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Asian Journal of Animal and Veterinary Advances

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www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 6 (2): 117-125, 2011
ISSN 1683-9919 / DOI: 10.3923/ajava.2011.117.125
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# Karyotype for Identification of Genetic Abnormalities in Cattle

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## ABSTRACT

Genetic abnormalities are not very common. About one third of total calves die due to various reasons, among these a significant number die from different disorder. When they do occur, they cause economic losses. One of the main constraints for cattle improvement is insufficient knowledge on chromosome inheritance pattern, different genetic disease and their chromosomal view. A slight slip in Chromosome number or structure brings obvious disorder though out the life, it may leads to death. Karyotype analysis is an effective way for identification of this disorder of chromosome. Identification of genetic disorder or unique character through karyotyping and possible remedy may protect the loss of cattle, which in turn increases the total productivity. Various abnormal karyotype, karyotype analysis, karyotype preparation were mainly reviewed in this study.

**Key words:** Karyotype, chromosomal disorder, cattle, genetic disease

## INTRODUCTION

Numerical errors found with both the number and structure of autosomes or sex chromosomes. It has been proved that those defects have a negative influence on the fertility and development of animals. Although it is assumed that autosomal chromosome aberrations are more numerous than those of sexual chromosomes, anatomical abnormalities caused by defects of sexual chromosomes are more common. The atrophy of zygote is due to autosomal anomalies whereas defects of sexual chromosomes usually influence the development and function of reproductive system (Gluhovschi and Bistriceanu, 1973). Genetic disease can hamper the production of animals. Many genetic diseases such as Turner syndrome, Klinefelter syndrome, Gaucher's disease, Familial polyposis of the Colon, Tay-Sachs, Twin calf, Robertsonian translocation etc. found in cattle.

New directions in animal husbandry demand raising of animal kind that are adjusted to intensive way of breeding. In order to accomplish these demands, beside known methods in selection, cytogenetic control of existing genotypes is needed that has been carried through karyotype analysis. Numeric and structural changes on animal karyotype influenced on reproduction disturbance, phenotype expression, as well as selection program and stability of genofond (Slavica *et al.*, 2006). Karyotype of Sahiwal, Red Sindhi, Brahman and Santa Gertrodis breeds of cattle and observed that these breeds had 60 chromosomes (Halnan *et al.*, 1981).

Karyotype test can both count the number of chromosomes and look for structural changes in chromosomes that may indicate genetic changes associated with increased risk for disease. The greatest number of karyotype examinations has been carried out on bulls since there is a danger

of spreading through artificial insemination, potential chromosomal abnormalities within the population. Cytogeneticists are now armed with powerful tools to characterize normal karyotypes and to discover more about fundamental basis for abnormalities. The chromosomal screening can reduce embryonic and foetal mortality up to 20-30% (Roberts, 1971). This review will give a detail for karyotyping and chromosomal abnormalities in cattle.

## KARYOTYPE ANALYSIS

Cattle have 60 chromosomes, 29 pair of autosomes and 1 pair of sex chromosomes (Krallinger, 1927; Makino, 1944; Melander, 1959). All of the autosomes are somewhat teardrop shaped with centromere at the end of the chromosome. The sex chromosomes have centromere in the middle of the chromosome, with the X being much larger than the Y. A karyotype is a full set of metaphase chromosomes from one diploid cell usually made from an enlarged photomicrograph arranged in descending lengths (Eldridge, 1985). Karyotyping using digital image is a simulation of cattle chromosomes from actual cattle genetic studies. It arranges chromosomes into a complete karyotype and interprets the findings just as if one is working in a genetic analysis program. Chromosome analyses on numeric and structural changes can be done according to ISCNDA internationals standards for kariotypisation of domestic animals. Degree of changes could be observed under light microscope 1000X with high-resolution camera (Di Beraradino *et al.*, 1990). The sex chromosomes were examined at 1000X and the sex of the embryo could be diagnosed as described in a previous study (King *et al.*, 1991). Cells are usually blocking at mitosis and staining the condensed chromosome with Giemsa dye. Karyotypes of Giemsa stained autosomes were arranged according to the relative lengths of the chromosomes. The standardization of banded karyotypes of domestic animals one of the most important steps in the history of animal cytogenetics, provided for the first time the 'standard' G-banded karyotype of cattle (*Bos taurus* L.) as well as that of other domestic species in a reading conference (Ford *et al.*, 1980).

