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Analysis of Chromosome and Karyotype in Bali Cattle and Simmental-bali (Simbal) Crossbreed Cattle

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Abstract: Chromosome analysis is very important part for the initial analysis of genetics. Some genetics abnormalities can be detected at the chromosome level and are usually associated with the inherited diseases. Accurate and prompt information need to be obtained for the purposes of the prevention of the genetics abnormalities genetics risk. This study was aimed to find out and to analyze the different size and morphological chromosome of Bali cattle and its crosses with Simmental cattle (Simbal cattle). Ten Simbal cattle (5 male and 5 female) and 5 female Bali cattle were used in this study. Five milliliter blood was collected using venous puncture through the jugular vein of each cattle. Chromosome was derived from white blood cells (lymphocyte) of peripheral blood. The Bali cattle and Simbal cattle have diploid chromosome (2n) of 60, with 29 pair of autosomes and one pair of sex chromosome. All autosomes are acrocentric with centromere index of 25.13 to 29.52% for Simbal cattle and 21.61 to 24.84% for Bali cattle. Sex chromosomes were sub-metacentric in Simbal cattle either male or female and metacentric in female Bali cattle. Average length of chromosomes of female Simbal, male Simbal and female Bali cattle were 0.29 ± 0.04 micron, 0.30 ± 0.05 micron and 0.24 ± 0.02 micron, respectively. Chromosome size of female Bali cattle was smaller than Simbal cattle.

Key words: Bali cattle, Simbal cattle, chromosome, karyotype, acrocentric, sub-metacentric and metacentric

INTRODUCTION

Crossbreeding between some breed of animal is expected to produce offspring which carry favourable traits but often was followed by disadvantages in genetics called genetic barrier. Problem was sometimes found in crossbreeding between Bali cattle (*Bos sondaicus*) and *Bos taurus* has a problem such as sterility in male crossbred cattle its female offspring there would be only mild fertility decrease (Harjosubroto, 1994).

Karyotype was defined as arrangement chromosomes according to its length and shape (Yatim, 1986). Karyotype plays, an important role in trait inheritance. Karyotype could be used to identify normality of anatomy, morphology or physiology. Karyotype analysis was aimed to identify chromosomal disorder related to genetic disorder (Khatun *et al.*, 2011), subsequently it is said that karyotype analysis was done to calculate quantity of chromosomes and to see structural changes in chromosome which indicate genetic change related to increase in illness risk.

A study on abnormality of chromosome could be used to answer reproduction disorder phenomenon

in crossbred cattle. Chromosome disorder could affect fertility either directly or indirectly in each hereditary factor influencing fertility by modifying animal ability to react toward various management or environmental factors. Although those were non hereditary factors but it is highly influential on fertility rate (Gary *et al.*, 1991).

Chromosomal disability either in quantity or autosome structure or sex chromosomes would caused the negative effect on fertility and development of animals (Khatun *et al.*, 2011), subsequently stated that infertility in male animal mostly would resulted from cytogenetics illness, particularly those that related with sex aneuploid chromosome. Structural and quantity changes in cattle's karyotype would affective reproduction disorder, phenotype expression and section program and genofond stability (Slavica *et al.*, 2006). In crossbreeding of different species (yak, bison and cattle) there is only the male offspring is infertile while the female is fertile (Stranzinger *et al.*, 2007).

The objective of this study was to study the arrangement, size and morphology of chromosome in Bali and Simmental-Bali crossbred cattle.

MATERIALS AND METHODS

Material: Material used in this Study were 10 Simbal cattle (5 male+5 female) and 5 female Bali cattle that were sampled for peripheral blood.

