

STUDIES ON CHROMOSOMAL HETEROMORPHISM IN DAIRY CATTLE

By

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in partial fulfilment of requirements for the degree of :

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in

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Dedicated to
My Parents

CERTIFICATE I

This is to certify that the thesis entitled "Studies on the chromosomal heteromorphism in dairy cattle" submitted for the degree of M.V.Sc., in the subject of Animal Breeding to the CCS Haryana Agricultural University, Hisar is a bonafide research work carried out by Mr. Sandeep Karwasra under my supervision and no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.



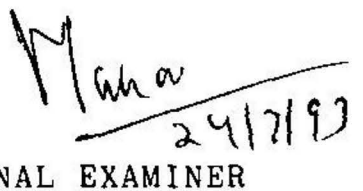
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CERTIFICATE II

This is to certify that the thesis entitled, "Studies on the chromosomal heteromorphism in dairy cattle" submitted to the CCS Haryana Agricultural University, by Mr. Sandeep Karwasra in partial fulfilment of the requirements for the degree of M.V.Sc., in the subject of Animal Breeding, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.


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1. INTRODUCTION

Chromosomes are repository of vital genetic information in living beings and have always been a subject of paramount importance. The chromosomes first observed by Nageli were named as cytoblast because of their observance in some cells and at certain times i.e. Mitosis and Meiosis. Late in 19th century these thread like chromosomes were rediscovered by German Anatomist Walter Flemming. The number as well as the morphology of chromosomes is same in all cells of an organism except in case of reproductive tissues. Generally, qualitative and morphological differences exist between chromosomes. Also the number of chromosomes is not sufficient for species identification as several species may have same number of chromosomes.

Chromosomal heteromorphism for autosomes and sex chromosomes generally help in understanding inter-relationship and evolutionary trends among allied and related species. Boer (1972) emphasised the potential of comparative cytogenetics in understanding relationships among related groups. The association of several forms of chromosomal abnormalities with certain diseases, reproductive impairment and development disorders in animals and human beings have added new dimensions to the study of chromosomes (DeGrouchy and Turlean, 1977; Yunis, 1977; Sutton, 1980). Research in the cytogenetics of farm animals remained neglected for a long time as the techniques

evolved in nearly stages were mainly applicable to the chromosomes of plants and insects and did not give satisfactory results in farm animals and other mammals.

Cytogenetic investigations on livestock and other farm animals gained momentum in early 50's after the introduction of lymphocyte culture techniques. These techniques were initially standardized for the display of human chromosomes (Lejeune et al., 1959). However, all these techniques now have been successfully applied to farm animals with promising results.

The Indian sub-continent is endowed with wide and varied forms of germplasm of cattle, buffaloes, goats, sheep and poultry (Sundaresan, 1975); Acharya and Bhat, 1984; Nagarcenkar and Sethi, 1984; Sahai, 1986). In India there are 26 well defined breeds of cattle which constitute around 18 per cent of total 192 million population (Taneja, 1989). Although, Indian breeds of cattle inhabiting different agro-climatic regions are better adapted for tropical stress and diseases yet their milk production potential is generally lower than that of American and European counterparts. Crossbreeding programmes in India were initiated in early 60's so as to increase milk production and to lower the age of maturity of Indian breeds of cattle by incorporating exotic germplasm. A number of studies have shown quantitative differences for the economic traits in crossbred/synthetic strains in relation to native Zebu or exotic breeds. But

literature on the comparison of chromosomal profiles among indigenous and exotic breeds is not adequate (Yadav, 1981). Benjamin and Bhat (1977) were the first to initiate cytogenetic research on Indian cattle. Chromosomal heteromorphism will be useful in cataloguing and comparing breeds of Indian origin from their exotic counterparts. In-depth studies will further strengthen the selection improvement programme in identifying chromosomes associated with Marker genes.

The present investigation was contemplated to study the cytogenetic divergence in Hariana, Holstein Friesian and crossbred animals with the following objectives:

- 1- To establish morphological and biometrical attributes of sex chromosomes and autosomes in purebreds and crossbreds.
- 2- To study chromosomal heteromorphism by application of linear differentiating banding techniques i.e. G and C bandings.
- 3- Identification of NOR sites using silver staining.
- 4- To study cytogenetic divergence for morphometric measurements on chromosomes in different genetic groups.

2. REVIEW OF LITERATURE

2.1 CHROMOSOME STUDIES IN BOVIDAE

Until 1931 there was a great discrepancy in the reports on number of chromosomes of cattle ranging between 50 and 60. This confusion was cleared when Makino (1944) conclusively showed that diploid number was 60. However, he could not observe any difference between karyotypes of Bos taurus (exotic cattle) and Bos indicus (Indian cattle). It was Keiffer and Cartwright (1968) who showed that 'Y' chromosome of Bos taurus was submetacentric while that of Bos indicus was acrocentric.

Domestic cattle belong to super family Bovidae which has five sub-families and these include 49 genera and 129 species (Morris, 1965). However, somatic chromosomes of nearly 65 species have been catalogued. The mammalian chromosomes atlas includes karyotypes of 32 representatives from Bovidae (Hsu and Benirschke, 1968). In the last two decades chromosomes of several breeds of cattle, goat, sheep, buffalo, pig and equine have been described (Manna and Talukdar, 1965; Keiffer and Cartwright, 1968; Benjamin and Bhat, 1978; Sahai and Saxena, 1978).

2.2 INNOVATIONS IN ANIMAL CYTOGENETICS

The revival of interest in the cytogenetics of mammals is due to innovations in cytogenetic methodology

particularly the technique of harvesting chromosome from cellular material grown in vitro. Numerous excellent reviews and publications have appeared on the simplified lymphocyte culture technique (Buckton and Evans, 1973; Lubs and Ledley, 1973; Sharma and Talukdar, 1974; Darlington and Lacour, 1980).

The various protocols followed reflect modifications in the basic working plan of lymphocyte culture described by Hungerford et al. (1967). The discovery of colchicine a mitotic arrester at metaphase stage also contributed in superior characterization of chromosome. The use of mitogen like Phytohaemaagglutinin (PHA) and Pokeweed mitogen have simplified lymphocyte culture technique (Nowell, 1960; Basrur and Gilman, 1964). These substances trigger cell division in culture and dividing cell can be harvested at an appropriate stage for display of chromosomes. Hsu and Pomerat (1953) discovered that treatment of cells with hypotonic solution before fixation results in distinct spreading of chromosomes which facilitate their identification. Sahai (1979) analysed various constraints in lymphocyte culture and suggested remedial measures.

