Abnormal acrocentric Y chromosome in crossbreed cattle bulls

Youdhveer Singh[1,2], AS Yadav[2], SK Niranjan[1] and B Prakash[1,3]*

[1]*ICAR-National Bureau of Animal Genetic Resources, Karnal*
[2]*Department of Zoology, Kurukshetra University, Kurukshetra*
[3]*ICAR- Central Institute for Research on Cattle, Meerut*

ABSTRACT

Reproductive efficiency is the most crucial issue faced by the dairy industries in India and abroad. A variety of factors affect the reproductive performance of farm animals. Chromosomal abnormalities cause a drop in reproductive performance or even complete infertility in the carrier animals. Present investigation was undertaken to detect chromosomal abnormality, if any, in 206 breeding bulls of exotic (*Bos taurus*, 2n=60), indigenous (*Bos indicus*, 2n=60) cattle breeds and crossbreeds maintained at Central Institute for Research on Cattle, Meerut and Animal Breeding Centre, Salon, Uttar Pradesh. Chromosomal preparations were made using standard blood lymphocyte culture from animals under study. At least 30 metaphase spreads were screened per animal to detect the chromosomal aberrations and prepare the karyotype. Giemsa staining of chromosome revealed that 98.06% cattle bulls possessed normal chromosome. However, 1.94% bulls showed abnormal Y chromosome complements in crossbreed bulls. Extensive use of breeding bulls in breeding programme through artificial insemination (AI) made it mandatory to screen for chromosomal anomalies before inclusion in breeding programme. It will not only check the quick spread of chromosomal abnormalities in animal population rather it will save the time and amount spent on rearing the abnormal animals. Various types of chromosomal anomalies have been reported in India but the frequency of chromosomal abnormalities is much less as compared to reported worldwide.

Keywords: Chromosomal abnormality, crossbred cattle, cytogenetic screening sterility, Y chromosome
***Corresponding author:** birhamp@gmail.com
Manuscript received: 26.9.2017; accepted: 16.11.2017

INTRODUCTION

Domestic cattle of *Bos taurus*, *Bos indicus* and their crosses possess a normal diploid number of 60 chromosomes, which comprises 29 pairs of autosomes and a pair of sex chromosomes (XX in females and XY in males). Structurally, all the 29 pairs of autosomes and the X chromosome are acrocentric and sub-metacentric, respectively, in both *Bos taurus* and *Bos indicus* cattle. The only difference is in the Y chromosome, which is sub-metacentric in *Bos taurus* but acrocentric in *Bos indicus* breeds (Yadav 1981, Prakash 1982).

Alteration in chromosome number and structure are the best known genetic based variations, which have direct effects on fertility and reproductive outcome in cattle (Maria and King, 2004). Chromosome abnormalities have been reported to be associated with reproductive performances in cattle (Patel and Khoda 1998) viz., infertility of carriers, degenerations of reproductive organs, poor semen quality (Ducos et al 2008).Chromosomal aberrations can affect a large population in two ways i) it can be transmitted to a large population through Artificial Insemination (AI) and ii) it can cause repeat breeding problems in females because of embryonic losses and poor semen quality in breeding bulls (Krumrych 2009).Reduced fertility and infertility are major concerns in the dairy animals in India which could be due to poor breeding, feeding and management. However, it could also be due to chromosomal aberrations. Chromosomal aberrations might be transmitted or generated spontaneously during mitotic or meiotic cell divisions. Therefore, complete eradication of chromosomal aberrations from the dairy animal population may not be possible. In view of this the regular chromosomal screening, especially of breeding bulls at the early age, ought to be done. This

Table 1. Details of bulls of different cattle breeds and crossbreeds screened for chromosomal abnormalities

Name of the organization	Cattle Breed					
	Holstein – Friesian	Jersey	Hariana	Sahiwal	Holstein – Friesian X Sahiwal	Jersey X Sahiwal
Central Institute for Research on cattle (CIRC), Meerut	-	-	-	-	176	-
Animal breeding Centre, Salon, Raibarelley, U.P.	13	07	02	02	03	03
TOTAL	13	07	02	02	179	03

practice will not only reduce the occurrence of chromosomal abnormalities in the dairy animal populations rather it will save the time and amount spent on rearing of abnormal animals. In most of the developed countries there is restriction on the import/export of semen/live breeding males without a certification of normal karyotype. On similar lines cytogenetic evaluation of all breeding males has been made essential under the National Programme for Cattle and Buffalo breeding (NPCBB) by Government of India to keep our farm animal species free of any chromosomal abnormalities.Cytogenetics in domestic animals was started in the early sixties and various abnormalities have been reported in Indian cattle (Prakash et al., 1995; Murlidharan et al., 2011) and in buffaloes (Balakrishnan and Yadav 1984; Yadav et al., 1990; Patel et al., 2006; Chauhan et al., 2009; Prakash and Singh, 2009).

