STUDIES ON THE KARYOTYPE OF THE NILI-RAVI BUFFALO

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ABSTRACT

The normal chromosomal pattern in Nili-Ravi buffalo was studied from the lymphocytic culture of 5 each of the female and male animals kept at Livestock Experiment Station, University of Agriculture, Faisalabad. Micro method for culturing lymphocytes was used for chromosome analysis with slight modification. The chromosomal spread trapped at metaphase stage revealed that 2n diploid number of chromosomes in female and male was 50. The female had 48 autosomes and 2 X-chromosomes. The male had also 48 autosomes along with one X and Y chromosomes. The number of meta/submetacentric and acrocentric autosomal chromosomes was 10 and 38, respectively in both the male and female animals. The sex chromosomes in male and female animals were acrocentric.

Key words: Keryotype, Micromethod, Nili Ravi Buffalo

INTRODUCTION

The buffalo is an important dairy animal in Pakistan as it provides about 71 percent of milk and 23 percent of meat in the country. Recent studies have revealed the problem of reduced fertility and infertility in buffaloes (Ahmad, 1983). Various chromosomal abnormalities associated with reduced fertility/infertility have been reported in buffaloes. Commonly reported chromosomal abnormalities are translocations, trisomies, monosomies, chimerism, pericentric inversion etc. (Balakrishnan *et al.*, 1981; 1985; Yadav *et al.*, 1990; Prakash *et al.*, 1992; 1994; Vijh *et al.*, 1994). Similar chromosomal anomalies might be responsible for reduced fertility and infertility in local buffalo. However, it cannot be definitely established until normal chromosomal idiogram/karyotype for the Pakistani buffaloes are prepared. The present study was thus, planned to establish the chromosomal norms of the Nili-Ravi buffalo which may ultimately help in better understanding of the problem of reduced fertility and infertility in buffaloes.

MATERIALS AND METHODS

In the present study, the micro and macro methods for culturing lymphocytes were used for chromosome analysis with slight modification. However, the micro method has been described briefly in this article. Blood samples from 10 animals of the Nili-Ravi buffaloes maintained at Livestock Experiment Station, University of Agriculture, Faisalabad were used to study the normal chromosomal pattern of the breed. About 5 ml of peripheral blood from the jugular vein of each animal (5 male and 5 female) was drawn aseptically in disposable syringe containing 0.1 ml sodium heparin (Leo). Gibco microtest chromosome culture kit (code 121-6706) was used in this study. The Gibco chromosome medium 1A was rehydrated with the diluent provided in the kit and was placed in a water bath to equilibrate temperature to 37 °C. About 8-10 drops of heparinised blood from each animal was inocculated and mixed in the medium 1 A vial. After 96 hours of incubation, 0.05 ml of colchicine solution was added to give colchicine concentration of 0.1 mcg/ml of medium and the vials were shaken gently. The culture vials were reincubated for another 1.25 hours at 37 °C. The tubes were centrifuged for 8 minutes at 750-1000 r.p.m. Almost all the supernatant was carefully removed leaving 0.25-0.5 ml of liquid on the cells. A 6.0 ml of chilled fixative (Methanol/glacial acetic acid 3:1) was added, 2.0 ml at a time, by mixing gently after each addition of fixative. The centrifuged tubes were tightly closed and kept at 2-8 °C in a refrigerator for about 30 minutes. Then the second fixation was given in the same way.

The microscopic slides were dipped in methanol for one hour and then cleaned with distilled water and stored in distilled water at 4 °C. On each slide, 3-4 drops of cell suspension were dropped at 2-4 places from a distance of 4-6 inches. The slides were dried by using hot plate (40 °C) to evaporate the fixative.

The slides were dipped in couplin jar containing diluted Giemsa stain for 5-10 minutes. The slides were removed, rinsed with distilled water gently and were allowed to dry. The slide were examined under low power (100X) to scan the mitotic spreads. The stained slides were saved permanently by using De.Pe.X solution (BDH).

The mounted slides were scanned at low power (100X) and selected metaphases were photographed under oil immersion (1000X and 1500X) using Black and White film (Fortepan 200). The Black and White photomicrographs were enlarged and the chromosomes were cut from these photomicrographs. The homologous chromosomes were arranged in pairs in their descending order of size according to Book *et al.* (1960) and the arranged chromosomes were rephotographed.

RESULTS AND DISCUSSION

Female Chromosome Analysis: The chromosome spread trapped at metaphase stage from the lymphocytic culture of one female buffalo is shown in Plate 1 (1000X). The number of chromosomes in respect of their centromere position in five buffalo females is given in Table 1. It is evident that there are 48 autosomes and 2 sex chromosomes. The sex chromosomes are the largest acrocentric chromosomes in female. Amongst the autosomes, the number of meta/submetacentric chromosomes is 10, whereas, the number of acrocentric chromosome is 38.

