The Comparison of Chromosome Analysis Result by Manual and Software Cytovision Image Analysis Using Simple G-Banding

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ABSTRACT

Chromosome analysis or karyotyping is one among powerful methods to characterize normal or abnormal genetic of animals. On the basis of the important chromosome abnormalities and their negative effect in the near future, chromosomal investigation of breeding bull especially for Artificial Insemination (AI) began in different countries. Chromosomal abnormalities are usually considered to be a plague and are to eliminate. In Indonesia, AI implementation in cattle have been started intensively, especially using imported bulls (bos Taurus) i.e. Limousine. A number of cattle breeds have been reported on of the 50 exotic breeds with the problem of 1/29 translocation, then the chromosome analysis to be important to execute. Method performed by collecting blood samples from first generation of crossing breed Madura Cattle vs. Bos Taurus. Sample was added to medium (Karyo MAX Gibco) then placed in CO_2 incubator at 38^o C. Colcichine was added aafter 70 hours and kept for 2-3 hours. Slides were prepared and dried then stained with Giemsa. Slides were examined under high phase-contrast microscope, chromosome analysis using cytovision software and manual analysis straightly captured under microscope then arranged. Result of both method of kariotyping may accepted for analysing method of abnormal/normal chromosome. It showed that the 2 N diploid number of chromosome was normal was 60, there were 58 autosome and 2 sex chromosome in all cattle observed. It was observed that all cattle tested in this research were normal categories. The karyotype analysis of all cattle showed that the chromosomes of one cell and different individual each breed varied in size, shape and position of centromere. It was recommended to performed chromosomal investigation of breeding bulls using advanced sophisticated tools of analysis like cytovision image analysis of fluorescent technique. Manual method was recommended only for analysis of normal or abnormal number of chromosome.

Keywords: bull; artificial insemination; cytogenetic; karyotype.

INTRODUCTION

The analysis of chromosome is one among powerful tolls or considered as an important method to characterize the genetic normality or abnormality of animals. Chromosomal abnormality could be identified and then it will be followed by selection and culled animals from population. Abnormal genetic of animals reflected on reduced fertility in both female and male carriers of this abnormality. It will be considered very important because of thousands of offspring that may derived from one bull per year with implementation of Košarčić et al. (2006) reported that numeric and structural changes on animal karyotype influenced on reproduction disturbance, phenotype expression and selection program. Different

aspects of reproductive disturbance were small litter, embryo mortality, frequent repeat breeding, abortion and mummified embryo, offspring with abnormalities' and also different kinds of sterility. Chamdi (2005) noted that on the basis of the important of chromosome abnormalities and their negative effect in the near future, chromosomal investigation of breeding bull and their progeny began in different countries.

In Indonesia, there are too many numbers of local cattle that need to be characterized for their genetic potential, especially for standard of karyotyping. Chromosomal abnormalities are usually considered to be a plague and are to eliminate. In Indonesia, where cattle Artificial Insemination (AI) implementation have started intensively, especially using imported bulls (*Bos taurus*) i.e. Limousine that have been reported on of the 50 exotic breeds with the problem of 1/29 translocation, then the chromosome analysis to be important to execute (Innayah, 2011). Therefore, chromosomal aberration can be identified, selected and culled from breeding program. It is important to think that cytogenetic control is an important selective measure choice of genetically health breeding bulls as a good guarantee for next generation of their genetic quality.

MATERIAL AND METHODS

Method performed by collecting blood samples from parents and first generation of crossing breed Madura Cattle vs. Bos Taurus. Each animals blood was collected in sterile heparinized tubes. The method for culturing blood cells (leucocytes) was used adopted from combination of several protocols (Miyake, 1996, Ahmad et al., 2004). Sample of 0.5 ml of blood was added to 5 ml medium (Karyo MAX Gibco), placed in incubator at 38℃. After 70 hours, add to 1 ml working solution of colchicines and kept for 2–3 hours, were centrifuge at 1,000 RPM for 10 minutes. Slides were prepared and dried then stained with with G banding technique (Giemsa 4% in phosphate buffer, pH=7.0) was carried out in order to identifiy the 30 pairs of cattle chromosome for normal metaphase scoring.

