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First case of centric fission in a Murrah buffalo bull

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ABSTRACT

Reduced fertility in humans and domestic animals has been associated with cytogenetic abnormalities. Examinations during the last four decades of the chromosome complements of various species of domestic animals have revealed the existence of a considerable number of chromosomal abnormalities. Routine cytogenetic investigations, in this study, of cattle and river buffalo males allocated for breeding revealed the presence of higher somatic chromosome number (51) than the normal (50) in all the cells of a young, normal-looking river buffalo bull selected for reproduction. Analysis of the somatic chromosome number, karyotypic features and morphology of chromosomes established that the enhanced chromosome number was the consequence of centric fission of chromosome 1. Though centric fission has been observed in a range of organisms and has been ascribed an important role in karyotype evolution, the present case is only the first incidence of centric fission in buffaloes.

Key words: Centric fission, karyotype evolution, Robertsonaian rearrangements

INTRODUCTION

The relevance of chromosomes to human health and diseases was recognized during 1950s when improvements in techniques facilitated unequivocal chromosome delineation. Even though the counterparts to a variety of human diseases and disorders are seen in domestic animals, clinical applications of veterinary cytogenetics have been less well exploited mainly because of the cost-driven nature of demand on diagnosis and treatment. An area where the potential of veterinary cytogenetics has been largely exploited is reproduction since an inherited aberration that impacts on reproductive efficiency can compromise the success achieved over the years in animal breeding (Basrur and Stranzinger 2008). It is convincing to note that such aberrations can now be tracked and tackled using sophisticated cytogenetic tools.

Different mechanisms for altering diploid chromosome numbers have been described or hypothesized for various plant and animal species. Roberstonian rearrangements, i.e. centric fusions of telocentric chromosomes or centromere splitting of metacentric chromosomes, are the most common interpretations of karyotype alterations which increase or decrease diploid chromosome numbers without altering the arm number (Nombre fondamental). Robertsonian translocations are the most frequent structural chromosomal abnormalities observed in humans (Nielsen and Wohlert, 1991) and cattle (Fries and Popescu, 1999). Heterozygous carriers of Robertsonian translocations generally have a normal phenotype but show variable decreases in fertility (Refsdal, 1976; Gustavsson, 1980; Roux *et al.* 2005). Indeed, these carriers can produce a significant percentage of unbalanced gametes which lead to recurrent spontaneous abortions (Munné *et al.* 1998; Roux *et al.* 2005). The 1/29 centric fusion (Robertsonian translocation) in cattle (Gustavsson and Rockborn, 1964) can be described as the single cytogenetic finding that for the first time illustrated the relevance of cytogenetics in terms of animal breeding. The 1/29 translocation has since then been observed in over 50 breeds of cattle studied by cytogeneticists all over the world (Ducos *et al.* 2008). Robertsonian fusion and centric fission are unique in being readily identified in comparative karyotype studies since both result in concomitant changes in chromosome morphology and chromosome number. The importance of these kinds of change in karyotype evolution is shown by their high incidence in animals.

We report centric fission of chromosome 1 in a healthy river buffalo male referred for chromosome analysis. Both fission products were mitotically stable. This centric fission of chromosome 1 appears to have no clinical significance for this bull.

MATERIAL AND METHODS

Blood samples were taken from jugular vein in sterile vacutainer tubes containing sodium heparin. The metaphase spreads preparations were obtained after the routine lymphocyte culture. The 72-hour lymphocyte culture was performed from whole blood in standard medium (RPMI 1640 – SIGMA, St. Louis, USA) supplemented with 15% of foetal calf serum. Penicillin and streptomycin were added (100 IU/ml and 0.1 mg/ml of culture medium, respectively), and pokeweed mitogen (2.5 µg/mL of culture medium, SIGMA, St. Louis, USA). For chromosome staining, conventional Giemsa staining was applied. A total of 136 Giemsa-stained metaphase spreads of the bull were analyzed. The karyotypes were prepared according to the recommendation of the Committee for the Standardized banded karyotypes river buffalo [CSKBB, 1994].

RESULTS AND DISCUSSION

During normal cytogenetic investigations of breeding males of cattle and buffaloes, a normal looking young breeding Murrah male (river buffalo; Bubalus bubalis L)) was found to carry 51 chromosomes in all the 136 metaphases examined.

