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SCREENING OF BREEDING BULLS OF DIFFERENT BREEDS THROUGH KARYOTYPING

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ABSTACT

A study of chromosomal analysis for 200 breeding bulls of different breeds of cattle (Jersey, Holstein Friesian, Sahiwal and Cross-bred) and Nili-Ravi buffalo, maintained at Semen Production Unit, Qadirabad and Livestock Experiment Station, Bhunikey (Pattoki) was carried out. Micromethod was adopted for leukocyte culture and chromosomes were trapped at metaphase stage. The diploid number of chromosomes in all breeds of cattle was found to be 60 (58 autosomes and 2 sex chromosomes: XY), while this number in Nili-Ravi buffalo bulls was 50 (48 autosomes and 2 sex chromosomes: XY). All the autosomes and sex chromosomes in males of both species were found normal.

Key words: Karyotyping, micromethod, cattle, buffalo, bulls.

INTRODUCTION

High productivity in farm animals can be achieved through selection and culling procedures. Karyotyping is one amongst different culling parameters. Cyto-geneticists are now armed with powerful tools to characterize normal karyotypes and to discover more about fundamental basis for abnormalities. The chromosomal screening can reduce embryonic and foetal mortality up to 20-30% (Roberts, 1971). Gustavsson (1979) described reduced fertility in female carriers of the 1:29 translocation. On the basis of his results, chromosomal investigations of breeding bulls began in different countries. Chromosomal abnormalities are usually considered to be a plague and are to be eliminated. So, it is very important to screen the breeding bulls for any chromosomal abnormality. Some chromosomal aberrations (Robertsonian translocation, tandem fusion) have marked effects on fertility (Gustavsson, 1980), leading to 10% loss in reproduction. Various chromosomal abnormalities linked with infertility in the buffaloes have been reported by various scientists (Yadav *et al.,* 1990; Prakash *et al.,* 1992; Vijh *et al.,* 1994). The normal chromosomal composition of cattle bull is 58, XY and that of Nili-Ravi buffalo bull is 48, XY (Halnan *et al.,* 1981; Ali *et al.,* 2001).

Chromosomal screening is beneficial in the selection of superior animals. The carriers of chromosomal aberrations can be identified and culled from breeding stock. This aspect of great importance has so far been neglected in Pakistan. It is desirable that the germ plasm introduced in the local population of cattle and buffaloes be screened out for any possible chromosomal abnormality.

The present study was, therefore, conducted to screen the breeding bulls of cattle and buffalo for any karyotypic abnormality, so that it can not be transmitted to the next generations through breeding.

MATERIALS AND METHODS

Blood samples from 200 bulls of Jersey, Holstein Friesian, Sahiwal, Cross-bred (SxHF) cattle and Nili-Ravi buffalo maintained at the Livestock Experiment Station (LES), Bhunikey (Pattoki), district Kasur and Semen Production Unit (SPU), Qadirabad, disrrict Sahiwal, Punjab, Pakistan were used to study the normal chromosomal pattern and any karyotypic abnormality (Table 1). About 5 ml of peripheral blood from jugular vein of each animal was collected in sterile heparinized tubes. The micromethod for culturing leukocytes was used in the present study. The procedure used was the combination of techniques of Moorhead *et al.* (1960) and Lin *et al.* (1977).

For this purpose, 5 ml of chromosomal medium per sample was taken in cell culture tubes and 0.5 ml of blood sample per animal was added up and placed in

Table 1: Detail of animals included in the study

Breed	Animals tested		Total
	LES, Bhunikey	SPU, Qadirabad	
Jersey	48	-	48
Holstein Friesian	80	-	80
Sahiwal	-	56	56
Cross-bred	8	-	8
Nili-Ravi	-	8	8
Total	136	64	200

incubator at 37°C. After 72 hours, culture tubes were removed from the incubator and 1 ml of cholchicine working solution per tube was added, and kept for 2 hours at 37°C. Then these tubes were centrifuged at 1000 RPM for 10 minutes using Phosphate Buffer Solution (PBS) (one tablet per 100 ml double distilled water) and supernatant was discarded. Two washings were given with PBS solution using 10 ml per tube, discarding the supernatant each time. Then 8 ml of potassium chloride solution (0.075M) was added per tube, shaked well and kept at 37°C for 30-35 minutes. After 35 minutes, 2-3 drops of fixative (1:3 ratio of acetic acid and methanol) per tube was added, shaked well and centrifuged for 10 minutes at 1000 RPM. The supernatant was discarded. Then 2 washings were given with fixative (10 ml/tube/washing), discarding the supernatant each time leaving behind tightly packed cells along with some solution of fixative. Slides were prepared by dropping the droplets of the cell suspension from a height of 4-6 inches on the glass slides with the help of a dropper, air dried and stained with Giemsa stain for 10 minutes. Stained slides were examined under high power phase-contrast microscope to study the chromosome spreads in single cells.

RESULTS AND DISCUSSION

Studies on chromosomal preparations showed that diploid number of chromosomes in Nili-Ravi buffalo and cattle of all breeds were 50 and 60, respectively. There is wide variation in the diploid number of chromosomes in different breeds of buffaloes (Miyake *et al.*, 1980). Family Bovidae and tribe Bovina has been classified in to three groups: Bovine (cattle), Syncerina (African buffalo) and Bubalina (Asiatic buffalo). Syncerina consists of red and black buffaloes which have 54 and 52 chromosomes, respectively. Bubalina consists of Anoa buffalo (2n=48), Tamarao buffalo (2n=46) and Asmi buffalo which further consists of swamp buffalo (2n=48), and river buffaloes (2n=50). All buffalo species have different chromosome karyotypes. Karyotypes of Nili-Ravi buffalo bull showed that there were 5 submetacentric and 20 acrocentric pairs (including sex chromosomes). The male has one X chromosome and one Y chromosome, which was one of the smallest acrocentric chromosome.

The present study on Nili-Ravi buffalo bulls showed a similar karyotype to that reported by de-Hondt *et al.* (1989). Toll and Halnan (1976) studied the karyotype of Australian swamp buffalo and reported that the Y chromosome was one of the smallest acrocentric. Gupta and Chaudhri (1978) made similar findings in Murrah buffaloes.

Naqvi and Baig (1994) reported that the Giemsa banded spreads of chromosomes of Nili-Ravi buffalo, trapped at metaphase stage, revealed 2n=50, both in male and female. The male had 48, XY chromosome complement, which is in line with the results of the present study. Ali *et al.* (2001) used different micro and macro methods for culturing lymphocytes of Nili-Ravi breed of buffalo with an incubation period of 94 hours and reported that 2n chromosome number was 50.

The karyotypic analysis of present study for cattle showed that the chromosomes of one cell varied in size, shape and position of the centromere. The 2n diploid number of chromosome was 60. Furthermore, there were 58 autosomes and 2 sex chromosomes in cattle. The sex chromosomes for male, were XY. The X-chromosome was the largest while the Y-chromosome was the smallest, both being submetacentric. However, all the autosomes were acrocentric. It was observed that all the samples tested in the laboratory were normal.

The results obtained in the present study are similar as narrated by earliers workers (Anis *et al.*, 1990; Lioi *et al.*, 1995). Halnan *et al.* (1981) studied the karyotype of Sahiwal, Red Sindhi, Brahman and Santa Gertrodis breeds of cattle and observed that these breeds had 60 chromosomes. Anis *et al.* (1990) reported that all the 58 autosomes were acrocentric.

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