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Review Article

APPLICATION OF CYTOGENETIC TECHNIQUES IN LIVESTOCK IMPROVEMENT

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ABSTRACT
Cytological techniques about last 20 years enable precise and detailed observations of the chromosomes of mammals and birds. Most of the therapeutic agents inserted to animals, as well as herbicides, pesticides, victual additives. Karyological methods are subsidiary to detect chromosomal damage or aberrant mitosis or meiosis which is bespeakers of toxicity activity. In animal genetics, cytogenetic techniques are applied to test postulations of theory. Incipient sources of genetic variation may be chromosomal aneuploidy, and euploidy. When used to cells through culture and cumulated with modern methods of molecular biology, cytogenetic technique can avail discover incipient loci.

KEYWORDS: chromosome, aneuploidy, euploidy, toxicity, incipient loci.

INTRODUCTION

An exuberance to observe chromosomes has characterized animal geneticists since it was apperceived that chromosomes are the conveyances in which the genes reside. The science of cytogenetics developed in the prospect of finding sodalities between morphology, or deportment and gross anatomical or physiological functions of animals holistically.

Cytogenetics

Chromosomal deportment during somatic cell division in magnification and development (mitosis) and germ cell division in reproduction (meiosis). Revelation of incipient techniques, amendments of subsisting techniques or incipient coalescences of well-established techniques is often followed by progress in the sciences.

Chromosomes and mundane chromosome complement

Chromatins are tenebrous staining materials present in the nucleus of a cell. At the time of cell division, these chromatin bodies condense into shorter and thicker threads called chromosomes, which carry the genes and functions in the transmission of hereditary information. In a mundane diploid cell, there are 46 chromosomes (23 chromosome pairs), where one of each dyad is derived from the father and the other from the mother of the individual. The first 22 dyads are called the autosomes (non-sex chromosomes) and the 23rd dyad is called the sex-chromosomes. In males, the 23rd dyad is XY and in females, it is XX. In the case of gametic cells (sperm and ovum), or otherwise called haploid cells, they have only single chromosome from each pair.

Banding techniques

The different banding techniques sanction precise identification of each chromosome as well as to detect structural chromosomal rearrangements. A coalescence of many banding techniques are obtaining the information compulsory for chromosomal analysis.

Q-banding

This banding technique does not require any prior treatment of the chromosomes. The Q-bands appear along each chromosome in alternating effulgent and dull bands with varying intensity. However, Q-banding does not sanction sempiternal preparations. Certain antibiotics like anthracyclines engender fluorescent bands homogeneous to Q-bands and are more stable than those engendered by quinacrine.

G-banding

G-bands are engendered by staining the chromosomes with a stain, Giemsa. This is done by treating the chromosomes with substances (customarily trypsin), that alters the structure of proteins followed by staining with a Giemsa solution (Rowley, 1973). It is the most prevalent method of banding, as it engenders the same banding pattern as quinacrine with even more preponderant resolution; it sanctions sempiternal preparations and does not necessitate the utilization of fluorescence microscopy. Thus, G-band patterns can be acclimated to pair and identify each of the human chromosomes accurately.

R-banding

R-bands are just the inversion of G-bands, which can be engendered by a variety of methods. Since the staining ability of the chromosomes is remotely lost due to heating, the utilization of phase contrast objectives gives a better contrast of the chromosomes for analysis.

C-banding

C-bands localize the heterochromatic regions of chromosomes. Pardue & Gall (1970) first reported C-

bands in 1970 when they discovered that the centromeric region of mouse chromosomes is opulent in perpetual DNA sequences and stains dark with Giemsa. The pristine method of Arrighi and Hsu (1971) involves treating the slides with 0.2 N hydrochloric acid followed by treatment with RNAse and sodium hydroxide. These polymorphic regions can be visualized optimally with C-band methods and are most often optically discerned on acrocentric chromosomes, the centromeric region of chromosomes 1, 9, and 16, and the distal portion of the Y chromosome. C-banding is withal utilizable to show chromosomes with multiple centromeres, to study the inception of diploid molar pregnancies and true hermaphroditism and to distinguish between donor and recipient cells in bone marrow transplantation.

T-banding

This method involves staining the telomeric (end) regions of the chromosomes. Dutrillaux (1973) treated the slides with either phosphate buffer or Earle's balanced salt solution and then stained utilizing commixed Giemsa solution to engender the T-bands.

CT-banding

Scheres, (1974) developed a method to stain both the centromeric heterochromatin as well as the telomere of chromosomes. He treated the slides with barium hydroxide to engender the CT-bands.

