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Blood cell chimerism (XX/XY) in Murrah buffalo bulls

S Katragadda, RK Patel*, R Kotikalapudi

Sandor Proteomics, Banjara Hills, Hyderabad-500 034, India

Abstract

To investigate the chemeric condition of bulls by cytogenetic analysis the present study was conducted. The whole blood culture, cell harvesting, conventional Giemsa staining and GTG banding were performed for the chromosomal studies. During cytogenetic investigation on 200 buffalo bulls, 3 young bulls exhibited sex chromosomal chimerism (XX/XY) in their blood cells. Hundred metaphase fields were screened for each bull. Cytogenetic studies on chimeric bulls indicated unusual higher ratio of XX chromosomes in 3 bulls. The fertility status of these bulls are not known as they are young and not in semen collection. As documented, the fertility of chimeric bulls is not grossly affected and the condition is not inheritable. Hence, the bulls may be used for breeding programmes if their semen quality is good.

Key words: Buffalo, chimerism, karyotyping, sex chromosomes

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Introduction

The freemartin condition represents the most frequent form of intersexuality found in cattle (Peretti et al. 2008), and occasionally in sheep (Wilkes et al. 1978), goats (Ilbery and Williams 1967), buffalo (Iannuzzi et al. 2005) and pigs (Bruere el al. 1968). Freemartinism is a distinct form of intersexuality which arises as a result of a vascular anastomosis of the adjacent chorioallantoic sacs of heterozygous fetuses in multiple pregnancies. It is generally assumed that 92% of heifers which are born as co-twins to bulls are sterile freemartins (Biggers and McFeely 1963). Vascular anastomosis occurs as early as 30 days of gestation; thus if there is death of the male twin of a heterozygous pair, it is possible for a single-born freemartin to occur. This has been demonstrated as a cause of infertility in heifers with apparently normal external genitalia but with sex chromosome female chimerism (Wijeratne et al. 1977).

Cytogenetically the freemartin shows a mixture of male and female cells (60,XX/60,XY) in cattle and (50,XX/50,XY) in buffaloes. The XX/XY chimerism could be detected after birth using cytogenetic techniques (Karyotyping) thus it could be used as a tool for early diagnosis of chimerism in animals (Kanawala et al. 1965). The factors whether environmental or hereditary for the development of placental anastomoses, are not clear. Some reports based on single case studies, suggest hereditary tendency to develop placental anastomoses between co-twins in sheep (Dain 1971). Pedigree analysis of 22 heterosexual litters demonstrating the leukocyte chimerism (XX/XY) suggested that development of placental anastomoses in sheep is genetically controlled (Szatkowska and Switonski 1996). Therefore, the objectives of this study was to investigate the chemeric condition of bulls by cytogenetic analysis.

Materials and methods

Heperinized blood samples were collected from 200 phenotypically normal mostly one year old Murrah buffalo bulls/calves stationed at different farms for routine cytogenetic investigation. The cytogenetic investigation is useful tool in early diagnosis of freemartinism in animals (Kanawala et al. 1965). Chromosomal preparations were performed using standard procedure. Briefly, whole blood was cultured in RPMI-1640 (Himedia) medium supplemented with antibiotics, 15% fetal calf serum and 1% pokeweed mitogen (Patel 1999). The whole blood cultures were incubated at 38°C for 69 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 harvesting. The cells were separated by centrifugation at 1500 rpm for 5 minutes followed by hypotonic treatment with 0.075 M KCl for 30 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. Conventional staining by Giemsa and routine GTG banding with little modification (Patel et al. 1995) were performed on chromosome slides. Finally, around 100 Giemsa stained and G-banded metaphase plates were screened per animal for chromosomal analysis using Olympus microscope

*Corresponding Author: **rkpatel46@yahoo.com**

attached with image analyzer system (Cyto-Vision).

Results and Discussion

Out of 200 Murrah bulls/bull calves screened during last 4 months as part of routine cytogenetic investigation from September to December, 2012, three bulls exhibited XX/XY chromosomes in their metaphase cells (Figure 1).

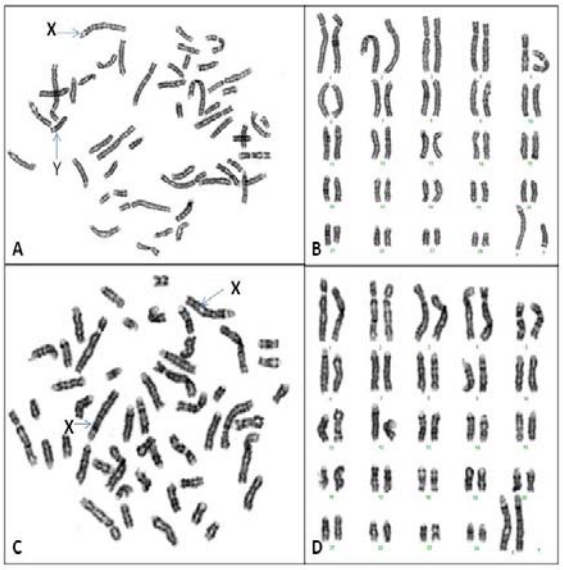

Figure 1. *A, Metaphase field exhibits 50,XY; arrows indicates sex chromosomes; B, Karyotype of same metaphase; C, Metaphase plate exhibits 50,XX; arrow indicates sex chromosomes and D- Karyotype of same metaphase*