The chromosome banding technique represent another noteworthy landmark in development of animal cytogenetics. The various banding techniques (Q, G, C, R and NOR etc.) have been discovered in recent years and voluminous literature is available on this subject (Schnedl, 1972;

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Sharma and Talukdar, 1974; Gustavsson and Hageltorn, 1976; Lin et al., 1977; Darlington and LaCour, 1980; Narki and Osterhoff, 1985).

The examination of banded chromosome has been found useful in karyotyping and in clinical genetics. The literature on banded chromosomes of farm animals is still inadequate (Masuda et al., 1974; Chakrabarti and Benjamin, 1976; Gustavsson and Hageltorn, 1976; Sahai, 1980; Yadav, 1981; Kho et al., 1982; Narki and Osterhoff, 1985).

The human cytogeneticists have laid criteria for identification of chromosomes by organizing a number of conferences. In domestic animals conflicting descriptions have appeared on banded karyotype. Accordingly, an International Conference for Standardization of banded karyotype of animals was convened by the University of Reading (U.K.) from 2nd to 6th August, 1976.

2.3 DIPLOID NUMBER OF CHROMOSOMES

The animals of family Bovidae possess diploid chromosomes numbers ranging between 38 and 60 (Wurster and Benirschke, 1967; Sahai and Mathur, 1981). However, majority of species have a diploid count of 60. The chromosomes are well defined but owing to striking similarities in the shape and size, it is difficult to

precisely pair and establish their karyotypes (Moraes and Mattevi, 1980; McGurk and Rivlin, 1983; Pinheiro, 1984; Nosach and Arakova, 1985; Takashima et al., 1985). With the improved cell culture techniques a diploid number of 60 chromosomes has been established in normal cattle (Nichols and Levan, 1962; Monnier Cambon, 1964; Popescu and Granciu, 1965; Basrur and Moon, 1967; Gupta et al., 1974; Yadav, 1981; Porswal, 1987).

2.4 SOMATIC CHROMOSOME OF CATTLE BY CELL CULTURE

Crossley and Clarke (1962) described a tissue culture technique for the elucidation of chromosomes of cattle (Bos taurus). In sixties, a number of publications appeared which systematically described techniques for the study of bovine chromosomes (Sasaki and Makino, 1962; Biggers and McFeely, 1963; Ulbrich and Pfeiffer, 1963; Basrur and Gilman, 1964). Nichols et al. (1962a) reported that female lymphocytes grew better than lymphocytes of male. However, other workers did not confirm this. Lymphocytes of cattle do not grow in culture under stress and mitotic index declines (Sahai, 1980).

A number of species of genus Bos have been examined cytogenetically viz. Bos indicus, Bos taurus, Bos grunniens, Bos bison (Bardeleben, 1892; Yosida and Lamontain, 1964; Monnier-Cambon, 1964; Manna and Talukdar, 1965; Gerneke, 1967; Wurster and Benirschke, 1967; Kieffer and Cartwright, 1968). A typical diploid complement of

60 chromosomes was reported in all of these. Bos taurus Gaur has 58 somatic chromosomes. The reduction in chromosome number is perhaps due to centric fusion of one pair. Heck et al. (1968), Wurster and Benirschke (1967) and Sahai and Mathur (1981) reported that family Bovidae is characterized by great stability of karyotype and majority of the living species retain primitive fundamental number. The primitive complement consists of 60 somatic chromosomes. The 'X' chromosome is large while 'Y' chromosome is smallest. The 'Y' chromosome was shown to be submetacentric by Crossley and Clarke (1962) in British breeds and Benjamin and Stringam (1969) in Herefords. Sasaki and Makino (1962), Nichols et al. (1962), Biggers and McFeely (1963), Ulbrich et al. (1963), Popescu and Granciu (1965), Basrur and Moon (1967), Kieffer and Cartwright (1968) described the 'Y' chromosomes as submetacentric in European breeds. Only Chiarelli et al. (1960) described the 'Y' chromosome of European cattle as acrocentric. The somatic chromosomes of various breeds of Bos taurus (e.g. Brown Swiss, Red Dane, Holstein Friesian, Jersey, Russian Black Pied) have been studied. Herzog and Hoh (1971), Pinherio (1984), Yeo (1984) and Ngah et al. (1985) reported that their karyotypes show great homology in the morphology of the chromosomes.

Literature on Asian and African humped cattle (Bos indicus) is in infancy. For the basic attributes of

chromosomes hardly six breeds of Indian Zebu have been studied (Chakraborty, 1976; Benjamin and Bhat, 1977; Gupta et al., 1978; Yadav, 1981; Porswal, 1987). The karyotype of various domestic mammals including Zebu was reported by Makino (1944).

2.5 CHROMOSOME BANDING

Elegant procedure for identifying chromosomes have appeared since 1970 which employ special staining procedures. The dyes binds preferentially to certain segment of DNA owing to chemical differences. Thus, bands are produced along the length of chromosomes. Caspersson et al. (1971) reported that certain quinacrine derivatives bind to chromosome and produce fluorescent band (Q bands). Arrighi and Hsu (1971) noted that denaturation and subsequent re-association followed by Giemsa staining can produce distinctive zones of high stain affinity. These are termed as 'C' bands ('C' for constitutive heterochromatin). Hsu (1973), Schwarzacha and Wolf (1974), Dutrillaux and Lejeune (1975), Yunis (1976), and Comings (1978) reported that by modified treatment schedules and Giemsa staining, a numerous bands have been developed and are called 'G' bands. Currently, various forms of bands like R, G-II, CT, CD are studied to understand the architecture of chromosome.

2.6 'C' BAND

According to Evans et al. (1973) absence of distinct staining in centromeric segment was a noteworthy feature in chromosomes of cattle. The absence of centromeric heterochromatin blocks in the sex chromosome of Zebu (Bos indicus) was reported by Gupta et al. (1974). The centromeric heterochromatin observed by a modified technique was located on the short arm of the submetacentric 'X' and 'Y' chromosomes (Popescu, 1973). Potter et al. (1979) reported that the 'C' bands on the autosome and 'X' chromosomes were similar in various bovines and crossbreds. The comparison of G and C banding was done by Raicu et al. (1978) in Capra hircus, Bos taurus and Ovis aries. It was found that several autosomes and the 'Y' chromosomes exhibited similar banding pattern. Evidence was presented on the basis of G-banding that 'Y' chromosome was metacentric in Bos taurus and telocentric in other species. Two translocations were reported in cattle by Ciupercaescu (1982) by the C band analysis. It was shown that in 1/20 translocation, fused chromosome showed a single constitutive heterochromatin block; whereas in 14/20 fusion formed dicentric. This account does not mention the breed of animals studied.