The chromosome spread at metaphase stage of one of the representative female was cut out and arranged in 4 groups (A, B, C & D) according to size and form and is shown in Plate 2. Group A comprised meta/submetacentric chromosomes (Pair 1-5), which were the largest amongst the autosomes.

The chromosomes in group B (Pair 6-12), group C (Pair 13-19) and group D (Pair 20-24) were all acrocentric. The size of chromosomes in group B, C and D was large, medium and small, respectively. The sex chromosomes were the largest amongst the acrocentric chromosomes and hence placed along with group B.

Plate 1: Meta phase spread (1000 X) of Nili-Ravi buffalo female.

Male Chromosome Analysis: The spread of chromosomes captured at metaphase stage from lymphocytic culture of one of the buffalo male is shown in Plate 3 (1000X). The number of chromosomes as counted from the plate is 50. The chromosomes also varied in size, shape and position of centromere. The number of chromosomes with respect to their centromere position is given in Table 1. The 48 autosomes are the same as that of female but there was

Table 1: Number of chromosomes according to the position of centromere in Nili-Ravi buffalo

Sex	No. of Animals	2n	Autosomes		Sex Chromosomes	
			m/sm	Acrocentric	m/sm	Acrocentric
Female	1	50	10	38	0	2
	2	50	10	38	0	2
	3	50	10	38	0	2
	4	50	10	38	0	2
	5	50	10	38	0	2
Male	1	50	10	38	0	2
	2	50	10	38	0	2
	3	50	10	38	0	2
	4	50	10	38	0	2
	5	50	10	38	0	2

m/sm=meta/sub metacentric

only one sex chromosome similar to that of female. The smallest acrocentric chromosome was designated as Y-chromosome. The number of meta/submetacentric and acrocentric chromosomes are same as described for the females.

The chromosome spread at metaphase stage of one of the representative male was also cut out and arranged in 4 groups A,B,C and D according to the size and form (Plate 4). The chromosomal arrangement was similar to that of female except for one X and one Y-chromosome. The Y-chromosome is the smallest acrocentric and hence placed in group D.

The results of the chromosome complement of Nili-Ravi buffaloes as obtained in the present study are similar to those of several workers (Fischer and Ulbrich, 1968; Fischer, 1974; Bongso *et al.* 1977; Bongso and Hilmi, 1982 ;Harisah *et al.*, 1989). As early as 1968, the river type Murrah buffalo was shown to have a chromosome complement of 2n = 50 (Fischer and Ulbrich, 1968). Fischer, (1974) conducted a study on the Karyotype of Swamp X Murrah crossbred buffaloes and reported that the Murrah buffalo had 50 chromosomes. Scheurmann *et al.* (1974) studied the indigenous Swamp buffalo of Sri Lanka and reported that it contained 50 chromosomes. Bongso *et al.* (1977) conducted studies on the karyotype of indigenous Sri Lankan buffalo and confirmed the findings of Scheurmann *et al.* (1974). It was further hypothesized by them that the Sri Lanka Swamp buffalo probably originated from the Indian river type and, in the course of time, when man's emphasis on the animals use shifted from milk to draught in marshy lands, it lost its dairy characteristics and acquired Swamp habits.

The buffaloes of Asia and Australia have been recently classified according to habitat and cytogenetic status (Cockrill, 1981). The river type Murrah buffalo was shown to have a chromosome complement of 2n=50 (Fischer and Ulbrich, 1968), while the Swamp type buffalo of the most Asian countries including Malaysian buffalo possessed 2n = 48 (Ulbrich and Fischer, 1967). The Australian Swamp buffalo believed to be descended from Asian buffalo, also possessed 2n = 48 chromosome complement. The results of the present study about the diploid number 2n = 50 chromosomes are not in agreement with those of several workers who reported that the chromosome number in the Swamp and African buffaloes were 48 and 52, respectively. Ulbrich and Fischer (1967) conducted a comparative study of chromosomes of the Asiatic buffalo (Bulbalus bubalis) and the African buffalo (Cyncerus caffer) and reported that the male and female water buffalo (Swamp buffalo) and African buffalo had 48 and 52 chromosomes, respectively. Similarly, Toll and Halnan (1976) studied the karyotype of the Australian Swamp buffalo and observed that the 2n diploid number was 48. Rommelt-Vasters *et al.* (1978) also observed that the diploid number of chromosomes for the Swamp buffalo was 48 which was not in line with the present study. The differences in the chromosome complement of Swamp and River buffalo was attributed to be the result of a balance autosomal 2/9 tandem fusion in the Swamp buffalo.

The present study, though of preliminary nature, has confirmed the earlier findings about the chromosome number of riverine buffalo (*Bubalus bubalis*). It was also observed that the methodology and procedures used for various species of livestock and human beings were not very effective in buffalo. Several problems in culturing and processing were encountered.

