International Journal of Veterinary Science

www.ijvets.com P-ISSN: 2304-3075 E-ISSN: 2305-4360 editor@ijvets.com

RESEARCH ARTICLE

Autosomal Mosaicism in Leukocytes of a Gaolao (*Bos indicus*) breed of cattle

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ARTICLE INFO

Received: January 04, 2014
Revised: January 07, 2014
Accepted: January 09, 2014

Key words:
Abnormality
Autosomal mosaicism
Chromosome
Gaoloa
Infertility

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ABSTRACT

Blood samples were obtained from phenotypically normal Gaolao cattle breeds mainly young bulls. Out of 70 animals, one young bull aged one year exhibited inconsistent chromosomal number during routine cytogenetic investigation. The whole blood culture was set up in RPMI-1640 (HiMedia) medium for 72 hrs, harvested to prepare chromosomal slides for conventional G- banding using trypsin and Giemsa (GTG). A total 105 metaphases plates were screened under microscope and we observed inconsistent number of chromosome in 28 cells (26.6%) exhibiting 61,XY/59,XY/58,XY/57,XY. Mosaicism is usually associated with reduced fertility in male and infertility in females. The bull calf in present study was one year old; therefore its fertility could not be estimated. However, it is advisable that bulls having mosaicism should be checked for the fertility before being used for artificial insemination and breeding programmes.

Cite This Article as: Kotikalapudi R, RK Patel, NN Sugali and H Medidi, 2014. Autosomal mosaicism in leukocytes of a Gaolao (*Bos indicus*) breed of cattle. Inter J Vet Sci, 3(1): 4-6. www.ijvets.com

INTRODUCTION

The Gaolao cattle are shorthorned, white or light-gray in colour, medium height, or rather light build and tend to be narrow and long. The breed is found mainly in the districts of Wardha, Nagpur (Maharashtra) and Chindwara (Madhya Pradesh) in India. There is a close similarity between the Ongole and the Gaolao breeds. The Gaolao cattle are dual purpose as the Marathas developed this breed for quick army transport in the hilly areas of Gondwana in Madhya Pradesh. Historical records show that the breed had fair milk-producing capacity (Joshi and Phillips, 1953). Zebu cattle of India are not often screened for detecting chromosomal aberrations as most of the sperm stations keep high yielding cattle breeds for use in Artificial Insemination (AI). However, some of the animal rearing farms in Maharashtra are promoting using indigenous breeds therefore; blood samples of zebu cattle breeds including Gaolao are regularly received by our Cytogenetics laboratory for karyptyping. Chromosomal mosaicism in somatic cells is one of the aberrations which are usually associated with reduced fertility and reproductive failure (Meinecke *et al*., 2007; Roldan *et al*., 1984; Patel, 2003).

The coexistence of two or more genetically distinct cell populations derived originally from a single zygote is known as mosaicism, which occurs due to mitotic or meiotic non-disjunction at any stage of development even from the two-cell stage (Los *et al*., 2004). Mosaicism could be developed due to sex chromosomes or autosomes and both. Mosaic somatic alterations are present in all multi-cellular organisms, but the physiological effects of low-level mosaicism are largely unknown. In humans, when chromosomal mosaicism arises during development of fetus, pregnancy outcome depends on tissue involved, and how much of that tissue is abnormal. Theoretically, cases with a relatively high proportion of aneuploidy cells (a change in chromosome number that is not the exact multiple of the haploid) are more likely to be associated with an abnormal outcome or congenital defects. If only a small fraction of any body tissue was involved, the aneuploidy would likely to have little effect on the growth and development. However, a very minor degree of mosaicism could still be important if a crucial tissue carries the abnormal cells, as an abnormal chromosome change confined to one part of the brain could theoretically impair neurological function (Gardner & Sutherland, 1996). For example mosaic Down syndrome can be associated with a less characteristic facial appearance and milder mental impairment than those with typical trisomy-21. Most of mosaicism cases reported in animals are based on lymphocyte blood cells (Roldan *et al*., 1984; Yadav *et al*., 1991; Patel and Patel, 1999; Patel, 2003; Meinecke *et al*., 2007; Patel *et al*., 2011; Patel *et al*. 2012; Kotikalapudi and Patel, 2013) whereas it may also be validated by other tissues of body.

