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Karyotype of Malayan Gaur (*Bos gaurus hubbacki*), Sahiwal-Friesian Cattle and Gaur x Cattle Hybrid Backcrosses

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Abstract: Interspecific hybridization has been reported for a wide variety of vertebrate species either spontaneous or by organized crossing of bovine species. The hybrids were often carrying intermediate characters genetically and phenotipically of the parents. Thus, status information of both aspects is valuable in animal production for selection and breeding management. The Gaur-cattle hybrids was reported to be superior in production value compared to their parent cattle but fertility status was still questionable. The project was abandoned due to their fertility issue and the hybrids were kept within the cattle in a dairy farm. Cytogenetic status and breeding record of the remaining herd were unavailable since then. The herd was then translocated to a deer farm (PTH Lenggong) and kept freely in the paddock. Recently, two female calves were born via *inter se* mating. Peripheral blood cultures of Malayan Gaur, Sahiwal-Friesian cattle and Gaur x cattle hybrid backcrosses were analyzed via Giemsa stained metaphase. The Gaur and cattle were having diploid chromosome number (2n) of 56 and 60, respectively. Interestingly, the backcrosses from the hybrids by cattle bulls were found to have two chromosome arrangements, which are 2n = 58 and 2n = 60.

Key words: Malayan Gaur, cattle, interspecific hybrids, selembu, karyotype

INTRODUCTION

Hybridization between species of animal is considered as important evolutionary process (Barton, 2001; Dowling and Secor, 1997) that may occur due to overlapping habitat or induce by human influence in captive breeding (Nijman *et al*., 2003). It is common that interspecific hybrids produced intentionally in breeding programs or sometime accidental mating of species sharing same area. Systematic program of breeding is often hoped to improve the desirable characteristics of original breeds via heterosis. Several hybrids have been reported for the Bovini such as Yakows; Yak x Taurine hybrids (Tumennasan *et al*., 1997), selembu; Gaur x domestic cattle hybrids (Bongso *et al*., 1988) and beefalo; bison x taurine catle hybrids (Basrur and Moon, 1967). Crossbreeding between taurine and zebu are very common to produce better breed for beef and dairy. The taurine were usually selected for their production performance but at the same time they are generally less compatible with the tropical atmosphere and diseases compared to the domestic species. The zebu has been found to improve tolerance of the taurine to hot environment and disease-resistant (Bradley *et al*., 1994) hence the hybrids embrace advantages of both decisive factors; performance and resistance. Although, interspecific hybrid offspring may combine desired properties of the parental species, but fertility is often restricted to homogametic sex (Forsdyke, 2000). Such phenomenon is a serious setback for development of hybrids but is able to be established by repeated back crossing until genetically balanced chromosome configuration is produced.

In early April of 1983, Malaysia saw the birth of the first interspecific hybrid from dairy heifers (Sahiwal-Friesian) resulting through chance mating with a wild Malayan Gaur bull (*Bos gaurus hubbacki*) that was stranded in a dairy farm of the Malaysian Veterinary Services at Kluang, Johor, Malaysia. The hybrid calves were then called SELEMBU. Nor Azman (1989) reported that the F1 hybrids show embodiment of vigor with huge body and sturdy limb and found to be superior in growth performance at all life stages compared to their cattle parent. The animal posses coat color of black with brown patches on the forehead and along the midline. The hybrids were kept with dairy cattle group in the same farm. Chromosome analysis of these calves confirmed that they were hybrids by having a diploid number (2n = 58), an intermediate of the parents (Bongso *et al*., 1988). Bongso *et al*. (1988) demonstrated that the karyotype of

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the hybrid consisted 28 pairs of autosomes and a pair of sex chromosome. The X chromosomes were smaller compared to the submetacentric autosomes and Y chromosome was the smallest. Best to our knowledge, no further karyotype and breeding report of these animals was available since then. The project was abandoned due to their fertility issue and the hybrids were kept within the cattle in the dairy farm. The herd was then translocated to a deer farm (PTH Lenggong) and recently, two female calves were born via inter se mating. Thus, earlier assumptions that the animals were sub fertile are now invalid. It may perhaps that these remaining descendants of the Gaur x cattle hybrid were produced through back crossing sired by the cattle. The objective of the present study was to reports the karyotype of the Malayan Gaur, Sahiwal-Friesian cattle and their hybrid backcrosses which will be beneficial for assessment and selection in further breeding program.

