanavalin A (Sigma Aldrich, Germany) as mitogen at 15 µg/ml final concentration. The cultures were incubated in 15 ml sterile tubes at 37°C for 72 hours in a dry incubator. At the end of incubation, 50µl of colcemid solution (10 µg/ml) (Biological industries, Israel) were added into each culture tube and the cultures were continued to incubation for an additional 30 minutes. The cells were harvested by starting hypotonic (0.075 M KCl) treatment. Following centrifugation at 1000 g for 8 min, the supernatants were removed and hypotonic solution (0.075 M KCl) was added up to 10 ml by vortexing using a Pasteur pipette. Cells were mixed using a Pasteur pipette, and then they were stored at 37° C for 10 min. To stop the hypotonic treatment, 1 ml of fixative solution (methanol/acetic acid 3:1) was added and centrifuged at 1000g for 8 min. The supernatants were removed. Following three additional fixation steps by fixative solution (methanol/acetic acid 3:1), metaphase spreads were prepared by dropping the cell suspension on to cold and wet slides. The chromosomes were banded using trypsin (Sigma Aldrich, Germany) and Giemsa dye (Merck, Germany) followed by aging of the chromosomes for three days at 37°C. One metaphase for female and one for male Anatolian water buffalo were chosen for preparation of the karyotypes. Karyotypes were arranged according to the standard karyotype (Iannuzzi., 1994).

Results and Discussion

GTG banded metaphase spreads from female (left) and male (right) cells of Anatolian river buffalo with relative karyotypes are shown in Fig. 1.

Chromosomes represent an important biological material in the genetic improvement of livestock by means of evolutionary, clinical, molecular, environmental cytogenetics (Iannuzzi, 2015). There is comparatively little information on Anatolian water buffalo breed raised in Turkey, because previous studies did not identified individual chromosomes as of GTG banded karyotype. The precise identification of banding patterns using different staining techniques constitutes a first step to explore the chromosomes so to reveal chromosome abnormalities such as translocations, autosomal and sex chromosome aneuploidies (Ali et al., 2010).

Clinical and cytogenetic studies on water buffalo have been reported in Italy and India (Iannuzi et al., 2005; Di Meo et al., 2008). The study of karyotype is an important step which should routinely be performed to all males and females addressed with reproduction problems. In addition, young females with male traits (large head and horns, prominent withers, tight pelvis) should be promptly investigated (karyotype, rectal palpation) to save time and money (Iannuzi., 2015).

Indeed, chromosomal abnormalities may reduce the genetic improvement program in water buffalo (Iannuzzi, 2015).

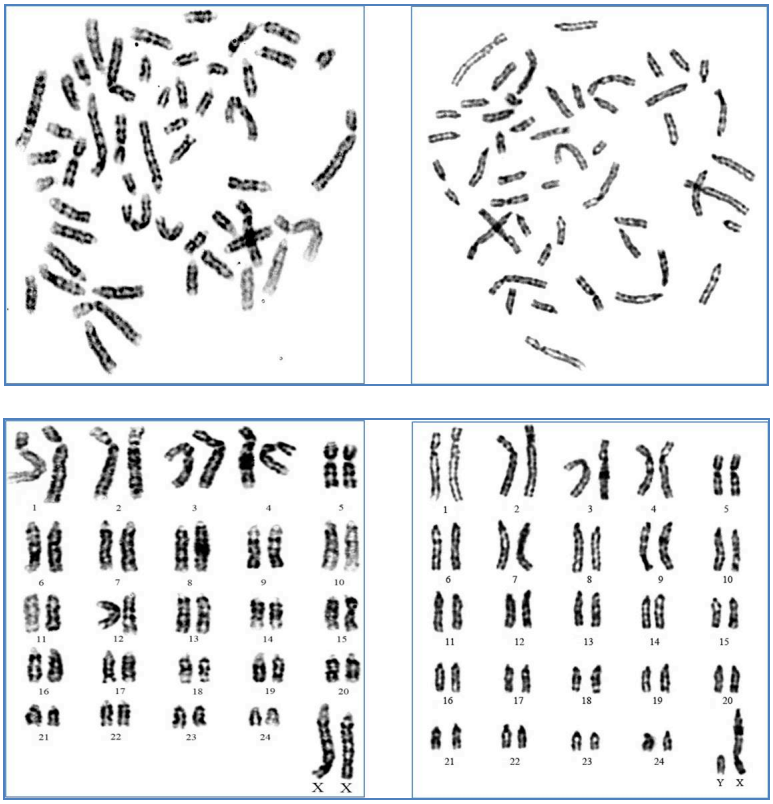

Figure 1. GTG banded metaphase spreads and karyotypes from female (left) and male (right) Anatolian river buffaloes

Since some numerical autosomal abnormalities could be visible, they can be eliminated by the breeders. On the other hand, sex chromosomal abnormalities, almost all related to sterility (or low fertility) especially in females, are generally not visible in the carriers. Structural chromosome abnormalities as deviation from shape and gene order are very important for the high percentage of carriers in cattle (i.e. centric fusion) which have normal body condition but reduced reproductive value (low fertility). A study done in Italy reported that all river Buffalo bulls examined so far showed normal karyotype by RBG banding with the only exception of one famous bull (Magnifico) which was found to be a carrier of complex chromosome abnormalities (Albarella et al., 2013). X trisomy (2n=51,XXX) and X monosomy (2n=49,X) revealed by FISH with X-chromosome specific probe in freemartin cases were also reported (Di Meo et al., 2008).

In conclusion, it is widely accepted that the sex chromosomal abnormalities are strictly related to the fertility, especially in the females. In this study, we report the GTG banded karyotypes of the Anatolian water buffalo which can be used as point of reference for future studies in Turkish buffaloes with infertility and/or with developmental abnormalities.

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