When karyotypes were first investigated, individual pair of chromosomes could be identified only according to their space and size. More recently, various methods of staining chromosomes have been developed to the stage where reproducible banding patterns on particular chromosomes can be obtained. The methods most commonly used are G, R, C, T and Q- banding. The first four involve the use of Giemsa dye. G-banding produces a particular pattern of light and dark bands and R- banding gives a pattern that is the reverse of G- banding, C- banding causes the constitutive heterochromatid to stain darkly, while T- banding stains the telocentric (end) regions and the centromeres. In Q- banding, the used of quinacrine dye results in alternating brightly and dimly fluorescent bands which can be viewed through an ultraviolet fluorescent microscope, with the bright bands corresponding to the dark bands of G- banding. The sequential GTG-RBA banding procedure, performed for the first time on chromosome of cattle, is suitable for a specific characterization of individual chromosomes of this species. Previous contributions on the RBA banding pattern in cattle chromosomes (Gustavsson and Hagelthorn, 1976; De Giovanni *et al.*, 1979, 1988; Di Berardino *et al.*, 1983, 1985a, b; Di Berardino and Iannuzzi, 1982) reported karyotypes which were based, as far as possible, on the Reading G-banded standard karyotype, but a direct correlation between G and R bands has not so far been reported. Recently, a G- and R-banding comparison of cattle prometaphase chromosomes arranged according to the Reading system reported by Iannuzzi (1990) but without use of a sequential G-R banding procedure.

## KARYOTYPE PREPARATION

**Preparations from fibroblast cell:** In general, any tissue aseptically removed, can give usable cultures. A series of steps involved in cell transfer. The first step is a biopsy, which can be obtained from any part of the body by surgical means, the most common one being skin culture. The more common method is to cut up tissue into small bits before placing culture. Usually the bits are fixed in position by using a plasma clot (Harnden, 1960; Lejeune *et al.*, 1959); or holding them down with perforated cellophane (Hsu and kellogg, 1960), or by pining under a cover slip (De Mars, 1963; Harnden and Brunto, 1965), but cultures can also be carried out without holding down the tissue (Davidson *et al.*, 1963). Puck *et al.* (1958) recorded the growth of cultures for a year without any deleterious effects, but Hayflick and Moorhead (1961) and Moorhead and Saksela (1963) observed gradual lose of cell multiplication and aneuploidy after a particular period in cultures. For chromosome preparation, attempts are made to obtain partially synchronous divisions by changing the medium or subcultures. The culture is allowed to become acidic and the pH restored to 7.4 by adding $NaHCO_3$ and embryo extract, resulting in a large number of mitosis after 16h. In most techniques however, colchicines is added to the culture to obtain metaphase configurations. Either the cell are grown on cover slips and processed while attached to them (Harnden, 1960), or the cells are suspended through trypsin digestion and then processed in any methods available for peripheral blood culture, the second method gives more satisfactory results. In fixation, acetic ethanol or acetic methanol (1:3) is commonly employed. The cells can be spread by the air- drying schedule used for blood cells, 75% acetic acid immediately before drying can be used for better spreading.

**Preparations from bone marrow cells:** Direct processing enables identification of cell line from which the analyzed cells are derived. Aspirate 0.5 mL bone marrow treat in two changes of 2-3 mL colchicine solution. Then transfer to watch glass with a few drops of 2% acetic-orcein; NHCL (9:1) mixture and heat gently (Tizio and Whang, 1965). Squash in a drop of 2% acetic-orcein or Giemsia: Seal and store: it can be made permanent by leukaemia cells (Priest, 1969). Blow on each droplet as soon as the cells are attached to the glass surface, to assist spreading. Dry and immerse 10-20 min in 2% acetic-xylol grades mount in permount. Feulgen reagent, Giemsa stain and crystal violet can be used as alternative stains (Rothfels and Siminovith, 1958). In short term culture, 1 mL bone marrow from the individual centrifuge and resuspend in isotonic glucose-saline mixed in equal parts with bovine serum. For immediate examination, intravenous injection of colchicine 2 h before external puncture, followed by direct treatment in hypotonic solution, fixation and staining (Bottura and Farrari, 1960). Medium term culture in liquid medium involves 1 mL bone marrow suspend in 3 mL culture medium: AB-serum, 35%; TC 199 medium, 60% and embryonic extract, 5%. Distribute in Petri dishes containing cover slips, incubate at 37°C for 24 to 72 h in an atmosphere containing 5% $CO_2$ and stain following the acein-orcein schedule (Fraccaro *et al.*, 1960; Hirsch-Horn and Cooper, 1961).

**Preparations from peripheral blood leukocyte:** In macromethod, draw in and eject 1 mL solution of 5000 IU heparin $mL^{-1}$ in a syringe, thus coating its wall completely with heparin. Draw 20 mL venous blood into heperinised syringe and separate supernatant containing exclusively WBC. Study an aliquot of the supernatant in medium containing 30-35% of the individual's serum and 65-70% of basal medium and add 0.2 mL bacto-phytohaemagglutinin per 10 mL mixture.

