The Karyotiping of Indonesian Local Cattle and Buffalo for Genetic Quality Standarization by Detection of Chromosome Abberration

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ABSTRACT

Chromosomal analysis of breeding bull should be performed, because of the importantce of chromosome abnormalities and their negative effect on production and reproduction performance. Chromosomal abnormalities are usually considered to be a plague and are to eliminate. Chromosomal abberation of candidate bull can be identified and culled from breeding program. It is neccessary that bull for straw production for Artificial Insemination (AI) purpose must be screened out for any possible chromosomal abnormality. The cytogenetic control is an important selective measure choice of genetically health breeding bulls.The chromosome analysis to be important to execute. Method performed by collecting blood samples from 3 local cattle breedsand 3 swamp buffalo with diferent phenotypic character of skin colors. Sample of 0.5 ml of blood/animal was added to 5 ml chromosomal medium (Karyo Max, Gibco), placed in incubator at 38^{o}C. After 70 hours, culture tube were added to 1 ml colchicines kept for 2-3 hours, then were centrifuge at 1000 RPM for 10 minute and supernatant was discarded, pellet cells added then by fixative solution. Slides were prepared by stained with Giemsa for 10 minute, were examined under high power phase-contrast microscope. Chromosomal analysis was performed with help of cytovision software image analysis.Results showed that the 2 N diploid number of chromosome of all three local cattle breed was considered normal was 60, there were 58 autosome and 2 sex chromosome in all breeds cattle observed. Meanhile, buffalo observed for diploid number chromosome 2N= 48, considered as normal swamp buffalo. It was observed that both cattle and buffalo tested were normal categories. The karyotype of all animals showed that the chromosomes of one cell and different individual each breed varied in size, shape and position of centromere. However, it was recommended to performed chromosomal investigation of breeding bulls and others Indonesian local breed using advanced sophisticated tools of image analysis technique especially for semen production and AI application.

Key Words: Local cattle, Buffalo, Karyotiping, Chromosome, Abnormalities

INTRODUCTION

The analysis of chromosome is one among powerfull tools to characterize the genetic normality or abnormality of animals. Chromosomal analysis or karyotiping of breeding bull and their progeny should be done, because of the importantce of chromosome abnormalities and their negative effect on production and reproduction of animals. Chromosomal aberration of candidate bull can be identified and culled from breeding program (Achmad, et al., 2004). It is neccessary that bull for straw production and insemination must be screened out for any possible chromosomal abnormality.

Chromosomal abnormality could be identified and then following by selection and culled animals from population. Abnormal genetic of animals reflected on reduced fertility in both female and male carriers of this abnormality. Kosarcic et al (2006) reported that numeric and structural changes on animal karyotype influenced on reproduction disturbance, phenotypeexpression 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. 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 shoul be done in relation to genetic improvement and breeding of animals.

In Indonesia there are too many numbers of local ruminant that need to be characterized for their genetic potential, especially for standard karyotyping. In Indonesia, where 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. It is important to think that cytogenetic control is an important selective measure choice of genetically health breeding bulls.

MATERIALS AND METHODS

Method performed by collecting blood samples from both 3 local breed (Madura, Bali and Brahman, 3 bulls each) and 3 buffalo with diferent phenotipic character of skin color (grey, black and white/albino). Sample of 0.5 ml of blood/animal was added to 5 ml chromosomal medium (Karyo MAX ,Gibco), placed in incubator at 38 ^{o}C. After 70 hours, culture tube were added to 1 ml colchicines kept for 2 – 3 hours, then were centrifuge at 1000 RPM for 10 minute and supernatant was discarded, doing for 2 times respectively. The pellet cells added then by fixative solution. Slides were prepared by dropping the cell suspension on the glass slide and dried then stained with Giemsa stain. Slides were examined under high power phase-contrast microscope. Chromosomal analysis was performed with help of cytovision software image analysis.

Each animal blood was collected in sterile heparinized tubes. The method for culturing blood cells was used adopted from combination of several protocols (Miyake, 1985, Ahmad, 2004). Sample of 0.5 ml of blood was added to 5 ml medium (Karyo MAX .Gibco), placed in incubator at 38 ^{o}C. After 70 hours, add to 1 ml working solution of colchicines and kept for 2 – 3 hours, were centrifuge at 1000 RPM for 10 minute. Slides were prepared and dried then stained with with G banding was carried out in order to identifiy the 30 pairs of animals chromosome for normal metaphase scoring. The metaphase cells selected for 10 spreading chromosomes were photographed and karyotyped. Each chromosomes was identified according to the International Standart Chromosome Analysis according both number and its banding pattern.

RESULTS AND DISCUSSIONS

Results showed that the 2 N diploid number of chromosome of all 3 local cattle breedswereconsidered normal was 60, there were 58 autosome and 2 sex chromosome in all cattle observed. Meanwhile, buffalo observed for diploid normal number 2N= 48, considered as swamp buffalo. It was observed that all animals tested were normal. The karyotype analysis showed that the chromosomes of one cell and different individual each breed varied in size, shape and position of centromere.with a biarmed chromosome, in addition to the X and X or Y (Figure 1) . The sex chromosome for bulls 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 fixation or chromosomspreading.