MATERIALS AND METHODS

This work was conducted at Department of Zoology, Kurukshetra University, Kurukstetra and molecular cytogenetic laboratory of National Bureau of Animal Genetic Resources, Karnal to investigate the chromosomal abnormalities in breeding bulls of different breeds and crossbreeds of cattle.A total of 206 blood samples were collected in sterile heparinized vacutainer tubes from phenotypically normal 13 Holstein–Friesian(HF), 7 Jersey, 2 Sahiwal, 2 Hariana, 179 Frieswal (i.e.Holstein Friesian x Sahiwal) crossbred and 3 Jersey crossbreed cattle bulls maintained by various organizations (Table 1).The 72-hour lymphocyte culture was performed from whole blood in standard medium (RPMI 1640-Sigma, St. Louis, USA) supplemented with 15% of fetal calf serum,penicillin and streptomycin (100 IU/ml and 0.1 mg/ml of culture medium, respectively), and pokeweed mitogen (2.5 µg/L of culture medium, SIGMA, St. Louis, USA). To arrest the somatic cells at metaphase stage, colchicine (Sigma, India) 2 µg/mL was added for one hour before harvest. The cells were harvested by centrifugation at 1000 rpm for 20 minutes followed by hypotonic treatment with 0.075 M KCl for 20 minutes at 37°C and fixed thrice in Carnoy's fixative (3:1 ratio of methanol and glacial acetic acid). Finally, cell suspension was dropped on slides and air dried. Slides were stained with 2% Giemsa stain and DPX mounted. At least 30 Metaphase spreads for each animal were analyzed under bright field microscopy and karyotyping was done by using automatic karyotyping software(Genus).

RESULTS AND DISCUSSION

In Cattle there are two well-known species: Bos taurus (humpless, taurus) and Bos indicus (humped, Zebu). Both possess a normal somatic chromosome number of 60 (2n = 60) comprising of 29 pairs of autosomes and one pair of sex chromosome. All the autosomes are acrocentric in decreasing order of size and X-chromosome is a large Sub metacentric while Y-chromosome is small sub-metacentric in Bos taurus and smallest acrocentric in Bos indicus(figure1).

***Figure 1**. Normal karyotype of a zebu (2n=60; left) and taurus (2n=60; right) male cattle showing difference in the morphology of Y-chromosome.*

Table 2. Details of bulls showing chromosomal abnormalities

Sr. No.	Station	Species/ breed	Abnormality found	No. of bulls with abnormality
1	CIRC Merrut	Cattle (HF X Sah) Frieswal	Abnormal -Y chromosome (acrocentric)	03
2	Animal Breeding Centre Salon, Raibarelley (U.P.)	Cattle (JY X SAH)	Abnormal -Y chromosome (acrocentric)	01

While screening the chromosomes of 206 males in this study, three crossbred Frieswal (HF X Sahiwal) bulls belonging to Central Institute for Research on Cattle, Merrut, U.P. and one Jersey X Sahiwal crossbred bull from Animal Breeding Centre, Salon, U.P. were found to have acrocentric Y- chromosome instead of the anticipated sub-metacentric. The results of the study are presented in table 2 from which it is evident that out of 206 breeding bulls of cattle screened, only 4 bulls with anomalous karyotypes were detected. All the 4 bulls were crossbred but having acrocentric Y-chromosome. Representative metaphase spreads form two bulls are shown in Fig. 2. Crossbreeding in India always involved crossing of indigenous zebu females with exotic Bos taurus males. The crossbreds so generated are then inter-crossed to generate further filial crossbred generations. Same breeding programme is followed by the two organisations. Thus the source of Y chromosome in all crossbred males is invariably of taurine origin which is sub-metacentric in morphology. All the crossbred males investigated in this study were expected to possess a taurine type sub-metacentric Y chromosome. Thus, the presence of zebu type acrocentric Y chromosome in the four bulls was considered abnormal.

