Cytogenetic Analysis of Dairy Animals in India: An update

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Abstract

Animal clinical Cytogenetics started during 1960s. The majority of laboratories in India and abroad developed and started cytogenetic screening of cattle and buffaloes during last 5 decades which was parallel to human Cytogenetics. These laboratories were created almost exclusively within academic research institutions with a focus on basic research worldwide. However, a Cytogenetic screening programme in India was launched by the apex body, the National Dairy Development Board responsible for dairy development and animal improvement programmes during 1990s. Reduced fertility and infertility are major concern in dairy animals in India which could be due to poor breeding, feeding and management. However, it could also be due to chromosomal aberrations. Chromosomal aberrations might be transmitted or spontaneous generated during mitotic or meiotic cell divisions. Therefore, complete eradication of chromosomal aberrations from the dairy animal population may not be possible. In view of this the regular chromosomal screening especially of breeding bulls at the early age ought to be done This practice will not only reduce the occurrence of chromosomal abnormalities in dairy animal population rather it will save the time and amount spent on rearing of abnormal animals. Various kind of structural and numerical chromosomal abnormalities have been reported in India which is very less as compared to reported elsewhere. The chromosomal aberrations are reported mainly by conventional methods (banding of chromosomes) by which it is difficult to detect microdeletion, microduplication, microshiting translocation of minute fragments of chromosomes. The new molecular technologies like probe based fluorescence in situ hybridization (FISH) and microarray may be applied to detect minor abnormalities so that they can be correlated with infertility problems.

Key words: Chromosomal abnormalities, Aneuploidy, Chimerism, Mosaicism, translocation.

Introduction

Animal Cytogenetics started during 1960s by realizing the advantages of human clinical Cytogenetics. The majority of laboratories in India and abroad developed and started cytogenetic screening of cattle and buffaloes during last 5 decades. These laboratories were created almost exclusively within academic research institutions with a focus on basic research worldwide. However, a Cytogenetic screening programme in India was launched by the apex body, the National Dairy Development Board responsible for dairy development and animal improvement programmes during 1990s. Chromosomes are present in all nucleated cells of the body or eukaryotes. Chromosomes are nucleoproteins of the cells which carry genetic materials (genes) from one to another generation. The number and shape and size of the chromosomes are different in different species of animals. In cattle, revereine buffaloes and humans they are 60, 50 and 46 in number.
respectively. Chromosomes are divided in two categories; sex chromosomes which determine sex of an individual, and others exist as homologous pairs and are known as autosomes. Arranging homologues pairs of chromosomes as per their shape and size is called karyotyping. A photomicrographs of an individual’s chromosomes arranged in a descending order is called Karyotype. Chromosomes are classified as per the location of their centromere; telocentric, acrocentric, submetacentric and metacentric. In dairy animals, chromosomes are usually acrocentric and submetacentric.

Some of very specialized chromosome banding techniques developed in human Cytogenetics (1, 2) allowed rapid progresses in the animal Cytogenetics. An international study group with the mandate of standardizing the karyotypes of most farm animal species including cattle, buffalo, was created in 1976 during the Reading Conference (3). The Reading standard formed on the basis of all subsequent nomenclature reports (4-10).

Reduced fertility / infertility are major concern in dairy animals (cattle and buffaloes) due to poor breeding, feeding and management practices. To little extent, chromosomal abnormalities also contribute for reproductive failure in animals. Cytogenetics has several applications in animal improvement and one of them is the detection of chromosomal abnormalities usually associated with reduced fertility, infertility, embryonic loss, fetal waste and internal or external genital malformation. Quite a few bulls reared for breeding cannot be used effectively due to chromosomal abnormalities related to fertility. Chromosomal defects can affect breedable animals in two ways: a) the animal having chromosomal defects like sex chromosome mosaicism, inversions, trisomy-X, XXY etc., can be infertile or sub-fertile and b) the animals particularly AI bulls having chromosomal defects like Robertsonian translocation, reciprocal translocation, sex chromosomal mosaicism etc can cause repeat breeding problem in females.

