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First Report on Standard G-Banded Karyotype of Nili-Ravi Buffalo, *Bubalas bubalis*

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Abstract.- The present study was planned to determine if chromosomal dysmorphology based on G band nomenclature is common in the Nili-Ravi buffalo which is the prime dairy breed thriving predominantly in the Punjab Province of Pakistan. Microscopic screening of 50 good quality metaphase cells from each of 34 male and female buffalo calves revealed the expected chromosome complement of 24 autosomal pairs and a pair of sex chromosomes XY and XX. A complete G-band homology was noticed between Pakistani Nili-Ravi and earlier investigated karyotypes for water buffalo in India and Italy. Morphology of all metaphase chromosomes examined was normal with five pairs being sub-metacentric chromosomes, whereas the other autosomes including X and Y were classed as acro-subacrocentric. During this study we observed that trypsinization time for optimal G-bands was highly variable and individual chromosomes were identified by marker G-positive and negative bands. X chromosome, the largest acrocentric chromosome, had light G positive-negative bands in the upper middle length distal to the centromere.

Key words: Nili Ravi buffalo, karyotype nomenclature, GTG bands.

INTRODUCTION

Buffalo, a prime dairy animal of Pakistan producing 29.25 million tons of high quality milk, is a major source of beef (Economic Survey of Pakistan, 2007–08). Due to market preference for buffalo milk and its potential ability to produce high value mozzarella cheese, buffalo can be a major source of cheese production for export to neighboring Gulf countries. However, productivity potential of Nili Ravi buffalo is not yet suitable for intensive commercial dairy farming without rapid genetic improvement of river buffalo based on information produced from genomic studies. Confirmation of normal chromosomal morphology is the first step towards implementing strategies to improve genetic merit for milk production, feed efficiency, reproductive efficiency and disease resistance.

Cytogenetic diversity within different livestock species is critical to delineate evolutionary genetic relationships and provide a basis to localize economically important genes on different chromosomes in the absence of genetic maps (Iannuzzi, 2007). There is little information on chromosomal morphology and dys-morphology of Nili Ravi buffalo in Pakistan, because previous studies did not identify individual chromosomes (Ali, 2001). The precise identification of banding patterns using differential staining techniques such as G, R, Q-banding constitute a first step to explore chromosomal dys-morphology such as translocations, autosomal and sex chromosome aneuploidy. Studies on clinical cytogenetics of buffalo have recently been initiated and reported in Italy and India (Di Meo *et al*., 2008; Iannuzzi, 2007; Patel *et al*., 2006). This information is important to shed some light on cytogenetic reasons of low fertility in carriers of chromosomal abnormalities due to production of unbalanced gametes during meiosis (Long, 1985). Specific reports on this subject are very rare and only a few reports have been cited in the contemporary literature on buffalo genome. We initiated this unique study to determine standard G-band nomenclature of Nili Ravi buffalo to identify possible chromosomes abnormalities that may be common to this breed.

MATERIALS AND METHODS

Whole blood sample of 34 buffalo calves were collected in heparinized vacutainers through jugular venepuncture from Buffalo Research Institute (BRI) Pattoki, District Kasur. The blood samples were immediately transported to the Molecular Cytogenetics and Genomics Laboratory

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of the University of Veterinary and Animal Sciences Lahore, in a flask maintained at 4°C. These blood samples were cultured using standard protocols (Long, 1985) with slight modifications after optimization of technique in this lab. The culture medium of choice was RPMI-1640 (Gibco) with 20 % fetal bovine serum, pokeweed mitogen and penicillin/streptomycin. Two drops of heparin were also added in the cultures to avoid blood coagulation of blood during incubation period (Iannuzzi, 2008)

To induce GTG-bands the slides were left in the oven at 60°C overnight for maturation before exposing to trypsin treatment. Each slide was exposed to a solution of 35 mg trypsin in 70 ml PBS buffer for 10–30 seconds at the room temperature. The trypsinized slides were then stained with 10% Giemsa Gurr buffer (Gibco) for 10 minutes, washed and blot dried to for microscopic analysis using Applied Imaging (AI) cytovision apparatus. A minimum of 20 well spread metaphase cells were scored for each animal and the selected ones were photographed for karyotype analysis. Each metaphase cell was examined at 1000x magnification under oil immersion.

