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Discovery of a new *de novo* originated Robertsonian translocation rob (11;22) in Indian cattle

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Robertsonian translocation especially rob (1;29) is the most common structural chromosomal aberration identified in different breeds of cattle (*Bos taurus*) throughout the world (Popescu 1978). Since its detection in Sweedish red breed of cattle by Gustavsson and Rockborn (1964) many rare translocations involving other chromosomes have also been reported widely in more than 60 different breeds of cattle (Popescu and Pech 1991). Compared to the European breeds of cattle (*Bos taurus*), fewer cases were reported in Indian cattle breeds (*Bos indicus*). On the other hand sex chromosome chimerism (60, XX/XY) is a very common chromosome abnormality in multiple pregnancy of cattle where a female calf born co-twin to a male calf (Kozubska-Sobocinska *et al.* 2011, Biswas *et al.* 2014). Evidences from the previous studies suggested that freemartin condition with serious reproductive disorder is the possible outcome of sex chromosome chimerism. However, occurance of Robertsonian translocation along with the sex chromosome chimerism is extremely rare event. The present study confirmed the occurrence of a new Robertsonian translocation, rob (11;22) in chimeric triplets (59, XX/XY) of Indian non-descript cattle (*Bos indicus*) originated "*de novo*" by cytogenetic investigation.

The present study was carried out in the karyotyping laboratory, Frozen Semen Bull Station, Haringhata Farm, West Bengal, India. Blood samples (5 ml) of each of triplet calves (two females and one male) along with their sire and dam of Indian non-descript cattle (*Bos indicus*) were collected in vacutainer tubes containing heparin from jugular vein of animals. Cytogenetic study of the somatic chromosomes was conducted by using the improved technique of in-vitro whole blood lymphocyte culture following the protocol of Halnan (1977) with some modifications. The lymphocyte culture media was composed of RPMI 1640, 15 % fetal calf serum, 0.25 mg lectin from phytolacca americana (pokeweed), 10 mg streptomycin and 6 mg penicillin and used after filtered through 0.2 µm filter. About 8 ml working culture media containing 0.5 ml blood in 15 ml culture tube was placed in an incubator at 37°C for 72 h. After 72 h incubation, harvesting the each culture in four slides and the slides having good metaphase spreads were subjected to conventional Giemsa staining (Gustashaw 1991), G-banding (Seabright 1971) and C-banding (Sumner 1972). The stained slides were examined under the differential interference contrast microscope by the use of ×100 objectives and photographed. A total of 100 metaphase chromosome plates from each animal were screened for chromosomes identification according to the recommendations of first international conference (Reading conference) for the standardization of banded karyotypes of domestic animals (Ford *et al.* 1980).

Cytogenetic study of the present investigation showed the normal diploid number of chromosomes (2n = 60) in sire and dam of all triplet calves but all the triplets calves exhibited the chromosome complement of 59, which are heterozygous carrier of a Robertsonian translocation, rob (11;22) and sex chromosome chimerism forming the karyotype 2n = 59, XX/XY, rob (11;22) (Fig.1). The karyotyping of G-banded chromosome revealed that one abnormal biarmed sub-metacentric chromosome, which was clearly identified to be Robertsonian translocation involving one member each of 11th and 22th acrocentric autosomes in all the cells (Fig.2). Further, C- banding pattern revealed that the centromere of the two acrocentric chromosomes were fused forming large centromere without reducing the amount of centromeric heterochromatin and appeared to be double indicating dicentric fusion in all the metaphase plates (Fig.3). This finding was consistent with the other published reports (Rubes *et al.* 1996, Tanaka *et al.* 2000).

Robertsonian translocations involving various chromosomes have been reported in different breeds of *Bos taurus* whereas it is very limited in Indian cattle breed. As far as we know this is the first case of a new centric fusion translocation, rob (11;22) of two acrocentric chromosomes

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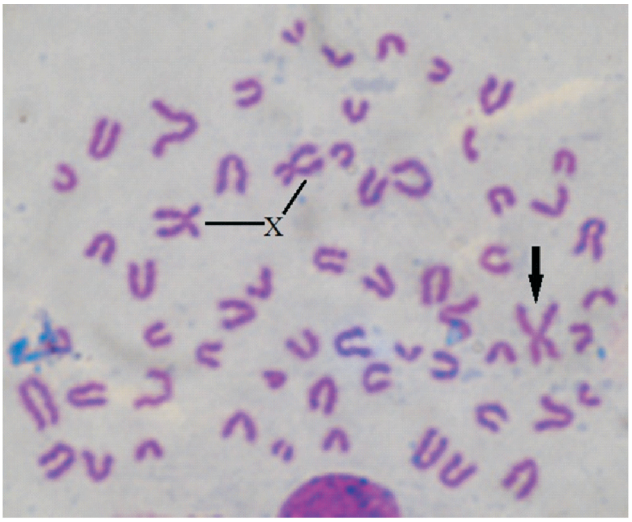

Fig. 1. Giemsa- stained metaphase plates showing the sub-metacentric translocated chromosome (11;22) (arrow). The sex chromosomes (X) are also highlighted.

