Ceylon Journal of Science (*Bio. Sci.*) 42(1): 63-66, 2013
DOI: 10.4038/cjsbs.v42i1.5900

SHORT COMMUNICATION

Metaphase Displacement and Anaphase Lagging during Induced Lymphocyte Culture in Cattle and Buffalo

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Received: 09 March 2013 / Accepted: 11 May 2013

ABSTRACT

Chromosomal aberrations are of great concern in dairy animals worldwide as they are usually associated with reduced fertility or infertility. There are multiple reasons which may cause chromosomal aberrations during mitosis and meiosis. To understand the probable cause of aberrations, a simple study was performed during induced lymphocyte culture. The lymphocyte cultures were setup using the blood samples of cattle and buffaloes. Samples were harvested without adding ethedium bromide and colchicine. The Giemsa stained chromosome slides were screened under the microscope (100X) and photographed with attached system.

Keywords: chromosome, cattle, buffalo, metaphase displacement, chromosome lagging

INTRODUCTION

Mitosis is a cellular event which takes place in a highly organized manner. During metaphase, some changes occur in cells, *i.e*., nuclear membrane disappears completely, the two pair of centrioles align at opposite poles of the cell, polar fibres or microtubules that make up the spindle fibres continue to extend from the poles to the centre of the cell. Chromosomes are held at the metaphase plate by the equal forces of the polar fibres pushing on the centromeres of the chromosomes. At metaphase, the spindle is at its maximum size and kinetochores of each pair of sister chromatids are positioned equidistant from the two poles, thus forming the metaphase plate. Traditionally, the chromosome alignment on the metaphase plate has been thought to be achieved by the balance between the two oppositely oriented pulling forces, acting on each of the kinetochores of the paired chromatids. The detachment of chromosomes from spindle fibres in metaphase is known as metaphase displacement of chromosomes, which can be usually seen at the centre of metaphase ring. Ford and Lester (1982) tested three hypotheses which led to the following conclusions: (1) Chromosomal displacement is a function of size-smaller chromosomes is displaced more often than larger ones, (2) The position of the centromere does not influence chromosome displacement, (3) The amount of centromeric heterochromatin in the chromosome has a highly significant effect on displacement; chromosomes with more heterochromatin are displaced more often than those with less. Ford and Roberts (1984) concluded that chromosome displacement is a function of chromosome size and does not reflect spatial ordering at metaphase.

Furthermore, it is suggested that many studies of apparent ordering at metaphase may merely reflect chromosome displacement. The analysis of displacement rates in all other chromosomes of the complement was undertaken in one of the translocation carriers (Ford and Roberts, 1984). This showed no alteration of relative displacement rates. Metaphase displacement with mitotic disturbances in animal chromosomes was also studied by Patel *et al.* (1997), who emphasized that abnormal chromosome may disturb metaphase cell stage causing metaphase displacement. Dosage imbalance of whole chromosomes is likely to result in inviability (Hassold and Hunt, 2001). However, a direct mechanistic link between extra centrosomes and chromosomal instability has not been established even in tumor cells where chromosome instability is a hallmark of many tumors cells associated with the presence of extra centrosomes (Ganem *et al.* 2009). The search for the origin of chromosomal aneuploidy started in the 1970s when cytogenetic analyses of human oocytes revealed meiotic chromosomal aneuploidy.

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Anaphase lagging appeared to be the major mechanism through which human embryos acquire a mosaic chromosome pattern during pre-implantation development to the blastocyst stage (Coonen *et al.* 2004). The current study was carried out to investigate chromosomal displacement at metaphase and chromosome lagging in anaphase in cattle and buffalo, and to understand their possible associations with numerical chromosome aberrations.

MATERIALS AND METHODS

The heparinized blood samples were collected from phenotypical normal cattle and buffalo for routine cytogenetic investigations. Standard whole blood cultures in RPMI-1640 (Himedia) medium supplemented with antibiotics, 15% fetal calf serum and 1% pokeweed mitogen were set and incubated at 38°C. To study the mitotic orientation of chromosomes and anaphase stage in these bulls, two cultures each from cattle and buffaloes were harvested at 69 hrs without the use of ethedium bromide and colchicine (Patel *et al.*, 1997). The cells were separated by centrifugation at 1500 rpm for 5 minutes followed by hypotonic treatment with 0.075 M KCl for just 5 minutes at 37°C and fixed in 3:1 ratio of methanol and acetic acid glacial. Finally the cell suspension was dropped on slides and air dried. Conventional staining by Giemsa was performed on chromosome slides to visualize the metaphase displacement and anaphase lagging under the microscope and photographed under the oil immersion objective (100X).

