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CYTOGENETIC STUDIES OF THE DAIRY BULLS

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In India, the dairy industries are mainly dependent on cattle and buffalo milk; therefore, cattle and buffalo breeding bulls are considered as dairy bulls. Infertility and reduced fertility in animals are because of many reasons but chromosomal aberrations could also be one of the reasons. Cytogenetic investigations were carried out on a group of 57 dairy bulls of different age group. Lymphocyte culture revealed that a few samples showed sporadic chromosomal aberrations like chromosome fragmentation, polyploidy and premature centromeric division (PCD) in metaphase chromosomes of dairy bulls which may not have direct relevance on fertility as such aberrations were not consistent.. However, one sample each of cattle and buffalo bull exhibited low degree of sex chromosome mosaicism (60,XY/61,XYY) and autosomal mosaicism (50,XY/51,XY) respectively. The bulls exhibiting mosaicism are mature and in semen collection at different places, indicating that both bulls are fertile. In such cases the fertility of bull as compare to normal bull, must be slightly reduced that is not probably noticed by the Manager of sperm stations as fertility index of bull based on their field AI performance are not regularly monitored.

Key words: Cattle, buffalo, breeding bulls, chromosome aberration, mosaicism

Animal Cytogenetics has several applications in animal improvement and one of these is the detection of chromosomal aberrations that are usually associated with reduced fertility, infertility, embryonic losses, fatal waste and internal or external genital malformation. Chromosomal abnormalities account for substantial loss in animal production and number of pedigreed bulls selected for breeding programmes can not be used effectively due to one or the other reasons related to infertility. Chromosomal aberrations (numerical or structural) associated with reduced fertility have caused concern in various countries and many of them have screened their breeding animals regularly (Kovacs and Szepeshelyi, 1987; Patel, 2000). The fertility problems can occur on different levels, and of course due to different factors like poor breeding, feeding and management. However chromosomal aberrations or abnormal karyotypes could also be one of the reasons for reproductive failure in dairy animals. The most common effect of unbalanced karyotype in a zygote is early embryonic mortality. It is therefore, desirable to keep breeding animals under cytogenetic check. In animal population where artificial insemination (Al) is used, chromosomal aberration can affect a large population in two ways**:** i**)** it can be transmitted to large population through Artificial Insemination programme and ii**)** it can cause repeat breeding problems in females because of embryonic losses, and poor semen quality in breeding bulls. Cytogenetics in domestic animals was started in the early sixties and various abnormalities have also been reported in Indian cattle (Prakash et al., 1995; Patel et al., 1997a; Patel, 1999a; Patel and Patel, 2000, Yadav, 2000; Patel, 2002 Patel, 2003; Patel et al, 2005; Chauhan et al, 2009) and in buffaloes (Balakrishnan and Yadav 1984; Balakrishnan et al., 1985; Yadav et al. 1987; Yadav et al. 1990; Prakash et al. 1992; Prakash et al., 1994; Patel et al., 1997b; Patel and Khoda, 1998; Patel, 1999b; Patel et al., 2006; Prakash et al., 2009) associated with reduced fertility or reproductive failure.

MATERIALS & METHODS

Heparinized blood was collected from a phynotypically normal 29 cattle bulls comprising of HF Crossbred, Jersey crossbred, Jersey and Gaolao (zebu) and 27 buffalo bulls mainly Murrah and Surti above 3 years of age for chromosomal screening. Chromosomal preparations were performed by using standard whole blood culture in RPMI-1640 medium supplemented with antibiotics, 15% fetal calf serum and 1% pokeweed mitogen (Patel et al, 1995). The blood culture was incubated at 37°C for 72 hours. To increase the relative frequency of prometaphase chromosomes, Ethidium bromide (Sigma) @10 µg/ml was added and to arrest somatic cell division at metaphase stage, Colchicine (Sigma) @ 2 µg/ml was added to the culture for 2 and 1 h respectively, prior to the harvesting. The cells were harvested by centrifugation at 150 g for 5 minutes followed by hypotonic treatment with 0.56% KCl for 20 minutes at 37°C and fixed in 3:1 ratio of methanol and acetic acid glacial. Finally, cell suspension was dropped on slides and air dried. Slides were conventionally stained in Giemsa stain for screening under the Nikon compound microscope attached with photographic system.

RESULTS AND DISCUSSION

The cattle normally possess 60 (2n) chromosomes. The karyotype composed of 29 pairs of autosomes and one pair of sex chromosomes. All the autosomes are acrocentric and sex chromosomes (XY) are submetacentric in *Bos taurus*, whereas Y chromosome in *Bos indicus* is acrocentric (fig. 1 & 2).

