B-5236
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RESEARCH ARTICLE Indian Journal of Animal Research

Occurrence Autosomal Mosaicism in Karan Fries Cattle and Murrah Buffalo with Poor Reproduction

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10.18805/IJAR.B-5236

ABSTRACT

Background: The present study was conducted at the Livestock Genome Analysis Laboratory (LGAL), ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India, to explore, chromosomal anomalies in reproductively inefficient animals. Chromosomal abnormalities are deviations in normal genetic architecture and lead to disorders in bearer individuals. These can be both in number and structure of autosomes or sex chromosomes, usually inflict adverse effects on anatomy, survival and reproductive performance in domestic animals.

Methods: Present report is on a female Murrah buffalo (*Bubalus bubalis*) and a Karan Fries cattle which were identified during a routine cytogenetic reproductively inefficient females at cattle yard, National Dairy Research Institute, Karnal. Metaphase chromosome preparations were prepared using standard lymphocyte culture method. Slides were stained with Giemsa and treated for R-banding using fluorochrome-photolysis-Giemsa technique (RB-FPG technique). Karyotypes were prepared and abnormal chromosome was identified.

Result: Routine examination using Giemsa-stained slides revealed two cell lines with 60 and 61 chromosomal constitutions in cattle and 3 cell lines in buffalo. Among 182 metaphase plates examined were examined for Karan fries which revealed two types of chromosome configuration, the frequency of cells carrying 60 and 61 chromosomes was 83.5% and 16.5%, respectively. R banding technique distinguished autosome 16 responsible for mosaicism. Cytogenetic evaluation of 407 metaphase spreads of Murrah buffalo revealed three types of chromosome configurations, *viz*. 49, 50 and 51 all with XX and the overall frequency was 16.7, 76.7 and 6.6 per cent, respectively. The R- bandings confirmed autosome 11 both in monosomy (2n=49) and trisomy (2n=51), respectively. This unusual chromosomal number might have arisen due to non-disjunction during early stage of zygotic development of the buffalo and cattle.

Key words: *Bubalus bubalis,* Cattle, Mosaicism, R-banding.

INTRODUCTION

Chromosomal anomalies are often reported to be associated with varied effects viz. anatomical malformation, spontaneous abortions, neonatal losses and other functional reproductive problems (Iannuzzi *et al*., 2021). However, when changes in chromosomes are large enough to be seen using a light microscope, they are called chromosome aberrations or disorders. Cytogenetic revelation of abnormal phenotypes and mosaic aneuploidies in domestic animals are quite limited compared to those in humans.

Mosaicism represents the presence of two or more cell lines with different chromosomal configurations in individuals. Cytogenetically chromosomal mosaicism can be identified. Identification of lower levels of mosaic cells is a tough job, as many cells have to be counted. Hook, (1977) reported that analysis of 20 cells (standard for routine chromosome analysis) will detect 14% mosaicism (in the tissue being studied) with 95% confidence. In human's mosaicism for almost all of the autosomes have been reported in either liveborn infants (usually ascertained because of physical, growth and developmental congenital defects) or in foetuses through prenatal testing (Jackson-Cook, 2011). The chromosome anomalies encountered can be inherited or *de novo* occurred during development of individuals. Cases of two cell lines such as 60, XY/61, XYY in cattle (Krumrych *et al*., 2002) and 50, XX/51, XX+5, in buffalo, (Yadav *et al*., 1991) were reported earlier.

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How to cite this article: Sen, S., Tonk, R.K., Sah, V., Yadav, B.R., Vandre, R.K., Singh, A., Sant, S. and Gupta, N. (2024). Occurrence Autosomal Mosaicism in Karan Fries Cattle and Murrah Buffalo with Poor Reproduction. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5236.

Submitted: 06-09-2023 **Accepted:** 28-03-2024 **Online:** 20-05-2024

The inherited mosaic aneuploidies arise in parental gonads during meiotic events, with formation of an abnormal zygote with gain of one copy of chromosome in some and loss in other cells. The *de novo* anomalies come up in a zygote, where during early embryonic development due to non-disjunction or anaphase lag both the chromosomes of a particular pair go to one daughter cell while the other lacks the same (Robinson *et al*., 1995). Thus, post fertilization disomy and non-mosaic trisomics indicate both gain and loss of a chromosome. In the cases where the meiotic non-disjunction occurs, it is likely that there is a trisomic configuration in the very early stages of development in one individual, while monosomic in the other. Alternatively, in the cases of mitotic origin of the trisomy, early development proceeds to whole body, while the trisomy originating subsequently along in development possibly affect only a subset of tissues. Research work has shown that there is a chromosome-specific bias in the proportion of meiotically to mitotically occurring non-disjunctions (Robinson *et al*., 1995). These different mechanisms usually cause gross chromosome anomalies in the affected foetus inflicting adversely the development or performance in life.