The normal standardized karyotype of river buffalo comprises of 50 chromosomes composed of 24 pairs of autosomes and a pair of sex chromosomes (CSKBB 1994). Of the 24 pairs of autosomes, 5 are biarmed (metacentric or submetacentric) and the remaining 19 pairs are acrocentric. The X chromosome is the largest acrocentric chromosome, almost 25% larger than the largest autosome pair and thus morphologically distinguishable. The Y chromosome is a

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small acrocentric chromosome and morphologically indistinguishable from smaller autosomal pairs. A metaphase spread and karyotype of normal river buffalo male is shown in Fig. (A).

The breeding male with 51 chromosomes possessed only 9 biarmed chromosomes as compared to 10 in the normal karyotype of river buffalo. However two acrocentric chromosomes replaced the loss of one submetacentric chromosome. This accounted for the one excess chromosome in the karyotype.

metaphase spreads and karyotypes of the affected bull are depicted in Fig. (B and C). The 5 pairs of river buffalo biarmed chromosomes are morphologically distinguishable based on comparative lengths of small and long arms. Chromosomes 1, 2 and 3 are almost of the same length. But chromosome 3 is a clear metacentric while 1 and 2 are submetacentric. Even chromosome 1 and 2 are morphologically identifiable as the short arms of chromosome 1 are comparatively shorter than that of chromosome