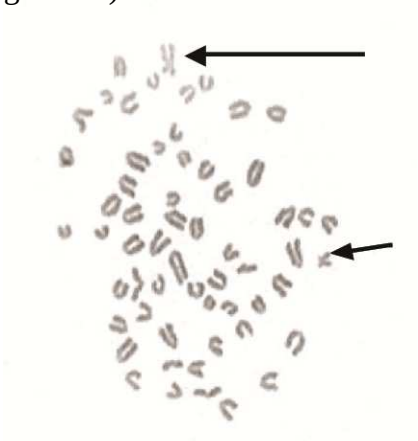

Fig.-1: Submetacentric Y chromosome

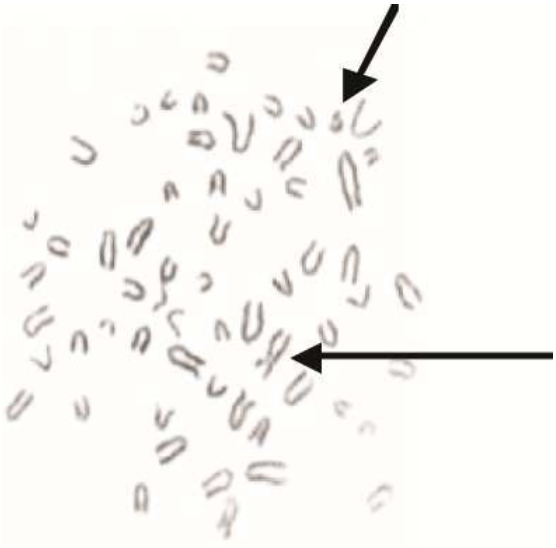

Fig.-2: Acrocentric Y chromosome
(Small arrow indicates Y chromosome and large arrow indicates X chromosome)

The riverine buffalo (*Bubalus bubalis*) possesses 50 (diploid) chromosomes. The karyotype composed of 24 pairs of autosomes and one pair of sex chromosomes. The first five pairs of autosomes are submetacentric whereas all other chromosomes including sex chromosomes are acrocentric. The X chromosome is largest acrocentric which could be easily identified even without GTG banding. The Y chromosome is among the smaller acrocentric chromosomes (Kumar and Yadav, 1991) and can be identified by conventional G and C- bandings, as these are best and simplest techniques which allows distinguishing sex chromosomes (fig. 3).

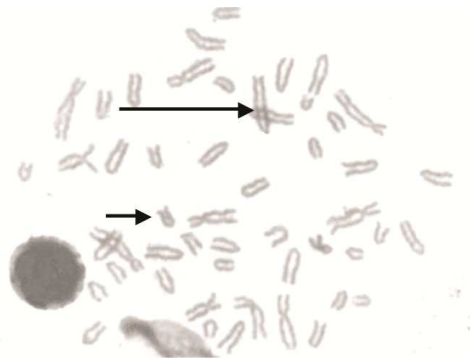

Fig.-3: Metaphase chromosome of buffalo. Large arrow indicates X chromosome and small arrow indicates probable Y chromosome.

As chromosome index was low in most samples, only 22 to 30 metaphase chromosome fields of all the cattle and buffalo bulls were screened in our study. Most of all samples exhibited no gross abnormalities except one each of cattle (60,XY/61,XYY) and buffalo bull (50,XY/51,XY) exhibited low degree of mosaicism (figure 4 to 7). Mosaicism of sex chromosome in cattle was found in 2 out of 30 metaphase fields (6.7%), whereas autosome mosaicism in buffalo bull was 3 out of 30 metaphase fields (10%) in our studies. The additional submetacentric chromosome in a mosaic buffalo bull was size as indicated by large arrows in figure 7. G-banding could not be performed on chromosomal slides because of poor metaphase index. It was also not possible to recollect the blood sample from the bull as sperm station was far away.

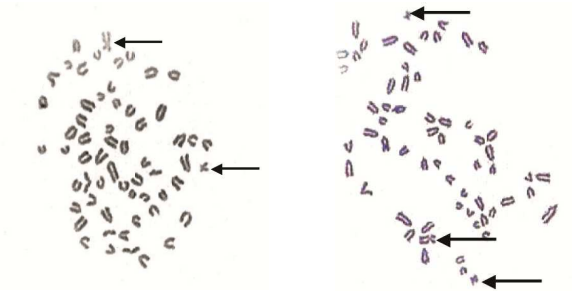

Fig.-4 and 5: Large arrows indicate X chromosomes and small arrows indicate Y chromosomes in both the metaphase plates.