Chromosome Isolation: Chromosome isolation procedure consisted of isolation of lymphocyte, washing and preparing preparat. Lymphocyte culture procedures were based on method used by Hayes and Dutrillaux (2000) with several modifications. Three to five drop of buffy coat of blood centrifugation sample was put into cultural tubes containing 10 ml medium consisting of RPMI 1640 (Gibco) with L-glutamine phosphate buffer saline (Medicago), Foetal Bovine Serum (Gibco), penicillin-streptomycin (Gibco) and Con-a (Sigma), samples were incubated for 72 h at a temperature of 37°C with 5% carbon dioxide. Colchicine (Gibco) 15 $\mu\text{g mL}^{-1}$ was added ten minute before harvesting, put into pellet 3 mL KCl 0.075 M, added with 2 mL mixture of methanol and glacial acetic acid (3:1) as fixative during harvesting process. A smear was made in glass object, paint with 10% giemsa (with in Sorensen mixture, pH 6.8) for 10 min.

Karyotype manufacture: Smear containing chromosome was checked under three-dimension microscope and pictures were taken for at least five chromosomes in the best metaphase condition. Pictures obtained would be printed in 10 R size and the arranged based on Reading Conference (Ford *et al.*, 1980) and International System for Cytogenetics Nomenclature of Domestic Animal (Di Berardino *et al.*, 1990). The arrangement was carried by ordering the chromosome from the longest to the shortest and were given number accordingly from 1 to 29 for the autosomes and X and Y for sex chromosomes.

Variable: Variables observed were the length of chromosome, long arm and short arm of chromosome, centromere index and arm ratio of chromosome of crossbreed of Bali cattle and Simbal cattle. Variable measurement was based on work done by Suryo (2007).

Statistical analysis: Data were analyzed using Analysis of Variance (ANOVA) and further test using Least Significant Difference. The differences between groups were considered significant at $p < 0.05$.

RESULTS

Karyotype: It is the description of size and morphology of chromosome in metaphase. During metaphase chromosome was easy to identify (Suryo, 2007). Metaphase chromosome of Bali cattle and Simbal cattle was illustrated in Fig. 1-3.

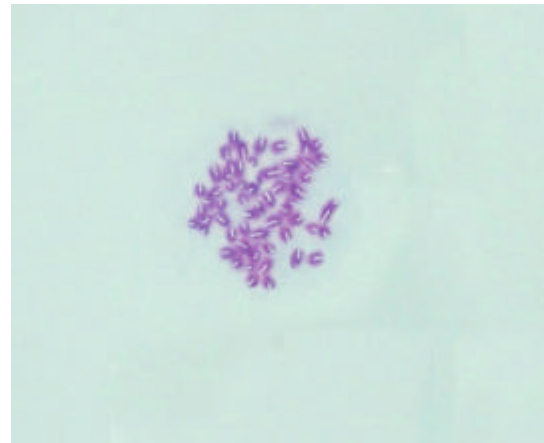


Fig. 1: Chromosome of female bali cattle

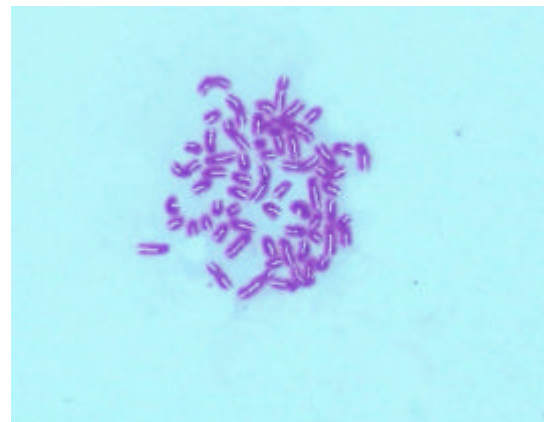


Fig. 2: Chromosome of male Simbal cattle

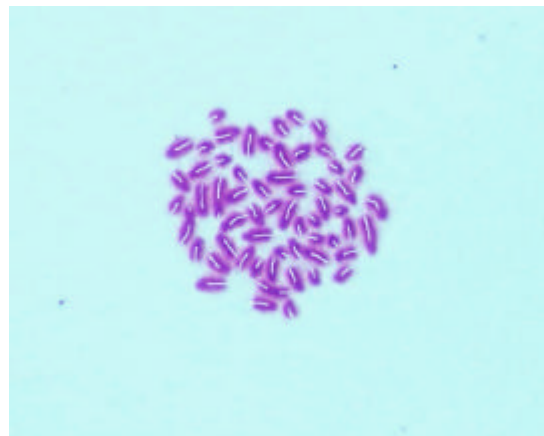


Fig. 3: Chromosome of female Simbal cattle

Karyotype description of Bali and Simbal cattle was shown in Fig. 4-6.

