Cytogenetic screening of cattle and buffalo breeding bulls

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ABSTRACT

Chromosomal abnormalities cause a drop in reproductive performance or even complete infertility/sterility in the carrier animals. Present investigation was undertaken to detect chromosomal abnormality, if any, in 155 breeding bulls of exotic (*Bos taurus*, 2n=60), indigenous (*Bos indicus*, 2n=60) cattle, their crossbreeds and Murrah buffalo (*Bubalus bubalis*, 2n = 50) maintained by various organizations. Mitotic chromosome spreads were prepared and analysed from cultured lymphocytes of 155 breeding bulls of different indicus, exotic and crossbred cattle (Holstein Friesian, Jersey, Red Sindhi and Sahiwal) and 41 Murrah buffalo bulls reserved for breeding and maintained at different stations of State Livestock Development Boards from the state of Haryana, Punjab, Uttrakhand, Jammu and Kashmir, Rajasthan and Assam. Giemsa staining of chromosomes revealed that 98.2% cattle bulls possessed normal chromosome complements. One Holstein-Friesian and one HF crossbred bulls were found to be sex chromosome chimeric (60, XX/60, XY). No chromosomal abnormality could be detected in any of the Murrah buffalo bulls studied. Such regular cytogenetic screening will not only reduce the occurrence of chromosomal abnormalities in dairy animal population but also will save the time and cost of rearing the abnormal animals.

Key Words: Chromosomal abnormality, infertility, sterility, sex chromosome chimeric, cytogenetic screening.
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INTRODUCTION

Reproductive efficiency is one of the corner-stone in enhancing productivity of dairy animals. Infertility and / or sub fertility are the most crucial issues being faced by the dairy industry. The infertility problem can occur at different levels and of course due to different factors like poor breeding, feeding management. However, chromosomal aberrations or abnormal karyotype could be one of the reasons for reproductive failure in dairy animals. Eliminating bulls with physical problems or reduced fertility from the breeding herd will improve overall reproductive efficiency of the herd (Jamir et al., 2015).

Chromosomal aberrations can affect a large population in two ways. It can be transmitted to a large population through artificial insemination (AI) and secondly it can cause repeat breeding problems in females because of embryonic loss and poor semen quality in breeding bulls (Krumrych 2009). Chromosomal abnormalities have been reported to be associated with reproductive performance in Indian cattle (Patel and Khoda 1998), which include infertility of carriers, degeneration of reproductive organs, poor semen quality (Ducos et al., 2008). Chimerism is very common in cattle (Peretti et al 2008) but also has been reported in buffalo (Balakrishnan et al. 1981; Iannunzzi et al. 2005; Kumar et al. 2009). When two or more populations (XX/XY) derived from heterosexual zygotes exist in the same individual, the condition is known as Chimerism (Patel and Patel, 2014). Chimerism usually affects fertility of females but males are not grossly affected. However, on the contrary many reports have been published on reduced fertility or infertility of chimeric bulls. Reduced fertility was

observed and many bulls were culled due to no semen ejaculation, low sperm count or a high incidence of abnormal spermatozoa (Dunn et al., 1979). Cytogenetic investigations in domestic animals were started in early sixties globally and various abnormalities have been reported in Indian cattle (Prakash et al., 1995; Patel and Patel, 2000; Yadav, 2000; Patel, 2002; Patel, 2003) and buffaloes (Chauhan et al., 2009; Prakash et al., 2009).

Due to wide spread use of AI, the risk of spreading genetic defects through bulls has increased manifold. There is therefore a pressing need for cytogenetic screening of bulls before using them for AI to keep the herd free from genetic defects. Even in India it is now mandatory to karyotype each and every cattle and buffalo bull before putting it into breeding programme under the recently formulated National Programme for Cattle and Buffalo Breeding (NPCBB) by DAHDF, Govt. of India. Any animal can be screened or karyotyped even at calf hood stage. Subsequently, the animal with chromosomal abnormality can be removed from breeding programme to avoid the transmission of chromosomal abnormality or genetic defects in progeny.

The present study was conducted to screen the breeding bulls of cattle and buffalo from different states of India for any karyotypic abnormality, so that bull can be culled at early stage before putting into breeding programme and such abnormality are not transmitted to the next generations through breeding.