In the event of occurrence of a chromosome abnormality, configuration of gene(s) gets altered thereby interruption in their functions. The basic problem is in genetic imbalance, the control of morphogenesis and growth is liable to be interfered with, most commonly giving rise to abnormal structure and hampered function. Although some deviations cause only slightly observable changes, however, affect certain physiological reactions leading to reduced performance of the carrier individual. Chromosomal errors occur both in the number and structure of autosomes or sex chromosomes. Chromosomal abnormalities constitute a major cause of embryonic loss in mammals due to their unbalanced configuration, only a few chromosomally unbalanced individuals are born alive in domestic animals or human beings (Jacobs, 1990; King, 1990).

The present study was restricted exclusively to these animals (one Karan Fries cattle and one Murrah buffalo) since there were only two cases of autosomal mosaicism among the various screened reproductively inefficient animals diagnosed as cytogenetically abnormal having numerical chromosomal anomalies.

MATERIALS AND METHODS

Present study was carried out on nine years old Murrah female buffalo and five-year-old Karan Fries heifer due to inefficient reproductive performance. Use of animals was reviewed and approved by the Institutional Animal Ethics Committee where the experiments were carried out. The breeding history at different parities exhibited reproductive problems such as irregular estrus cycle, repeat breeding and anoestrus. Animals were investigated through per rectal examination and evaluated cytogenetically for ascertaining the cause. Pedigree record was scrutinized to correlate chromosomally abnormal females with their parents, half sibs and other relatives.

Chromosome preparations

Blood sample of about 10 ml under strict aseptic conditions was collected from the external jugular vein of the Murrah buffalo and Karan Fries cattle in standard heparinised vacutainer tube. Whole blood was used for setting up of cultures as per the routine method (Yadav and Balakrishnan, 1985). An aliquot of 0.5 ml blood sample was added to each culture vial of 30 ml capacity containing 6 ml medium consisting of RPMI 1640 (as per instructions of manufacturer), contents such as antibiotics (streptomycin and penicillin) (Sigma) and phytohemagglutinin (PHA) (Sigma) 1 ml each with final concentration (20 units/ml) and (10 µg/ml), respectively were added, one drop of sterile sodium heparin to prevent the coagulation problems. Multiple and repeat cultures were set up to obtain abundant cells growth and high mitotic index for confirmations and were incubated for a duration of 96 hours at 37.5±0.5°C. Seven hours prior to harvesting of cultured cells, late BrdU (20 mg/ml) incorporation in each culture bottle allows obtaining more elongated chromosomes (higher frequency of prometaphase cells) and a better band-interband contrast. Subsequently, 45 minutes prior to harvesting of cultured lymphocytes 2 drops (10 µg/ml) of colchicine solution was added to each culture bottle to arrest the mitosis. Cultures were harvested with routine method of 0.075 M potassium chloride hypotonic solution treatment and fixation with methanol and acetic acid (3:1). Chromosome preparations were obtained on clean glass slides with air-dried method.

Slides were stained with Giemsa (2% at pH 6.8) stain for conventional chromosome examination. Evaluation of a total of 182 in Karan Fries cattle and 407 metaphase spreads in Murrah buffalo was done. In order to identify the anomaly slides were subjected to obtain R-banding with RB-FPG technique (Mukherjee and Yadav, 2004) because R-banding are the most commonly used techniques for chromosome identification (karyotyping) and for identifying abnormalities of chromosome number. Karyotypes were constructed on the basis of 'standard karyotypes' of the river buffalo (Iannuzzi, 1994) while for cattle R banding comparison of cattle prometaphase chromosomes arranged according to the Reading system reported by Iannuzzi (1990). Details of band patterns of autosomes and X chromosomes were compared and matched with standard karyotype to identify chromosome involved in mosaicism.

RESULTS AND DISCUSSION

The present study on autosomal mosaicism with three cell lines in Murrah buffalo 5321 is the first report, since no information of such case is available in the literature in farm animals. The cytogenetic screening revealed autosomal mosaicism. Results on Karan Fries cattle

revealed two cell lines represented as 60, XX/61, XX +16, while in Murrah, three cell lines, represented as 49, XX -11/ 50, XX/51, XX +11, consisting of 49, 50 and 51 chromosomes, respectively. This condition may be due to non disjunction of chromosomes. In literature there are many reports on the occurrence of trisomy and monosomy, however, reports are also available on mosaicism though quite scanty.