Nucleolar Organizing Region-banding

Nucleolar organizing region (NOR)–banding is a technique that stains NORs of chromosomes (Matsui & Sasaki, 1973). NOR-bands may represent structural non-histone proteins that are categorically linked to NOR and bind to ammoniacal silver. Goodpasture *et al.* (1976) developed a simple silver nitrate staining technique that is now used widely. NOR-banding is utilizable in clinical practice to study certain chromosome polymorphisms, such as double satellites. This method is withal subsidiary to identify satellite stalks that are infrequently visually perceived on non-acrocentric chromosomes.

The cull of banding technique

For routine analysis, the banding technique utilizing trypsin and Giemsa became the most accepted ecumenical (Seabright, 1971). Since the banding pattern enabled the detection of sundry structural aberrations like translocations, inversions, expunctions, and duplications in juxtaposition of the already well-kenned numerical aberrations, not only potentially unbalanced cases (patients) could be studied but additionally salubrious individuals as possible carriers of a balanced aberration. For instance, salubrious family members of already kenned carriers and couples suffering from perpetual spontaneous abortions were cytogenetically investigated (Dominique FCM Smeets, 2004).

High resolution banding

Despite the above banding patterns, resolution of chromosome studies remained relatively circumscribed with an approximate count of 500 bands per haploid genome (resolution ≈ 6 million base pairs ≈ 50 genes per band) because the total numbers of bands engendered on metaphase chromosomes are less and it is arduous to detect rearrangements involving modicums of chromosomes due to exorbitant condensation. High resolution cytogenetics assignments of gene loci, more preponderant than with earlier techniques, since analysis of tardy prophase sub-banding reveals more than twice the number of bands visually perceived at metaphase (Sawyer & Hozier, 1986). By applying this technique, several already well-kenned clinical syndromes like Prader Willi and Angelman syndrome with an expunction at the proximal long arm of chromosome 15, Smith-Magenis and Miller-Dieker syndrome with (different) expunctions in the short arm of chromosome 17 and DiGeorge/Velo Cardio Facial (VCF) syndrome with expunctions in the long arm of chromosome 22 could be linked to diminutive chromosome aberrations and the concept of the micro-effacement or contiguous gene syndrome was born (Schmickel, 1986).

Sex chromatin analysis

This is obtained by taking buccal smears on an immaculate slide followed by fine-tuning them in ethanol, air drying, hydrolysing in hydrochloric acid, washing in distilled dihydrogen monoxide to abstract the acid and then determinately staining utilizing crystal violet. The presence of a chromatin mass, called the "Barr body" denotes a chromatin positive cell.

Sister Chromatid Exchange (SCE)

It is accomplished in cell cultures by incorporating BrdU (bromodeoxyuridine) (in lieu of thymidine) into replicating cells for 2 cell cycles. This engenders an acridine fluorescence pattern in which one chromatid fluoresces more brightly than the other chromatid. The biologic consequentiality of SCEs is skeptical, but some mutagens increase their frequency (Perry & Evans, 1975).

MOLECULAR CYTOGENETICS

Fluorescent in situ Hybridization (FISH)

Even with the technique of high resolution chromosome banding, it was arduous to visualize the aberrations at the cytogenetics level. In 1986, Pinkel *et al.* (1986) developed a method to visualize chromosomes utilizing fluorescent-labeled probes called FISH. FISH sanctioned chromosomal and nuclear locations of concrete DNA sequences to be optically discerned through the microscope. FISH technology sanctions the detection of categorical nucleic acid sequences in morphologically preserved chromosomes, cells and tissues. FISH probes are generally relegated by where they hybridize in the genome or by the type of chromosome anomaly they detect. These techniques are subsidiary in the work-up of patients with sundry congenital and malignant neoplastic disorders, especially in conjunction with conventional chromosome studies. Fluorescent tags are safer and simpler to utilize, can be stored indefinitely, give higher resolution which opened up prospects for simultaneously locating several DNA sequences in the same cell by labelling them with different fluorochromes (Barbara J Trask, 2002). Utilizing FISH, cytogeneticists could detect chromosomal abnormalities that involved minuscule segments of DNA. Even more importantly, FISH opened up the nuclei of non-dividing cells to karyotype analysis. Utilizing FISH and chromosome-concrete probes, cytogeneticists could enumerate chromosomes, simply by counting spots in each nucleus.

