

CYTOGENETIC STUDIES IN THE GREEK BUFALLO (*BUBALUS BUBALIS*)

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Abstract

The main purpose of this work was to describe the karyotype of the Greek buffalo. Cytogenetic studies were carried out on a group of 42 Greek buffaloes (21 females and 21 males) reared in 5 Greek farms. The collected blood samples arrived to Romanian cytogenetic laboratory in the same day and two types of cell cultures were performed: normal cultures and cultures with addition of analogue bases during the last hours of incubation to get improved R-banding patterns and SCEs test. Slides from both cultures were stained with acridine orange or giemsa. The metaphase plates were studied under a Nikon microscope, captured with a CCD camera and were processed by specific image software. The RBA-banding karyotype for male (2n=50,XY) and female (2n=50,XX) have been done. The present study revealed that 21 males and 20 females had normal karyotype and one female was found with chromosomal instability, showing frequent mono- and bi-chromatidic breakages on autosomes and heterosomes. For each investigated animal the cytogenetic analysis bulletin has been delivered.

Key words: Greek buffalo; karyotype; chromosomal instability

INTRODUCTION

Taking into account that the cytogenetic studies represent an useful tool for the genetic evaluation of farm animal, the purpose of this work have been to obtain and describe the karyotype of a small nucleus of Greek buffalo. Since buffaloes are very important for their milk and meat products, the cytogenetic investigations applied to this species should receive much more attention. As well as the artificial inseminations in buffaloes increased, it is very important to keep animals under cytogenetic control. A longstanding interest is to investigate the chromosomal abnormalities. Very important are the structural abnormalities because the carriers have normal phenotypes but the structural alteration of chromosomes

generates chromosomal instability and various level of infertility. In river buffalo, although several studies have been undertaken, clinical cytogenetics is relatively recent. Clinical cytogenetics applied to domestic species should receive much more attention because the costs for cytogenetic control are less than the economical losses which arise when the animals with aberrations are used for breeding. The following report presents the results of our observations concerning the karyotype of Greek buffalo (*Bubalus bubalis*).

MATERIALS AND METHODS

The cytogenetic investigation was carried out in a group of 42 Greek buffaloes (21males and 21females) reared in 5 different Greek farms. The collected blood samples arrived to Romanian cytogenetic laboratory in the same day. Peripheral blood lymphocytes were cultured for about 72 hours at 38,5°C in Pb-

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Max complete medium (Gibco). Two types of cell cultures were performed: normal cultures (without addition of any analogue base) and culture with addition of 5-Bromodeoxyuridine (BrdU) and Hoechst 33258 during the last 6 h to get improved R-banding patterns and SCEs test. Slides from both cultures were stained with acridine orange or giemsa. The metaphases were studied under a Nikon microscope, captured with a CCD camera and transferred on PC in order to be processed by specific software of image analysis.

RESULTS AND DISCUSSIONS

The cytogenetic investigation carried out in the group of 42 Greek buffaloes revealed normal karyotype, $2n=50,XY$ (fig. 1A) for 21 males. In the group of the 21 buffalo females, 20 females presented normal karyotype, $2n=50,XX$ (fig. 1B) and one buffalo female (individual number 620214000415) was identified with abnormal chromosomal configurations expressed by a higher percentage of mono- and bi-chromatidic breaks on the metacentric, acrocentric and sex chromosomes (fig. 2A,B). In this case the cytogenetic diagnosis was *chromosomal instability*.

The RBA banded karyotype have been done, according with the standard karyotype [4], for male (fig. 3) and female too (fig. 4). The normal karyotype of Greek buffalo has five biarmed autosomes and all the others are acrocentric, including X chromosome (the longest acrocentric) and Y chromosome (among the smallest acrocentrics). This configuration belongs to the *river buffalo* type.

For every one of the 42 investigated Greek buffaloes the *Cytogenetic analysis bulletin (CAB)* has been delivered.

SCEs test applied on male and female normal karyotype metaphases showed the normal number (7-8 SCEs/cell) of Sister Chromatid Exchanges for buffalo species (fig. 5).