The mosaicism in present case was also not validated by other tissue as taking biopsy of tissue could cause injury and infection. However, newer technologies like fluorescent in situ hybridization (FISH), microarray etc are being used mainly in human to detect mosaiciam (Baugher *et al*., 2013). The present study shows a case of mosaicism in an indigenous young bull belonging to Gaolao breed.

MATERIALS AND METHODS

Heperinized blood samples were collected from 70 Gaolao young bulls from bull rearing centres in Maharashtra for karyotyping during last one year (2013). Chromosomal preparations were performed using standard procedure (Patel, 1999). Briefly, whole blood was cultured in RPMI-1640 (Himedia) medium supplemented with antibiotics, 15% fetal calf serum and 1% pokeweed mitogen. The whole blood cultures were incubated at 38°C for 69 hours. To increase the relative frequency of prometaphase chromosomes, ethidium bromide (Sigma) @10 µg/ml was added and to arrest somatic cell division at metaphase stage, Colchicine (Sigma) @ 2 µg/ml was added to the culture for 2 and 1 h respectively, prior to harvesting. The cells were separated by centrifugation at 1500 rpm for 5 minutes followed by hypotonic treatment with 0.075 M KCl for 30 minutes at 37°C and fixed in 3:1 ratio of methanol and acetic acid glacial. The cell suspension was placed on slides and air dried. Conventional staining by Giemsa and routine G-banding using trypsin and Giemsa (GTG) with minor modification (Patel *et al*, 1995) was performed on chromosome slides. In present case of mosaicism in Gaolao cattle, around 105 G-banded metaphase plates were screened for chromosomal analysis using Olympus microscope attached with image analyzer system.

RESULTS AND DISCUSSION

Mostly, around 30 metaphase spreads were screened under microscope for normal animals, whereas, 100 or more metaphase plates were screened when cells exhibit numerical or structural abnormalities or for uncertain cases. In the present study, out of 105 metaphase spreads, 77 spreads (73.4%) exhibited normal karyotypes (60,XY) (Fig. 1: A & B), whereas 28 cells (26.6%) exhibited either 2n+1 or 2n-1/2n-2/2n-3 (Table 1). Normal cells consisted of 58 acrocentric autosomes and 1 submetacentric X-chromosome and one acrocentric Y-chromosome in a male zebu (*Bos Indicus)* cattle. After screening metaphase chromosomes, it was observed that 6 cells exhibited 61,XY viz; three cells with 60,XY+27, one cell with 60,XY+29, one cell with 60,XY+28 (Fig. 1: C & D). In one cell the additional chromosome could not be identified due to lack of banding. Similarly, 5 spreads exhibited 59,XY-27 (Fig. 2: A & B), 3 cells 59,XY-29 (Fig. 2: C & D), 10 cells exhibited 59,XY but missing chromosome could not be identified due to lack of banding. However, 3 cells were found with 58,XY and one with 57,XY respectively. Over all none of cells exhibited mosaicism due to sex chromosome, only small autosomes numbers 27, 28 and 29 played a role in aneuploidy cells causing mosaicism in blood cells. However, it cannot be ruled out the error in identifying the extra/missing chromosome in aneuploidy cells because of ambiguity in banding and small size of chromosomes.