RESULTS AND DISCUSSION

For each species of cattle and buffaloes, a minimum of 25 metaphase rings were observed. During screening, the number of anaphase stages was also observed in both species. The samples considered for metaphase displacement and anaphase lagging exhibited normal karyotypes during routine cytogenetic investigation.

Slides prepared for metaphase displacement and anaphase lagging revealed many chromosomes displaced at metaphase stage as shown in figures 1 and 2. Arrows in the Figure 1 (a) and (c) indicate the displaced chromosomes in metaphase, whereas no displacement of chromosomes is illustrated in figure (b) and (d). Moreover, Figure 1 (d) indicates premature centromeric division almost in all chromosomes causing chromosomal displacement. Anaphase initiation (pre-anaphase) and various stages of anaphase are depicted in Figure 2 (a), (b), (c) and (d) respectively. However, in Figure 2 (c), the arrow indicates the lagged chromosome.

All chromosomes at metaphase stage are attached with spindle fibres and therefore, they normally move to their respective centrioles during anaphase. The chromosomes which are detached from the spindle fibres due to disturbances during cell cycle may be displaced at metaphase. Such displaced chromosomes are unable to move to their respective centrioles or lag behind other chromosomes during anaphase, causing aneuploidy.

Premature Centromeric Division (PCD) of chromosome(s) will also cause aneuploidy or polyploidy. The PCD in all chromosomes [Figure 1 (d)] may cause polyploidy as none of the chromosomes will move to their centrioles. A previous study has revealed that chromosomal malorientations during cell division may cause non-disjunction which in turn leads to aneuploidy (Janicke *et al.*, 2007).

Chromosomal instability is a hallmark of many tumors and correlates with the presence of extra centrosomes. It has been proposed that extra centrosomes generate chromosomal instability by promoting multipolar anaphase, a highly abnormal division that produces 3 or more aneuploid daughter cells (Ganem *et al.*, 2009). Anaphase lag describes a delayed movement during anaphase, where one homologous chromosomeinmeiosis or one chromatid in mitosis fails to connect to the spindle apparatus, or is tardily drawn to its pole and fails to be included in the reforming nucleus. As a result, the lagged chromosome forms a micronucleus in the cytoplasm and is lost from the cell (Gardner and Sutherland, 2004). The lagging chromosome is not incorporated into the nucleus of one of the daughter cells, resulting in one normal daughter cell and one with monosomy (Gardner and Sutherland, 2004). Anaphase lag is one cause of aneuploidy, which can also cause a rescue of the daughter cell if the cell was originally a trisomy (Gardner and Sutherland, 2004). Metaphase displacement and anaphase lagging could also be attributed to spindle displacement which is an important event of the cell cycle, causing many cells to divide unequally (Campbell *et al.*, 2009).