In the case of buffalo female (nr. 620214000415) with chromosomal instability when we treated the chromosomes for SCEs we observed 11-13 SCEs/cell (fig.6) comparative with the normal situation in buffalo (7-8SCEs/cell). These results suggest that the chromosomal instability identified at the female with a high rate of SCEs could be related with the presence of different

environmental toxic agents. As well as this kind of chromosomal abnormalities may cause reproductive disorders with different level of infertility, it is important to repeat the cytogenetic examination and to investigate the reproduction activity of this female. Many years before, a very much attention has been given to the investigation of chromosomal gaps and breaks in the cultured lymphocytes [15]. Chromosomal instability has been observed in human and animals. Human fragile X chromosome has been associated with the inherited mental retardation [8]. Spontaneous and induced fragile sites have been identified in domestic animals: cattle [3] and goat [7]. It has been suggested that fragile sites are identifiable as non-staining gaps of variable width in the metaphase spreads and may be involved in chromosome breakages and recombination events. The cytogenetic expression of a fragile site is the genomic instability. Although this instability might lead to the gap seen in cytogenetics preparations, it is possible that the consequences are more significant. There is growing evidence that some common fragile sites predispose their surrounding region to the localized chromosomal instability seen in certain cases. The current challenge is to understand the mechanisms of this instability and its biological significance. Concerning the mechanism, it is possible that the common fragile sites and perhaps the rare ones arise because of incompletely replicated DNA sequences that do not package completely for mitosis [15]. Late replication regions of DNA – i.e. initiate replication in the late S phase or are slow to replicate, therefore the chromosomal breaks and gaps observed in metaphase chromosomes are due to un-replicated DNA. The fragility of chromosomes and their relation with chromosome rearrangements were carried out in Holstein-Friesian and Creole cows [6, 14]. These types of abnormalities were related with development and reproductive disturbances [10, 11]. Chromosomal fragility has been identified in two sheep flocks dioxins during pasturage in the Campania region of Italy [5]. Cytogenetic investigations carried out in the Romanian cattle and buffaloes revealed also that, the chromosomal instability, most often, influence the reproductive performances [9, 10, 11, 12].