Hundred metaphase fields were screened for each bull to assess the ratio of XX and XY cells as mentioned in table 1. In all chimeric bulls the ratio of XX cells was very high ranging from 94 to 97%, similar to unusual blood chimerism reported earlier in cattle bulls sired normal males and females (Kosaka et al. 1969; Patel et al. 1997). Unusual chimerism with 100% XY cells in chimeric female cattle was also reported by Balakrishana et al. (1979). In addition to chimerism, aneuploidy cells, chromatid breaks and gaps, chromosomal fragmentations, pulverization, polyploidy, premature centromeric division (PCD) etc. were also observed (Figure 2) in some of metaphase field (Table 1) but not observed consistently in all 3 bulls. However, our observations on numerical and structural abnormalities in buffalo are similar to earlier reports in cattle (Basrur and Stoltz 1966; Glazko et al. 1992; Patel and Khoda 1997; Patel et al. 1997; Peretti et al. 2008). Most cases of chimerism were observed only in cattle worldwide. A case of chimerism was reported in heterosexual Murrah buffalo triplets by Balakrishna et al. (1981) in India and a few cases by Iannuzzi et al. (2005) in Italy. They found 10 freemartins (8 females and 2 males) out of 42 buffaloes with variable percentages of male and female blood cells, the majority however showing similar percentages of both. Other numerical or structural chromosomal abnormalities are well documented (Patel et al. 2012) which usually occur in buffaloes.

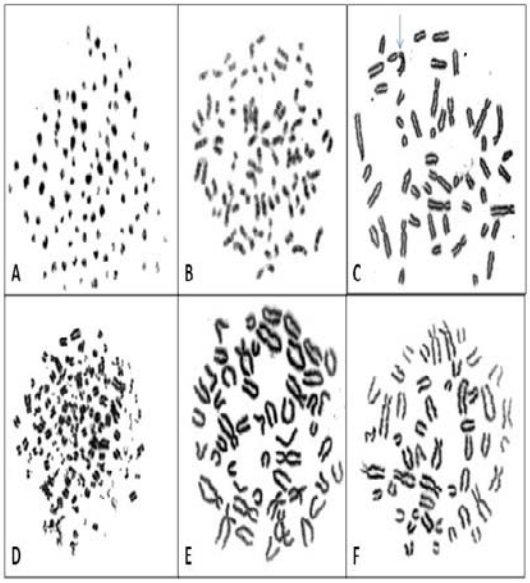

Figure 2. A, Chromosomal fragmentation; B, Polyploidy due to PCD; C, Gap in X-Chromosome; D, Pulverization; E, Aneuploidy with 51 chromosomes; F, Aneuploidy with 49 chromosomes

Table 1. Sex chromosome chimerism with various chromosomal aberrations

Sl. No.	Sample No.	Date of Birth	No. cells screened	Karyotype	No. cells with XY	No. cells with XX	Other chromosomal aberrations				
							Fragmentation & Pulverization	PCD	Polyploidy	Aneuploidy (2n-1/2n+1)	Chromatid gap & break
1	901	20.8.11	100	50,XY/50,XX	6	94	5	2	2	8	-
2	912	2.8.11	100	50,XY/50,XX	3	97	11	-	-	3	-
3	914	7.6.11	100	50,XY/50,XX	5	95	3	-	-	2	5

In the present study, the fertility of these bulls is not known as they are young and not at the stage of semen collection. The published literatures showed that the fertility of most chimeric bulls in cattle and buffaloes are not grossly affected and therefore could be utilized for the breeding/ artificial insemination programmes if giving normal semen (Teplitz et al. 1967; Patel et al. 1997; Patel and Khoda 1997). However, some of chimeric bulls were reported with reduced fertility as compared to normal bulls (Dunn et al. 1968). It is therefore, advisable to check the fertility of chimeric bull and its semen qualities before it is used for breeding.

Conclusion

It can be concluded that though chimerism occurs occasionally in buffaloes as compare to cattle, it provides a great opportunity of conducting further research on fertility of buffalo chimeric males. Although the fertility of most chimeric bulls in cattle and buffaloes is not grossly affected but it is always advisable to check the fertility of such bulls and examine their semen qualities before they are inducted for buffalo improvement programmes.

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