Karyotype result, showed chromosome of Bali cattle's and its crossbreed was paired or diploid with 60 in number ($2n = 60$) consisting of 58 autosomes and 2 sex chromosomes. Average value in differences of chromosomal size between female Bali cattle and its crossbreed can be seen in Table 1.

The result of chromosomal set analysis showed significantly difference, not all chromosomal pairs

showed significant difference because there was six pair of chromosomes was similar between female Bali cattle and female *Simbal* cattle which was pair chromosome of 1, 16,17, 18, 28 and 29. While for female Bali cattle with male *Simbal* cattle there were five similar pair of chromosome that was pair chromosome of 14, 15, 17, 18 and 29.

Based on the measurement of long arm and short arm of chromosome, it could be created an ideogram which is a comparison between long arm and centromere position. Ideogram of female Bali cattle and its crossbreed with male Simmental cattle could be viewed in Fig. 7-9.

Figure 7-9 shows, the longest chromosome lies in X chromosome either in Bali or Simbal cattle and also the length of chromosome in Bali cattle and its crossbreed were highly vary with enough gap between longest chromosome and the shortest one. In Bali cattle, the longest chromosome was $0.36 \pm 0.06 \mu$ and the shortest was $0.14 \pm 0.01 \mu$ while for Simbal cattle, the longest chromosome was $0.47 \pm 0.09 \mu$ and the shortest was $0.17 \pm 0.04 \mu$.

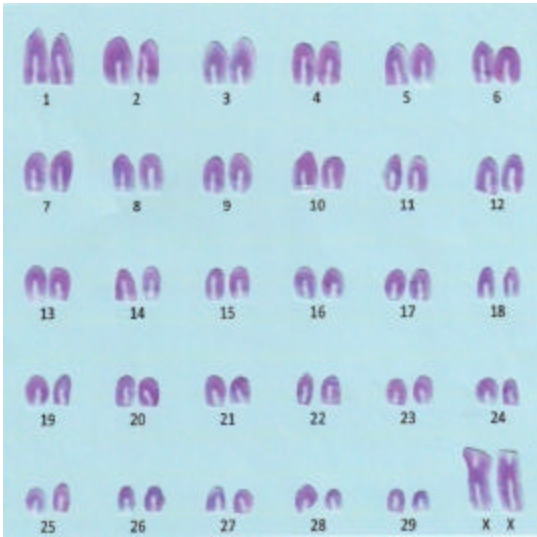


Fig. 4: Karyotype of female Bali cattle

Table 1: Average value of length, long arm, short arm and centromere index of chromosome in Simbal and Bali cattle

Parameter	Simbal female	Simbal male	Bali female
Chromosome length (μ)	0.29 ± 0.04^a	0.30 ± 0.06^a	0.24 ± 0.02^b
Long arm (μ)	0.21 ± 0.03^a	0.21 ± 0.04^a	0.18 ± 0.02^b
Short arm (μ)	0.08 ± 0.02^a	0.08 ± 0.03^a	0.06 ± 0.01^b
Arm ratio	0.40 ± 0.29^a	2.74 ± 0.56^b	3.27 ± 0.77^b
Centromere index	0.28 ± 0.02^a	0.28 ± 0.04^a	0.25 ± 0.04^b

Different superscript in the same row indicated significant different at $p < 0.05$