The abnormal Y-chromosome detected in the four bulls could also be due to wrong pedigree or undetected false mating. But the two organizations strictly follow AI and bulls are never kept in the vicinity of females, the possibilities were ruled out. While screening the literature, it was discovered that similar abnormality in HF crossbred bulls was detected by Yadav et al (1984). Analysing the comparative structure of Y chromosome in Bos taurus and B. indicus by FISH using region-specific, microdissected, and locus-specific DNA probes, Goldammer et al (1997) indicated that the Y chromosomes of B. indicus (BIN Y) and B. taurus (BTA Y) differ by a pericentric inversion. Similarly, using comparative FISH-mapping among Y chromosomes of cattle (Bos taurus, 2n = 60, BTA, submetacentric Y chromosome), zebu (Bos indicus, 2n = 60, BIN, acrocentric Y chromosome), river buffalo (Bubalus bubalis, 2n = 50, BBU, acrocentric Y chromosome), sheep (Ovis aries, 2n = 54, OAR, small metacentric Y chromosome) and goat (Capra hircus, 2n = 60, CHI, Y-chromosome as in sheep), Di Meo et al. (2005) concluded that BTA-Y and BIN-Y differed as a result of a centromere transposition or pericentric inversion since they retained the same gene order along their distal chromosome regions and had chromosome arms of different size. It was therefore, assumed that the existence of acrocentric Y chromosome in the four crossbred bulls in this study was due to pericentric inversion in the sub-metacentric Y chromosome leading to an acrocentric Y chromosome(Fig.3A). Similarly, an acrocentric chromosome can evolve into a sub-metacentric chromosome through pericentric inversion (Fig. 3B).

Figure 2. *Representative metaphase spreads of crossbred bulls (HF X SAH, left) and (J X SAH) having acrocentric Y chromosome.*

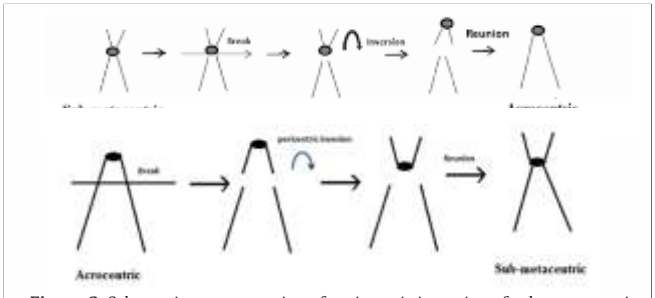

Figure 3. *Schematic representation of pericentric inversion of sub-metacentric chromosome leading to acrocentric chromosome (A) and evolution of sub-metacentric/metacentric chromosome from acrocentric through pericentric inversion (B)*

Thus, in the present study three Frieswal bull (i.e.Holstein Friesian X Sahiwal) and one crossbred (Jersey X Sahiwal) bulls, otherwise physically normal were found to have abnormal acrocentric Y-chromosome. These findings are perplexing because at these institutes crossbreeding has been carried out using males of only exotic breeds with zebu females and subsequent mating amongst the crossbreds. Hence all F1 hybrids must possess only sub-metacentric Y. Similar presence of acrocentric Y chromosome in HF X Tharparkar (Karan fries) cattle bulls was reported by Balakrishnan and Yadav (1987) and they hypothesized the instability of Y chromosome in crossbred bulls. Therefore, a hypothesis of pericentric inversion has been set up for such acrocentric Y chromosome in cattle crossbred bulls which is consistent with the interpretations of Yadav et al 1984, Di Meo et al 2005.

Breeding centers/stations from where samples were collected have been informed regarding the results of cytogenetic evaluation and accordingly the bulls may be culled or retained for AI programme in the view of above results. Extensive experience of chromosomal abnormalities during last six decades has explicitly demonstrated that most of the chromosomal anomalies have a negative impact on the phenotype /production or reproductive efficiency of the carrier animals. It is thus advisable to submit the reproductively inefficient animals to cytogenetic evaluation. More specifically the breeding bulls, which are a source of faster spread of any chromosomal anomaly due to their extensive use in AI, need to be essentially evaluated before putting them into any breeding programme. Cytogenetics can be very handy and cost effective in eliminating this risk and prevent spread of genetic disorders.

