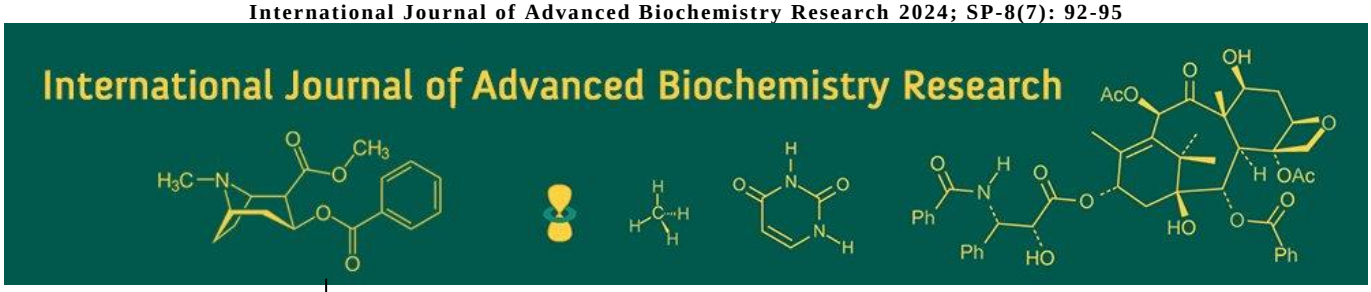

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Screening of Gaolao cattle for chromosomal analysis

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Abstract
This study aimed to screen Gaolao cattle for chromosomal abnormalities, focusing on both numerical and structural aberrations that impact animal breeding and reproductive value. A total of 150 Gaolao cattle (50 males and 100 females) were selected from breeding farms in Maharashtra, India. Blood samples were collected and cultured for cytogenetic analysis using RPMI-1640 medium, fetal bovine serum, and pokeweed mitogen. Giemsa staining and GTG-banding techniques were employed to examine chromosome number and morphology. The diploid chromosome number in Gaolao cattle was confirmed to be 2n = 60, consisting of 29 pairs of autosomes and one pair of sex chromosomes (60XX/XY). Autosomes displayed acrocentric morphology, while the X chromosome was metacentric and the Y chromosome was acrocentric. The study also confirmed the presence of Robertsonian translocation (1:29), a known cause of reduced fertility. This research underscores the importance of cytogenetic screening in breeding programs to enhance reproductive efficiency and genetic health of cattle populations.

Keywords: Gaolao cattle, chromosomal abnormalities, numerical aberrations, structural aberrations, cytogenetic analysis

Introduction
There are numerical and structural abnormalities observed in animals. Numerical chromosomal aberrations play important role in animal breeding because the breeding bulls which are using for AI should be free from the said disorders to avoid further transmission. Structural abnormalities of Chromosomes have also equal importance in animal breeding. The carriers show normal body conformation but have reduced reproductive value due to unbalanced gamets which give rise to unbalanced zygote and embryos which will die in early embryonic life. Cytogenetic defects like structural and numerical chromosomal abnormalities also play an important role in dairy industry. Therefore, the major emphasis is being given on testing of cattle and buffalo breeding bulls for cytogenetic defects prior to introduction at AI station. Chromosomal aberrations like chromosome fragmentation, polyploidy, Trisomy, Monosomy (Turner syndrome), Translocation, Chromosome mosaicism in sex as well as autosomes could also be one of the reasons for reduced fertility in dairy animals. 1:29 Robertsonian translocation is the well-established chromosomal abnormality and known for reduced or sub fertility in farm animals.