Slides were examined under high power phase-contrast microscope. The metaphase cells on the slides, selected for 10 spreading chromosomes each sample, were photographed and karyotyped (manually/ nondirect photography and using Inverted DIC camera with Genus CytoVision Image software ver. 4.5.1). Each chromosome was identified according to the International Standart Chromosome Analysis according both number and its banding pattern (Yamanaka, 1977) and analysis on numeric and structural changes was done according to International System for Cytogenetic Nomenclature of Domestic Animals (ISCNDA, 1989).

RESULTS AND DISCUSSION

Result analysis by both method of manual and cytovison images analysis showed that with G-banding may possible to analysis normal or abnormal chromosomes base on the number of chromosome of good quality of spreading out chromosome preparation. The number of cattle normal chromosome was 60 (2 N diploid), there were 58 autosomes and 2 sex chromosomes in all cattle observed. It was observed that all cattle tested in this research were normal categories. All the breed cattle observed (3 breeds, 150 Giemsa stained metaphases we examined had 58 chromosomes with a biarmed chromosome, in addition to the X and Y (Figure 1). G-banded provide a clearly chromosome band which represent in black (dark band) as G-positive and white (light band) regions as G-negative on chromosome.

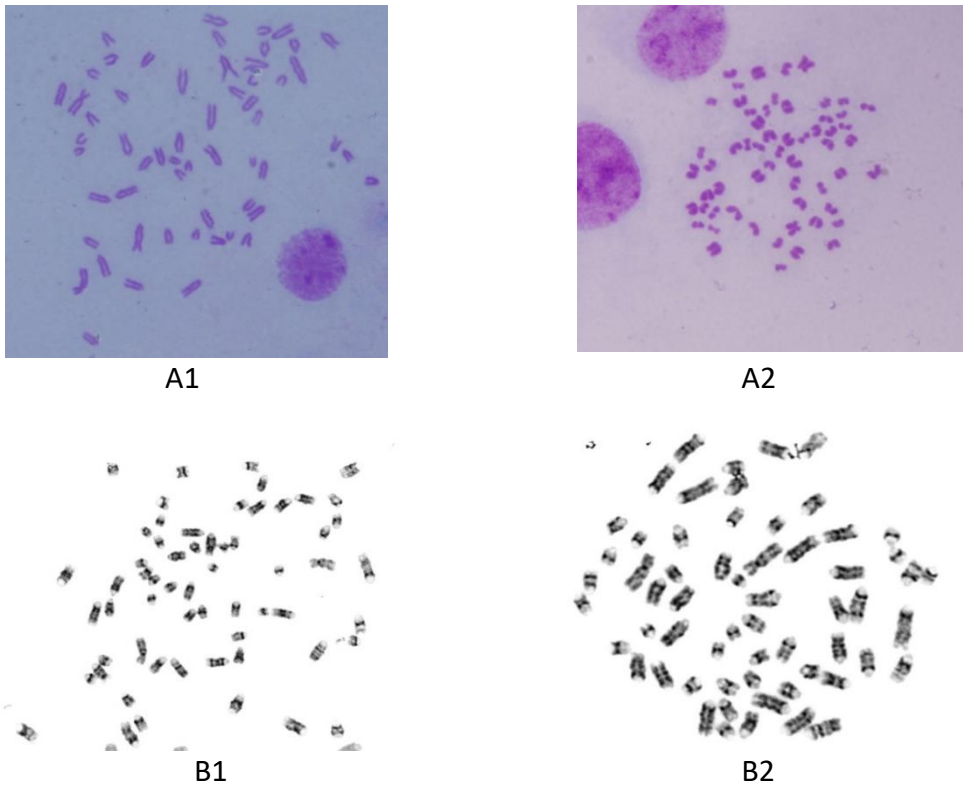

Figure 1. Cattle Chromosome Showed Spreading Variation Result. Each Spreading Chromosome Resulted in Different Size And Shape of Chromosomes. A: Manual Photography (1,000X) B: Captured using Inverted Camera With Cytovision Software (1,000X), 1: Male/Bull 2: Female/Cow.

The sex chromosome for male were XY, which the X-chromosome was the largest while the Y-chromosome was the smallest, both were categorized as submetacentric. The karyotype analysis of all cattle showed that the chromosomes of one cell and different individual each breed varied in size and shape. Such variation may be caused by some physical factors during cells preparation, fixation or the spreading of the chromosome on a slide.