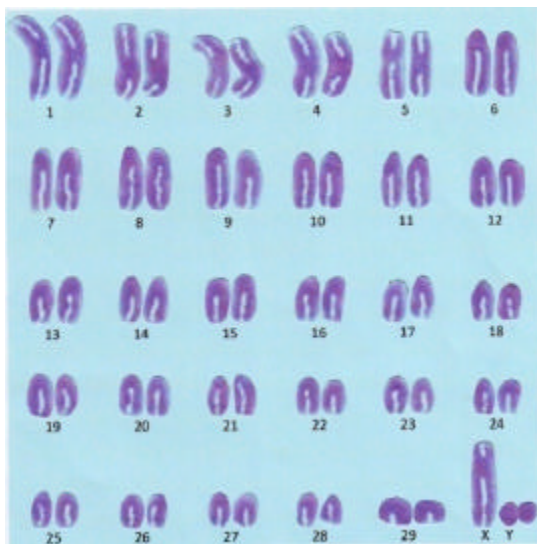


Fig. 5: Karyotype of male Simbal cattle

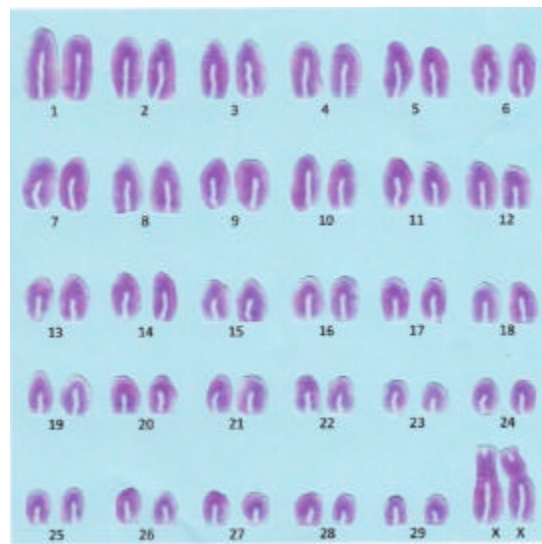


Fig. 6: Karyotype of female Simbal cattle

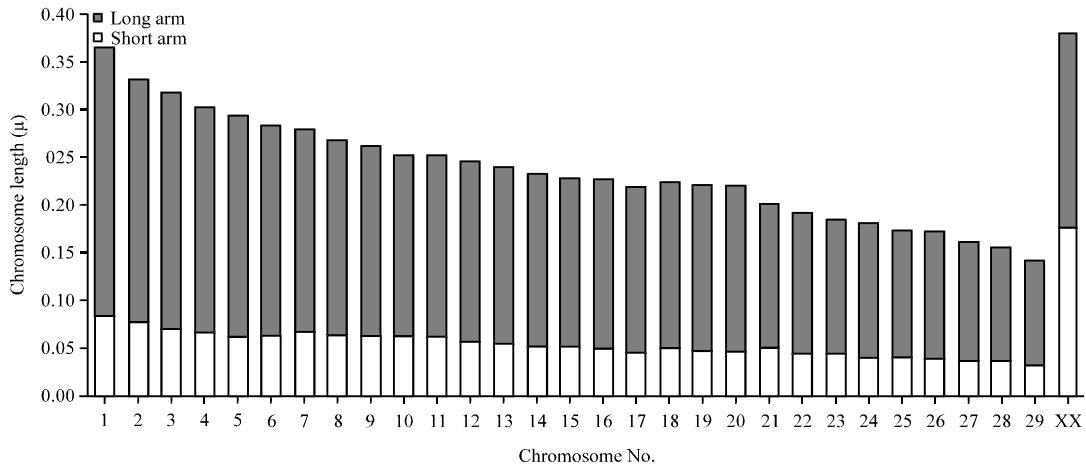


Fig. 7: Idiogram of chromosome morphology of female Bali cattle

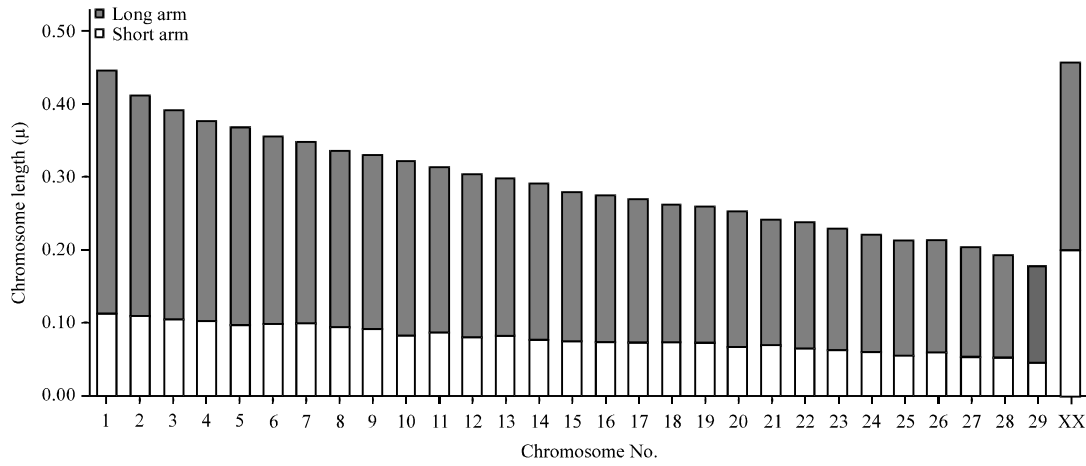


Fig. 8: Idiogram of chromosome morphology of female Simbal cattle

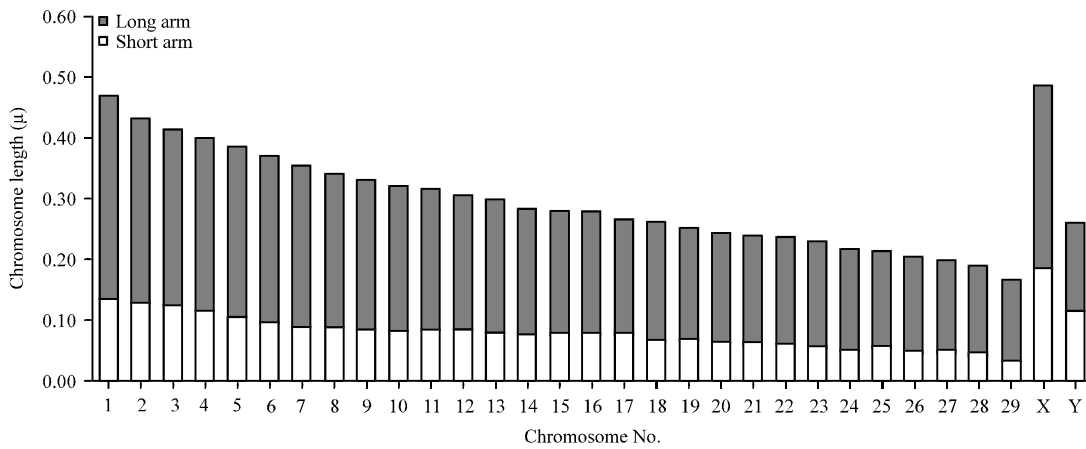


Fig. 9: Idiogram of chromosome morphology of male Simbal cattle

DISCUSSION

Bali and Simbal cattle's chromosome identified in this study was in pair or diploid and it was consisted of 29 pairs of autosome and one pair sex chromosome. Result of this study was in line to preliminary study that reported by Lightner (2008) which was karyotype of *Bos taurus*, *Bos indicus* and *Bos sondaicus* with 60 chromosome. Cattle has 60 chromosomes, 29 pairs of autosome and a pair of sex chromosome (Basrur *et al.*, 2001; Issa *et al.*, 2006; Abdullah *et al.*, 2009; Khatun *et al.*, 2011). Then this results were similar to the result of reported by Matsuda *et al.* (1980) that Bali cattle chromosome in numeric or structural manner was hard to distinguished to those of European breeds (*Bos taurus*), since 29 pairs of autosome were acrocentric, Y chromosome was smaller and sub-metacentric.