MATERIALS AND METHODS

Two Malayan Gaur, two Sahiwal-Friesian cattle and six Gaur x cattle backcrosses were sampled for peripheral blood. The animals were kept at three different locations, which are Animal Production Centre (PTH Kluang) for the cattle, Animal Production Centre (PTH Lenggong) for the selembu and Gaur Conservation Centre Jenderak for the Gaur. Procedure of short-term lymphocyte culture is based on method by Hayes and Dutrillaux (2000) with minor modifications. Three to five drops of buffy coat from centrifuged blood sample was added to a 50 mL plastic culture flask containing 10 mL medium. The medium was prepared containing 9 mL RPMI 1640 (Gibco®), 1 mL heat inactivated Foetal Calf Serum (Gibco®), 0.1 mL penicillin-streptomycin (Gibco®) and 0.1 mL pokeweed (Gibco®) as mitogen. The flasks were capped tightly and incubated at 37.5°C for 72 h under 5% of carbon dioxide environment and shaken gently twice daily. Colcemid (0.1 mL) was added one hour before harvesting and hypotonic solution (0.075 M KCl) was added to swollen the cells. Fresh cold methanol/glacial acetic acid (3:1) was used as fixative during harvesting process and slide preparation. The slide was conventionally stained with 5% stock Giemsa's solution for 5 min. Thirty good metaphase spreads were analyzed and counted using a photomicroscope. Karyotype was constructed using Video Test Karyo 2.1 software based on chromosome morphology and size with the autosomes was arranged in pair from the largest to smallest.

RESULTS AND DISCUSSION

Thirty conventionally stained good metaphase spread were examined for each animal. The chromosome complement (2n), Fundamental Number (N.F.) and morphology of the autosomes and the sex chromosome of the animals are summarized in Table 1 and representative karyotype in Fig. 1a-d. The Malayan Gaur (*Bos gaurus hubbacki*) is having 2n = 56 comprises of 2 pairs of submetacentric, 25 pairs of acrocentric autosomes and 2 sex chromosomes. The cattle karyotype (2n = 60) made up of 58 acrocentric autosomes and 2 sex chromosomes which described as the primitive karyotype for the bovid species (Buckland and Evans, 1978; Wurster and Benirschke, 1968). Diploid chromosome number (2n) for the backcross hybrid is 60 except for a female, with 2n = 58. Backcrosses with 60 chromosomes hold the chromosome arrangement that is equivalent to the cattle where autosomes are acrocentric and X chromosomes are large submetacentric. The female backcross hybrid with 2n = 58 hold similar chromosome configuration that is intermediate of the parent, comparable to as reported by Bongso *et al*. (1988). It consists of 2 non-homologous submetacentric, 54 acrocentric autosomes and a pair of X chromosome.

Generally, the backcross hybrids resemble their cattle parent phenotype and the coat colors were varies from light to dark brown. The animals were observed to inherit characteristic of the Gaur such as dark brown dorsal midline and they are alert and sensitive to disturbance in the surrounding (Fig. 2a, b). The male backcross possesses an acrocentric Y chromosome; which is one of a character for the *Bos indicus* (Gallagher and Womack, 1992). However, the breeding records were unavailable since the project was abandoned and remaining selembu was kept with the group of Sahiwal-Friesian cattle since then. We assume that the remaining selembu were backcrosses to cattle based on the following: (1) the male

Table 1: Chromosome classification of gaur, cattle and the hybrid

				Autosome		Sex chromosome	
Animals	♂:♀	2n	N. F.	SM	A	X	Y
Malayan gaur	1:01	56	62	4	50	SM	SM
Sahiwal-Friesian cattle	1:01	60	62/61	-	58	SM	A
Backcross selembu (F1 Hybrid type)	0:01	58	62	2*	54	SM	-
Backcross selembu (Cattle type)	1:04	60	62/61	-	58	SM	A

♂: Male, ♀: Female, 2n: chromosome compliment, NF: Fundamental No., A: Acrocentric, SM: Submetacentric, *: Non homologous