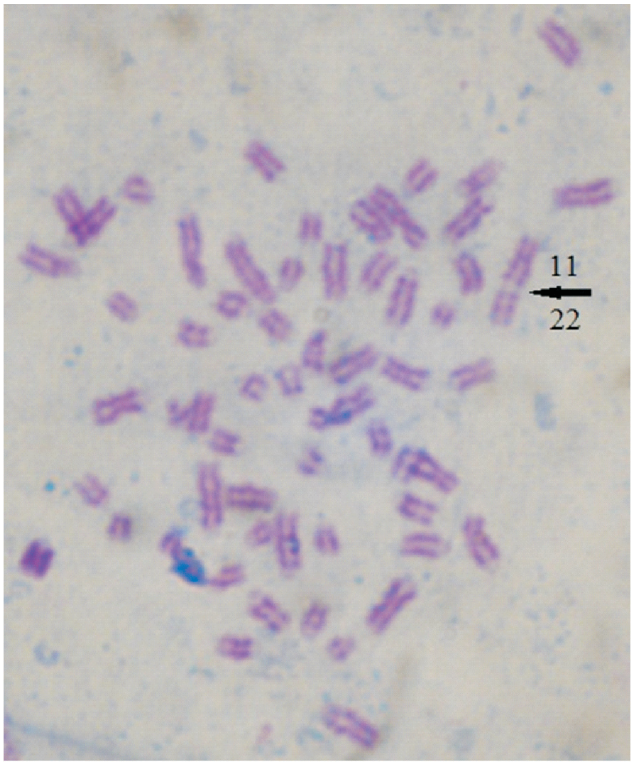

Fig. 2. G- banding pattern of chromosomes showing the fusion of two acrocentric chromosomes (chromosome 11 and 22) indicated by arrow.

identified in Indian non-descript cattle. In the present study, the triplets carrying the new Robertsonian translocation rob (11;22) was phenotypically normal resembling with various published reports elsewhere (Rubes *et al*. 1996).

In the present study, the karyotype of sire and dam was normal indicating that this is not a heritable translocation from either of the parent. Post-zygotic translocation resulting from a centric fusion between chromosome no. 11 and no. 22 would be denied because translocated

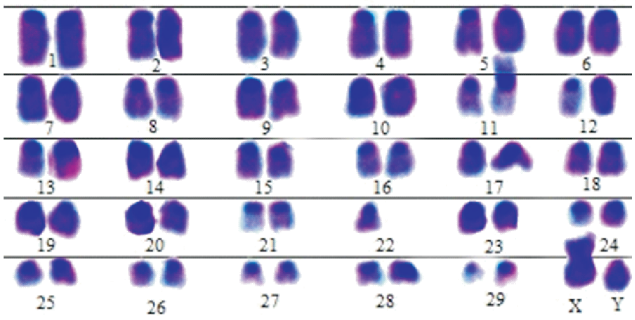

Fig. 3. C-banded karyotype of a male calf carrying a centric fusion involving chromosome 11 and 22.

chromosome was detected in all the triplets. In this study, as all the triplets have translocated chromosomes so they may be of either parent produced gametes with translocated chromosomes at the time of meiosis (i.e., dam during oogenesis or sire during spermatogenesis). Fertilization of these gametes produced all the calves with translocated chromosomes indicating that this unique translocation was originated de novo.

The triplets in the present study also have shown the mixture of two cell lines (XX and XY) i.e. the sex chromosome chimerism (59, XX/XY). The female calf no.1 showed 80% XX and 20% XY cells, female calf no. 2 had 73% XX and 27 % XY cells whereas male calf had 72% XX and 28% XY cells. Similarities in chimeric ratio and preponderance of female cells (XX) in this study resembled with the other reports in multiple pregnancy (Basrur and Kanagawa 1969). The result of this investigation and numerous other studies provide evidence that the sex of the offspring can have an influence on the chimeric ratio in multiple pregnancies. However, it is not clear whether there is any correlation between Robertsonian translocation and sex chromosome chimerism in this study. Nevertheless there may be a possibility of reduced fertility in triplets and development of freemartin condition in the female calves showing Robertsonian translocation rob (11;22) along with the sex chromosome chimerism in the present investigation. Evidence from the numerous published reports overwhelmingly supports this interpretation.

SUMMARY

Blood samples were collected from phenotypically normal triplets along with their sire and dam of Indian non-descript cattle (*Bos indicus*) for chromosomal screening. Cytogenetic investigation by conventional Giemsa staining, G-banding and C-banding revealed dicentric fusion translocation involving chromosome number 11 and 22 in chimeric triplets forming karyotype 2n = 59, XX/XY, rob (11;22) in all metaphase field. The normal diploid chromosome number of sire and dam (2n= 60) indicate that the translocation was originated "*de novo*". Our study showed for the first time the Robertsonian translocation rob (11;22) in bovine species in general and Indian cattle in particular. It is advisable not to use these triplets for artificial insemination programme as there may be a

possibility of embryonic death and freemartin condition due to imbalance of chromosomal number in the gametes and sex chromosome chimerism.

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