Spectral Karyotyping and Multicolor FISH (M-FISH)

After the advent of FISH, where a single copy gene could

fluoresce, a more potent technology called WELKIN or M-FISH was developed. M-FISH sanctions all the 24 human chromosomes to be painted in different colours. By making utilization of sundry amalgamations and concentrations of fluorescent dyes, it is even possible to give every single chromosome a different color (EMPYREAN) which can be of particular use when dealing with intricate aberrations often associated with sundry types of solid tumors. WELKIN or M-FISH enables engenderment of chromosome-categorical 'paints': amalgamates fluorochromes to engender 24 colour cumulations, one for each chromosome (Ried *et al.* 1992) and hence multicolour analyses. FIRMAMENT paints the entire chromosome in the same colour, whereas in the case of M-FISH, sundry fluorescence dyes to represent different painting probes at the same time are utilized. These imaging systems can be programmed to relegate each chromosomal segment automatically and they offer the first authentic hope of automated karyotype analysis. EMPYREAN and M-FISH have proved to be prodigiously utilizable in detecting translocations and other involute chromosomal aberrations.

Comparative Genomic Hybridization (CGH)

While FISH investigations have proved to be benign in many ways, it withal has demerits. Like all probes, it has to be hybridized and later microscopically analyzed. Moreover such procedures were time-consuming and arduous to automate. This led to the development of technique of FISH called CGH. Later, a further ameliorated technique was developed which was an array predicated on comparative genomic hybridization (Sabina Solinas-Toldo *et al.* 1997; Albertson & Pinkel, 2003). In contrast to analysis carried out on banded chromosomes, CGH does not require preparation of metaphase chromosomes from the cells. In lieu of hybridizing a labeled probe to human chromosomes on a slide, we now have the potential to print thousands of different and well-characterized probes on a glass slide. Subsequently, consummate isolated and fragmented DNA from the patient is labeled in a certain color and commixed with precisely the same amount of DNA of a mundane control (or a commix of controls) which is labeled in a different color. This DNA commix is then hybridized to the denatured probe DNA on the glass slide. After several washing steps, the fluorescence pattern of each spot can be analyzed and the ratio of test (patient) over reference (control) is quantified. The array-CGH is even more promising than the conventional CGH (Pinkel *et al.* 1998).

Prenatal genetic diagnosis

Prenatal diagnosis of chromosomal aberrations requires cytogenetic analysis of amniotic fetal cells (Verma *et al.* 1998). Amniocentesis is an invasive, well-established, safe, reliable, and precise procedure performed during pregnancy to detect chromosomal abnormalities as well as other categorical genetic diseases. Fuchs and Riis (1956) reported the first utilization of amniotic fluid examination in the diagnosis of genetic disease in 1956 in their seminal article in "Nature". The tenaciousness of fetal sex led to the prenatal management of patients with Haemophilia in 1960 and Duchenne brawny dystrophy in 1964. Cytogenetic investigation of spontaneous pregnancy losses provides the rudimentary information for precise genetic counseling (Neus Baena *et al.* 2001). The prenatal genetic diagnosis is indispensable in cases where the sonographic findings leads one to doubt on the chromosomal disorders, especially the syndromes associated with sundry trisomies. It is withal warranted in individuals with a high risk of trisomic pregnancies predicated on pedigree analysis for chromosomal disorders to ken the family history of trisomy, incremented maternal age, and incremented incidence of meiotic or mitotic non-disjunction and couples who are suspected or kenned to be carriers of inherited genetic disorders.

CONCLUSION

So far, no system can relegate banded chromosomes as robustly and accurately as an adroit cytogeneticist, despite the millions of dollars that have been invested in automated karyotype analysis since 1968. Routine banded karyotype analysis can now be amalgamated with M-FISH and other molecular techniques leading to more precise detection of sundry syndromes in children. Through the analysis of chromosome banding patterns, thousands of chromosomal abnormalities have been associated with inherited or de novo disorders, engendering many leads to the underlying molecular causes of these disorders and today, when high resolution genetic linkage analysis can be conducted facilely, the revelation of a patient whose disorder is caused by a gross chromosomal abnormality is heralded as a valuable resource for locating the disease gene. Solid tumors additionally present a myriad of intricate chromosomal aberrations and each is a possible clue to tumor initiation and progression. In other words, chromosomal abnormalities subsist as nature's guide to the molecular substructure of many unexplained human disorders. Hence, cytogenetics perpetuate to remain as indispensable implements for the diagnosis of sundry genetic disorders which gives an overall picture of the whole genome for analysis. This could possibly additionally pave a way for treatment and management cognate to chromosomal disorders.

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