Distribute in test tubes, filling them to one-third of their volume. If required, inject a mixture of 5% $CO_2$ and air before sealing the tube. Incubate at 37°C for 72 h; add two drops of isotonic colchicine solution at concentration at 0.4% per mL of the medium 2 h before harvesting. Another alternative method, micromethod was developed by several workers used only a few drop of blood (Edwards, 1962; Froland, 1962; Arakaki and Sparkers, 1963; Grouchy *et al*., 1964). The micromethod for culturing leukocytes (Moorhead *et al*., 1960; Lin *et al*., 1977) was used in chromosomal analysis for 200 breeding bulls of different breeds of cattle (Jersey, Holstein Friesian, Sahiwal and Cross-bred) and Nili-Ravi buffalo, maintained at Semen Production Unit, Qadirabad and Livestock Experiment Station, Bhunikey (Ahmad *et al*., 2004).

## ABNORMAL KARYOTYPE IN CATTLE

Abnormal karyotypes arise from error in chromosome replication, fertilization or early cleavage divisions of the fertilized egg. In many cases, abnormal karyotypes have been observed in healthy and/or highly productive animals and likewise, many diseased and/or unproductive animals have completely normal karyotypes. The most important effect of abnormal karyotypes is their contribution to lowered reproductive performance through decreasing ability or complete failure to produce functional gametes, or death of embryos.

**Abnormal chromosome number:** Infertility and sterility in animals that have never been fertile is often the result of cytogenetic disease, particularly with regard to sex chromosome aneuploidy (e.g., XO, XXX, XXY genotypes). Cytogenetic analysis of such individuals is often necessary if an abnormality is detected, further diagnostic efforts can be avoided. As an indication of the importance of chromosome abnormalities in reproductive failure, 29 cases study of infertility in cattle summarized in a report (Blue *et al*., 1978). The lack of one X chromosome, the most common abnormality was reported in 12 cases. Animals that lack a chromosome are said to be monosomic for that chromosome. These 12 cattle are therefore said to be monosomic for the X chromosome. Their karyotype is written as 59, XO, with the O indicating absence of an X chromosome the condition is called Turner's syndrome. All XO individual have a more or less normal female external phenotype (Gustavsson, 1980). The most common type of chromosomal abnormality is autosomal or sexual chromosome trisomy. Another abnormality detected in one of the 29 infertile cattle was the presence of three X chromosomes rather than the normal two. This example of trisomy for the X chromosomes and in the case of cattle with the external X, the condition is written as 61, XXX. Cytological examination revealed nondividing cells of X trisomics were seen to contain two Barr bodies, thus indicating that only one X in each cell remained active. Although the XXX cattle in that study was infertile, XXX individual were often fertile and had been reported as giving rise to offspring with normal karyotypes in cattle (Sysa *et al*., 1988, 1998; Switonski, 1992, 1998). Two 61, XX and one t (1:29) was reported from 57 aborted fetuses of six cattle breeds (Salam *et al*., 2002).

XO and XXX individuals arise from non-disjunction, which failure of the chromosomes or chromatids to disjoin during meiosis. Non-disjunction can occur with any of the autosomes or with the sex chromosomes. In the letter case, results of non-disjunction differ depending on the sex of the individual in which meiosis is occurring and in cattle, whether non-disjunction occurs during meiosis I or meiosis II. With males, however, four different types of unbalanced sperms can result, depending on when non-disjunction of sex chromosomes occurs. As an indication of the range of different types of sex chromosomes aneuploidy that have been detected in domestic species.

Aneuploidy of sex chromosomes is usually associated with sexual abnormalities. Non-disjunction of the sex chromosomes has been shown to exist in cattle but has not been readily documented. Non-disjunction is caused by failure of chromatids to separate during meiosis (Eldridge, 1985). This condition is comparable to the Klinefelter syndrome (XXY) in humans. It has been found in humans that Klinefelter syndrome originates from non-disjunction during maternal or paternal meiosis (Lorda-Sanchez *et al.*, 1992). In bulls, it is characterized by underdevelopment of the testicles and the animal is usually sterile (Eldridge, 1985). Cows over 9 years old have higher risk of abortion because they are more prone to non-disjunction and numerical chromosome problems (Arifuzzaman, 2003).