Autosomal trisomy represents a very low incidence in liveborn as most autosomal aneuploidies are eliminated prior to embryo implantation (Hare *et al*., 1980). There are several studies associated with separate numerical anomalies. Earlier studies of non-banded chromosomes have reported trisomy in association with lethal brachygnathia (Mori *et a.l*, 1969; Dunn and Johnson, 1972). Lioi *et al*. (1995) also reported trisomy of autosome 20 in a 1-week-old calf associated with lower brachygnathia, deformations of anterior and posterior legs, an opistotonous neck, bilateral blindness and absence of external genitalia. It was also suggested that trisomy might be associated with chromosomal instability as high incidence of abnormal cells found in conventional culture. Schmutz *et al*. (1996) reported trisomy of chromosome 22 in all the cells female Angus newborn calf showed multiple malformations, including hypoplasia of palpebral fissures, cleft palate, kyphoscoliosis and arthrogryposis. In other studies, the extra chromosome was not always clearly identified (Long, 1984; Schmutz *et al*., 1987). Chromosome 22 trisomy in a calf was also reported by Mayr *et al*. (1985). Trisomy 24 has been reported by Makinen *et al*. (1987). In a Sahiwal calf with arthrogryposis (in all the four legs) trisomy of 27 autosome has been reported (Yadav, 2000). Norberg *et al*. (1976) reported trisomy of chromosome X in a heifer with underdeveloped ovaries and no changes in reproductive tract.

The reproductive observation of both abnormal cattle and buffalo in the present study showed that morphologically they were normal, body conformity and appearance was similar to the contemporary females. The Karan Fries cattle was a heifer while the Murrah buffalo screened was nine years old female with three parities completed. Gynaecological examination revealed normal sized ovaries, presence of corpus luteum (CL) and was normally cycling. Though morphologically and gynaecologically normal, these animals had long history of different types of reproductive problems including anoestrus, irregular heat cycle and repeat breeding. Although both the animals showed different types of reproductive problems they were categorised under one broad cytogenetic abnormal group of animal that is a numerical anomaly (autosomal mosaicism) as the different reproductive problems in both cattle and buffalo may be due to different chromosomes and animal species involved in autosomal mosaicism leading to involvement of different genes associated with reproduction which can be confirmed using further developed molecular techniques such as Fluorescent *in situ* hybridization (FISH). Several studies with varied degree of reproductive abnormality have been reported in different numerical and structural chromosomal anomalies.

The breeding history of Karan Fries cattle showed age at puberty in Karan Fries 7263 cattle was 658 days (1.8 yr) similar to other contemporaries in the herd. The Karan Fries cattle conceived after four AIs followed by abortion during first trimester of pregnancy, which was unnoticed and was recorded during per rectal examination. Animal exhibited postpartum oestrus after abortion and was attended for next AI. Animal underwent seven AIs after abortion yet failed to conceive. Swartz and Vogt (1983) reported 59, XO/60, XX/61, XXX mixoploid in Pinzgauer x Angus heifer in frequency of normal vs abnormal cell lines was equal in proportion and 59, XO/60, XX/61, XO (with iso-X) mixoploid in Charolais heifer which showed failure to reproduce and had reduced fertility.

The breeding history of Murrah buffalo covered a period of seven years, which included three times calving and age at puberty was 3.6 yrs. On reaching puberty conceived after 4 unsuccessful AIs and age at first calving (AFC) was at 48 months of age. During first lactation it conceived like other normal contemporary buffaloes *i.e.* after 80 days of first calving female showed signs of oestrus and conceived with two AIs (consecutive months). However, during second lactation conceived after 6 unsuccessful AIs from semen of different bulls. Inter calving period between second and third calving was about 3.5 years (1225 days). Subsequently, after third calving it did not show any signs of oestrus in a period of more than one year, turned out to be anoestrus and was disposed off from herd. Animal were inseminated more than four times on exhibiting signs of oestrus during each oestrus cycle without any deviation in inter estrus period i.e. normal estrus cycle but failed to conceive. This finding is in accordance with several studies conducted in repeat breeding animals found to have mosaicism of two cell lines; one with normal chromosome complement, 50, XX and the other with an extra chromosome, 51, XX+5 (fifth submetacentric autosome) in 8 year old Murrah buffalo (Yadav *et al*., 1991) and mosaicism for various structural abnormalities of chromosomes such as gaps and breaks (Hanada and Muramatsu, 1980).