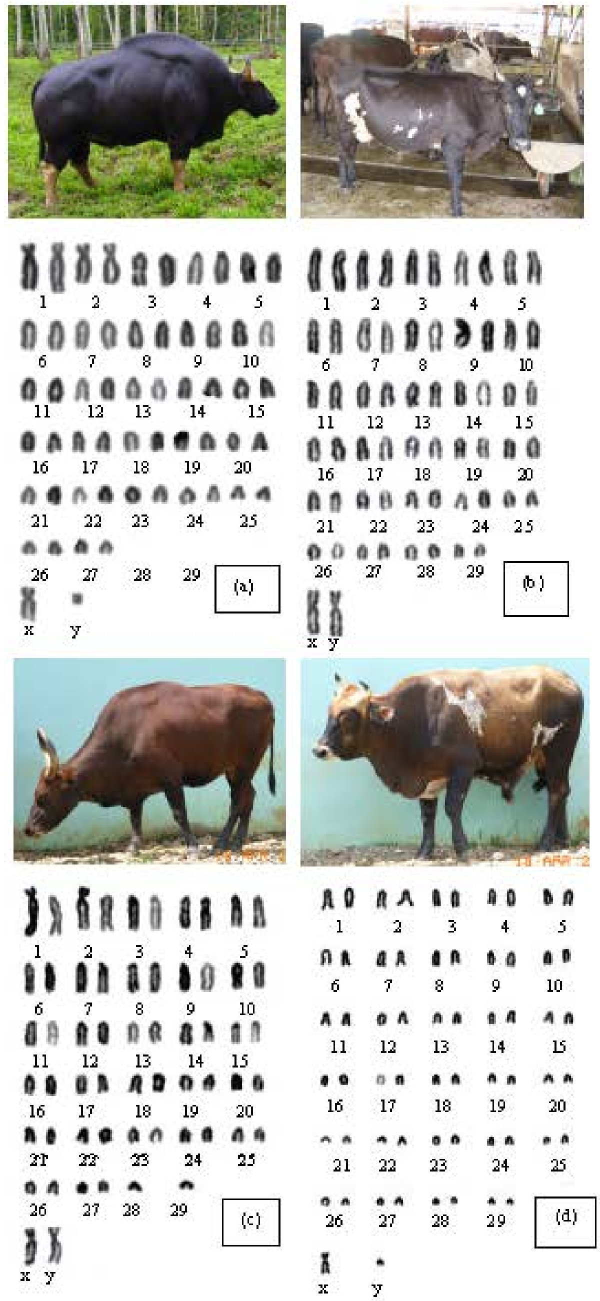

Fig. 1: Picture and karyotype of (a) Malayan Gaur, (b) Sahiwal-Friesian cattle, (c) and (d) Gaur x cattle hybrids backcrosses

F1 hybrids were sub fertile (Nor Azman, 1989); (2) no available Gaur bull in the herd; and (3) Y chromosome of male backcrosses were acrocentric; which is similar to the cattle but submetacentric in F1 hybrids (Bongso *et al*., 1988) and Gaur. The acrocentric Y chromosome has resulted that the fundamental number of male cattle and backcross selembu decreased to 61. Animal with submetacentric Y chromosome as seen in the Malayan Gaur and taurine cattle (Gallagher and Womack, 1992) were having 62 chromosome arm.

The submetacentric autosomes were differ in size significantly and were inherited from the Malayan Gaur of chromosome 1 and 2, pairing with an acrocentric chromosome 1 and 2 of the cattle. Chromosome 28 and 29 were unpaired, which inherited from the cattle while the Gaur lack of both. Chromosomes 3 to 27 were homologous

Fig. 2: (a) Dark brown of dorsal midline and (b) Tempered backcross bull towards disturbance

diploid acrocentric while the X chromosomes were submetacentric with comparable size of each other and significantly smaller than the submetacentric autosomes. Similar karyotype arrangement was reported for Burmese and Thai Gaur (*Bos gaurus readei*) (Vadhanakul *et al*., 2004) while Indian Gaur (*Bos gaurus gaurus*) comprised of 2n = 58 (Gallagher and Womack, 1992; Wurster and Benirschke, 1968).

Extensive similarities of chromosome 2 in the Indian Gaur with chromosome 2 and 28 of the cattle reported by Gallagher and Womack (1992) and in agreement with important role of centric fusion during evolution in Bovid (Buckland and Evans, 1978; Wurster and Benirschke, 1968). The Malayan Gaur is lack of chromosome 28 and 29 with 2 pairs of biarmed chromosome 1 and 2 compared to the cattle karyotype. Centric fusion between chromosomes 1 and 29; 2 and 28 of the ancestral cattle karyotype are believed to give rise to the Gaur karyotype and remain similar genetic material and the fundamental number. This evolutionary fixed centric fusion may provide reasonable ability of this two different species to produce interspecific offspring naturally.