2.7 G BAND

There are few publications on the G-banded chromosomes of cattle (Schnedl, 1972; Evans et al., 1973; Masuda et al., 1974; Logue, 1978; Potter et al., 1979; Halnan et al., 1981; Krutzler et al., 1986). G-banding is an extremely important for identification and pairing of homologous chromosomes. In bovine this technique shows special significance as majority of chromosomes are similar morphologically (Gustavsson, 1980; Ciupercescu, 1982; Krutzler et al., 1986). G-banding technique is quite helpful for the identification of chromosome involved in Robertsonian translocation. G-bands of 21st and 22nd pair of chromosomes were very similar and 25th pair lacked distinct bands (Masuda et al., 1974). A competitive evaluation of the banding pattern described by various authors showed considerable discrepancy. It was realised that some of standardization of banded karyotype should be considered. So, an International Conference was convened at University of Reading, Reading in August, 1976 (Ford et al., 1982). A number of similar conferences to standardize human karyotypes have been held at Denver (1960), London (1964) Chicago (1966) and Paris (1971).

2.8 NOR BAND

Ten NOR's in cattle, goat and sheep were reported by Henderson and Bruere (1979). The similarity in the number

of NOR's in these species was attributed to the basic similarity of karyotype. According to some reports, NOR's in cattle are located in chromosome number 2, 3, 4, 5 and 28 (Mayr and Czeke, 1981; Kuo-Aipu et al., 1982; Pinheiro et al., 1982; Mayr and Gruber, 1987; Mayr et al., 1987; and Shashikanth et al., 1991). However, due to breaks and possible translocations shifting of NOR's can take place (Sikka et al., 1988).

2.9 CHROMOSOME ABERRATIONS

Despite well known stability of genetic system abnormal chromosomes have been reported in animals and human beings. So, various syndromes related to nervous and reproductive disorders have been associated with various forms of cytogenetic changes (Jacob et al., 1974; Hook and Porter, 1977; Borgaonkar, 1977; Yunis, 1977; Berardino et al., 1983; Bhatia and Shanker, 1991).

Several forms of chromosomal abnormalities reported in cattle have been associated with impaired growth, reproductive and birth defects. Among various forms of abnormalities, trisomy, chromosomal mosaicism and Robertsonian translocations have been specifically linked with reproductive disorders and impaired production. Popescu (1972) showed pericentric inversion of autosome in a Normandy bull having low fertility. This abnormality was inherited as simple dominant character (Popescu, 1977).

The affected chromosome was later identified as 19th pair of the autosome.

Robertsonian translocation in a bull having low sperm count and reduced fertility was reported by Lajda et al. (1976). A number of publications have appeared on Robertsonian translocations (Gustavsson, 1969; Hansen and Berton, 1970; Herzog and Hohn, 1971; Froget et al., 1972; Harvey, 1972; Popescu, 1977; Yadav, 1981). In this abnormality the number of chromosome is altered but the fundamental number remain unaffected. A translocation between 1 and 2 chromosome in Gurnsey bull with low fertility was reported by Bongso and Basrur (1976). Popescu (1969) reported that the Scandinavian bulls carrying 1/29 translocation did produce fertile progeny. Falaschini (1984) reported 1/29 translocation in Romogga cattle. In a report of Animal Physiology Institute, U.K. (1986), 1/29 translocation was identified after C-banding. Yadav (1981) reviewed 15 other types of translocations involving different chromosomes. Autosomal translocations involving 25th and 27th pair of chromosomes was reported in the five Alpine Grey bulls (Giovanni et al., 1975). Berardino et al. (1979) referred to two different translocations in a Todolian female. It was found that fusion involved 1/29 and 14/26 chromosomes. Wurster and Benirschke, 1968; Sahai and Mathur, 1981) suggested that Robertsonian fusion was an important mechanism in bovine specification. The Robertsonian trans-

locations and fusion between non-homologous increased heterozygosity in an individual with deleterious effects (Bruere and Chapman, 1973).

Lajda et al. (1976) and Dunn et al. (1977) reported chromosomal mosaicism in 23 per cent progeny of a bull having hypoplasia and small testes. Chromosomal analysis of female heterosexual twins in cattle lead Eldridge and Balazak (1977) and Balakrishnan et al. (1979) to infer that for early diagnosis of freemartin condition chromosomal examination is essential. A case of free martin heifer, which was a chromosomal mosaic (XX/XY) has been reported by Miyake et al. (1980).

A Friesian bull showed trisomy of the sex chromosomes (61,XXY). Due to an extra 'X' there were 61 chromosomes (Harvey, 1972). Testicular hypoplasia was noted in Hereford bull by Dunn et al. (1979). The karyotype of this animal revealed an extra X chromosome, Norberg et al. (1976) showed trisomy in a Norwegian Red Heifer (61,XXX) with impairments of reproduction.

Kanagawa et al. (1965), Hersehlar and Fecheimer (1967) reported the presence of 'Y' chromosome in a female cattle possibly caused hermaphroditism..

Bongso and Basur (1976) associated low fertility in some bulls to chromatid breaks and chromatid gaps.

Deletions in one or both chromosomes in 14th and 26th pair in Zebu and Hereford was reported by Halnan (1972). El-Nahos et al. (1974) noted breaks, gaps and deletion in 'X' chromosomes of Black Pied German Cattle resulting in sterility. The effect of chromosome abnormalities was reviewed by Cribiu and Popescu (1977). Congenital ataxia in Black Pied cows having 58 chromosomes was reported by Zhigachev and Goldman (1975). Gorin and Budevich (1976) observed euploidy incidence between 8.4 to 12.2 per cent in chromosome harvested by in vitro technique.