The time recommended for arresting the chromosomes at metaphase stage by colchicine/colcimid treatment is from 1 hour to 2 hours (Lin *et al.*, 1976; Lin *et al.*, 1977; DiBerardino *et al.*, 1981; Bongso and Hilmi, 1982). A great difficulty was observed in determining the proper treatment time, since the prolonged treatment resulted in shortened and thickened chromosomes. However, through several repetitions it was found that the mitotic spread was better when the colchicine treatment was for 1.25 hours.

The other problem pertained to the time required for hypotonic treatment. The hypotonic treatment time as used by various workers in domestic animals ranged from 12 to 25 minutes (Lin *et al.*, 1976; Lin *et al.*, 1977; DiBerardino *et al.*, 1981; Bongso and Hilmi, 1982). In the present study it was noticed that the chromosomes overlapped when the hypotonic treatment was for a shorter time but they scattered widely when the hypotonic treatment was prolonged. The chromosomal spread was the best in the present study when the hypotonic treatment was for 20 minutes.

Two to three fixations of cells have been used in domestic animals by various workers (Lin *et al.*, 1976; Lin *et al.*, 1977; DiBerardino *et al.*, 1981; Bongso and Hilmi, 1982) but in the present study the mitotic spread was observed to be excellent when the smears were made after first fixation of cells. The subsequent fixations resulted into the scattered chromosomes.

It is evident that the methodology and procedures used for various species of livestock and human being are not very effective in buffaloes and need refinement. It is also suggested that further studies using banding techniques for chromosomal deviations may also be under taken to elucidate the problem of reduced fertility/infertility in buffalo.

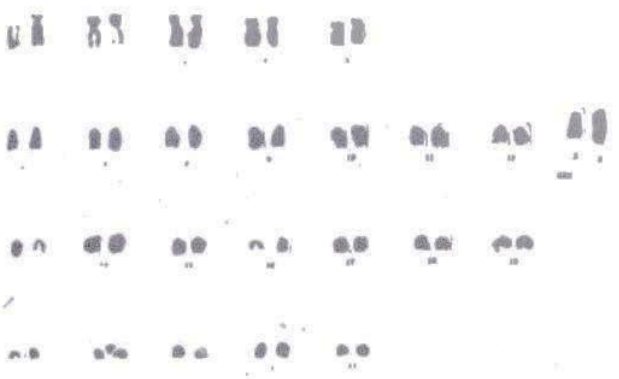

Plate 2: karyotype of Nili-Ravi buffalo female.

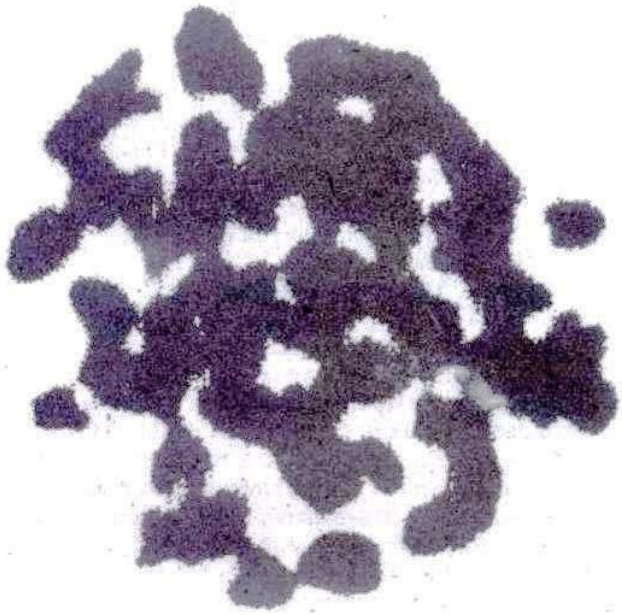

Plate 3: Meta phase spread (1000 x) of Nili-Ravi buffalo male.

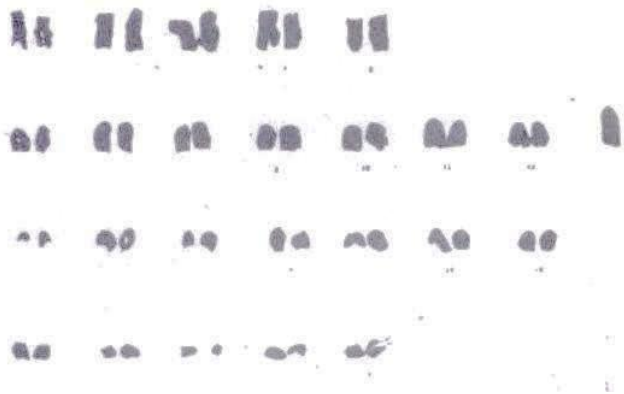

Plate 4: karyotype of Nili-Ravi buffalo male.

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