Nevertheless, it has been shown that aneuploidy cells accounts for the numerical abnormalities in animals and humans (Torres, *et al*., 2008). Many case of mosicism especially due to sex chromosome effect reproductive system or fertility. A case of (60,XX/90,XXY) in Holstein were associated with an aplastic vulva, penis and clitoris agenesis, a male-like urethra located in a pseudoprepuce opening between the mammary complexes and a well developed M. Rectipeninus (reproductive disorder) (Meinecke *et al*., 2007). Similarly cases like (60,XX/60,XX, t(12 q;15 q), inv (6) found to be associated with low fertility in Holstein cow which delivered three calves during 11 years of age (Roldan *et al*., 1984). Cases of mosaicism (60,XY/61,XYY) in a Jersey crossbred bull (Patel and Patel, 1999) and 60,XX/61,XXX in HF cow (Patel, 2003) were found associated with reduced fertility and infertility respectively. Yadav *et al.*, (1991) reported a case of

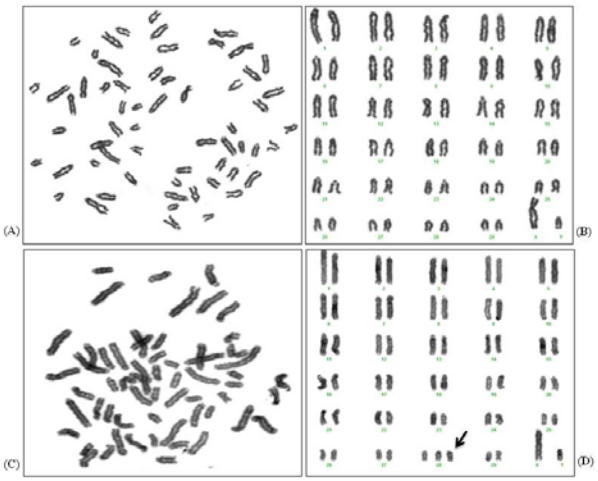

Fig. 1 (A & B): Normal metaphase spread (50,XY) and its Karyotype; **(C & D):** Numerical abnormal metaphase (61,XY+28) and its Karyotype.

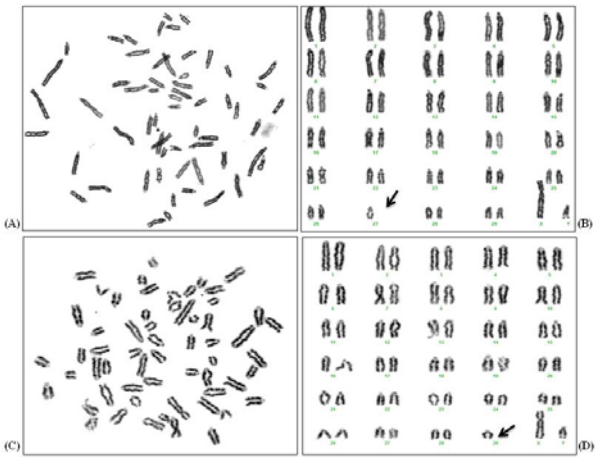

Fig. 2 (A & B): Numerically abnormal metaphase, 59,XY-27 and its Karyotype; **(C & D):** Metaphase with 59,XY-29 and its karyotype

Table 1: Aneuploidy cells with their inconsistent number of chromosomes

Total cells screened	60,XY	61,XY+27	61,XY+29	61,XY+28	61,XY+?	59,XY-27	59,XY-29	59,XY-12	59,XY-?	58,XY-?	57,XY-?
105	77	3	1	1	1	5	3	1	9	3	1
%	73.4	2.86	0.95	0.95	0.95	4.76	2.85	0.95	8.57	2.85	0.95

mosaicism (50,XX/51,XX) in an 8 year old buffalo having irregular breeding history. Their cytogenetic investigations revealed additional 5th chromosome (51,XX+5) in 22.67% cells. Most of cases were also reported in young bulls; XXY/XYY in Jersey crossbred and 50,XY/51,XY+4? in Murrah buffalo where these numerical aberrations could not be correlated with reproductive performance (Patel *et al.*, 2011) . Our observations in the present investigation are similar to the mosaicism recently reported by Kotikalapudi and Patel (2013) in a Gir young bull. The fertility in this case could not be estimated as male was just one year old at the time of blood collection. However, it is advisable that the bull having mosaicism should be checked for the fertility before use in artificial insemination and breeding programmes.

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