In present case buffalo and cattle carrying numerical anomaly were morphologically and gynaecologically normal without exhibiting any malformation of external or reproductive organs. In literature, similar reports are available with no abnormality in reproductive tract and ovaries observed upon gynaecological examination. This may be due to lesser proportion of cells carrying abnormal chromosomal configuration resulting in lower level of mosaicism detected when the number of normal cells is increased, however analysis of more cells is not normally carried out unless there is a suspicion for chromosomal mosaicism. In addition, for some types of mosaicism, the abnormal cells as well as the normal cells may not divide, so

analysis of metaphases might provide a biased view of the true chromosome constitution of this individual (Conlin *et al*., 2010). It has also been established that complete autosomal trisomic embryos rarely survive until birth and are malformed (Herzog *et al*., 1977). Mosaics survive more easily and even attain a normal appearance (Gustavsson, 1980).

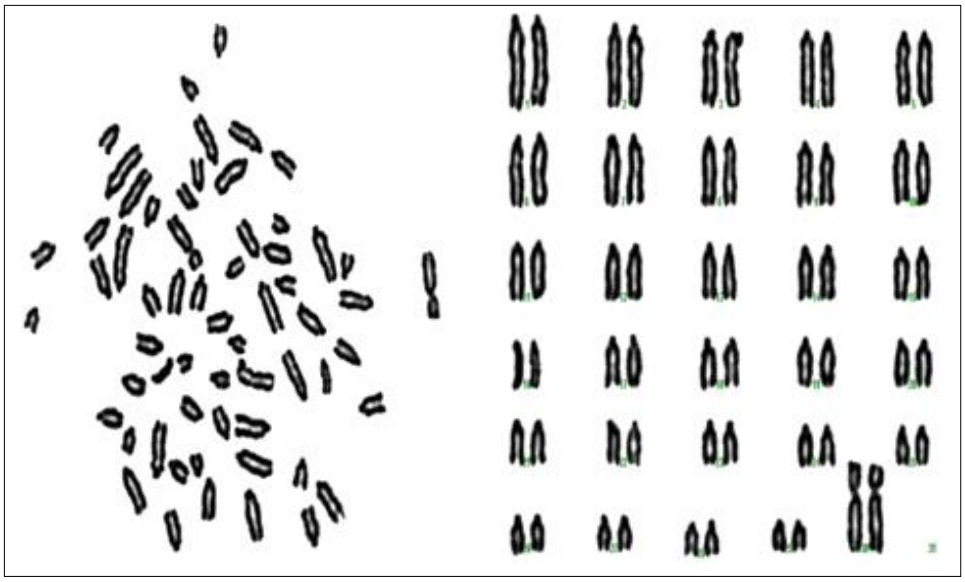

Fig 1: Giemsa stained metaphase spread (left) and its karyotype (right) of karan fries 7263 cells carrying 2n=60 chromosome.

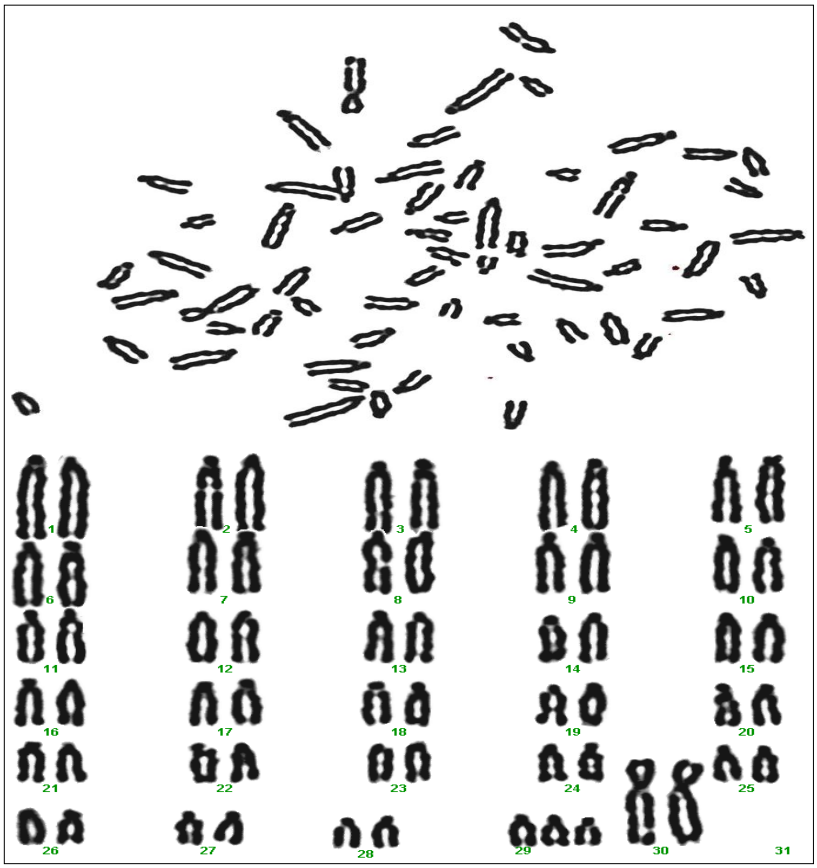

Fig 2: Giemsa stained metaphase spread (above) and karyotype (below) of Karan Fries 7263 carrying a supernumerary chromosome (2n=61).