In India, it has become mandate to screen breeding bulls before they are inducted in semen collection to avoid spread of such aberrations and repeat breeding. It is always advisable that the animal soon after birth may be screened for chromosomal aberrations so that the time and money spent on rearing of defective breeding animals can be saved. The routine chromosomal screening, to an extent, has been eliminating such animals which could have otherwise created reproductive failure in a large population. Routine screening of chromosome can only eliminate the abnormal one but cannot totally eradicate chromosomal aberrations from animal population as these abnormalities are spontaneous generated mainly during cell cycles (mitosis and meiosis). The chromosomal preparation and various banding pattern from lymphocytes culture can help us in routine investigation (11,12). Chromosomal aberrations could be numerical or structural or both in an individual. Many chromosomal abnormalities (numerical / structural / both) have been reported in cattle and buffaloes in India, which are described as under:

Chimerism is very common in cattle (13) and sheep (14) but also observed in buffalo (15), goat (16) and pigs (17). When two or more cell populations (XX/XY) derived from heterosexual zygotes the condition is known as chimerism. Developmental of placental anastomoses during pregnancy between heterosexual twins causes altered sexual development of a female co-twin due to the action of male hormones produced by a male co-twin. The factors whether environmental or hereditary for the development of placental anastomoses, are not clear. Some reports based on single observation, suggest hereditary tendency to develop anastomoses between co-twins in sheep (18). The freemartin syndrome occurs in about 92% of heterosexual twins (19), which can be diagnosed by leukocyte sex chromosomes chimerism, XY/XX. Chimerism usually affects fertility of females (13,20) but males are not grossly affected (21,22). However, on the contrarily many reports were published on reduced fertility or infertility of chimeric bulls.
Reduced fertility was observed (23) and many bulls were culled due to no semen ejaculation, had a low sperm count or had a high incidence of abnormal spermatozoa. Besides, the 7 chimaeric bulls were culled because of poor reproductive performance and testicular degeneration. Similar performance was also observed during studies of semen qualities in 15 chimeric bulls (24). They analyzed semen which included volume of ejaculate (ml), motility of spermatozoa (%) and sperm concentration/ml, and observed significant differences with regard to the volume of ejaculate and highly significant differences with regard to the motility of spermatozoa and sperm concentration. These parameters were lower in the group of chimeric bulls. There ratio of sex chromosomes (XX or XY) in blood cells have not been consistent irrespective to sex of animals (25). Around 20 river buffaloes (18 females and 2 males) were reported chimeric (26). All female carriers were sterile. Similar observation was observed in 8 female buffaloes (15). Some of chimerism cases were also reported with other chromosomal anomalies gave rise to different reproductive problems, as in case of a Friesian calf with an elongated urethra and without a vulva was born twin to a dead bull calf. Blood cell chimaerism and Mosaicism (XY/XXY) was found in the skin tissue (27).

Several cases of chimerism have been reported in Indian cattle (22,28,29,30) which were mostly in fertile males but their fertility was not evaluated. A few cases of buffalo chimerism (50,XY/50,XX) were also reported in India (31,32). Besides, many cases of chimerism in cattle and buffaloes probably have not been reported. By observing the impact of chimerism worldwide, it is advisable to examine the quality of semen of chimeric bulls before they are considered for semen collection centres.

Chimerism in cattle (33, 34) and other ruminant (35) may also be detected by polymerase chain reaction (PCR) by identifying amelogenine genes (AMELX and AMELY) responsible for tooth development (36) present on both X and Y chromosomes. The AMELY allele of Y chromosome contains a 63-bp deletion in the fifth exon as compared to the AMELX allele on the X chromosome. A PCR technique based on amplification of the region of AMX/Y containing this deletion uses a single primer pair to amplify a 280-bp fragment from the X chromosome and a 217-bp fragment from the Y chromosome (37). Different PCR based protocols have been used for sex determination. Some of the existing protocols can identify specific sequence on Y-chromosome such as sex determination region Y (SRY) gene (38,39) and testis-specific protein Y-encoded (TSPY) gene (40) or repeated sequences (41). Cytogenetic studies of leukocyte chimerism was performed with the fluorescent in situ hybridization (FISH) technique by using probes painting sex chromosomes to distinguish cell lines present in animals (42,43,44).