MATERIALS AND METHODS

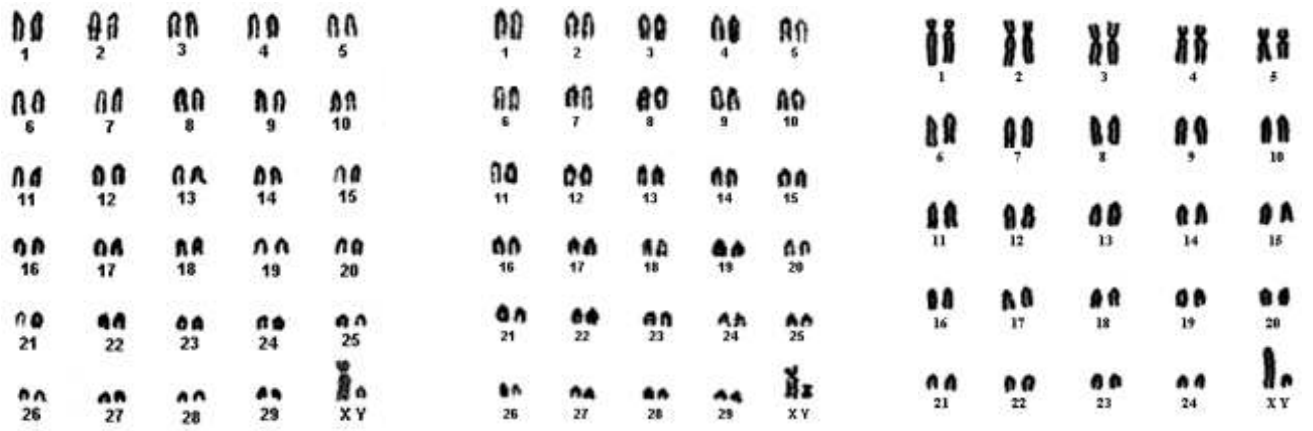
This work was conducted at the Department of Zoology, Kurukshetra University, Kurukshtra and molecular cytogenetic lab of ICAR-National Bureau of Animal Genetic Resources, Karnal to investigate the chromosomal abnormalities in breeding bulls of different breeds and crossbreds of cattle and buffalo.

A total of 155 blood samples were collected from in sterile heparinized vacutainer tubes from phenotypically normal 69 Holstein–Friesian (HF), 7 Jersey, 6 Red Sindhi, 6 Sahiwal, 20 HF crossbred, 6 Jersey crossbreed bulls and 41 Murrah buffalo bulls (Table 1) maintained at different stations of State livestock development boards from Haryana, Punjab, Uttrakhand, Jammu and Kashmir, Rajasthan & Assam.

Whole blood lymphocyte cultures were set-up for 72 hours at 37^0C for each bull in standard medium (RPMI 1640-Sigma, St. Louis, USA) supplemented with 15% fetal calf serum, Penicillin and streptomycin (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). To arrest the somatic cells at metaphase stage, colchicine (Sigma, India) 2 µg/mL was added for one

Table 1 Details of bulls of cattle and buffalo breed wise screened for chromosomal abnormalities.

Name of the Station	Cattle Breed								Buffalo Breed
	HF	Jersey	Red Sindhi	Sahiwal	HF X Sahiwal	Jersey x Sahiwal	HFX Gir	Jersey X Red Sindhi	Murrah
1. Semen Bank, Hisar (Haryana)	05	-	-	-	02	-	-	-	20
2. Animal Breeding Centre, Salon(UP)	13	-	03	01	08	-	02	03	10
3. Semen Bank Nabha (Punjab)	13	-	-	-	05	-	-	-	02
4. Semen Bank, Roper (Punjab)	12	01	-	03	03	-	-	-	-
5. Deep Frozen Semen Production Centre, Rishikesh, (Uttrakhand)	08	01	03	02	-	02	-	-	09
6. Kashmir Livestock Development Board, Kashmir (Jammu and Kashmir)	05	-	-	-	-	-	-	-	-
7. Rajasthan Livestock Development Board, Jaipur (Rajasthan)	12	-	-	-	-	-	-	-	-
8. Frozen Semen Bank, Jagadhari (Haryana)	01	-	-	-	-	-	-	-	-
9. Assam Livestock Development Board, Guwahati(Assam)	00	05	-	-	-	01	-	-	-
TOTAL	69	07	06	06	18	03	02	03	41



A B C

Figure 1. Normal karyotype of zebu (A; 2n:60), taurus (B; 2n:60) and river buffalo (C; 2n:50) males

hour before harvest. The cells were harvested by centrifugation at 2000 rpm for 20 minutes, followed by hypotonic treatment with 0.075 M KCl for 20 minutes at 37°C to lyse the RBCs and lymphocytes were fixed in Carnoy's fixative (3:1 ratio of methanol and glacial acetic acid). Finally, cell suspension was dropped on slides and air dried to prepare chromosome spreads. Slides were stained with 2% Giemsa stain and DPX mounted. At least 30 metaphase spreads for each animal were analyzed under bright field microscopy under oil immersion (100X magnification) and karyotyping was done by using automatic karyotyping software (Genus).