This early study of G-banding preparation showed in some improper preparation, a clear banding pattern could not be obtained, because the bands were unclear or chromosome spread were too close each other. Using manual technique, also reduce the quality of band pattern, like poorly focused during take a shoots and sharp quality or pixel from manual camera is lower.

Technique of photography and combined with manual karyotype were also less supported this analysis. Yamanaka (1977) suggested that in order to be identified each chromosome for karyotype analysis in cattle, the number, intensity, width and disposition of each band, as well as size of chromosomes should be considered.

The early result study of normal-abnormal chromosome obtained are relatively similar to other researchers (Anis et al, 1990) that studied the numbers of cattle that reported that cattle had 60 chromosomes. All of the 30 pairs displayed poor characteristic banding pattern (Figure 2). However, with the help of cytovision software the autosome and sex chromosome could be identified on the basis of their numbers, size and banding patterns. This technique may be very valuable in cytogenetical analysis of chromosomal abnormality in cattle**.**

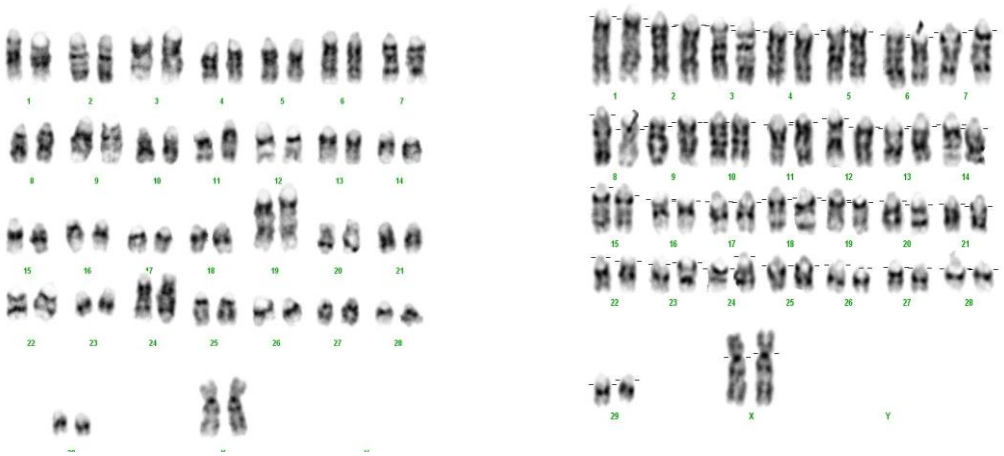

Figure 2. Cattle Chromosome showed a normal numbers of 30 pairs. xB, Kariotyping using software image of cytovision.

Ahmad et al, (2004) mentioned that screening of breeding bull of different breeds throught karyotyping is important, especially bulls mainteined at semen production unit or Artificial Insemination Center (AIC). Karyotyping is one amongst different culling parameters. Chromosomal screening is beneficial in the selection of superiors animals. Gustavsson (1979) and Schmutz et al (1997) described reduced fertility in female carriers of the 1:29 translocation. There are many types of chromosome abnormalities in domestic animals and these abnormalities are closely related to the reproductive disorders (Miyake, 1996, Gallagher,et al, 1999, De Luca et al, 2007, Munoz et al, 1994). An abnormality of chromosome could reflected on reduced fertility in both female and male carriers of this abnormality and chromosomal aberration can be identified and culled from breeding program.

Advanced research using karyotiping is determining the idiogram (Iannuzzi, et al., 1996). A direct comparison of manual and using Genus CytoVision Image software ver 4.5.1. As reported in the present study, Cytovision software obtained a better characterization of the banding pattern of chromosomes as well as to construct good idiograms. Actually, manual method was recommended only for analysis of normal or abnormal number of chromosome.

CONCULISON

It was concluded that both methods of karyotyping are considered as valuable protocol for genetic normal-abnormal base on the number of chromosomes. This study performed of chromosomal investigation of all cattle were normal, both structure and number. However, the genetic control and timely exclusion of chromosome abnormality is necessary. On state level, especially in Artificial Insemination Center, it is necessary to introduce this karyotyping on selection program, for chromosomal control of breeding bulls. It was recommended to performed better chromosomal investigation of breeding bulls using advanced sophisticated tools of analysis Genus CytoVision Image software analysis.

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