Repeat breeding may be due to early embryonic death (EED) which remains unnoticed. EED has been attributed to irregular LH and progesterone profiles that induce failures in the maintenance of CL (Swanson and Young, 1990). EED is associated with poor quality of gametes and zygotes, uterine alterations, hormonal imbalances and defects in the immune mechanisms (Bruyas *et al*., 1993). The EED occurs between days 8 and 16 post-mating (Diskin and Sreenan, 1980), before cow returns to estrus. As a result, no variation in inter estrus interval is observed and clinicians cannot differentiate between embryonic resorption and other pregnancy failures. The incidence of EED is highly variable, from 10.6 to 39.7% in cattle.

Cytogenetic revelation of abnormal phenotypes and mosaic aneuploidies in domestic animals are quite limited compared to those in humans. Routine examination of Karan Fries cattle and Murrah buffalo Giemsa-stained slides (Fig 1 to Fig 5) revealed two and three cell lines respectively, resulting in autosomal mosaicism. Giemsa stained 182 metaphase plates examined in Karan Fries cattle showed two cell lines (Fig 1 to Fig 2) and frequency of cells carrying 60 and 61 chromosomes was 83.5 and 16.5%, respectively. Presence of two simultaneous cell line is a very rare event (Di Berardino *et al*., 1979). Sex chromosome mosaicism such as 60, XY / 61, XYY in cattle (Krumrych *et al*., 2002), 59, XO/60, XX/61, XXX mixoploid in Pinzgauer × Angus heifer and 59, XO/ 60, XX/ 61, XO (with iso-X) mixoploid in Charolais (Swartz and Vogt, 1983) have been reported. Critical examination and counting of chromosomes in Murrah Giemsa stained slides revealed

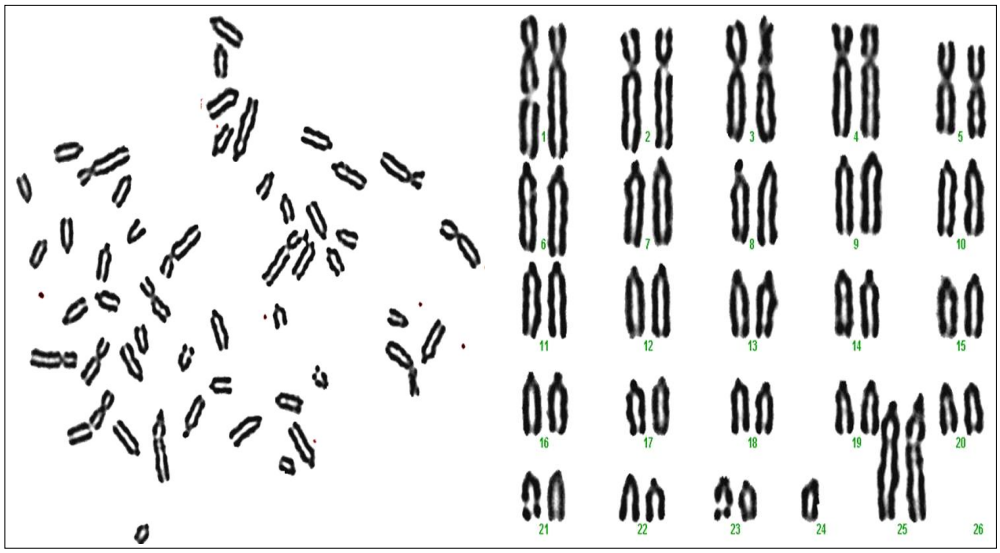

Fig 3: Giemsa stained metaphase plate (left) and karyotype (right) of Murrah buffalo revealing monosomy (2n=49, XX).