RESULTS AND DISCUSSION

Domestic cattle of *Bos taurus* (humpless taurine), Bos indicus (humped, Zebu) and their crosses possess a normal diploid number of 60 chromosome (2n = 60), 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 submetacentric, respectively, in both *Bos taurus* and *Bos indicus* cattle. The only difference is in the Y chromosome, which is submetacentric in Bos taurus but acrocentric in *Bos indicus* cattle (Lightner, 2008). A normal karyotype of male zebu and Taurus cattle is shown in Fig.1 A-B.

Normal Karyotypic profile of a Murrah buffalo (*Bubalus bubalis*) comprises 50 chromosomes composed of 24 pair of autosomes and a pair of sex chromosomes (CSKBB1994). First five pairs of autosomes are biarmed (metacentric or submetacentric) and the remaining 19 pair are acrocentric. The X chromosome is morphologically distinguishable because it is largest acrocentric chromosome, almost 25% larger than the largest autosome pair (Prakash et al., 2009). The Y chromosome is amongst the smaller acrocentric chromosome and not distinguishable from smaller acrocentric autosome pairs (Kumar and Yadav, 1991). A normal karyotype of male river (Murrah) buffalo (*Bubalus bubalis*) is shown in Fig.1 C.

The results of the study are presented in Table 2. Out of the 155 breeding bulls of cattle and buffalo screened, only 2 cases of anomalous karyotype were detected. Both were cattle bulls which were chimeric with respect to 60, XX/60,XY. First case was a crossbred i.e. Frieswal (HF x Sahiwal) belonging to Animal Breeding Centre, Salon (U.P) and the other was a pure HF bull maintained at Semen Bank, Nabha of Punjab Livestock Development Board.

Both the bulls showed the presence of male (60,XY) as well as female (60,XX) cells in the chromosome preparations obtained from blood leukocytes. One bull showed 17% female cells while the other had 28% female cells (60,XX). Presence of male and female cells in the two bulls is shown in Fig. 2. At present, Sex chromosome chimerism (XX/XY) may

Table 2. Details of bulls having chromosomal abnormalities

S.N.	Station	Species/ breed	Abnormality found	No. of bulls afflicted
1	Animal Breeding Centre Salon, Raibareilly (U.P.)	Cattle (HF X Sahiwal) Frieswal	XX/XY (Chimeric)	01
2	Semen Bank, Nabha (Punjab)	Cattle (H.F.)	XX/XY (Chimeric)	01

be diagnosed by karyotyping (as in the present study), blood typing, polymerase chain reaction (PCR) or fluorescence identification of Y-chromosome directed probes (FISH).

Chromosome chimerism occurs in cattle (60,XX/60,XY) as well as buffalo (50,XX/50,XY) heterosexual twins. The female co-twin of such twins is usually defined as a sterile female (Freemartin) calf that shows underdeveloped or misdeveloped genital tract as a result of early development of vascular anastomoses between fetuses of different gender. As consequence of placental anastomoses between the heterosexual twins, blood chimaerism occurs (60, XX/XY) and passage of male gonad determinants or hormones (such as Anti-Mullerian hormone and androgens) are responsible for disrupted differentiation of the female embryonic gonads and disturbed genital tract development (Padula, 2005; Schlafer and Miller, 2007). Compared to the dramatic changes observed in genital differentiation in the freemartin heifer, the male co-twin only displays minimal gross defects, though a decrease in male fertility has been reported (Dunn et al., 1979; Padula, 2005). The pedigree record of the two bulls in the present study could not be confirmed, but the findings suggest their births as co-twin to female partners.

