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RESEARCH NOTE

Ascertaining the paternal lineage in crossbred calves

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Abstract. Karyotyping is one among the culling parameter used for taking up culling decisions. Cytogenetic screening of breeding bulls has been recommended to screen for chromosomal abnormalities before semen production in artificial insemination. The chromosomal analysis of a Holstein Friesian crossbred bull revealed the presence of acrocentric Y-chromosome, which was further confirmed by CBG-banding. The shape of the Y-chromosome determining that male line used for crossbreeding was from indigenous origin. Karyotyping is a best and reliable technique for the identification of crossbred calves born to the indigenous bulls.

Keywords. paternal lineage; Holstein Friesian crossbred bull; acrocentric Y-chromosome.

Introduction

The process of evolution and domestication has led to mutations and rearrangements in chromosomes, and played an important role in speciation. Despite the common ancestor, the divergence of *Bos taurus* and *B. indicus* had occurred between 10,700 to 7000 BC long before domestication but the chromosomal number is same in both the subspecies ($2n = 60$). The sex chromosome polymorphism is well known in mammals (Stranzinger *et al*. 2007). The chromosomal morphology of autosomes and X-chromosome would be similar, whereas Y-chromosome exhibits differences in morphology (Parada *et al*. 2018). In *B. indicus*, Y-chromosome is acrocentric, while in *B. taurus* it is metacentric. The differences in morphology of the Y chromosome in both the subspecies is explained as pericentric inversion or transposition of centromere (Iannuzzi *et al*. 2001). The structural differences in the morphology of the Y chromosome helps in the determination of the male lines used in the crossbreeding programmes (Cauveri and Sivaselvam 2015; Suresh *et al*. 2015).

In India, most of the genetic improvement programmes in cattle are aimed at increasing the milk production by crossing indigenous cows with exotic bulls. The calves / young bulls induced for semen production should be screened cytogenetically for identification of chromosomal abnormalities, as these anomalies, if present, would be transmitted to the next generation. With this backdrop, the present study was carried out to identify the animals with abnormal chromosomal complement and also to determine the paternal origin, as exotic bull inheritance is important for high genetic merit.

Materials and methods

Blood samples were collected from the 20 crossbred Holstein Friesian (HF) and five crossbred Jersey bull calves which were produced under field progeny testing programme, for cytogenetic screening. The samples were collected aseptically in heparinized tubes and transported to laboratory. Chromosome preparations were made by using short-term lymphocyte culture technique standardized in the cytogenetic laboratory of Department of Animal Genetics and Breeding, MVC with slight modifications of (Moorehead *et al*. 1960).

CBG-banding was performed as per the technique given by Sumner *et al*. (1972) with some modifications. Freshly prepared slides were aged for 7–10 days at 37°C. Aged slides were incubated in 0.2 N HCl for 1 h at room

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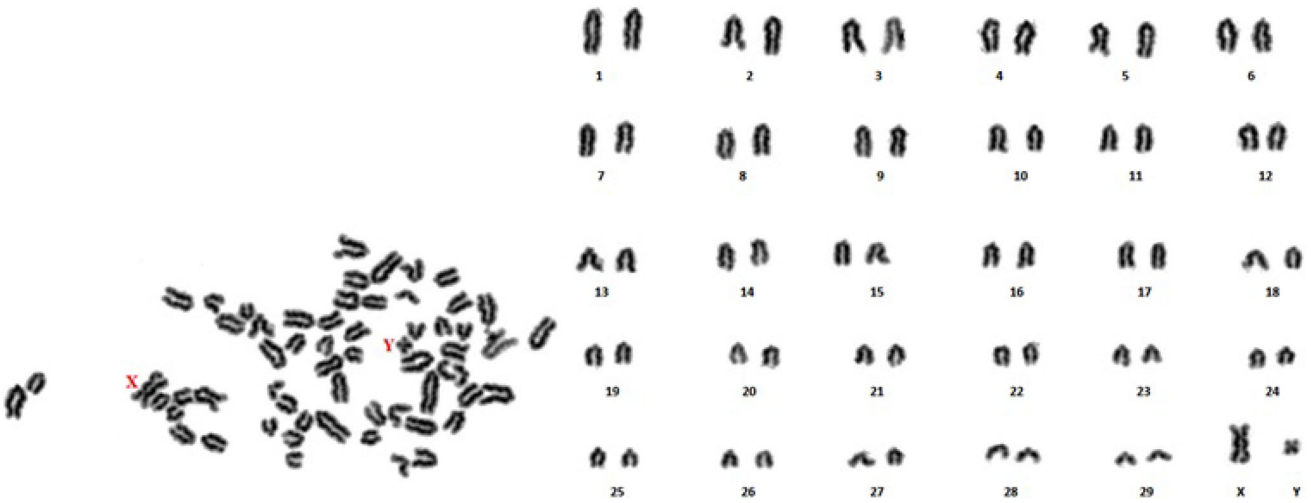