Mosaicism occurs due to mitotic nondisjunction during development. The coexistence of two or more genetically distinct cell populations derived originally from a single zygote is known as mosaicism. Mosaics may arise at any stage of development, from the two-cell stage onward, or in any tissue which actively proliferates thereafter. Mosaicism could be developed due to sex chromosomes or autosomes and both. The phenomenon is commonly observed in many species of animals and plants. Mosaic somatic alterations are present in all multi-cellular organisms, but the physiological effects of low-level mosaicism are largely unknown. Most mosaic alterations remain undetectable with current analytical approaches, although the presence of such alterations is increasingly implicated as causative for disease. In humans, when chromosomal mosaicism arises during development, pregnancy outcome depends on which tissue, and how much of that tissue is abnormal. In theory, cases with a relatively high proportion of trisomic cells are more likely to be associated with an abnormal outcome than those with a low proportion of trisomic cells. If a majority of the cells are abnormal then human development is likely to be abnormal. If only a tiny fraction of some tissue were involved,
the aneuploidy would likely to have little effect on growth and development. Perhaps many people carry a tiny and completely unimportant abnormal cell line somewhere in their body. However, a very minor degree of mosaicism could still be important if a crucial tissue carries the abnormal cells. An abnormal chromosome change confined to one part of the brain could theoretically impair neurologic function (45), for example mosaic Down syndrome can be associated with a less characteristic facial appearance and milder mental impairment than those with typical trisomy-21. Chromosomally abnormal cells may also arise with age and contribute to such health problems as the occurrence of cancer in human. However, most age-related chromosome changes are likely either eliminated due to poor cell growth or have no obvious harmful effect, for example, 45,X0 cells are increasingly common in female blood cells as they age, but appear to have no harmful effect. Similarly, the effect of mosaicism on fertility of animals varies due to degree of mosaicism. A rare case of sex chromosome mosaicism (60,XX/90,XXY) in Holstein were associated with an aplastic vulva, penis and clitoris agenesis, a male-like urethra located in a pseudoprepuce opening between the mammary complexes and a well developed M. rectipeninus (46). An another case of mosaicism (60,XX/60,XX, t(12 q;15 q),inv (6) found to be associated with low fertility in Holstein cow which delivered three calves during 11 years of age (47). There are no much more reports on mosaicism and their effects especially in cattle and buffaloes. Recent report (48) presented the Parent-of-Origin-based Detection (POD) method for chromosomal abnormality detection in trio-based SNP microarray data. The method and software provide a significant advancement in the ability to detect low-level mosaic abnormalities, thereby opening new avenues for research into the implications of mosaicism in pathogenic and non-pathogenic processes.

In India, Sex chromosome mosaicism (XY/XXY) reported in a Jersey crossbred bull (49) associated with reduced fertility, 60,XX/61,XXX in an infertile HF cow (50) and XXY/XXY in a Holstein crossbred young bull (51). However, a low degree of Y chromosome mosaicism (sub-metacentric/acrocentric) was also reported in Holstein crossbred young male (52). Similar case of Y chromosome instability was reported in three crossbred cattle (53) but its clinical significance has not been documented. Cases of mosaicism in Indian buffaloes are not much documented except a few cases. A case of mosaicism (50,XX/51,XX) was reported in 8 year old buffalo having irregular breeding history (54). Their Cytogenetic investigations revealed additional 5th chromosome (51,XX+5) in 22.67% cells. The autosomal mosaicism (50,XY/51,XY+4?) in 10% of the cells in a young bull which was phenotypically normal, was observed (51). A case of mosaicism was observed in a phenotypically normal young calf of Gir (Bos indicus) cattle which exhibited inconsistent number of chromosomes 60,XY/58,XY/59,XY/61,XY/61,XYY/61,XY+17/3n in 21 out of 111 metaphase spreads (18.2%) (55).