RESULTS AND DISCUSSION

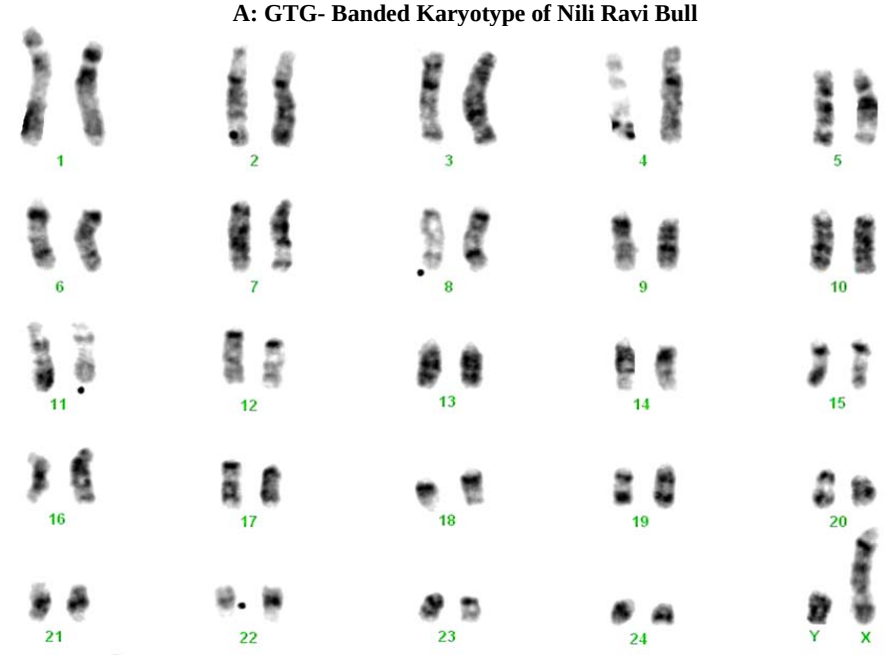
G-band pattern of metaphase chromosomes corresponds to that of chromosomes observed without treatment at pachytene stage of meiosis (Hungerford and Hungeford, 1980). G-banding method either uses the effect of a proteolytic enzyme or mild denaturation, which affects the interaction between proteins and nucleic acid components of the chromatin structure. The G-band mechanism is based mainly upon differences in protein composition and organization (Holmquist, 1988), and the suggested trypsin treatment leads to unfolding of protein loops and permits the protein structure associated with the alignment of AT rich sequences (Popescu *et al.,* 2000).

The GTG banding results revealed that the trypsin exposure time was highly variable to obtain optimal G-bands. Trypsin treatment time of 10 – 30 seconds was found to be generally satisfactory to achieve optimal G-bands at different times. The optimal treatment time was determined by running couple of time course experiments (data not shown). The satisfactory level of G-bands was defined to allow precise identification of individual chromosomes by visualization of bands at 1000× magnification under oil immersion.

The results of this trial were highly inconsistent in generating satisfactory induction of G-bands to allow the precise identification of individual chromosomal bands. Factors affecting staining included relative humidity, room temperature and variable extent of slide maturity during overnight incubation (Wiscovitch *et al.*, 1974). However, there was a general trend of unsatisfactory banding results with humid and cold weather, while trypsinization worked more effectively on a dry and sunny afternoons. In addition, a peculiar difficulty was encountered to grow cytogenetic cultures in case of buffalo. In about 20 culture settings in a series, the blood content of the culture flask would congeal and formed a lump of blood at the bottom even after repeated mixing of the cultures. This made it very difficult to successfully harvest the cultures in the newly established cytogenetic lab. However, this difficulty was controlled by adding two drops of heparin in each flask at the time of culture preparation (Iannuzzi, 2008). This problem may be exacerbated by the high content of heme in buffalo blood.