2.10 NATIVE TRACT OF HARIANA AND SYNTHESIS OF CROSSBRED STRAINS OF DAIRY CATTLE

India possesses 26 well defined breeds of dairy cattle which constitute 18 per cent of total cattle population. The Zebu cattle were domesticated as early as 4000 B.C. Remains of ancestors of Bos indicus were found in Sindh (Pakistan), Gujarat, Rajasthan and Shivaliks (Acharya and Bhat, 1984). Zebu cattle originating from tropical regions have been reported for disease resistance and adaptations to the environmental fluctuations. Oliver (1938) was perhaps the first to record breeds of Indian cattle. Raghvan (1960), Kaura (1961) and Bhat et al. (1981) have defined characteristics of native breeds of cattle and buffaloes. Haryana cattle have their home tract in the districts of Rohtak, Hisar,

Gurgaon, part of Karnal and Delhi province (Kaura, 1961). Haryana animals are proportionately built with compact phenotypic appearance and greyish white in colour (Plate 1). Haryana animals have long narrow face with flattened forehead. Bullocks are considered as good work animals.

Cattle breeding policies for the improvement of native breeds were taken up with the introduction of exotic germplasm through cross-breeding. Temperate dairy breeds like Holstein Friesian, Jersey and Brown Swiss were imported which were used for synthesis of cross-breeds of different cattle breeding farms, State Farms, and AICRP Units. Different genetic groups were evolved by crossbreeding local Haryana breed with exotic ones was taken up at the Hisar Centre of AICRP. Taneja (1989) reviewed the results of various crossbreeding experiments and reported that Holstein crosses were superior to other temperate breed crosses for growth and milk production.

Indian breeds are better adapted, resistant to diseases and can perform under low inputs and harsh environment in comparison to exotic ones. There is a need to identify the differences at the genetic level. In the present investigation attempts have been made to study chromosomal profiles of Indian and exotic breeds of cattle as well as their crosses. The intra and inter breed differences at genomic level will further help in the

3. MATERIALS AND METHODS

Cytogenetic studies were conducted on indigenous and exotic breeds of dairy cattle as well as their crosses maintained at animal farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar and Government Livestock Farm, Sector-3 of Animal Husbandry Department (Haryana). A total of 77 animals (36 Haryana, 29 crossbreeds - $\frac{1}{2}$ F + $\frac{1}{4}$ J + $\frac{1}{4}$ H and 12 Holstein Friesian) were analysed. Whole blood cultures were set up from these samples at the Animal Cytogenetics Laboratory of Animal Breeding Department, CCS Haryana Agricultural University (CCS HAU), Hisar. The details regarding distribution of experimental animals are given in Table 1.

3.1 COLLECTION OF BLOOD SAMPLES AND TRANSPORTATION TO LABORATORY

Blood (5-7 ml) was collected from the external jugular vein of each animal using Heparin coated vacutainer tubes. The vacutainer tubes were gently shaken for few seconds to prevent clotting of blood. Blood samples collected at GLF, Sector-3 were transported in a double jacketed iced container (cool pack) at 5°C. Samples were kept in a plastic beaker and crushed ice was filled in another plastic beaker. The two beakers were kept apart so as to avoid the chilling effect. Blood samples were stored in refrigerator for three hours before the setting up of cultures.

Table 1. Distribution of experimental animals.

Breed	Total No.of samples collected	Males	Females
* Hariana	36	15	21
** Crossbreds	29	12	17
***Friesian	12	5	7
Total	77	32	45

* Pure indigenous Hariana animals kept at Animal Farm (Animal Breeding Department).

** Synthetic crossbred dairy animals of 3/4th exotic inheritance.

*** Pure Holstein Friesian animals belonging to GLF Sector 3, Hisar.

3.2 PREPARATORY STEPS INVOLVED IN SETTING UP OF CULTURE

The essential glassware, equipments and chemicals were stocked in advance before the commencement of culture work.

3.2.1 Washing

Glasswares used in tissue culture work were soaked in liquid detergent for 24 hours. These were then scrubbed and washed in running tap water. After washing, glasswares were immersed in a trough containing Potassium dichromate solution (1%) for 12 hours. After a thorough washing in running tap water, these were rinsed in distilled water and dried at 150°C for two hours.

3.2.2 Sterilization by Autoclaving

All the glasswares and media filtration assembly used in setting up of culture were wrapped in aluminium foil. Pipetts were packed in metallic case. All these were autoclaved for 30 minutes at 15 pounds per sq. inch pressure (1.05 kg/cm²). The autoclaved glasswares were kept in laminar flow hood.

3.2.3 Serum Separation from Blood

The blood (250-300 ml) was collected from healthy young males in a sterile, empty and pre-evacuated glucose transfusion bottle by connecting one end of needle to bottle

and other end to jugular vein of animal. The bottle was left undisturbed for an hour to allow the clotting of blood. Later the bottles were kept overnight in refrigerator. The serum was gently drained into sterile centrifuge tubes. To obtain clear straw coloured serum, tubes were centrifuged at 3000 r.p.m. for 15 minutes. Serum bottles/tubes were stored at -20°C for subsequent use.

3.2.4 Formulation and Disbursement of Tissue Culture Medium

The working tissue culture medium of following composition was prepared :

1.	Glass distilled water (Pre-autoclaved)	150 ml
2.	Medium TC 199	1.629 gm
3.	Tryptose phosphate broth	236 mg
4.	L-Glutamine	15 mg
5.	Penicillin (6 mg/5 ml)	0.2 ml working/100 ml
6.	Streptomycin (100 mg/5 ml)	0.2 ml working/ 100 ml
7.	Pokeweed mitogen (Sigma)	2.0 ml working/ 100 ml
8.	Bovine serum	40 ml.

The pH of medium was adjusted to 7.2 by adding drop-wise an aqueous solution of sodium bicarbonate. The medium was

filtered through Seitz filtration assembly (Filter pore size 0.1 μ) after connecting it to vacuum pump. The filtered medium (5.0 ml) was dispensed into each screw capped culture tube under strict aseptic conditions in laminar flow hood. The tubes containing medium were used either immediately or stored in freezer (-20°C) for subsequent use.

3.2.5 Inoculation of Blood

The tubes containing media were removed from refrigerator and kept in laminar flow hood under the UV light for one hour. An aliquot of 0.5 ml whole blood was added to each culture tube. The cap of tube was replaced tightly after flaming. The inoculation was carried out under strict aseptic conditions. The remaining blood was again stored in refrigerator till the results of first culture were available. The stored blood was used for setting more cultures. Culture tubes were incubated for 72 hours in a slightly inclined position at 38°C ($\pm 0.5^{\circ}\text{C}$). These were shaken after every 24 hours to improve the dispersion of cells.