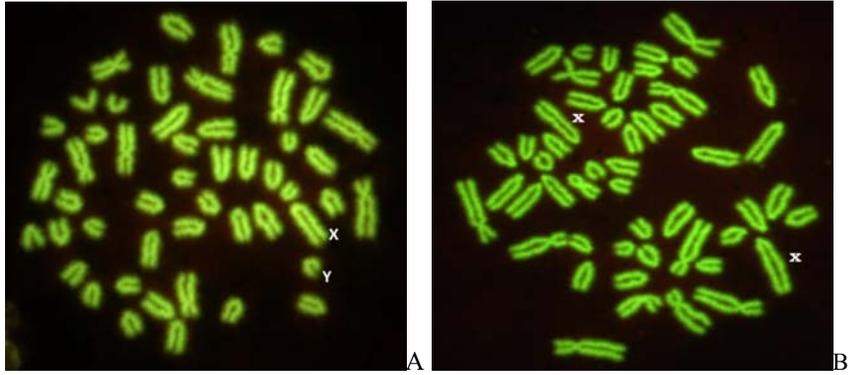


Fig. 1,A,B Normal metaphases of male (A) and female(B) buffalo

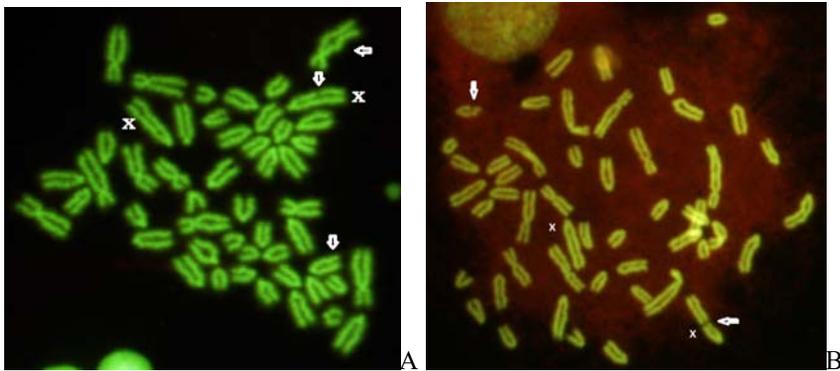


Fig. 2,A,B Female buffalo metaphases with chromosomal instability

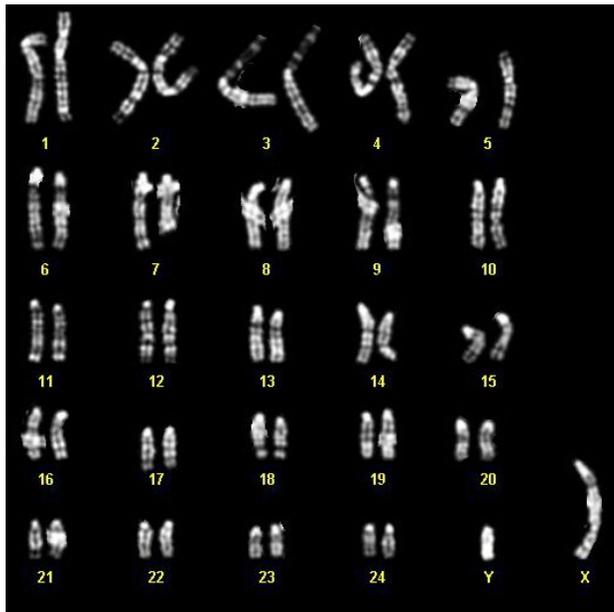


Fig. 3 RBA-banded male buffalo normal karyotype

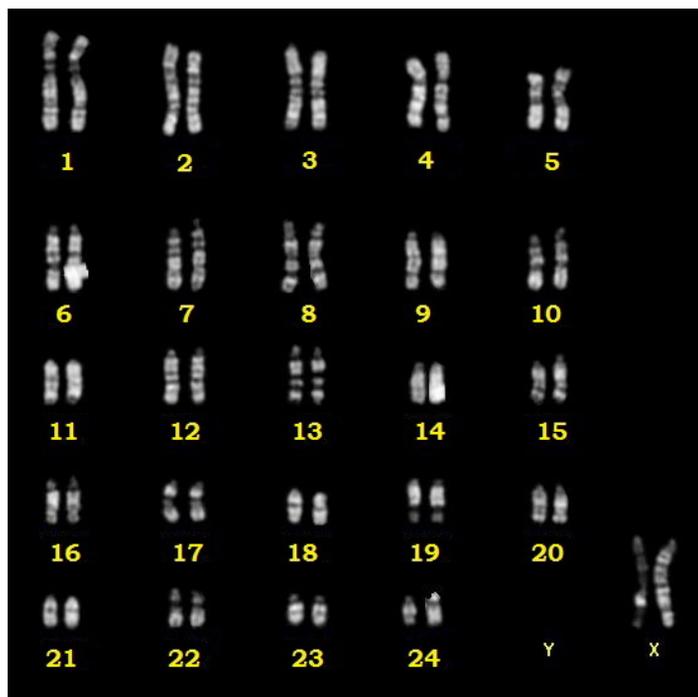


Fig. 4 RBA-banded female buffalo normal karyotype

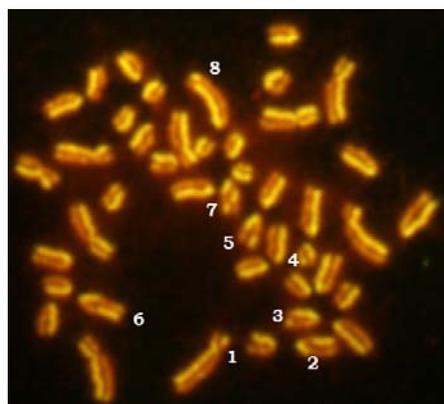


Fig. 5 Buffalo metaphase with normal SCEs



Fig. 6 Buffalo metaphase with abnormal SCEs

CONCLUSIONS

The Greek buffalo belongs to the *river buffalo* type.

The normal karyotype of Greek buffalo has five biarmed autosomes and all the others are acrocentric, including X chromosome (the longest acrocentric) and Y chromosome (among the smallest acrocentrics).

The RBA-banding chromosomes revealed normal karyotype ($2n=50,XY$) for 21 male and for 20 female ($2n=50,XX$). One female has been characterised by *chromosomal instability*. SCEs test applied on male and female metaphases showed the normal number of Sister Chromatid Exchanges for buffalo species (7-8SCEs/cell), excepting the female with chromosomal instability who

presented 11-13 SCEs/cell comparative with the normal situation in buffalo.

The results and the observed configurations give us the reason to continue our investigations in order to elucidate the causes of chromosomal instability identified and to point out the effects on reproductive performances of the carrier.

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