Cytogenetic study of embryo transfer born cattle and their progenies

Vaishali Sah, BR Yadav, AK Gupta, S Sen and S Dash

Received: 02 July 2015 / Accepted: 24 January 2016

Abstract A cytogenetic study of cattle born through Embryo transfer and their progenies was conducted in order to determine the incidence of chromosomal anomalies in animals born through this assisted reproductive technology. Out of animals produced from ET, only 7 animals physically available along with their 26 progenies and 9 dams. They were cytogenetically screened using short term lymphocyte culture, C and R banding as well as karyotyping. The progenies and dam were also screened to detect the transmission pattern of anomaly if any. Out of seven, two males were detected with autosomal translocation which was confirmed with C and R banding. However the chromosomal location of translocation could not be ascertained. The same anomaly was found to be transmitted to progenies sired by these males as well. While one ET born was confirmed to be a free martin carrying chromosome complement 60 XX/XY, other four animals and their progenies were cytogenetically normal.

Keywords: Embryo transfer, translocation, chromosomes, Sahiwal, cattle, chromosomal anomalies

Currently assisted reproductive technologies (ARTs) and biotechnology have provided new ways to breeders and animal geneticists to design, direct and alter the reproductive course, inculcate desired traits and accelerate genetic improvement. The potential genetic consequences of these approaches are worth the same degree of meticulous evaluation that is extended to the procedures used for overcoming their ‘technical’ inefficiencies. Commercial embryo transfer has become a large international business in animal species of human interest directly (Betteridge, 2006). The use of ET as a tool for enhancing reproductive efficiency has still some limitation of high cost of production of embryos and problems associated with poor freezability especially in vitro obtained embryos, abnormal fetuses and calves with altered sex ratios (Hasler, 2000; Thompson and Peterson, 2000; Van Wagendonk-de Leeuw et al., 2000; Peterson and Lee, 2003). The average loss of embryos due to chromosomal aberrations could be up to 10% of the fertilized ova (Viuff et al., 1999). Different types of alterations occur in chromosomes due to irregularities in the replication and cell division processes in most cell types and tissues. Such alterations occur and affect chromosomes in many ways at different region and stages. Retrospective studies of the chromosomal make-up of the embryos have shown that chromosomal alterations are mostly influenced by the methods of embryo production and handling (King et al., 2006). The frequency of mixoploidy was found to increase with time after ovulation in in vivo produced embryos obtained from superovulated cattle (Viuff et al., 2001). In superovulated cattle delayed or interrupted ovulation leads to the production of oocytes of inferior quality (Greve et al., 1984) ending up in inferior zygotes and thus embryonic mortality.

In cattle, structural anomalies similar to Robertsonian translocation (ROB) is known to cause subfertility (Dyrendahl and Gustavsson, 1979; Long, 1985), female carriers with these translocations exhibit repeat breeder tendency (Gustavsson, 1971; King and Linares, 1983; Swartz and Vogt, 1983; Popescu, 1990). As reported the reduction in fertility in cows was observed not only for ROB (1;29) but also for other forms of ROB translocation such as ROB (26;19) (Ducos et al., 2008). The effects of other types of structural aberrations on the fertility of carrier bulls and cows are not well documented. Apart from this assisted reproductive technologies (ARTs) in general are restricted in its widespread use largely due to Large offspring syndrome (LOS) and its consequences. Growth factors in culture media and hormonal interventions (progesterone) at the time of superovulation have been shown as the causal factors of LOS (Blanco et al., 2011).

Numerical chromosome aberrations and unbalanced structural aberrations do not represent a big economical problem in animal breeding, because they produce such great adverse phenotypical effects for their carriers that they are not inherited. On the contrary,
balanced structural chromosome aberrations are more important, because the carriers have a normal body conformation and can transfer the structural aberration in both form to the next generation. Without cytogenetic screening, balanced structural aberrations can be easily distributed in a population particularly when artificial insemination is used (Iannuzzi et al., 2001). Therefore, the progeny and dam were screened to detect the transmission pattern of anomaly if any in the present population.

The study was carried on ET born cattle and their progenies produced at an organized dairy farm. Out of the cattle produced through ET only 7 comprising Sahiwal (3), CB (4) along with 9 dams and 26 progenies were included in the study. 10 ml of blood was collected from the jugular vein of each animal in vaccutainer tubes containing 143 USP units of sodium heparin per tube. 0.5 ml of blood/6ml of medium was used for setting up of cultures. The culture medium prepared using Hams F-10 (@ 2.94gm/300ml Milli – Q water), antibiotics (streptomycin and penicillin), Pokeweed mitogen 1 ml (@ (1ml/100ml Milli – Q water), adjusting pH to 7.2, 20% FCS followed by filtration through millipore membrane (0.22 µm) fixed assembly. The culture bottles were transferred to a 5% CO₂ incubator at 37.5°C (±0.5°C) for a period of 96 hours. Seven hours before the end of culture period BrdU (20 μg/ml,) was added for labelling late replication regions. 45 minutes prior to actual harvesting procedure 2 drops (0.5µg/ml) of colchicine solution (Sigma) was added to each culture bottle. Cultured contents were transferred to centrifuge tubes (15 ml) after 45 minutes of incubation with colcemid treatment. This was followed by several centrifugation steps and treatment with hypotonic solution (0.075 M KCl) and fixative. The pellet thus obtained mixed by gentle pipetting to obtain a slightly milky cell suspension. About 3-4 drops of cells suspension was dropped on clean slide and allowed to air dry at room temperature for 10-15 minutes. The slides were allowed to stay in the staining solution for 30 minutes. Subsequently, they were rinsed thoroughly with distilled water and dried and screened on Leica microscope. Normally 100 well spread metaphase were selected for photography. Selected metaphase plates were photographed using digital camera fitted on microscope. The images were stored in PC for evaluation and construction of karyotypes.