Materials and Methods
Total 150 Gaolao cattle (Male-50 and Female-100) were selected from Cattle Breeding Farm, Hettikundi, Bull Mother Farm Pohara of Wardha and Amaravati Districts maintained by Department of Animal Husbandry, Maharashtra State. Five ml of blood sample were collected from jugular vein in sodium heparin for cytogenetic investigations using all possible aseptic precautions. The samples were transported to the laboratory by maintaining cold chain.
For setting of culture, 15 ml sterile, graduated disposable centrifuge tubes were used. Five ml of RPMI-1640 was transferred to the each culture tube. One ml (20 per cent of culture) of Fetal Bovine Serum was added to provide essential nutrients, which may not be present in the synthetic medium. To stimulate cell division Lectin (Pokeweed) (1.5 per cent of culture) was added in each culture tube along with 80 µl of antibiotic Penicillin Streptomycin (100

IU/ml of medium) solution to avoid microbial contamination. Finally, about 0.5 ml blood was added to each culture tube.

The contents of the tubes were mixed thoroughly and caps of these tubes were tightened. The culture were incubated at 37 °C in CO_2 incubator for a period of 72 hours. The whole procedure was carried out under strictly aseptic conditions.

After 71 hours of incubation, 75 µl of spindle inhibitor colchicine (10 µg /ml) was added to each culture tube; mixed gently and further incubated for one hour at 37 °C in CO_2 incubator.

Finally, after 72 hours of incubation the culture tubes were removed from the CO_2 incubator and centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded leaving about 0.5 to 1.0 ml cell button and medium. Further, 7 ml of prewarmed hypotonic solution (0.075 M KCL) was added to these tubes and cells were resuspended in the solution. These tubes were kept at 37 °C in water bath for 20 minutes. Tubes were removed from the water bath and 2 ml of freshly prepared fixative solution (3: 1 Methanol and Glacial Acetic Acid) was added to each tube. These tubes were then centrifuged at 1000 rpm for 10 minutes and the supernatant hypotonic solution was removed leaving a small white button of cells and 0.5-1 ml of hypotonic solution. The cells were resuspended in 7 ml of fixative and the tubes were centrifuged at 1000 rpm for 10 minutes. After that, 2-3 new washes were given by chilled fixative until a white cell button appeared at the bottom of the tube. The cell button was resuspended with 0.5 ml of fixative for slide preparation.

Dropping was performed by holding the slides at the angle of 45° and 2-3 drops of cell suspension were dropped from the height of 2.5 - 3 feet on each slide. These slides were air dried and labeled. Two sets of slides were prepared from each sample, one set for giemsa staining and other for G banding. First set of slides were stained with 4 per cent Giemsa solution for 10 minutes. The slides were washed with distilled water and dried on the hot plate. The stained slides were mounted in DPX mountant and other set of slides were kept for aging for GTG- banding.

Results and Discussion

Chromosome number and morphology

The peripheral blood samples of all animals were subjected for cytogenetic investigations. Plate 1 and 3 represent Giemsa stained metaphases of male and female Gaolao cattle, respectively. Plate 2 and 4 represents Giemsa stained male and female karyotype of Gaolao cattle, respectively. The diploid chromosome number in Gaolao cattle was found to be 2n = 60. The average chromosomal complement of Gaolao cattle comprises of 29 pairs of autosomes and one pair of sex chromosomes (60XX/XY). Autosomes confirmed the acrocentric morphology of cattle chromosomes. Di Berardino *et al*. (1991) [3] in Bos *Taurus*, Balaji *et al*. (2006) [2] in Deoni cattle, Kumararsamy *et al.* (2006) [8] in Ongole cattle, Kumararsamy *et al*. (2008) [9] in Umblachery cattle, Mamat-Hamidi *et al.* (2009) [10] in Sahiwal-Friesian cattle; Patel *et al.* (2011) [11] in dairy bulls, Patel *et al*. (2012) [12] in HF and HF crossbred animal observed diploid chromosome number (2n=60) in Punganur, Vechur, Kasaragod, Malnand Gidda and Gangatiri rare zebu cattle breeds. Melander (1959) [13] reported 2n=60 chromosome number in Holstein (*Bos taurus*) cattle. Similar observations were reported by Anis *et al.* (1990) [1] in Sahiwal cattle,