3.3 HARVESTING OF CULTURES

3.3.1 Colchicine Treatment

An hour before termination of incubation one drop of aqueous colchicine (0.004 %) was added to each culture

tube with the help of 22 g needle to arrest dividing cells at metaphase stage.

3.3.2 Hypotonic Treatment and Fixation

The contents of each culture tube were transferred into 15 ml centrifuge tube and swirled at 1000 r.p.m. for 15 minutes. The cells deposited at bottom of tube formed a packed button. The supernatant was discarded and cell pellet was resuspended in 5-6 ml of potassium chloride (0.075 M) for hypotonic treatment at 38°C (\pm 0.5). After 8-10 minutes, hypotonic treatment was terminated by adding one ml of freshly prepared chilled fixative (3:1, Methanol : acetic acid) to each centrifuge tube. Each tube was again centrifuged for 10 minutes (1000 r.p.m.) and cell pellet was resuspended in cold 5 ml of fixative. This process of centrifugation and fixation in acetic methanol was repeated thrice so as to get a clear whitish pellet.

3.3.3 Preparation of Slides and Staining

A small quantity of cellular suspension was drawn into a long nozzled pasteur pipette and 3-4 drops were splashed on a clean, moist slide from a height of 2-3 feet. The slides were immediately warmed over spirit lamp for two seconds and stored. Thoroughly dried slides were stained with 2% phosphate buffered Giemsa solution (pH 6.8) for 30 minutes and rinsed in distilled water.

These were left for 24 hours in an incubator (38°C) for complete drying. Dried slides were cleared in Xylene (10 minutes) and mounted in D.P. X. (Distrene-plastisizer-Xylene).

3.4 STAINING FOR G-BANDING

The protocol of Sea Bright (1971) was suitably standardized for G-band staining. Freshly prepared slides by air drying technique were immersed in a jar containing trypsin (0.025 %) already maintained at 38°C for 45-50 seconds and washed in phosphate buffer saline solution. Slides were later stained in 2 % Giemsa for 2-3 minutes, cleared in xylene and mounted in D.P.X. The bioactivity of trypsin treatment and temperature are critical factors in the successful G band staining. Several trials were made to standardise the procedure.

3.5 STAINING FOR C-BANDING

Usually one week old slides were employed for C-band staining after modifying the methods of Arrighi and Hsu (1971); Summer (1972). The slides were immersed in 0.2 N hydrochloric acid for one hour at room temperature and repeatedly rinsed in normal saline solution. These were transferred for 8 minutes into a saturated solution of Barium hydroxide (56°C) already kept in a water bath. After 3 or 4 washings in normal saline solution slides were

incubated in 2 X SSC for two hours at 64°C, washed in distilled water and finally stained for 1½ hours in 2 % Giemsa.

3.6 STAINING FOR NOR's

The technique of Howell and Black (1980) was suitably modified for locating the Ag-NOR sites. Three drops of freshly prepared aqueous gelatin and two drops of silver nitrate (50%) were placed on slide floating on a wooden plank in water bath at 70°C. Slides covered with cover glasses were left for 15-20 minutes till the colour turned golden brown. The cover glasses were gently removed and slides were passed from photographic hypo solution for 5-6 seconds and rinsed in distilled water. Dried slides were later cleared in xylene and mounted in D.P.X.

3.7 SCREENING OF SLIDES

Slides were screened under Olympus Binocular Microscope using oil immersion objectives (X 1000). For each breed and sex, a total of 300 well spread metaphases were carefully observed for various types of chromosomal aberrations, if any. The chromosomal aberrations were classified (Gebhart, 1970) into structural and numerical aberrations as follows :

A. Structural aberrations

i. Chromatid type aberrations

Chromatid gaps (CG)

Isochromatid gaps (ICG)

Chromatid breaks (CB)

Deletions (D)

Acentric fragments (ACF)

Pulverizations (PULV)

ii. Chromosomal type aberrations

Acentric and Centric rings (RG)

Terminal deletions (TD)

Translocations (reciprocal translocations)RT

Centric fusion (CF)

B. Numerical aberrations

Polyploidy (POLY)

Endoreduplication (ER)

3.8 KARYOTYPING

Photomicrographs of well spread and distinctly stained metaphase plates were taken under oil immersion (X 1000) on Olympus Binocular Microscope using 125 ASA ORWO Films (35 mm). The chromosomes were cut carefully and homologous pairs were pasted on a sheet sequentially. The pairing of homologous chromosome was done on the basis of their identical morphology and maximum approximation

in length. The chromosomes were arranged in a descending order of their length after following the recommendations of an Atlas of mammalian chromosomes (Hsu and Benirschke, 1968). The karyotypes of banded chromosomes were made following the specific recommendations of reading conference (2nd to 6th August, 1976) applicable to domestic animals (Ford et al., 1976).

3.9 RELATIVE LENGTH MEASUREMENTS AND IDIOGRAM OF CHROMOSOMES

Ten karyotypes from each sex in three different breeds were selected for the measurements of relative length. The length of chromosomes were measured by Dial type Caliper. In case of female the mean length of homologous paired 'X' chromosome was added and relative length of each chromosome expressed as percentage of total. In case of male mean length of homologous pairs of autosomes, X-chromosome and Y-chromosomes were taken as total and relative lengths were calculated using the following formula:

$$\text{Relative length of chromosome(\%)} = \frac{\text{Length of chromosome}}{\text{Total length of genome}} \times 100$$

Diagrammatic representation of chromosomes known as idiogram was prepared for both the sexes among three breeds where each chromosome has been shown as thick vertical bar (Figs. 1 and 2).

3.10 MORPHOMETRIC MEASUREMENTS OF CHROMOSOMES

Arm ratio and centromeric index were calculated for sex chromosome 'X' in females after measuring the 'p' and 'q' lengths from the mid-point of centromere using Dial Caliper in millimeters.

$$\text{Arm ratio} = \frac{\text{Length of long arm of chromosome ('q')}}{\text{Length of short arm of chromosome ('p')}}$$

$$\text{Centromeric index (\%)} = \frac{\text{Length of short arm of chromosome ('p')}}{\text{Total length of chromosome (p+q)}}$$

3.11 STATISTICAL ANALYSIS

The average relative length of each chromosome for three breeds were analysed by method of one way classification. Mahalanobis D^2 -statistic was applied to measure the cytogenetic divergence between the three breeds for different traits viz., average relative lengths of autosome number 1 and sex chromosomes (X and Y).

3.12 COMPOSITION, METHOD OF PREPARATION OF REAGENTS AND STAINS

1. 0.2 N Hcl solution

1 ml concentrate Hcl was diluted with 54 ml double distilled water.