The Gaur x cattle hybrid demonstrated intermediate karyotype consists of a haploid set of each parents (Bongso *et al*., 1988). During meiosis, the submetacentric autosome within each set is likely to pair with either one of the members within the set. The possible meiotic products are those shown in Fig. 3, which include a large proportion of genetically unbalanced gametes. These gametes would not survive the gametic selection, thus accounts for the very low sperm counts (Nor Azman, 1989) and therefore reduced fertility. A similar explanation has been provided for the reduced fertility observed in 1/29 translocation in cattle (Gustavasson, 1979) and in buffalo interspecific hybrid (Hilmi, 1984). It is conceivable that only those genetically balanced gamete carrying 1/29 and 2/28 or 1, 2, 28 and 29 or 1, 2/28 and 29 or 2, 1/29 and 28 will survive the gametic selection. This suggests that four type of gametes carrying n = 28, n = 29 (two type of combination) and n = 30 are being produced by the F1. The result of fertilization of these gametes would produce offspring with four of different karyotype via back crossing the F1 with one of the parent species. Theoretically, expected karyotypes of backcrosses are:

Backcross with the gaur:

- 2n = 56; with pair of rob(1;29) and rob(2;28)
- 2n = 57; with unpaired of rob(2:28), 2 and 28; pair of rob(1;29) and missing 29
- 2n = 57; with unpaired of rob(1;29), 1 and 29; pair of rob(2:28) and missing 28
- 2n = 58; consisting unpaired of rob(1;29), rob(2;28), 1, 2, 28 and 29

Backcross with the cattle:

- 2n = 58; with unpaired of rob(1;29), rob(2;28), 1, 2, 28 and 29
- 2n = 59; with unpaired of rob(1;29), 1 and 29
- 2n = 59; with unpaired of rob(2;28), 2 and 28
- 2n = 60; similar to the cattle karyotype

Currently, five backcrosses including the calves holding 2n = 60 and only one female with 2n = 58. Based on the principle of segregation and independent assortment, the findings are deviating of expected karyotype ratio 1:1:1:1. This may perhaps be due to: (1) a small population of back crossed examined or (2) affinity of n = 30 gametes in the F1 are being favoured against n = 58 and n = 59. However, the backcrosses with 2n = 60 may perhaps have reached balance and stable gametic material that improved their fertility.

Hybridization of these cattle and Gaur of which are with different chromosome constitution may due to

1, 29 1/29 2, 28 2/28	0	1	29	1/29	1 29	1 1/29	1/29 29	1 1/29 29
0	**26** 0	**27** 1	**27** 29	**27** 1/29	**28** 1 29	**28** 1 1/29	**28** 1/29 29	**29** 1 1/29 29
2	**27** 2	**28** 1 2	**28** 2 29	**28** 2 1/29	**29** 1 2 29	**29** 1 2 1/29	**29** 2 1/29 29	**30** 1 2 1/29 29
28	**27** 28	**28** 1 28	**28** 29 28	**28** 1/29 28	**29** 1 29 28	**29** 1 1/29 28	**29** 1/29 28 29	**30** 1 1/29 28 29
2/28	**27** 2/28	**28** 1 2/28	**28** 2/28 29	**28** 1/29 2/28	**29** 1 2/28 29	**29** 1 1/29 2/28	**29** 1/29 2/28 29	**30** 1 1/29 2/28 29
2 28	**28** 2 28	**29** 1 2 28	**29** 2 28 29	**29** 2 1/29 28	**30** 1 2 28 29	**30** 1 2 1/29 28	**30** 2 1/29 28 29	**31** 1 2 1/29 28 29
2 2/28	**28** 2 2/28	**29** 1 2 2/28	**29** 2 2/28 29	**29** 2 1/29 2/28	**29** 1 2/28 29	**30** 1 2 1/29 1/28	**30** 2 1/29 1/28 29	**31** 1 2 1/29 2/28 29
2/28 28	**28** 2/28 28	**29** 1 2/28 28	**29** 2/28 28 29	**29** 1/29 2/28 28	**30** 1 2/28 28 29	**30** 1 1/29 2/28 28	**30** 1/29 2/28 28 29	**31** 1 1/29 2/28 28 29
2 2/28 28	**29** 2 2/28 28	**30** 1 2 2/28 28	**30** 2 2/28 28 29	**30** 2 1/29 2/28 28	**31** 1 2 2/28 28 29	**31** 1 2 1/29 2/28 28	**31** 2 1/29 2/28 28 29	**32** 1 2 1/29 2/28 28 29

Fig. 3: Possible number of meiotic elements and chromosome rearrangement in a nucleus of F1hybrid gamete after chromosome segregation during the meiotic division. (1) Cells enclosed by thick border contain a balanced configuration. (2) Bold number in a cell indicate number of meiotic elements and rest indicate chromosome number (3) 1st row shows the possible segregation of the trivalent element consisting of chromosomes 1, 1/29 and 29 (4) 1st column shows the possible segregation of the trivalent element consisting of chromosomes 2 , 2/28 and 28

genetic similarities as both species was descended from same ancestor. This study is still in progress to show that the chromosome of the Gaur, Sahiwal-Friesian cattle and their backcross hybrids are sharing the similar genetic material via chromosome banding. If the hypothesis is correct, the chromosome of these species should allocate similarities of the banding characteristic of homologous chromosome.

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