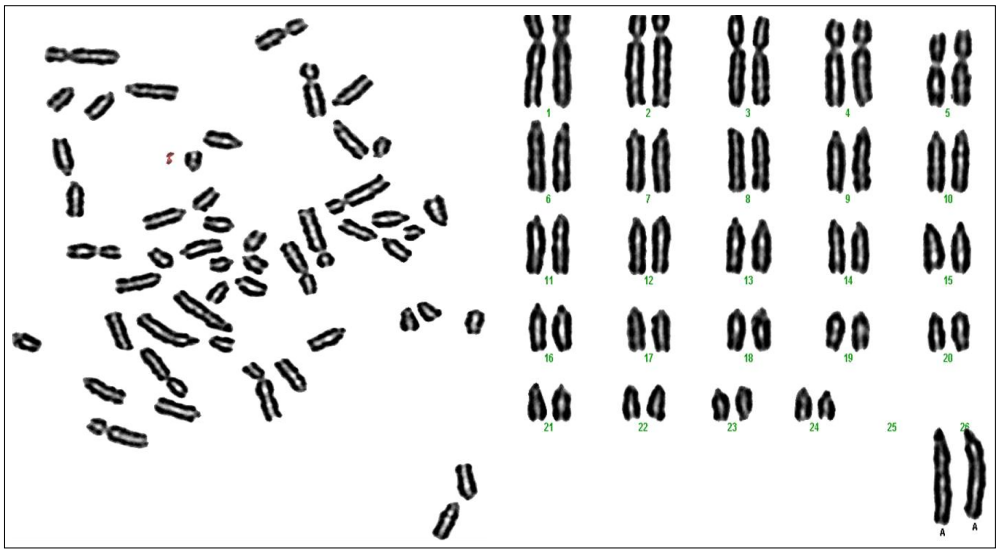

Fig 4: Giemsa stained metaphase plate (left) and karyotype (right) of Murrah buffalo revealing a normal 2n=50, XX.

three different types of metaphases with either 49 or 50 or 51 configurations (Fig 3 to Fig 5). Margaret *et. al.* (2010) identified 3 cell lines in one 17-year old female in humans, where chromosomal analysis revealed 45,X (4%)/ 47,X,i(X)(q10), i(X)(q10)(8%)/ 46,X,i(X)(q10)(88%).

RB-FPG technique distinguished further extra chromosome responsible for mosaicism. A total of 25 karyotypes with RB-FPG technique banded patterns were prepared and the supernumerary chromosome in metaphase plates containing 61 complement was

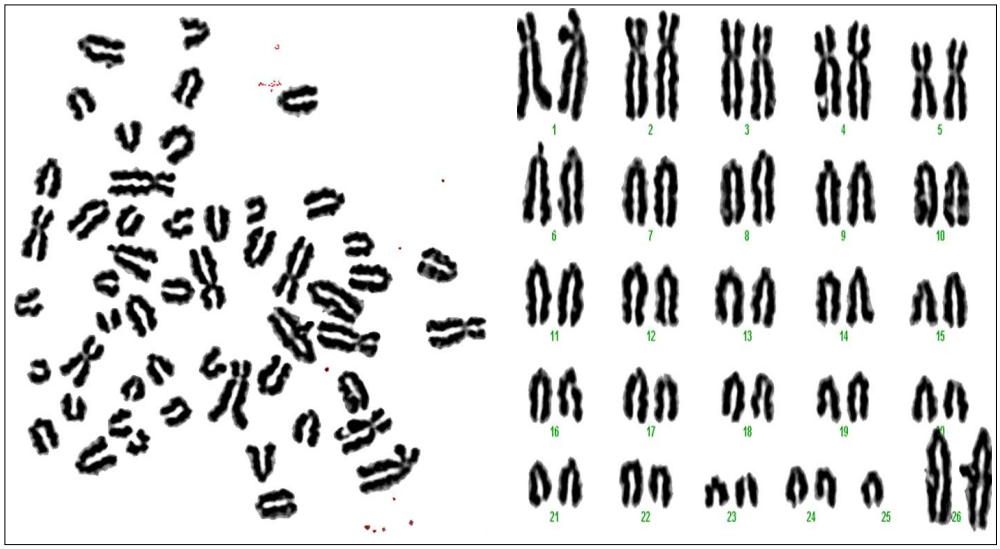

Fig 5: Giemsa stained metaphase plate (left) and its karyotype (right) of Murrah buffalo revealing trisomy (2n=51, XX).