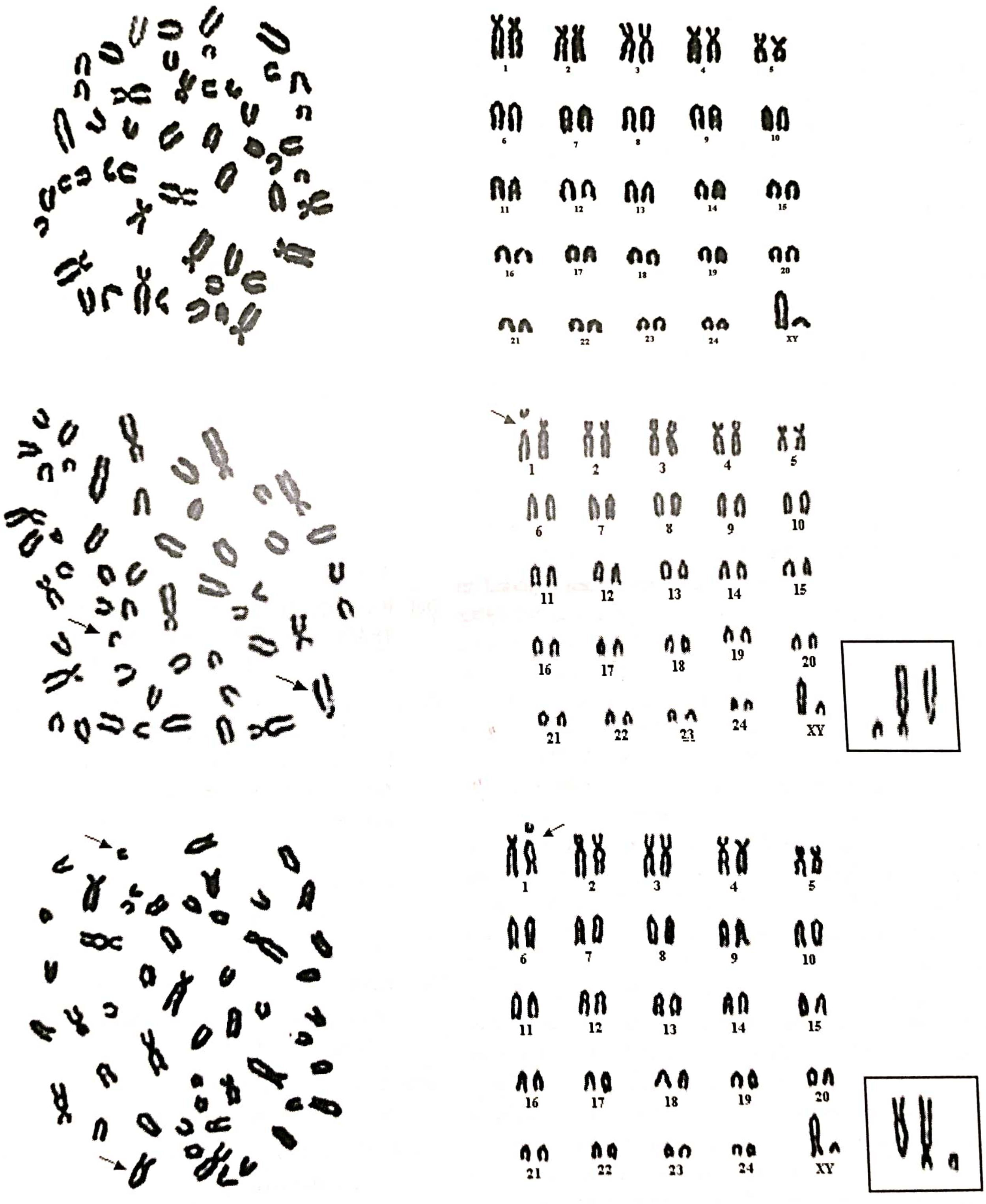

Fig. A =Metaphase spread and karyotype of a normal river buffalo male (2n=50), B & C= Metaphase spreads and karyotypes of the bull with 2n = 51 (centric fission of chromosome 1)

2 and thus unambiguously distinguishable. Based on these morphological distinguishing features it was deduced that the abnormality is due to centric fission of chromosome 1.

This is only the first case of centric fission observed in buffaloes. Due to non-availability of authenticated pedigree and breeding records, fertility status of the bull could not be evaluated. Carriers of this kind of balanced abnormality have a normal phenotype but can present reproduction troubles due to gametogenesis disorders and/or unbalanced gametes production. Centromere (centric) fission, also known as transverse or lateral centric misdivision, has been defined as the splitting of one functional centromere of a metacentric or submetacentric chromosome to produce two derivative acrocentric chromosomes. While centric fusions occur more frequently, centric fission occurs rarely in mammals and has been observed in a range of organisms (Nash z. 2001, Myka *et al.* 2003, Alaoui *et al.* 2004, Perry *et al.* 2005) and has been ascribed an important role in karyotype evolution.

Four cases of apparent centric fission have been described in humans (Perry *et al.* 2005). A centric fission of chromosome 4 was described in a child and his mother, both phenotypically normal (Del Porto *et al.* 1984). Centric fission of chromosome no. 7 was confirmed after G-, Q-, and C-banding of blood lymphocyte chromosomes in a healthy human female, referred for chromosome analysis because of three previous abortions. The same abnormality was found in a further five family members (three females, two males) and had been transmitted over three generations. Similarly de novo centric fission of chromosome 11 was detected in a healthy female referred for chromosome analysis due to recurrent miscarriages (Shim *et al.* 2007).

Few cases of centric fission have been reported in equines. Cytogenetic analysis of forty-two specimens, belonging to five different Spanish breeds in risk of disappearing, revealed that all specimens, except one, presented 2n = 62 chromosomes. Variation in chromosome number in one animal (2n = 63) was because of a fission in one chromosome of pair no. 3, resulting in two acrocentric chromosomes (Alaoui *et al.* 2004). The rearrangement detected suggested a fission tendency in the homologous E. asinus no. 3 chromosome in Equidae species. A mother-daughter pair of donkeys was found to have identical karyotypes with a diploid number of 63. The difference from the species karyotype (2n =62) could be explained by a centric fission event in the third largest autosomal pair (Trommershausen-Bowling and Millon 1988). First time chromosome analyses on 15 animals in a pedigree of endangered species, the Somali wild ass, Equus africanus somaliensis showed that the diploid number ranged from 62 to 64. Numerical chromosomal variation was the result of a centric fission, which was accompanied by a heterochromatic deletion. The centric fission polymorphism involved acrocentric elements 19 and 21 as determined by G-banding. These autosomes are homologous to those involved in centric fission/fusion polymorphisms in other equids: E. asinus (domestic donkey), E. hemionus (onager), E. kiang (kiang), and E. burchelli (common zebra) (Houck *et al.* 1998).

The genus Equus is unusual in that five of the ten extant species have documented centric fission (reverse of Robertsonian translocation) polymorphisms within their populations, namely *E. hemionus onager, E. hemionus kulan, E. kiang, E. africanus somaliensis*, and *E. quagga burchelli* (Myka *et al* 2003). Centric fission describes a rather poorly molecularly defined process of the transverse division of a functional centromere to result in two new centric chromosomes (Perry *et al.* 2004). Centric fission is the consequence of a break within the centromere of a single metacentric/submetacentric producing initially two structural telocentrics whose raw ends have the capacity to fuse after replication. This process produces an isochromosome, but where the mutation succeeds, telomere sequences are added to the ends. This in turn gives rise to two stable chromosomes of telocentric appearance.

No case of centric fission has been reported in domestic species of family bovidae. The present finding is, thus, the first case of this type of chromosomal abnormality not only in buffaloes but also in domestic bovids.

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