Figure 1. Metaphase spread and karyotype of normal crossbred bull (60, XY).

temperature and then treated with 5% Barium hydroxide for 2–3 min at 52°C. Later, the slides were incubated in 2× SSC buffer for 1 h at 60°C and stained with 4% Geimsa for 90 min. Further the slides were examined under Olympus microscope (USA) and good metaphase spreads were photographed using applied spectral imaging software.

Results

The diploid chromosomal number in all animals screened cytogenetically was found to be 60, XY, indicating the male sex of the animals. All the autosomes were acrocentric, X-chromosome was the largest sub-metacentric and Y-chromosome was the smallest metacentric in all the bulls except in one HF crossbred bull, which possessed acrocentric Y-chromosome. Metaphase spreads with respective karyotypes of crossbred bulls of exotic and HF bull with acrocentric Y-chromosome are shown in figures 1 & 2.

The presence of acrocentric Y-chromosome in HF crossbred bull was further confirmed by CBG-banding. CBG-banded chromosomes revealed all autosomes exhibiting a distinct centromeric heterochromatin region, i.e. a positive CBG-band whereas X-chromosome was CBG-band negative and Y-chromosome was darkly stained along the entire length. In HF crossbred bull, the C-banded metaphase spread revealed presence of acrocentric Y-chromosome (figure 3).

Discussion

Screening of breeding bulls through karyotyping is of primary importance, as the phenotypic selection would not guarantee the normal chromosomal complement. Besides, helping in the selection of superior and genetically healthy

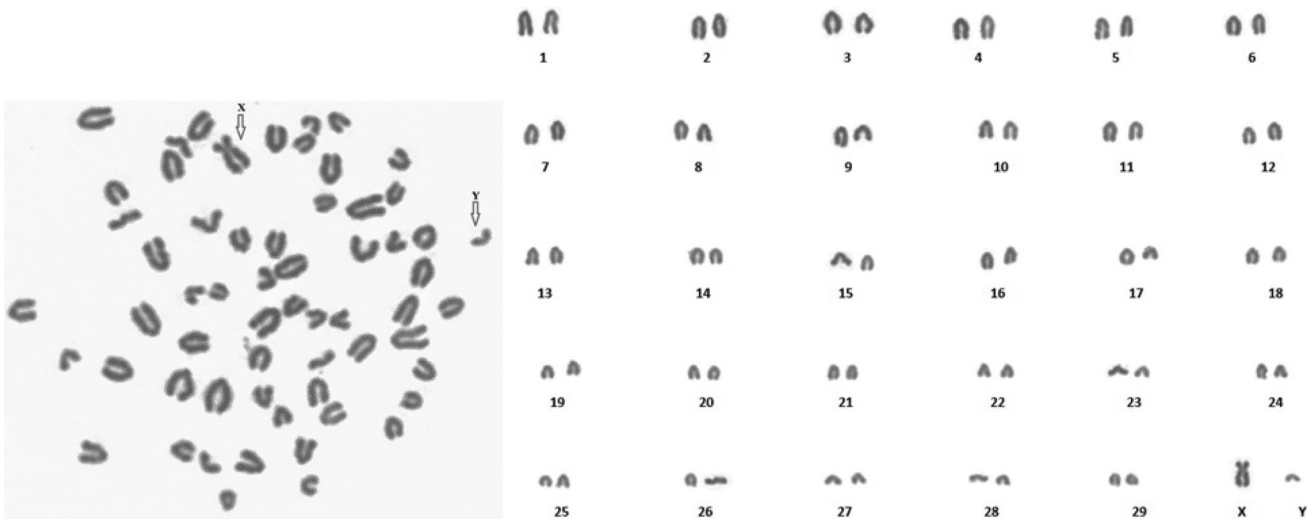

Figure 2. Metaphase spread and karyotype of HF crossbred bull with acrocentric Y-chromosome.