2. 5% Barium Hydroxide [Ba(OH)₂]

5 gm Barium hydroxide [Ba(OH)₂] was dissolved in 100 ml distilled water.

3. 2 X Sodium chloride and Sodium Citrate mixture (2 X SSC):

- a) 1.75 gm Sodium Chloride (NaCl) was dissolved in 100 ml of distilled water.
- b) 0.882 gm Sodium Citrate was dissolved in 100 ml of distilled water. The solutions 'a' and 'b' were mixed together to have 2 X SSC.

4. Normal Saline solution:

1.70 gm Sodium chloride was dissolved in 200 ml of distilled water.

5. Trypsin (Difco) solution:

Weighed 0.025 gm and dissolved in 100 ml of normal saline solution.

6. Giemsa stain (2%):

47 ml of distilled water + 2 ml phosphate buffer and 1 ml of Giemsa stain.

7. Gelatin:

Weighed 2 gm of gelatin powder and dissolved in the 100 ml of double distilled water and added 1 ml of formic acid, then mixed thoroughly till the formation of uniform solution.

8. Silver Nitrate [AgNO₃(50%)] :

4 gm of Silver Nitrate (AgNO₃) was dissolved in 8 ml double distilled water and stored in an amber coloured bottle. To protect it against effect of light black paper was also wrapped.

4. RESULTS AND DISCUSSION

Cytogenetic studies of domestic mammals are rare in India. The main reasons are, lack of technical knowhow as well as non-availability of culture materials. Bovine cytogenetics is presently gaining importance in order to catalogue the chromosomal profiles of various breeds and their crosses (Basrur and Moon, 1967; Kieffer and Cartwright, 1968; Pinheiro et al., 1980; Bhat, 1983). Comparative karyology will help in understanding inter-relationships and evolutionary trends among allied and related species.

Cross-breeding programmes in India were initiated in early 60's for increasing milk production and to lower the age of maturity of Zebu cattle by incorporating exotic germplasm. Taneja (1989) reviewed the results of crossbreeding on the basis of quantitative traits only. So far comparison of chromosomal profiles among indigenous and exotic breeds there is paucity of literature. The present study was made to identify chromosomal heteromorphism in Haryana, pure Holstein Friesian and their crosses on the pattern of their morphometric parameters.

4.1 DIPLOID CHROMOSOME NUMBER IN CATTLE

The family Bovidae includes 49 genera and 129 species (Morris, 1965). Normal karyotypes of nearly 65 species have been documented with diploid number of

chromosomes varying between 30 to 60. However, a vast majority contain 58 acrocentric chromosome and one pair of sex chromosome (Wurster and Benirschke, 1968; Sahai and Mathur, 1981). Several breeds of domesticated cattle (Bos taurus, Bos indicus) have been examined cytologically. Practically all the established breeds studied so far revealed a diploid complement of 60 chromosomes (Nichols and Levan, 1962; Popescu and Granciu, 1965; Basrur and Moon, 1967; Benjamin and Bhat, 1978; Sahai and Saxena, 1978). Masui (1919) reported 33 chromosomes in male germ cell of cattle. Chromosomal dimorphism with 37 and 38 in male and female cattle was noted by Wodredalek (1922). Karallinger (1931) noted 60 chromosomes in somatic cells. Although the chromosomes are well defined, striking similarities in shape and only imperceptible differences in size made their identification difficult. Somatic metaphase chromosomes of Haryana, Holstein Friesian and crossbred animals were carried out to establish the diploid number (Table 2). There was preponderance of metaphase cells having 60 chromosomes. However, few plates with slightly varying counts (57 to 61) were also observed. There were due to the inclusion or exclusion of chromosomes from other metaphases in vicinity. The study confirms the presence of similar diploid number of 60 ($2n = 60$) in all the three breeds, respectively.

Table 2. Frequency distribution of cells with various chromosomal counts.

Breed	Sex	No. of Metaphase cells scored	Chromosome count					
			57	58	59	60	61	62
Hariana	Male	300	-	5	14	278	3	-
	Female	300	-	-	11	287	2	-
Crossbreds	Male	300	17	-	12	269	2	-
	Female	300	5	4	16	273	2	-
Friesian	Male	300	4	2	5	291	-	-
	Female	300	1	8	7	283	1	-

4.2 NORMAL KARYOTYPES OF CATTLE

Precise and unequivocal characterization of each chromosome is absolutely essential for the construction of reliable karyotypes. However, even with excellent quality of chromosomes preparation using ordinary conventional stains, the visual identification of chromosomes became subjective and arbitrary. The karyotypes constructed on these observations are of limited value. The examination of linear length of chromosomes in relation to centromere, therefore, provides a simple and more rational basis for chromosome identification and pairing of homologues. Generally, the chromatid arms are subjectively sub-divided into two parts, a proximal part and distal part, each part roughly comprising half the chromosome. The proximal part is the area from centric region to the centre of chromatids and distal part consist of terminal regions. The chromatid arms of chromosomes are given the normal designation of 'p' for shorter arm and 'q' for longer arm.

Model karyotypes of Hariana, Holstein Friesian and crossbred dairy cattle were constructed from the well spread complete metaphase cells (Plates 2 to 9). Benjamin and Bhat (1977) karyotyped Hariana cattle for the first time. They described the autosomes as telocentric because of the terminal location of centromere. However, Biggers and McFeely (1963), Basrur and Moon (1967), and Kieffer

and Cartwright (1968). Gupta et al. (1974) observed bovine chromosomes (autosomes) as acrocentrics. In the present investigation autosomes in Haryana, Holstein Friesian and crossbred cattle were acrocentric type while the shape of 'X' chromosome was found to be submetacentric and similar in all the breeds. The 'Y' chromosomes, however, showed heteromorphism. It was observed as a small acrocentric and comparable in length to 25th autosome in Haryana animals. Pure exotic animals and their crosses with Haryana were having small metacentric 'Y' chromosome. Livescu (1976) classified the autosomes of cattle into 16 pairs of subtelocentric and 13 pairs as acrocentric but the autosomes of Haryana, Friesian and crossbred have been observed as acrocentric type and cannot be classified into groups due to similar morphology (Plates 2 to 9).