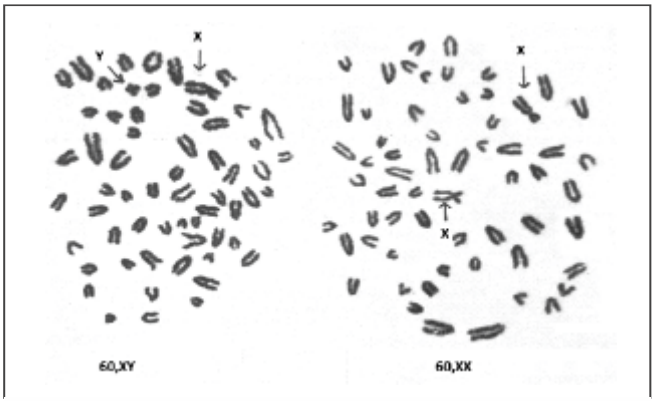


Figure 2. *Presence of male (60,XY) and female (60,XX) cells in two bulls*

As a rule, heterosexual twins have to be considered abnormal and should be identified as early as possible to cull them from the breeding stock. Twining seems to have some genetic background and varying incidence of twining among breeds has been reported (Table 3). Additionally, twinning incidence may vary with men-imposed artificial selection, either by culling or by intentionally using cows with higher twinning rates (Gregory et al., 1997), or even as a consequence of multiple non-sexed embryo transfer, where the deposition of two or more embryoes is currently performed (Padula, 2005). Multiple pregnancies are strongly affected by age and parity, but only slightly influenced by season. Multiple pregnancies in cattle also have some other drawbacks like the prolonged postpartum resumption of the ovarian cyclic activity, an increase in the number of abortion, the still birth and the premature births, and the predisposition to dystocia.

A male born as co-twin to a freemartin calf rarely exhibits gross morphological deformities (Kovacs et al.,1977, Schlafer and Miller, 2007). However, reports of associated male infertility and poor libido exists (Dunn et al., 1979). Still, the basis for infertility remains contentious. Some of the reports describe the presence of focal areas of testicular degeneration (Dunn et al., 1979) and of testicular hypoplasia (Meinecke et al., 2003) could lead to infertility. In addition, the presence of spermatogonial XX/XY chimerism (Redjuch et al., 2000) associated with chromosomal fragility, as demonstrated by Peretti et al. (2008), or to increased degenerative changes could also contribute to loss of fertility in males born co-twin to a female. XX germ cells do not survive in male gonads (Willier, 1921). In some situations, infertility has also been associated to changes in the quality of sperm (motility, concentration, morphology and viability) co-existing with of

Table 3. Reported incidence of twinning in different cattle breeds

S.N.	Breed	Twinning (%)	Reference
1	Holstein Friesian	3.40	Rutledge, 1975
2	Holstein Friesian	4.75	Cady and Van Vleck, 1978
3	Holstein Friesian	2.91	Silva del Rio et al., 2006
4	Jersey	1.30	Rutledge, 1975
5	Jersey	1.83	Cady and Van Vleck, 1978
6	Simmental	4.6	Weber, 1944
7	Guernsey	2.33	Cady and Van Vleck, 1978
8	Swedish Friesian	1.95	Johansson et al., 1974
9	Swedish Friesian	2.57	Gregory et al., 1990
10	Swedish Red and White	1.47	Johansson et al., 1974
11	Brown Swiss	8.90	Rutledge, 1975
12	Brown Swiss	4.08	Cady and Van Vleck, 1978
13	Hereford	0.40	Rutledge, 1975
14	German Fleckvieh	3.16	Silva del Rio et al., 2006
15	Angus	1.10	Rutledge, 1975
16	Santa Gertrudis	0.40	Rutledge, 1975
17	Brahman	0.20	Rutledge, 1975

From Esteves et al. (2012)

acrosome defects (Redjuch et al., 2000).

Cytogenetic screening should be used as diagnostic tool to improve the herd quality by selecting superior animals and culling the abnormality carrier from breeding programmes. Chromosomal abnormalities reduce the reproductive potential of farm animals through decreasing ability or complete failure to produce viable gametes and death of embryos. Moreover, there is a pressing need to establish a proper monitoring and regulatory system to ensure that the males (local or imported) selected for breeding are regularly screened against cytogenetic abnormalities. This would intensify benefits of the farming industry by reducing associated reproductive failure. In the present study one Frieswal bull and one Holstein–Friesian bull were found to be chimeric which were otherwise physically normal. Because of a number of reports indicating low fertility in chimeric bulls (Dunn et al., 1979), the concerned organizations were informed to exclude the afflicted bulls from the breeding programme in the view of the results of this investigation.

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