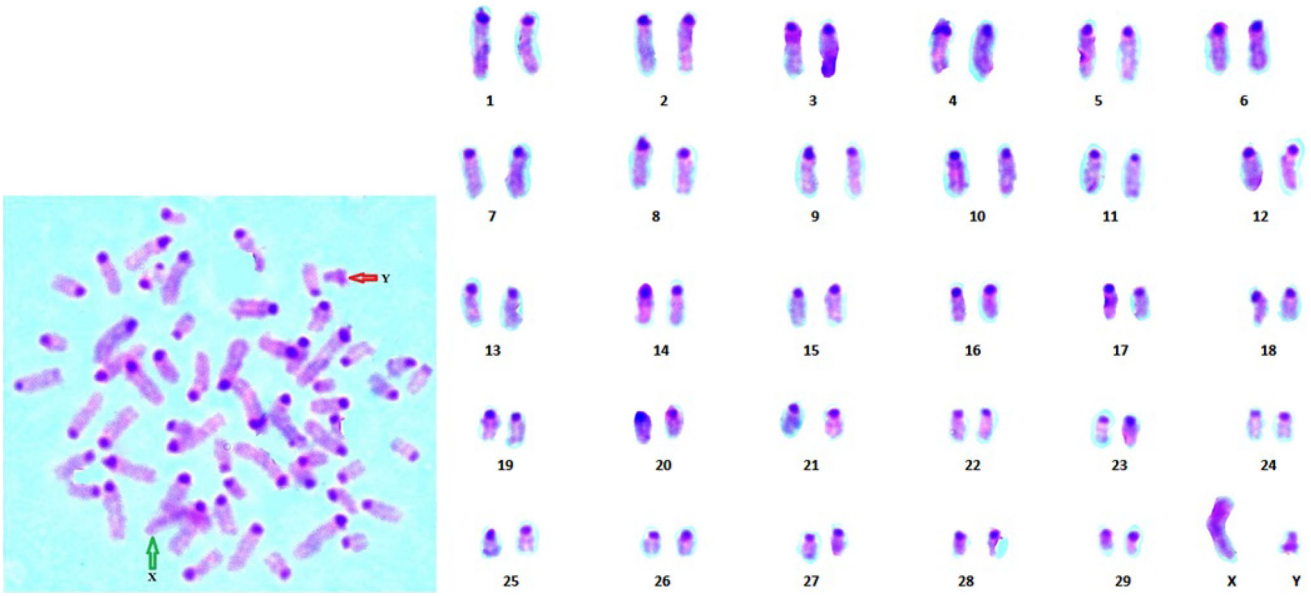

Figure 3. CBG-banded metaphase spread and karyotype of HF bull.

animals (Ciptadi *et al*. 2014), karyotyping enables the identification of paternal origin of Y-chromosome in the crossbred.

In *B. taurus* and *B. indicus*, the differences in the karyotype lies in the morphology of Y-chromosome (Parada *et al*. 2018), i.e. meta/sub-metacentric and acrocentric, respectively. In the present study, one HF crossbred bull was having acrocentric instead of meta/sub-metacentric Y-chromosome, which is indicative of that, the inheritance of Y-chromosome from indigenous native cattle, which is unacceptable from the breeders point of view. Because, in bovines crossbreeding programme, the sire line should be maintained with the exotic cattle, either Jersey or HF. In this case, mismating of crossbred cow would have occurred with the bull of indigenous origin (*B. indicus*) when the animal was sent for grazing and subsequent fertilization of ovum resulted with indigenous bull spermatozoa (Cauveri and Sivaselvam 2015). Similar finding was also reported earlier by Cauveri and Sivaselvam (2015) in a Jersey crossbred bull in Tamil Nadu.

Karyotyping is one among the parameters used for taking up culling decision. The crossbred bull calves used for semen production either in frozen semen banks or in original breeding farms should be of high genetic merit and must have exotic sire line. Cytogenetic analysis helps to identify such crossbred calves of undesirable parentage and these calves should be culled at an early stage of selection which could save time and amount spent towards genetic improvement programmes.

In conculsion, the cyto screening study is able to identify the gross chromosomal variations among the indigenous, exotic and crossbred cattle, helpful in taking up early decision on culling. This analysis supports the recommendations laid down in the minimum standard protocol of Government of India, in the production of frozen semen.

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