An example of a typical GTG-banded chromosome spread arranged by karyotype is illustrated in Figure 1. The diploid chromosome complement was 50 *i.e.,* 24 autosomes pairs and one sex chorosome pair, which is, XX in the females and XY in males. The largest five chromosome pairs were classed as sub-metacentric chromosomes having prominent ***p*** and ***q*** arms with highly distinct banding patterns. X chromosome was the largest acrocentric and the Y chromosome was also amongst the smaller acrocentric. Chromosomes pairs 6 – 24 were classified as acrocentric with a descending length gradient. At the cytogenetic level, identification of all Nili Ravi chromosomes was distinctively recognizable due to specific G-band pattern of each chromosome. The diploid chromosome complement of *Bubalas bubalis* and the G-band pattern did not show any significant

A: GTG- Banded Karyotype of Nili Ravi Bull



B: GTG- Banded Karyotype of Nili Ravi Female

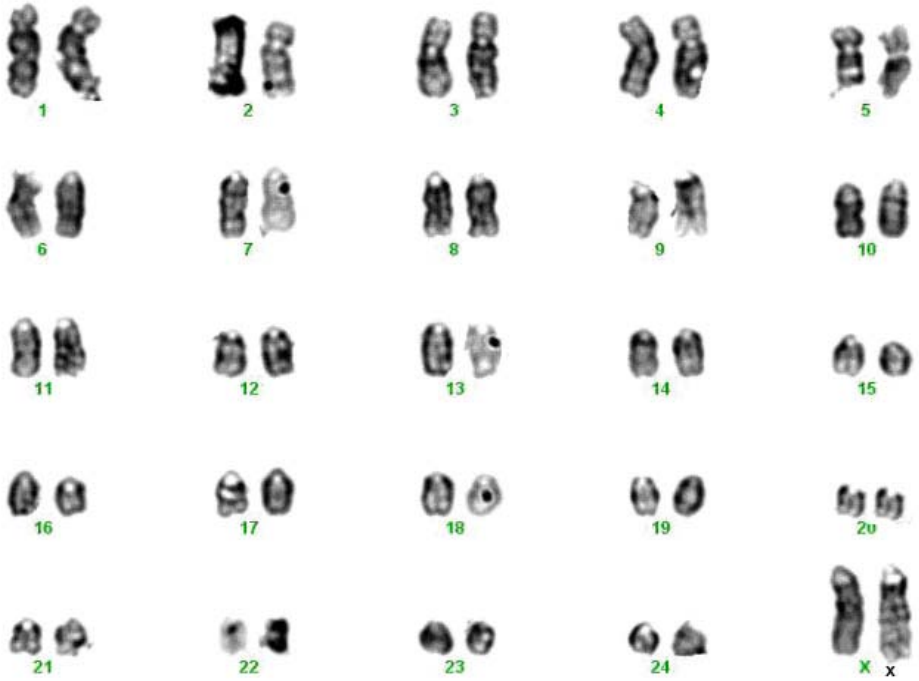


Fig. 1. A GTG-banded Karyotype of Nili Ravi bull (A) and Nili Ravi female (B).

variations across all samples analyzed. Furthermore, when compared with the published G-band nomenclature of river buffalo (Iannuzzi, 2007; Patel *et al*., 2006) there was no significant variation found in marker G-negative and G-positive bands. In the light of homology in G-band pattern, it would be interesting to explore existing chromosomal abnormalities in Nili-Ravi buffalo and to investigate the impact of chromosomal aberrations on fertility and performance of Pakistani buffalo.

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