In the present study all autosomes were found to be acrocentric and X chromosome was the largest metacentric whereas Y chromosome was acrocentric in nature. This study was in affirmation with Balaji *et al*. (2006) [2] in Deoni cattle where X chromosome was observed to be submetacentric in morphology and Y chromosome was acrocentric. Similar findings were reported by Halnan *et al.* (1981) [6] in Sahiwal, Sindhi Brahman and Santa Gertrodis breeds, Anis *et al*. (1990) [1] in Sahiwal, Kumarasamy *et al*. (2006) [8] in Ongole cattle, Kumarasamy *et al*. (2008) [9] in Umblachery cattle reported the similar observations. Moreover similar findings were observed by Jorge (1974) [14] in *Bos taurus taurus* (Charolais, Chianina and Jersey) and in *Bos taurus indicus* (Gyr, Guzerat and Red Shindi) purebred animals.

Plate 1: Giemsa stained metaphase of Gaolao male

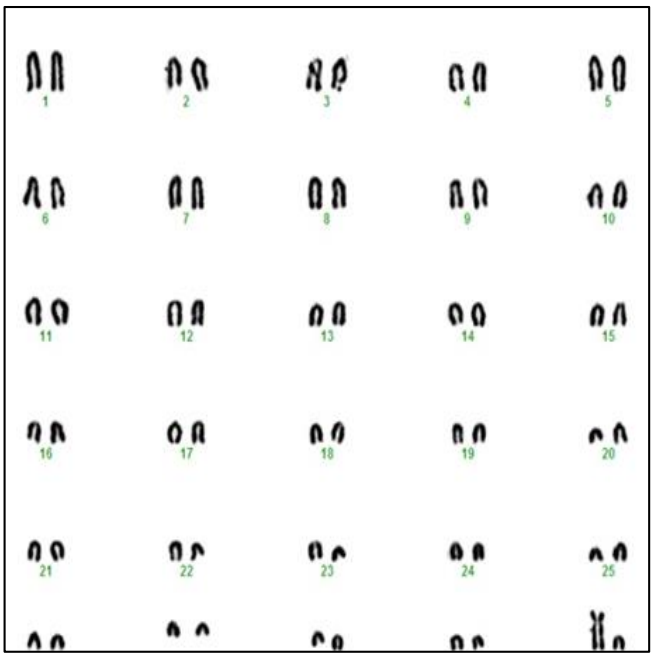

Plate 2: Karyotype of Gaolao male

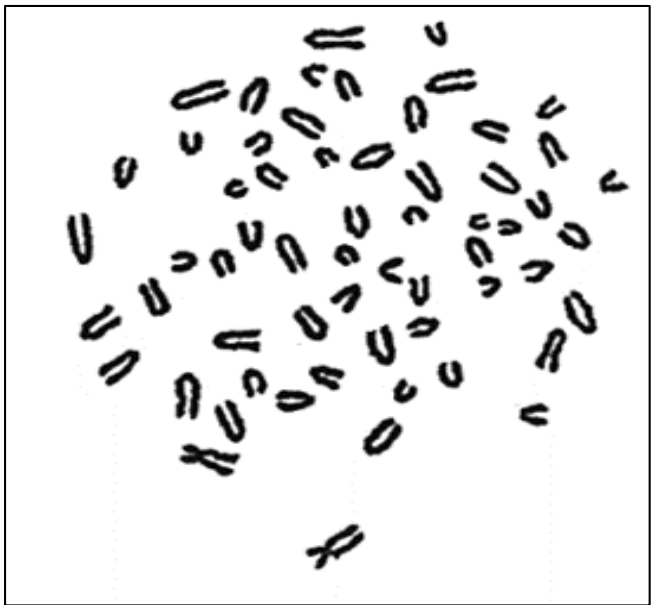