Sex chromosome aneuploidy occurs due to non-disjunction during meiosis or the early cleavage stages (56). The condition of sex chromosomal aneuploidy could be trisomy-X, XXY, XO and very rarely XYY which is observed in many species of animals including cattle and buffalo. However, the sex chromosome aneuploidy has deleterious effect on fertility as it was found associated with degradation of the seminiferous tubules (57), testicular hypoplasia (58) in bulls, abortion of six month male fetus (XXY) (59) and spontaneous abortions and neonatal losses in cattle (60). In one of testing surveys, 2 heifers with trisomy-X and 2 bull calves with XXY were found with serious abnormalities (61). River buffaloes with XO and Trisomy-X have been found to be associated with reproductive failure (26).

Many cases of XXY were reported in Indian cattle. A case of 61,XXY was reported (62) in an infertile Jersey crossbred bull which was ejaculating semen with more than 90% dead sperms. A Jersey calf with poor body development exhibited 61,XXY during routine cytogenetic investigation (63). Two Holstein crossbred calves...
also exhibited 61,XXY (64,65). However, their fertility could not be estimated as they were young and culled from the breeding programmes. Some cases of sex chromosomal aneuploidy (monosomy-X or XO) in buffaloes (66,67) associated with sterility and gonadal dysgenesis and trisomy-X with sterility (68) were reported in India.

Autosomal aneuploidy occurs due to non disjunction in autosomes. Autosomal aneuploidy typically alters the shape and proportions in characteristic ways. Plants tend to be somewhat more tolerant of aneuploidy than animals. Many still births caused by autosomal trisomy like 61,XY+27, 61,XY+?, 61,XY+21 were found in a cytogenetic survey of aborted fetuses (69). This indicates that animals with autosomal aneuploidy may not survive, as such cases are not reported in live animals. An autosomal aneuploidy in a still born Sahiwal zebu calf was also reported in India (70).

Most of anueploidies in animals were detected by conventional cytogenetic techniques whereas molecular diagnosis is being applied especially in prenatal diagnosis in humans. All molecular techniques are believed to be accurate and carry a low risk of misdiagnosis (71). DNA probes used in prenatal diagnosis of aneuploidy by FISH on interphase nuclei provide an initial rapid screen preceding the full cytogenetic evaluation (72). Multiplex PCR (73) and the PCR methods suitable for the detection of fetal aneuploidy. Siota et al. (74) used sex chromosome painting probe to identify XXY in 8-month old Polish Red breed.

Centric fusion was first observed by WRB Robertson in grasshoppers (75), hence popularly known as Robertsonian translocation. When two acrocentric chromosomes fuse at or around centromere, they produce such chromosomal aberration. Translocations are considered as structural as well as numerical aberration as centromere fusion to changes the structure of chromosome and resulting in reduction in numbers. It is also most common chromosomal rearrangement to occur during the karyotype evaluation of the Bovidae (76). The effect of translocation to animal breeding was realized four decades ago when Gutavsson (77) reported reduction in fertility of female carriers of 1;29 Centric fusion translocation (CFT) in Swedish Red cattle that encouraged investigators to extend their studies to various breeds. It has been found in various frequencies in about 60 different breeds of both Bos taurus and Bos indicus during 1964 to 1990 (78). Since then in various countries worldwide regular cytogenetic screening became mandatory to all the AI bulls before used for breeding programmes. Translocations are categorized in different types; i) centric fusion translocation in which the centromeres of two acrocentric chromosomes fuse to generate one large metacentric or submetacentric chromosome. The karyotype of an individual carrying a centric fusion has one less than the normal diploid number of chromosomes. ii) Reciprocal translocation occurs between two non-homologous chromosomes in which two chromosomes break and then exchange the fragments. Individuals carrying such abnormalities still have a balanced complement of chromosomes and generally have a normal phenotype, but with varying degrees of subnormal fertility. The subfertility is caused by problems in chromosome pairing and segregation during meiosis. Translocations can be balanced means an even exchange of material with no genetic information extra or missing, and ideally full functionality, or unbalanced where the exchange of chromosome materials are unequal resulting in extra or missing part of chromosome. Various kinds of translocations especially centric fusion translocation reported worldwide in various breeds of cattle and buffaloes.

