



Sex chromosome chimerism in heterosexual Sahiwal dizygotic twins

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Chimerism in heterosexual bovine dizygotic twins is a chromosome abnormality in cattle in which heifer calf born co-twin with a bull calf. Presence of both XX and XY chromosome in male and female born co-twin is known as sex chromosome chimerism (60, XX/XY). Sex chromosome chimerism in cattle is widely investigated (Kanaguwa *et al.* 1965, Smith *et al.* 1977, Peretti *et al.* 2008, Kozubska-Sobocinska *et al.* 2011). Chimeric dizygotic twins arise due to fusion of foetal membrane leads to reciprocal exchange of haematopoietic stem cells at some stage in early embryonic life (Kozubska-Sobocinska *et al.* 2011). Passage of hormone and other factors from one foetus to the other through the vascular communication established by the fusion of the chorioallantoic membranes of the bovine foetuses also another cause of chimerism (Kanaguwa *et al.* 1965). The freemartin syndrome in cattle occurs in about 82–95% of heterosexual twins (Kozubska-Sobocinska *et al.* 2011). Thus the objective of the present study was to investigate the chromosome constitutions of heterosexual Sahiwal dizygotic twins in blood cultures. An attempt was also made to observe the effect of chimerism on the development of female reproductive organs.

One male (bull) and female (heifer) Sahiwal calf born as dizygotic twins on 17 June 2009 at Bull Mother Farm, Haringhata Farm by embryo transfer technique were presented at Quality Control and Karyotyping Laboratory, Frozen Semen Bull Station, Haringhata Farm, West Bengal, for Karyotyping. These two calves were phenotypically normal. They were karyotyped in 2 separate batches along with other male calves with an interval of 1 month. Cytogenetic analysis of the somatic chromosomes was carried out by using the improved technique of *in-vitro* whole blood lymphocyte culture following Halnan (1977) with some modifications. The working tissue culture media for whole

blood bovine lymphocyte culture was prepared by RPMI 1640 medium, foetal bovine serum, lectin (Pokeweed), streptomycin and benzyl penicillin. At each time after 72 h incubation, harvesting the culture in four slides from each bull and heifer calf and stained by Giemsa stain. The slides were examined under the Differential interference contrast microscope by the use of oil immersion lens and photographed. A total of 200 metaphase chromosome spreads from each calf were analyzed using a computer according to the international system of human cytogenetic nomenclature (ISCN 2013) recommendations. Gynaecological examination of heifer was performed by per-rectum palpation.

The diploid number (2n) of chromosomes of both the bull and heifer calf in this study was found to be 60 with 58 autosomes and 2 sex chromosomes. The morphology of all the autosomes were found to be acrocentric, the X and Y chromosomes being sub-metacentric and acrocentric respectively. It transpires from the metaphase spreads that the bull calf as well as heifer calf showed the mixture of two cell lines of either XX or XY in both the animals i.e. the sex chromosome chimerism consisting of 60, XX/XY. The bull calf showed 40% cells or metaphase spreads to have 60, XX and 60% metaphase spreads to have 60, XY chromosomes. On the other hand the heifer calf showed 64% cells or metaphase spreads to have 60, XX and 36% spreads to have 60, XY chromosomes. Andrological study of the bull calf was not possible because it was culled from the Bull Mother Farm immediately after detection of abnormal karyotype at very younger age. The heifer did not show any symptom of heat till the age of 28 months and had poor developed udder and teat. Gynaecological examination of the heifer calf studied in this investigation revealed marked arrest in development of vagina, cervix, uterus and ovaries. Uterus and ovaries could not possible to palpate except as thin remnants of the normal structure. External genitalia were typically female, Clitoris was very prominent and vagina was almost half of the normal length.

In the present study the presence of XY chromosomes in heifer calf and XX chromosomes in bull calf confirmed the sex chromosome chimerism. Kozubska-Sobocinska *et al.*

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(2011) reported 34% and 58% XX cells in two freemartin heifers. On the other hand Kanaguwa *et al.* (1965) reported 21 to 23% XX cells in freemartin which was a modified female and in some male 12 to 39% XY cells although these males were complete male in phenotype. He also reported one heterosexual non-freemartin heifer twin having 100% XX cells. The abnormal development of reproductive organs in freemartin heifer is also reported by Kanaguwa *et al.* (1965) and Kozubska-Sobocinska *et al.* (2011). The actual reasons of abnormal development of female reproductive organs in freemartin heifer are unknown. However, according to Lillie (1916) the main cause is the androgen hormones or anti-mullerian hormone (AMH) or the H-Y antigen on Y-chromosome or the oncogenes from male co-twin inhibits the formation of female sex organs. Although Smith *et al.* (1977) in cattle and Sudik *et al.* (2001) in human reported a case of fertility in female born co-twin with a male.

SUMMARY

It is inferred from this study that presence of XX and XY cells in heterosexual dizygotic twins produce chimerism. Chimerism may be produced due to vascular anastomoses of foetal membranes during early embryonic life before the critical period of sex differentiation. In this study 60 to 64% pure cell lines were observed in both sexes. With this pure cell lines abnormal development of reproductive organs was found in freemartin heifer.

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