Melander (1959) showed that 'X' chromosome in Bos taurus was metacentric. A number of other workers have confirmed this finding (Chiarelli et al., 1960; Crossley and Clarke, 1962; Nichols et al., 1962). The 'X' chromosome was largest and submetacentric in three breeds and was close to first and second pair of autosomes in size. Similar report was given by Sasaki and Makino (1962) and Porswal (1987). It was found to be of original type as 'X' chromosome contributed 5% of the haploid genome (Ohno et al., 1964). Zhivalev and Goldman (1973) observed

variations in the length 'Y' chromosome in Russian Brown Swiss breed. Halnan and Watson (1982) reported that 'Y' chromosome in Bos taurus and Bos indicus was lying between 22nd and 25th pair. On the basis of relative length it has been suggested that among the animals studied there was no variation in size of 'Y' chromosome (Plates 2, 3, 4, 6, 8).

4.3 MORPHOMETRIC MEASUREMENTS OF CHROMOSOMES IN CATTLE

The relative length of crossbred cattle in relation to exotic and Hariana breeds were expressed for both the sexes (Tables 3, 4). The average relative length (%) among male karyotypes ranged from 1.82 to 5.15 per cent in Hariana; 1.72 to 5.30 per cent in crossbreds and 1.88 to 5.12 per cent in Friesian cattle for autosomes. The relative length of 'X' chromosome was higher in crossbreds (5.01%) than Hariana (4.83%). 'Y' chromosome among three breeds also depicted the similar but non-significant pattern in their relative lengths. Sarkhel and Katpatal (1989) compared the sex chromosome variability in Bos taurus, Bos indicus and crossbred cattle. It was reported that 'X' chromosome contributed higher in exotic breed than native Gir (5% vs. 4.87%). The present results showed similar chromosomal variability of 'X' chromosome in Hariana and crossbred cattle.

Table 3. Relative length (%) of chromosomes in male of different breeds of cattle.

Chromo- some No.	Hariana	Crossbreds	Friesian	M.S.due to breeds
	R.L. \pm S.E.	R.L. \pm S.E.	R.L. \pm S.E.	
1.	2.	3.	4.	5.
1.	5.150 \pm 0.302	5.301 \pm 0.267	5.129 \pm 0.264	1.135
2.	4.672 \pm 0.279	4.717 \pm 0.199	4.727 \pm 0.175	0.176
3.	4.464 \pm 0.277	4.471 \pm 0.167	4.417 \pm 0.118	0.214
4.	4.311 \pm 0.240	4.317 \pm 0.140	4.304 \pm 0.145	0.012
5.	4.020 \pm 0.267	4.169 \pm 0.150	4.197 \pm 0.136	0.106
6.	4.080 \pm 0.234	4.019 \pm 0.243	4.053 \pm 0.113	0.336
7.	3.913 \pm 0.216	3.929 \pm 0.141	3.825 \pm 0.457	0.343
8.	3.847 \pm 0.216	3.821 \pm 0.078	3.726 \pm 0.426	0.519
9.	3.723 \pm 0.200	3.738 \pm 0.063	3.754 \pm 0.165	0.101
10.	3.617 \pm 0.191	3.637 \pm 0.097	3.638 \pm 0.136	0.068
11.	3.455 \pm 0.206	3.511 \pm 0.123	3.573 \pm 0.126	0.437
12.	3.345 \pm 0.211	3.389 \pm 0.087	3.415 \pm 0.137	0.532
13.	3.221 \pm 0.173	3.198 \pm 0.087	3.296 \pm 0.123	1.501
14.	3.153 \pm 0.170	3.114 \pm 0.072	3.191 \pm 0.131	0.857
15.	3.064 \pm 0.146	3.029 \pm 0.049	3.118 \pm 0.112	1.652
16.	2.964 \pm 0.113	2.967 \pm 0.046	3.024 \pm 0.123	1.142

contd...

1.	2.	3.	4.	5.
17.	2.887±0.142	2.840±0.130	2.932±0.105	1.319
18.	2.803±0.187	2.787±0.131	2.791±0.098	0.033
19.	2.762±0.177	2.691±0.096	2.736±0.088	0.809
20.	2.694±0.146	2.592±0.112	2.634±0.109	1.484
21.	2.598±0.202	2.513±0.109	2.565±0.108	0.839
22.	2.524±0.203	1.452±0.124	2.448±0.112	0.792
23.	2.459±0.192	2.372±0.160	2.391±0.102	0.853
24.	2.362±0.178	2.298±0.173	2.294±0.112	0.574
25.	2.235±0.184	2.206±0.103	2.238±0.103	0.173
26.	2.113±0.166	2.086±0.062	2.180±0.121	1.503
27.	2.020±0.164	1.989±0.107	2.108±0.119	2.158
28.	1.952±0.152	1.852±0.134	2.016±0.103	3.934
29.	1.820±0.157	1.729±0.182	1.886±0.157	2.284
X	4.830±0.309	5.019±0.203	4.831±0.253	1.765
Y	2.220±0.243	2.838±0.985	2.342±0.173	3.031

Chromosome No.	Hariana R.L.±S.E.	Crossbred R.L.±S.E..	Friesian R.L.± S.E.	M.S. due to breeds
1.	2.	3.	4.	5.
1.	4.762±0.503	4.920±0.204	5.056±0.476	1.212
2.	4.476±0.344	4.510±0.275	4.553±0.325	0.148
3.	4.112±0.622	4.309±0.201	4.286±0.174	0.759
4.	4.165±0.306	4.183±0.141	4.173±0.171	0.016
5.	4.056±0.325	4.066±0.096	4.087±0.175	0.050
6.	3.982±0.344	3.972±0.124	3.963±0.204	0.015
7.	3.862±0.298	3.878±0.154	3.839±0.212	0.074
8.	3.801±0.297	3.752±0.125	3.741±0.180	0.220
9.	3.669±0.324	3.670±0.138	3.670±0.146	0.000
10.	3.601±0.299	3.606±0.101	3.572±0.148	0.085
11.	3.507±0.303	3.523±0.072	3.460±0.139	0.275
12.	3.441±0.282	3.402±0.102	3.295±0.087	1.745
13.	3.336±0.252	3.298±0.100	3.184±0.108	2.193
14.	3.267±0.251	3.165±0.126	3.084±0.082	2.922
15.	3.153±0.219	3.045±0.084	3.029±0.099	2.084
16.	3.091±0.195	2.983±0.306	2.915±0.155	3.546*
17.	2.986±0.193	2.825±0.143	2.805±0.146	3.716*

contd...