In India, 1;29 translocation in Jersey crossbreds (79,80), 7;16 translocation (81) in Holstein crossbred, 16;20 translocation in Deoni zebu cattle breed (82) and unbalanced 1;9 translocation in a Gir (Bos indicus) bull calf (83) were reported. However a few cases of translocations were reported in Indian buffaloes.
Vijh et al. (84) reported a partial translocation 50,XY,t(3q-;6q+) in Murrah buffalo male but the effect of the abnormality on fertility was not described. One case of unusual translocation (XXY chromosome complement due to X;X-translocation) was also reported in Murrah buffaloes (85) which was associated with azoospermia (86).

New molecular based techniques to identify precise chromosomes involved in translocations are being practiced in humans and animals. Fluorescence in situ hybridization (FISH), with the use of locus-specific BAC probes, facilitated description of the translocation (87). A rare case of centic fission, rob (1p;23) in buffalo was identified with the help of molecular markers (88).

Structural chromosomal abnormalities include deletion, duplication, inversion, ring chromosome, terminal deletion, shifting, isochromosomes, chromatid gaps and breaks, fragile sites, variant chromosome, marker chromosome, etc. which are observed more in human than animals. Some of these abnormalities have been found associated with reproductive failure (89,90,91,92) and congenital anomalies (93).

Many structural aberrations are reported in Indian cattle and buffaloes with their significant association with reproductive failure. Variant chromosome 3 (3p+) in river buffalo males and females (94,95) were found associated with reduced fertility, presence of secondary constriction on 24 chromosome (24q+) in buffalo bull was found with abnormal sperms (96). Fragile sites in number of chromosomes (97,98) were found in a group of sub-fertile cattle and buffalo bulls. Spontaneous pericentric inversion in buffalo (99) was associated with reduced fertility. Chromosome fragmentations, pulverization, premature centromeric division (PCD), polyploidy (3n and 4n), endoreduplication, fragile site etc were also observed by many workers in their published (32,51,100) or unpublished work.

However, microdeletions, duplications, terminal deletion etc. may not be detected by conventional G-banding. Such abnormalities can be detected by molecular techniques (101). Chromosome Micro Array (CMA) technique which is being used in detection of minor chromosomal aberrations specially deletions or duplications syndromes, as well as the pericentromeric and subtelomeric regions, is boon to understand the roles played by segment of chromosome or locus of specific genes thereon in the development of disease in human. Chromosomal Micro Array can detect genomic errors for each of the disorders that are usually identified by karyotype analysis, including Down syndrome (102), trisomy 13, trisomy 18, sex chromosomal abnormalities, and other rare genetic disorders reported to be associated with mental retardation and/or physical problems (103). CMA is more sensitive than older methods of chromosomal analysis, and is able to detect abnormalities that would not have been identified by karyotype analysis. Though Karyotype testing currently is still considered the standard testing but microarray test may replace karyotyping in the near future as it has the capability to zoom in on regions of the chromosomes that are too small to visualize using a microscope, allowing for a higher resolution to determine if smaller regions of the chromosome are extra or missing.

Conclusions

Over the past 50 years, hundreds of scientific publications reporting original chromosomal abnormalities generally associated with clinical disorders (mainly fertility impairment) have been published. Most of chromosomal abnormalities observed in India were basically part of the routine investigation and part of small research studies carried out by the post graduate students in various institutions and universities. Therefore, the number of animals karyotyped in India is very less as compared to cattle and buffalo population. The chromosome screening should be performed at large scale even in indigenous cattle and buffaloes so that it can be compared with exotic cattle for their occurrences. Besides,
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many of chromosomal screening findings are not published due to lack of confirmation of the chromosomal abnormalities by various banding patterns, different techniques and their association with infertility/reduced fertility. It is advisable to screen especially all breeding bulls before inducted in Artificial Insemination programmes preferably at calf hood stage so that the time and money spent on rearing of abnormal bulls can be saved. Though the chromosomal aberrations in large animals are less reported but as far as possible the chromosomal abnormalities observed, and their fertility performance may be correlated. The aborted materials and stillborn calves must be utilized for the cytogenetic studies to know the possible reason of fetal waste. The new techniques based on molecular biology, FISH, chromosomal painting, microarray etc may be developed for routine cytogenetic screening of dairy animals.

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