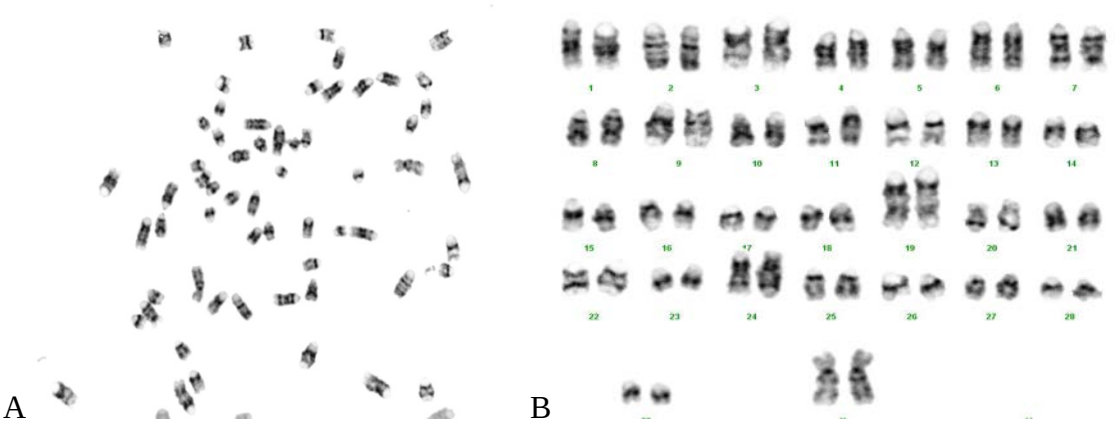

Figure 1. Cattle Chromosome showed a normal numbers of 30 pairs. A.Spreading chromosome resulted in different size and shape of chromosomes. (1000 X).B, Kariotyping using software image of cytovision

The results of chromosome study obtained are relatively similar to others researchers (Anis et al, 1990, Lio et al, 1995) that studied the numbers of breeds cattle that reported that cattle had 60 chromosomes. All of the 30 pairs displayed good qualities characteristic of G-banding pattern. (Figure 1). 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. So this technique may be very valuable in cytogenetical analysis of chromosomal abnormality.Cytovision software is very helpfull for having more acurate result.With cytovision, possible in metaphase counting/numbering and chromosome segmentation (split, overlap, and join) tools.

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. 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).

These early study of G banding preparation showed in some improper preparation, a clear banding pattern sometimes could not be obtained, because the bands were unclear or were too close each other. Technique of photography and manual karyotype were also less supported these analyses. (Yamanaka, 1977) suggested that in order to 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.

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CONCLUSION

These study performed of chromosomal analysis of all cattle were clasified as normal.It was recommended to performed chromosomal investigation of breeding bulls before using these bull for semen production for artificiell insemination. It was recommended to performed chromosomal investigation of breeding bulls and Indonesia local breed using for AI.

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REFERENCES

Ahmad,I, K. Javed and A. Sattar. 2004. Screening of breeding bull of different breeds through karyotyping. Pakistan Veterinaire Journal 24 (4), 2004: 190-192.

Annonymous, 2009. Cytovision, Case management, Bright field Capture and Analysis. Genetix. Guide Manual Book.

Anis,M., S. Ali , Z . Ahmad and M.A. Khan. 1990. Studies on the karyotipes of Sahiwal cattle. Pakistan Vet Journal 10 (2): 88 – 91.

De Luca,J.C., P. Sister, A. Prando, A. Baldo and G. Giovambattista. 2007. Absence of 1/29 translocation in aberdeen Angus Breed.J. analecta veterinaria 2007; 27 (2).

Gallagher, D. S., Jr.B.C. Lewis, M. De Donato, S.K. Davis, J.F. Taylor and J.F. Edwards. 1999. Autosomal Trisomy 20 (61,XX,+20)in malformed Bovive Fetus. Journal Vet. Pathol, 36: 448 – 451.

Gustavsson, Z. 1979. Distribution and effect of the 1/29 robertonian translocation in cattle. J. Dairy Science 62 (5); 825-835.

Kosarcik, S., M. Kovacevic, M. Jovicin, M Zivkoz Balos and D. Kosarcik. 2006. Changes in Karyotype in domestic animals discovered on the rarms in Vojvodina and their influence on reproduction. J. Genetika Vo. 39 No. 2, 121 – 128.

Lioi, M.B., M.R. Scarfi., D.Di Berardino and Di D. Berardino. 1995. An autosomal trisomi in cattle. Genetic Selection Evolution, 27: 437-476.

Miyake , Y.I. 1996. Study on Reproductive disorders related to chromosome abnormalilies in Domestic Animals. Journal Reproduction and Development Vo. 43 No. 6.: 111-118.

Schmutz S.M, JS. Moker V. Pavlyshyn, B. Haugen and EG. Clark. 1997. Fertility Effect of the 14; 20 Roberstonnian translocation in cattle. J. Theriogenology Vol 47 (4) : 815 – 823.

Yamanaka, H. 1977. The possibility of cattle chromosome classification, identified by G-Banding patterns. Jap Journal veterinaire Research 25: 52-58.