Chromosome morphology of female Bali and Simbal cattle showed that all autosome in female Bali cattle and male or female Simbal cattle was an acrocentric shape, while its sex chromosome shaped sub-metacentric in male and female Simbal cattle, while in female Bali cattle its sex chromosome has metacentric type. Eldridge (1985) stated that sex chromosome (X and Y) of *Bos taurus* was sub-metacentric shape and easy to identified relatively.

Chromosomal length of female Simbal cattle was higher in compared to female Bali cattle. Difference in chromosomal length was caused by different in genetic composition between Bali and Simbal cattle in which Simbal, consisted of 50% its Simmental and 50% coming from Bali cattle. In another words, genotype trait expressed the combination of both its parents. Bali cattle assumed originated from domestication of Banteng from Asia and categorized to sub genus Bibos is highly different with *Bos taurus* that come from Europe. Chromosome could lead changing order or its genetic ingredients, resulting phenotypic alteration, changes in set of genes and changes in ratio expected for its offspring (Suryo, 2007).

Size of chromosome resulted in this study was shorter than result of Stranzinger *et al.* (2007) who reported Simmental chromosome size about $0.39 \pm 0.05 \mu$ in pair chromosome 29. Although, each pair of homolog chromosome normally has same size and shape in certain phase of cell cycle, however, in pair chromosome of non-homolog and between chromosomes of different species it is normally found some differences (Suryo, 2007). The gap between chromosomes pairs in this study was not too salient and even some of chromosome pairs have a very small differences.

Average value and statistical analysis of centromere index could be viewed in Table 1. Centromere index

comparison between Bali cattle and its crossbreed showed a significant difference, female Simbal cattle has larger centromere index compared to female Bali cattle. Centromere index with highest value in chromosome of female Bali cattle was in chromosome pair 21 with the value of 25.81% while the smallest lies in chromosome pair 17 with the value of 21.61%. While for female Simbal cattle, the highest centromere index was 28.76% in chromosome pair 7 and the smallest centromere index was 25.30% in chromosome pair 29. Average of centromere index of male Simbal cattle was 27.77% with the highest value was 29.52% in chromosome pair 3 while the smallest index lies in chromosome pair 24 with the value of 24.09%.

Chromosome morphology of female Bali and Simbal cattle showed that all autosome in female Bali cattle and male or female Simbal cattle was an acrocentric shape, while its sex chromosome shaped sub-metacentric in male and female Simbal cattle, while in female Bali cattle its sex chromosome has metacentric type. This was similar to the result of Matsuda *et al.* (1980) who pointed out that Bali cattle chromosome and *Bos taurus*' autosome had an acrocentric shape and Y-chromosome in sub-metacentric. Eldridge (1985) stated that sex chromosome (X and Y) of *Bos taurus* was sub-metacentric shape and easy to identified relatively.

Comparison between chromosomal long arm and its short arm of chromosome was stated as chromosomal arm ratio. Largest arm ratio in female Bali cattle was obtained in chromosome 16 with the value of 3.80 while the smallest was 2.94 in chromosome 21. Meanwhile, female Simbal cattle has the largest chromosomal arm ratio in chromosome 27 with the value of 2.95 and the smallest value was 2.48 in chromosome 7. For male Simbal the largest chromosomal arm ratio was obtained in chromosome 24 with the value of 3.26 and the smallest value was 2.54 in chromosome 3. Statistical analysis result showed that chromosomal arm ratio of female Simbal cattle was lower than male Simbal or female Bali, chromosomal arm ratio of female Bali was not significantly difference to chromosomal arm ratio of male Simbal. This indicated that female Bali chromosomal length was longer than female Simbal for the same short arm size.

CONCLUSION

Female Bali cattle chromosome in morphology was similar to Simbal chromosome either male or female, it has 60 diploid chromosome ($2n = 60$) with 29 pair of autosomes shaped acrocentric and 1 pair of sexual chromosomes shaped sub-metacentric. Simbal chromosome has larger size than Bali chromosome and there was no other chromosomal disorder in Simbal cattle.

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