ACKNOWLEDGEMENT

The authers thank to Chairman, Department of Zoology, Kurukshetra University, Kurukshetra and Director, National Bureau of Animal Genetic Resources, Karnal for providing permission and necessary facilities to carry out this research. The auther also thanks the Director, Central Institute for Research on Cattle, Meerut and Animal Breeding Centre, Salon, Uttar Pradesh for his necessary support during collecton of blood samples.

REFERENCES

Balakrishnan CR and Yadav BR. 1984. Normal and abnormal chromosome in the Indian River buffalo bull. Buffalo Bullet. 3: 13-17.

Balakrishnan CR and Yadav BR. 1987. Instability of Y chromosome in crossbred males of cattle. NDRI Diamond Jubilee Commemorative. 3:424-430.

Di Meo GP, Perucatti A, Floriot S, Incarnato D, Rullo R, Caputi Jambrenghi A, Ferretti L, Vonghia G, Cribiu E, Eggen A and Iannuzzi L. 2005. Chromosome evolution and improved cytogenetic maps of the Y chromosome in cattle, zebu, river buffalo, sheep and goat. Chromosome Research 13:349–355.

Chauhan JB, Patel RK, Singh KM and Soni KJ. 2009. Impact of a novel Cytogenetic finding (unusual X; X translocation) on fertility of a buffalo bull (Bubalus bubalis). Buffalo Bulletin 28(3): 151-153.

Ducos A, Revay T, Kovacs A, Hidas A, Pinton A, Bonnet-Granier A, Molteni L, Slota E, Switonski M, Arruga MV, Van Haeringen WA, Nicolae I, Chaves R, Guedes-Pinto H, Anderson M and Iannuzzi I. 2008. Cytogenetic screening of livestock populations in Europe: an overview. Cytogenetic and Genome Research 120: 26-41.

Goldammer T, Brunner RM and Schwerin M. 1997. Comparative analysis of Y chromosome structure in Bos taurus and B. indicus by FISH using region-specific, microdissected, and locus-specific DNA probes. Cytogenetics and Cell Genetics 77(3-4):238-41.

Krumrych, W.I.E.S.L.A.W. 2009. Overview of the cytogenetic evaluation of young bulls bred in the north-western part of Poland in 1999-2008. Bull Vet Inst Pulawy, 53, 669-672.

Maria IN and King WA. 2004. Genetic factors that affect normal reproduction and fertility in Domestic Cattle. Veterinary Bulletin, 74 (11): 147.

Muralidharan P, Reddy C, Jain N, Reddy SK and Patel

RK. 2011. A case of centric fusion translocation in a Deoni (*Bos indicus*) Indian cattle bull calf. IIOAB Journal 2 21-23.

Patel RK, Singh KM, Soni KJ and Chauhan JB. 2006. Novel cytogenetic finding: Unusual translocation X:X in Mehsana buffalo (Bubalus bubalis). Cytogenetic and Genome Research 115 186-188.

Patel RK and Khoda VK. 1998. Presence of Variant chromosome 3 in the infertile water buffaloes (Bubalus bubalis). Veterinary Review 13, 25-27.

Prakash B. 1982. Studies on nucleolar organizer regions in chromosomes of cattle and buffaloes. Ph.D. Thesis, Kurukshetra University Kurukshetra (NDRI, Karnal).

Prakash B, Deepika and Singh S. 2009. First case of centric fission in Murrah buffalo bull. Journal of Livestock biodiversity. 1: 56-57.

Prakash B, Balain DS, Lathwal SS and Malik RK. 1995. Infertility associated with monosomy-X in a crossbred cattle heifer. Veterinary record 137(17), 436-437.

Yadav BR. 1981. Studies on chromosomes and their abnormalities in cattle and buffaloes. Ph.D. Thesis, Kurukshetra University Kurukshetra (NDRI, Karnal).

Yadav BR, Balakrishnan CR and Tomer OS. 1984. Chromosomal screening of male cattle and buffaloes. Indian Journal of Animal Sciences 54(6): 519-523.

Yadav BR, Kumar P, and Tomer OS, Kumar S and Balain DS. 1990. Monosomy –X and Gonadal dysgenesis in buffalo Heifer. Theriogenology 34: 99-105.