Plate 3: Giemsa stained metaphase of Gaoalo female

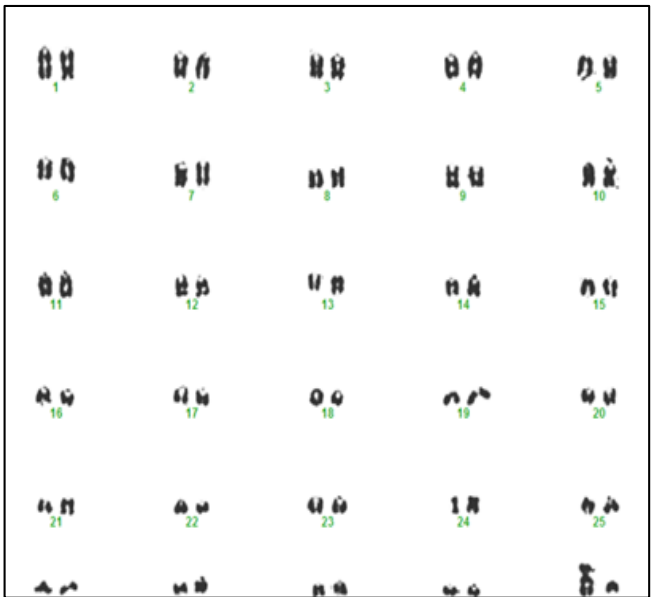

Plate 6: Karyotype of GTG banded Chromosome of Gaolao male

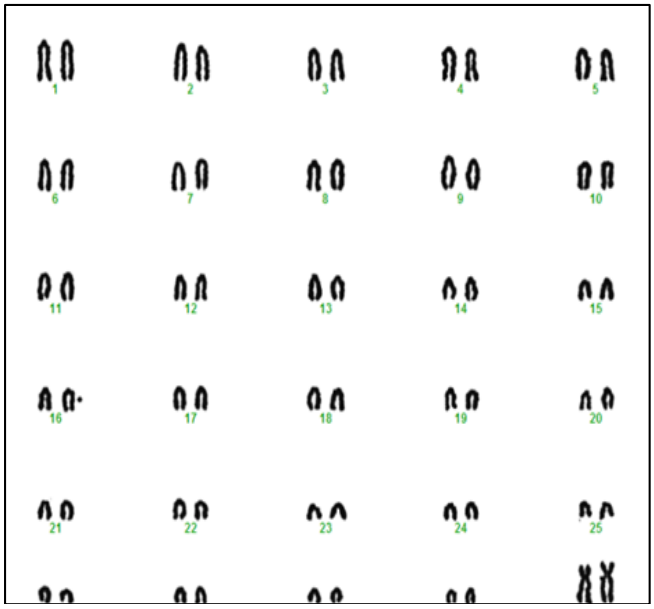

Plate 4: Karyotype of Gaolao female

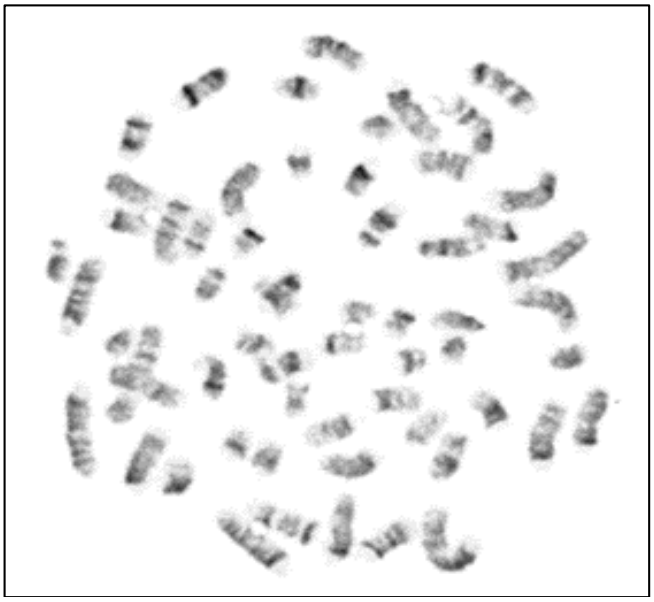

Plate 7: GTG- banded metaphase of Gaolao female

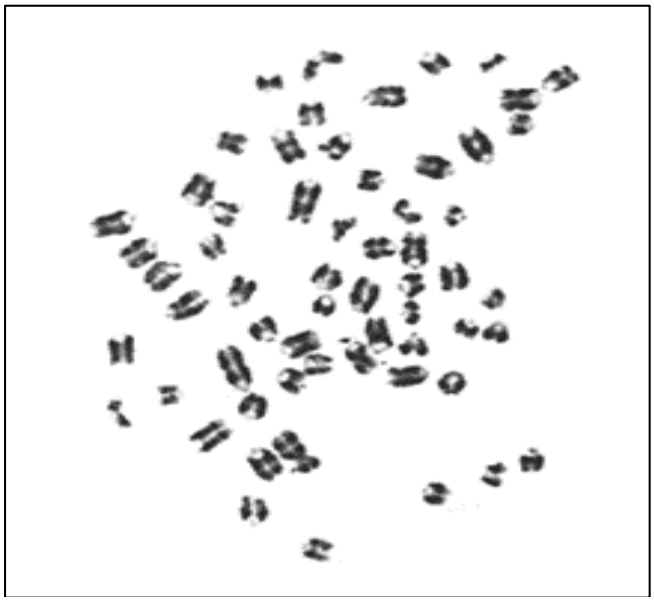

Plate 5: GTG -banded metaphase of Gaolao male

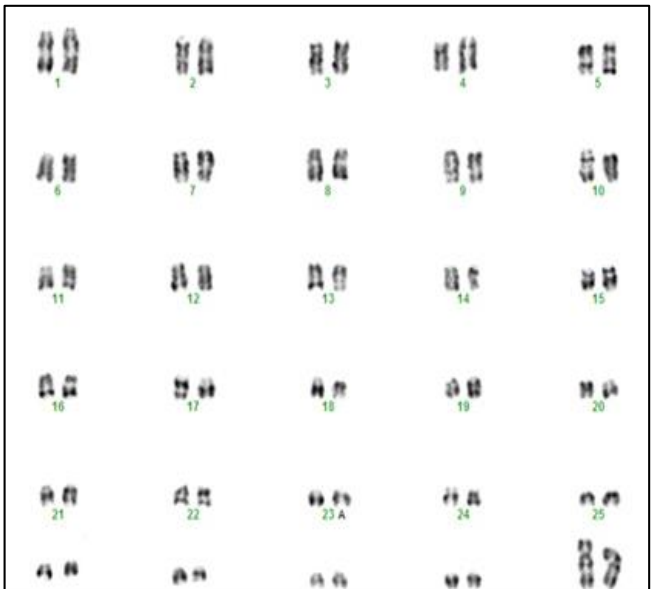

Plate 8: Karyotype of GTG banded chromosome of Gaolao female

GTG banding

Plate 5 and 7 represents GTG banded male and female metaphases of Gaolao cattle, whereas Plate 6 and 8 represents GTG banded karyotype of male and female Gaolao Cattle, respectively. All the acrocentric autosomes presented according to GTG band pattern. The X chromosome was easily recognized by its typical morphology in G-band pattern. Similarly, the Y chromosome in Gaolao male was identifiable as a small acrocentric chromosome with G-positive band in q arm. The GTG-banding of Gaolao cattle (*Bos indicus)* showed similar chromosome morphology to that of Ongole cattle reported by Kumarasamy *et al.* (2006) [8], Dangi cattle reported by Faske *et al*. (2009) [5], reported similar findings in Punganur, Vechur, Kasaragod, Malnand Gidda and Gangatiri cattle.

Conclusion

The cytogenetic investigation of Gaolao cattle revealed a diploid chromosome number of 2n=60, with 29 pairs of acrocentric autosomes and one pair of sex chromosomes (XX/XY). The X chromosome was the largest metacentric, and the Y chromosome was acrocentric. These findings align with previous studies on various cattle breeds, confirming the acrocentric nature of cattle autosomes. GTG banding further validated these results, showing distinct banding patterns for the X and Y chromosomes. This study enhances our understanding of Gaolao cattle's chromosomal morphology and genetic structure, providing valuable insights for breeding and conservation efforts.

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