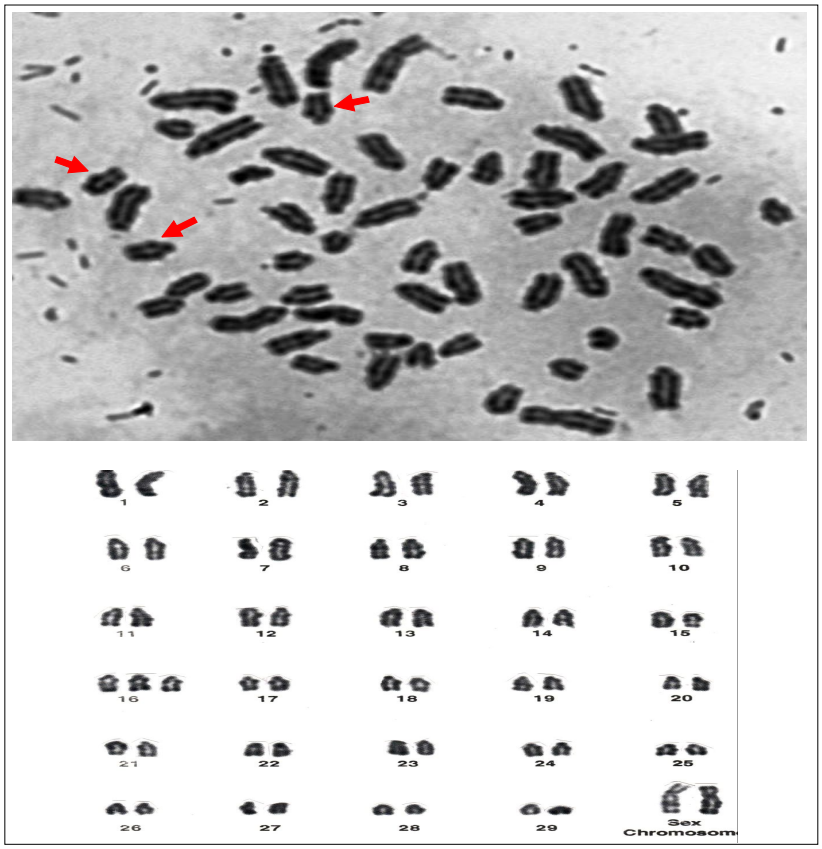

Fig 6: Metaphase spread showing trisomy of autosome 16 (top) and its karyotype (bottom) of Murrah buffalo revealing 2n=51, XX +16.

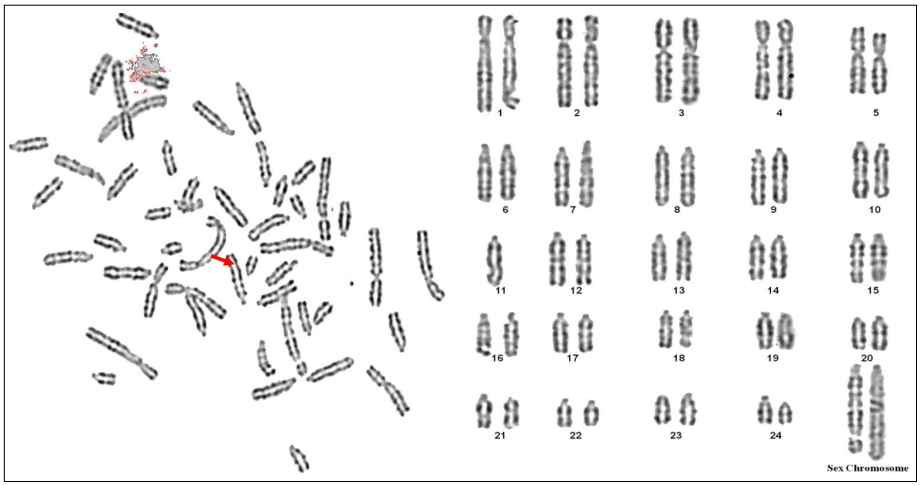

Fig 7: R- banded metaphase spread (left) and its karyotype (right) of Murrah buffalo revealing monosomy (2n=49, XX -11).

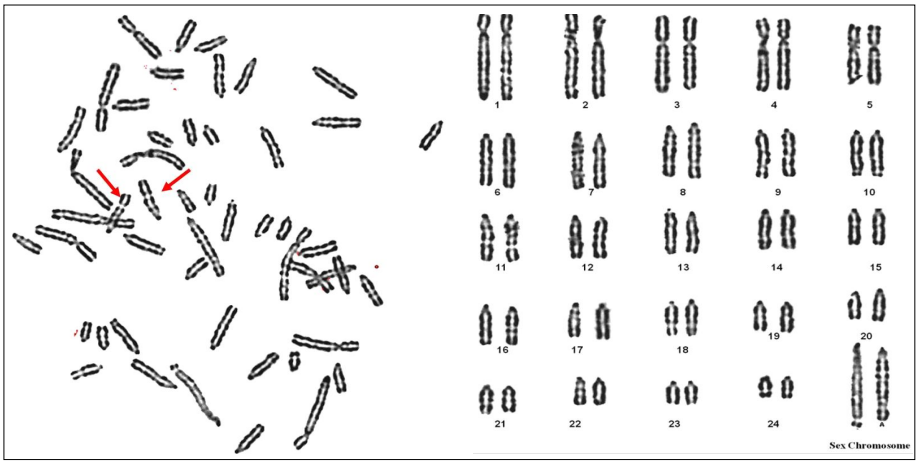

Fig 8: R- banded metaphase plate (left) and its karyotype (right) of Murrah buffalo revealing a normal 2n=50, XX.