Twinning in cattle is a complex trait that is associated with health and productivity of animals. Twinning has been associated with economic loss such as abortion, dystocia, lower birth weights and reduced neonatal calf survival (Markusfeld, 1987; Fricke and Wiltbank, 1999; Karlsen *et al.*, 2000). About 92% of heterosexual twin females show an absolute infertility and abnormal developments of sexual organs. By chromosome karyotyping from leucocytes, sex chromosomal chimerisms are distinctly observed, although the ratio of XX/XY is different in each case (Swartz and Vogt, 1983).

**Abnormal chromosome structure:** A reciprocal translocation involves each of two non-humongous chromosomes breaking into two segments, followed by exchange of segments between the two chromosomes. Since no part of any chromosome was lost during the formation of translocation individuals carrying it still have a normal, balanced complement of genetic material and usually have normal phenotype. The chromosome structures resulting from translocation between non-homologous chromosomes are many and varied. A type of translocation that has generated considerable interest is one in which the centromeres of two acrocentric chromosomes fuse to produce one metacentric chromosome which is known as a centric fusion individuals that are homozygous for centric fusion translocations will have two less than the normal number of chromosomes but they have also normal phenotypes because they have a complete genome (Gustavsson and Rockborn, 1964). Ever since centric fusions were discovered in animals, their effect on reproductive ability has been a controversial issue, in contrast to reciprocal translocations which clearly reduce fertility. Meiosis in individuals heterozygous for a centric fusion is certainly unusual, because three chromosomes have to synapse, forming a trivalent. If the centric fusion chromosomes disjunction from the other two, then balanced gametes will result. Any other type of disjunction will lead to unbalanced gametes, which would be expected to lead to reproductive failure (Gustavsson, 1966).

Centric Fusion (CF) (Robertsonian translocation) is an inherited cytogenetic condition involving the permanent joining of heterologous chromosomes. There is no obvious explanation for the apparent in the effect of centric fusions in sheep and cattle. The most important practical question is whether or not the 1/29 centric fusion should be removed from cattle population because of its undesirable effect on productive ability. It has been argued that eradication of the 1/29 centric fusion in cattle would produce substantial financial benefits in some cattle populations in terms of increased fertility (Gustavsson, 1979). The effect of eradicating a centric fusion such as the 1/29 in any breed of cattle will depend on its frequency in that breed; only if it is relatively common will its eradication have a noticeable effect on overall breed fertility. Population in which eradication is having a significant effect on fertility the frequency of the 1/29 centric fusion on fertility, various artificial insemination centers throughout the world conduct regular karyotype screening programs.

Some centers prohibit the use of centric fusion heterozygotes. Given the expense regular karyotype screening and the apparent absence of the1/29 centric fusion from certain popular breeds such as Herefords and Holstein-Friesians and its low incidence in many other breeds, it is unlikely that regular screening programs of cows and natural service bulls would be economically justifiable in most breeds in most countries. If, however, a centric fusion is common in a particular breed in a certain country, then it may be worthwhile screening all bulls of that breed entering artificial insemination centers in that country. Robertsonian translocations were examined in 767 Holstein sires, 1010 Czech Pied (Simmental) sires, 142 beef sires and 48 dams. Of these, 10 sires of Czech Pied breed, 5 beef sires and 13 females were found to be positive. The monitoring of Bovine Leukocyte Adhesion Deficiency, Complex Vertebral Malformation and Robertsonian translocations is recommended (Citek *et al.*, 2006). Presently, more than 25 different types have been reported in approximately 70 cattle breeds (Revay *et al.*, 2004). With fused chromosomes, significant unequal partitioning of genetic materials can occur during meiosis, theoretically resulting in a 2/3 reduction in fertility in cattle (Gustavsson, 1969), rather than the 5 to 21% reduction observed (Dyrendahl and Gustavsson, 1979; Schmutz *et al.*, 1991). A crossbred bull heterozygous for a double centric fusion of 14 months of age produced normal semen volume with sperm characteristics. Cytologic and ultrastructural evidence of spermatid degeneration was found coincident with abundant, apparently normal, sperm formation (Weber *et al.*, 2007).

The final types of structural abnormalities that we shall discuss are inversions and deletions. As their name suggests, inversions arise when a segment of chromosome becomes inverted following the breakage of a chromosome in two positions. If the segment includes the centromere is not includes the centromere, the inversion is said to be pericentric and if the centromere is not included the inversion is paracentric. Deleltions arise if following the breakage of a chromosome in two positions, the segment between the two break-points is lost. Deletions usually produce serious abnormalities, as the individual is effectively monosomic for the segment of chromosome deleted. Inversions and deletions are the least frequently observed of chromosomal abnormalities in domestic animals.

**ACKNOWLEDGMENTS**

The authors would like to thank supporting staff of Animal Biotechnology Division, National Institute of Biotechnology for their cooperation in the preparation of manuscript.

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