The slides were incubated in 0.2N HCL for 1 hour at room temperature and rinsed with distilled water. Subsequently, the slides were incubated in 1% Ba(OH)2 solution for 6-8 minutes at 56°c. The slides then rinsed thoroughly in glass distilled water at 56°c and incubated for 2 hours in 2X SSC solution and stained for 1.5 hour in 2% Giemsa solution at pH 6.8. Dried slides kept in jar filled with Xylene for 5-15 minutes.

The 3-7 days aged slides slide were treated stained for 15 minutes in 0.5% Hoechst dye 33258 (bisbenzimide). After rinsing in water slides immersed in 2X SSC solutions and placed on a hard surface upon which a stand fitted with blue-black fluorescent tube light kept at a distance of 2 or 3 cm. Slides exposed for 2 h and the whole assembly was kept covered with a black cloth. Then the slides were thoroughly rinsed and transferred to a 2% Giemsa solution for 5 min and resulting pattern were evaluated under a light microscope.

Slides were screened on Leica microscope. Selected metaphase plates photographed and stored in PC for evaluation and construction of karyotypes. The karyotypes were prepared using CytoVision Software.

Chromosomes were counted and critically examined in well spread metaphase plates. Among the ET born females examined one was found to be freemartin with some metaphase spreads have shown 60, XY (Fig. 1) which was later on confirmed by its breeding history and phenotypic appearance. Translocation was observed in two phenotypically normal bulls where one of the chromosome was larger in size compared to others in all the 100 metaphase spreads examined (Fig. 2) with no change in the chromosome number (60,XY). Both translocation carrier males belonged to Sahiwal breed, and were monozygotic twins. To rule
out the possibility of sex chromosomes in the translocation C-banding was done. The banding showed one chromosome to be largest in the complement while one appeared quite small, revealing only the centromeric portion (Fig 3). The involvement of sex chromosomes was thus excluded with confirmation of autosomal translocation. With no change in the chromosome number this appeared to be genetically balanced, in conjunction with finding that structural changes in the chromosome not causing any loss or gain in the genetic material can be said to be genetically balanced and not expressed in the phenotype of the carrier but likely to be disseminated in the population if not detected (Popescu, 1989). Studies in the past suggest that translocation carriers with a balanced karyotype cause spreading of the anomaly and the males and females heterozygous for the same have reduced fertility (Gustavsson, 1980). All the crossbred ET born animals were found to be normal in terms of their cytogenetic profile.

Prior to the inception of this study the two translocation carrier males were already selected, used for semen collection and had sired progeny. Nino-Soto & King (2004) reviewed factors affecting fertility and concluded that structural anomalies like translocation cannot be detected by the regular reproductive assessment and has the potential of causing important economic losses, especially through the use of affected males in AI programs. Keeping this in view further studies were planned on the progeny sired by these two bulls to assess the transmission pattern. Out of total 50 progenies sired by these males only 21
(11 and 10 from male 1& 2 respectively) were available for cytogenetic study. The available dams were also screened for any possible anomaly. After critical screening of the metaphase plates it was found of total 21 progeny 5 progeny (four progenies sired by male 1and one by male 2) exhibited translocation wherein one of the acrocentric autosomes was larger than rest of the chromosomes. The chromosome complement in all the metaphase spread examined was 60, XY and 60,XX for males and females respectively out of which 58 were acrocentric autosomes and two were sex chromosomes (fig.4). The dams were found to be cytogenetically normal. The percentage in which the translocation from the males was being inherited by their progenies was found to be 36.36 and 10 % for males 1 and 2 respectively.

C- banding of the progenies of these bulls in which the apparently larger translocated chromosome was not found revealed that these progenies inherited the other small chromosome containing only a centromeric region (dot like) from which probably the translocation took place and the possibility of sex chromosome translocation was ruled out (Fig.5). The well spread metaphase plates having good index and translocation were subjected to R-banding. (Fig 6). The band patterns were used to identify the two chromosomes involved in translocation. However, it could not be completed. The male progenies carrying translocation showed stunted growth and debility and one of the female (as per the records) in which translocation was found was a repeat breeder.

Conclusions

Out of seven, two males were detected with autosomal translocation which was confirmed with C and R banding. The same anomaly was found to be transmitted to progenies sired by these males as well. While one ET born was confirmed to be a free martin carrying chromosome complement 60 XX/XY, other four animals and their progenies were cytogenetically normal. Studies showed that it is advisable to cull and avoid using such bulls for semen production as any kind of translocation can have an adverse effect on fertility, apparently due to the production of chromosomally unbalanced gametes.

Acknowledgements

The authors are thankful to the Director, National Dairy Research Institute (NDRI) for providing necessary infrastructure and facilities for the successful completion of research work. Special thanks to Indian Council of Agricultural Research (ICAR), Ministry of Agriculture, Govt. of India for proving Junior Research Fellowship to the first author for conducting the study.

References

Dyrendahl I, Gustavsson I (1979) Sexual functions, semen characteristics and fertility of bulls carrying the 1/29 chromosome translocation. Hereditas 90(2):281-289
Gustavsson I (1971) Early DNA replication patterns of the normal sex chromosomes and a presumptive X-autosome translocation in cattle (Bos taurus L). Nature 229:339-341