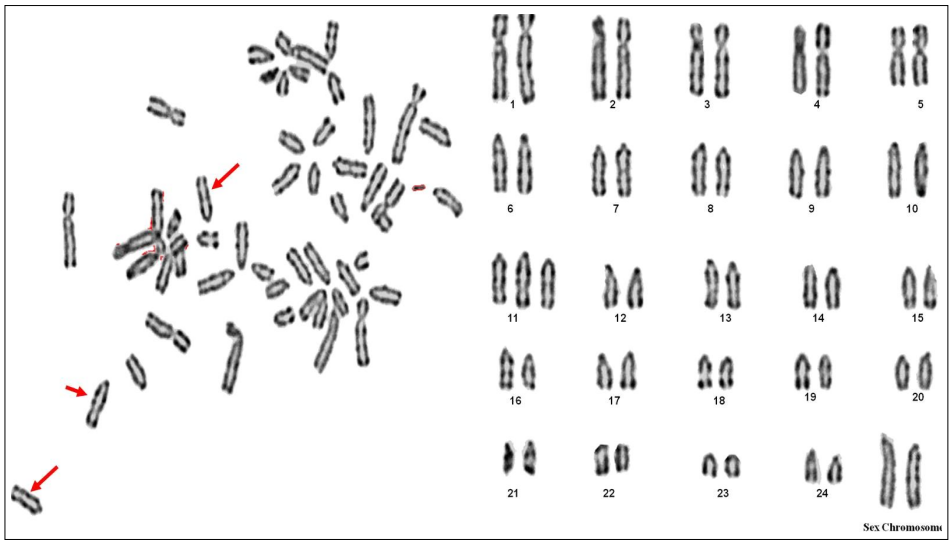

Fig 9: R- banded metaphase plate (left) and its karyotype (right) of Murrah buffalo revealing trisomy (2n=51, XX +11).

identified as autosome 16 in Karan Fries cattle (Fig 6) and autosome-11 was attributed to mosaicism in the affected buffalo (Fig 7-9). Yadav *et al*. (1991) reported mosaicism of two cell lines involving autosomes consisting of two cell line such as 50,XX/51,XX+5 in 8 year old Murrah buffalo as in present case of Karan Fries 7263. Chromosomal configuration of the buffalo in monosomic, normal and trisomic metaphase is represented as 49, XX (-11)/50, XX /51, XX (+11), respectively. Exclusive occurrence of autosomal trisomies represents a very low incidence in liveborns as most autosomal aneuploidies are eliminated prior to embryo implantation (Hare *et al*., 1979).

CONCLUSION

Karan Fries cattle and Murrah buffalo investigated in the present study carried a rare numerical chromosomal anomaly with two and three different types of chromosome configurations, respectively. The three different types of chromosomal configurations of 49, XX (-11A)/50, XX/51, XX (+11A) in Murrah buffalo unlike the known conditions in livestock species. However, the cattle and buffalo did not show any anatomical and/or morphological anomaly but had problems in reproduction. The reproductive problems of the affected individuals varied enormously. Mosaic trisomy condition in present animal might have raised due to mitotic non disjunction with a normal zygote and a subsequent non-disjunction or anaphase lag during a somatic division. In the cases of mitotic origin of the trisomy, early development proceeded normally, with trisomy originating further along in development and possibly affecting only a subset of tissues. Now a day this metaphase biasness against abnormal cells is corrected using new techniques: array analysis by comparative genomic hybridization or SNP array analysis, used widely in case of human. The intrinsic cause of meiotic aneuploidy remains, as yet, unknown, nevertheless mechanism for mitotic aneuploidy non-disjunction is considered. The observations revealed such cases can be fertile and produce progeny at young age, however, later become anoestrous and infertile. The present study on autosomal mosaicism is the first report, since no information of such case is available in the literature in farm animals, particularly water buffalo.

The research concludes that incidence of long uncured reproductive problems should be identified and subjected to routine cytogenetic screening to prevent over expenses in terms of management, which results in poor economy of farm. The molecular cytogenetic techniques such as primed *in situ* labelling (PRINS), fibre FISH, comparative genomic hybridization, chromosome micro-dissection, spectral karyotyping (SKY), Multiple colour FISH (M-FISH), colour banding, FISH with multiple sub-telomeric probes and array based CGH should be used as complementary tool to conventional cytogenetics to improve efficiency of chromosomal anomalies diagnosis to 15-20% compared to conventional which is 10%.

Ethical consideration

Present study involved use of cattle and buffalo farm animals, the ethics approval for this was granted by institutional ethical committee.

Conflict of interest

The authors declare that they have no competing interests.

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