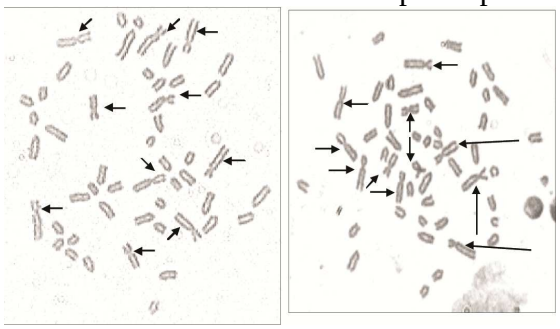

Fig.-6 and 7: Showing 10 and 11 submetacentric chromosomes respectively

Some samples of cattle and buffalo bulls showed sporadic chromosomal aberrations like chromosome fragmentation (fig. 8), polyploidy (fig. 9) and premature centromeric division (PCD) in figure 10 which has no relevance as they were not consistent.

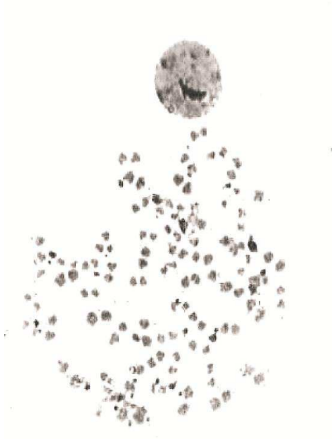

Fig.-8: Chromosomal fragmentation of cattle

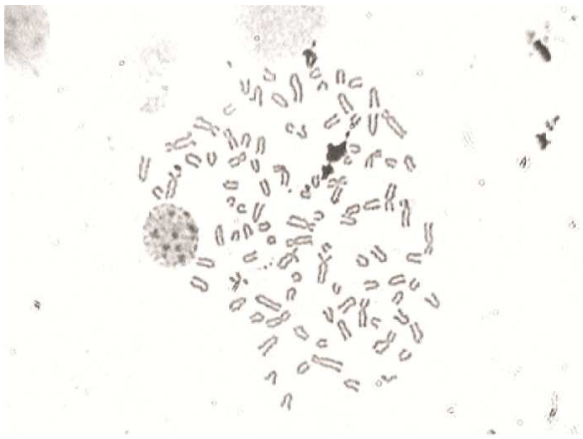

Fig. -9: Polyploidy cell of buffalo

Fig.-10: Premature centromeric division (PCD) in buffalo

Contradictory to human, majority of cattle and buffalo with various kind of sex chromosomal anomalies do not show abnormal body conformation, although these chromosome abnormalities are associated

with reduced fertility or infertility, especially in the cattle females (Patel and Patel, 2000; Iannuzzi et al, 2000, 2001, 2004, 2005; Patel, 2003). The effect of mosaicism on development depends on the timing of the non-disjunction event, the chromosome anomaly, the fraction of the body cells that are carrying the anomaly and the tissues affected.

The defects of sexual chromosomes usually influence the development and function of reproductive system (Gluhovschi and Bistriceanu, 1973). However; defects in autosomes are usually lethal except mosaicism and translocations (Patel, 2001; Vijh et al.,1994;. Patel et al., 2006). Chromosomal disorders such as XO (Yadav et al. 1990; Prakash et al. 1992; Iannuzzi et al. 2000), XXY (Prakash et al., 1994; Balakrishnan and Yadav, 1984), translocation (Chauhan, et al, 2009) reported in buffalo can reduce the fertility or hamper the breeding of animals. The minor structural changes in autosomes like variant chromosome No. 3 and 24 have indicated reduced fertility in buffalo male & females (Balakrishnan et al., 1985; Patel et al, 1997b; Patel and Khoda, 1998; Patel, 1999b) and centric fission (Prakash et al., 2009; Meo et al. 2011).

The bulls exhibiting mosaicism are mature and in semen collection at different places, indicating that both bulls are fertile. In such cases the fertility of bull as compare to normal bull, must be slightly reduced that is usually unnoticed by the Manager of sperm stations as fertility index of bull based on their field AI performance are not regularly monitored. It is advisable to confirm the mosaicism in the cells of other tissue(s) of the body which could not be possible in our studies. In view of the above, the sperm stations are informed to monitor fertility index and accordingly the bulls may be culled or retained for field AI.

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