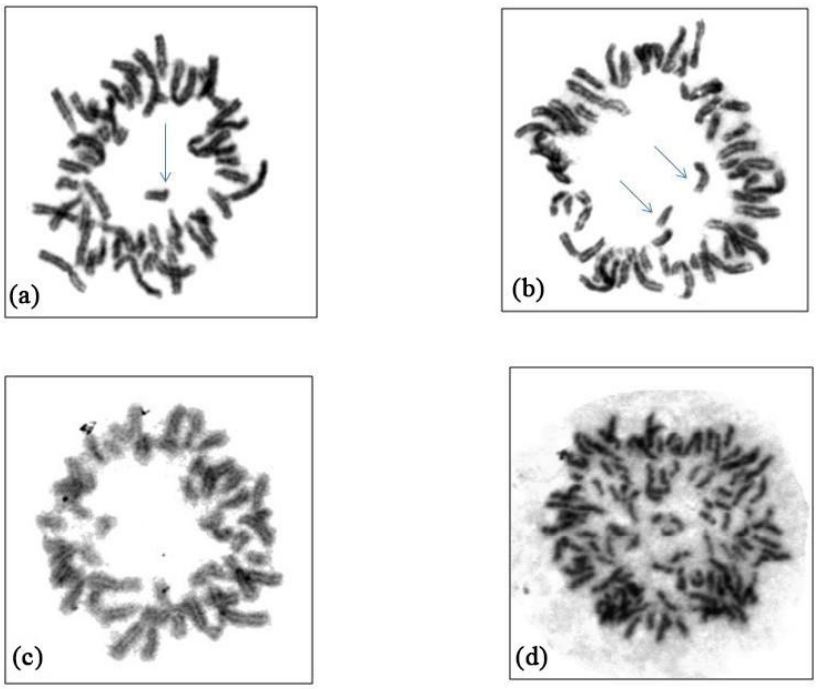

Figure 1. (a) & (b) Displaced chromosome in cattle metaphase (indicated by arrows), (b) Normal cattle metaphase (without displacement of chromosome) and (d) Premature Centromeric Division (PCD) in all the chromosomes of buffalo blood cells.

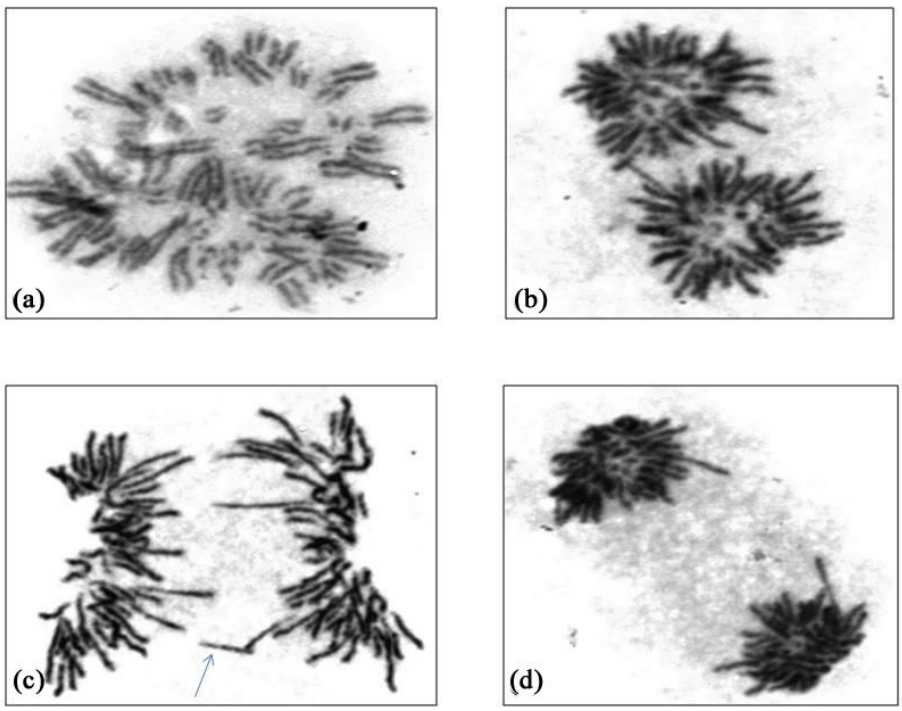

Figure 2. (a) Initiation of anaphase, (b) Progression of anaphase, (c) Lagged chromosome (indicated by the arrow) and (d) Latter stage of anaphase in buffalo blood cells.

More chromosome displacement and anaphase lagging may cause mosaicism and aneuploid cells (Coonen *et al.*, 2004). Therefore, the metaphase displacement and anaphase lagging studies may be used as an important tool for detecting numerical chromosomal abnormalities (Patel *et al*. 1997).

CONCLUSION

The chromosomal displacement during metaphase and chromosomal lagging during anaphase provide understanding of the probable cause of numerical chromosome aberrations occurred during cell division, which may be due to cell cycle errors or PCD. Hence, such techniques may be used as important tools for validating numerical chromosome anomalies in cattle and buffaloes.

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