Table 4 contd.. (ii)

1.	2.	3.	4.	5.
18.	2.910±0.168	2.729±0.119	2.767±0.124	4.705*
19.	2.813±0.160	2.635±0.128	2.669±0.071	5.711**
20.	2.752±0.152	2.557±0.133	2.598±0.060	7.141**
21.	2.657±0.147	2.427±0.104	2.557±0.069	10.790**
22.	2.581±0.130	2.369±0.109	2.463±0.085	9.359**
23.	2.482±0.151	2.238±0.155	2.381±0.097	7.968**
24.	2.377±0.170	2.188±0.155	2.313±0.082	4.591*
25.	2.298±0.191	2.121±0.137	2.252±0.097	3.900*
26.	2.212±0.176	2.025±0.143	2.128±0.120	4.010*
27.	2.124±0.186	1.937±0.125	2.060±0.121	4.162*
28.	2.066±0.163	1.856±0.094	1.890±0.211	4.782*
29.	1.966±0.139	1.760±0.076	1.767±0.233	5.160*
X ₁	4.240±0.389	4.698±0.274	4.867±0.321	9.562**
X ₂	4.313±0.427	4.871±0.362	5.207±0.358	13.832**

* P / 0.05

** P / 0.01

The relative lengths of autosomes and sex chromosomes in karyotypes of crossbreds females showed small significant differences for chromosome numbers 16th and 29th and 'X' chromosome ($p \leq 0.05$ and $p \leq 0.01$) in comparison to Haryana and Friesian. The results corroborates the reports of Sarkhel and Katpatal (1989) and Gupta and Gupta (1991) where it has been reported that indigenous breeds differ from exotic ones in their shape and size.

Idiograms of the crossbred females depicted them to be intermediate to exotic and native Haryana cattle (Fig. 2). But in case of crossbred males 'X' and 'Y' chromosomes contributed maximum to the haploid genome (5.01 and 2.83). Balakrishnan et al. (1989) explained that gross morphology of autosomes and X chromosomes in exotic and Indian cattle is same while Y chromosome is quite different. This statement does not rule out fine structural variations between chromosomes of two species. The Y chromosome variation is already causing problems of poor fertility in crossbred males as in one generation male the 'X' is from dam (Indian) Y chromosome from sire (exotic) but in second cross the Y will remain same, the X may be of Indian cattle or exotic cattle. Regarding autosomes in first generation one chromosome in each pair is exotic while the other member is Indian but in second cross wide variation is expected in the proportion of

IDIOGRAM OF CATTLE (Males)

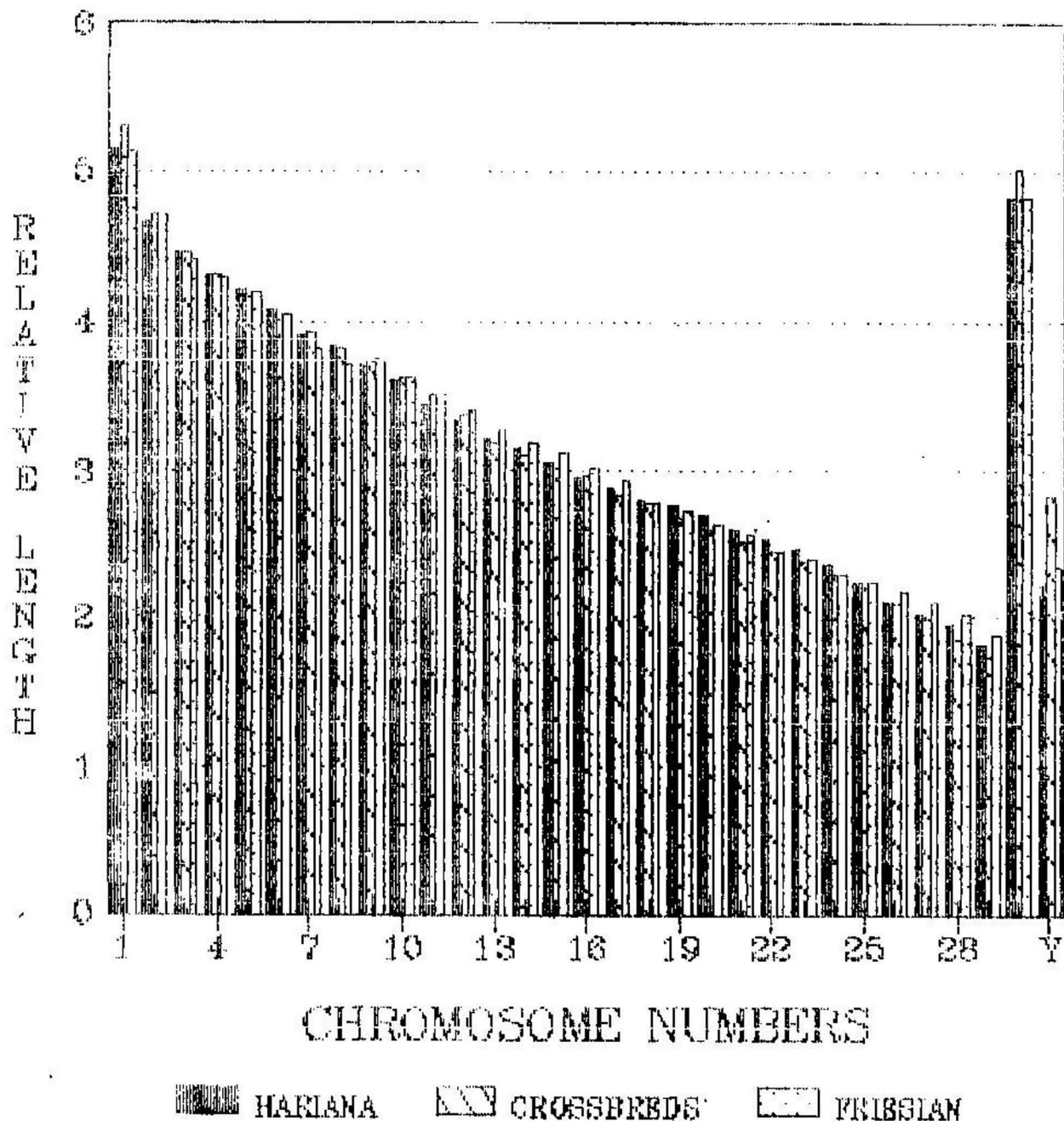


Fig 1. Idiogram in dairy cattle.

IDIOGRAM OF CATTLE (Females)

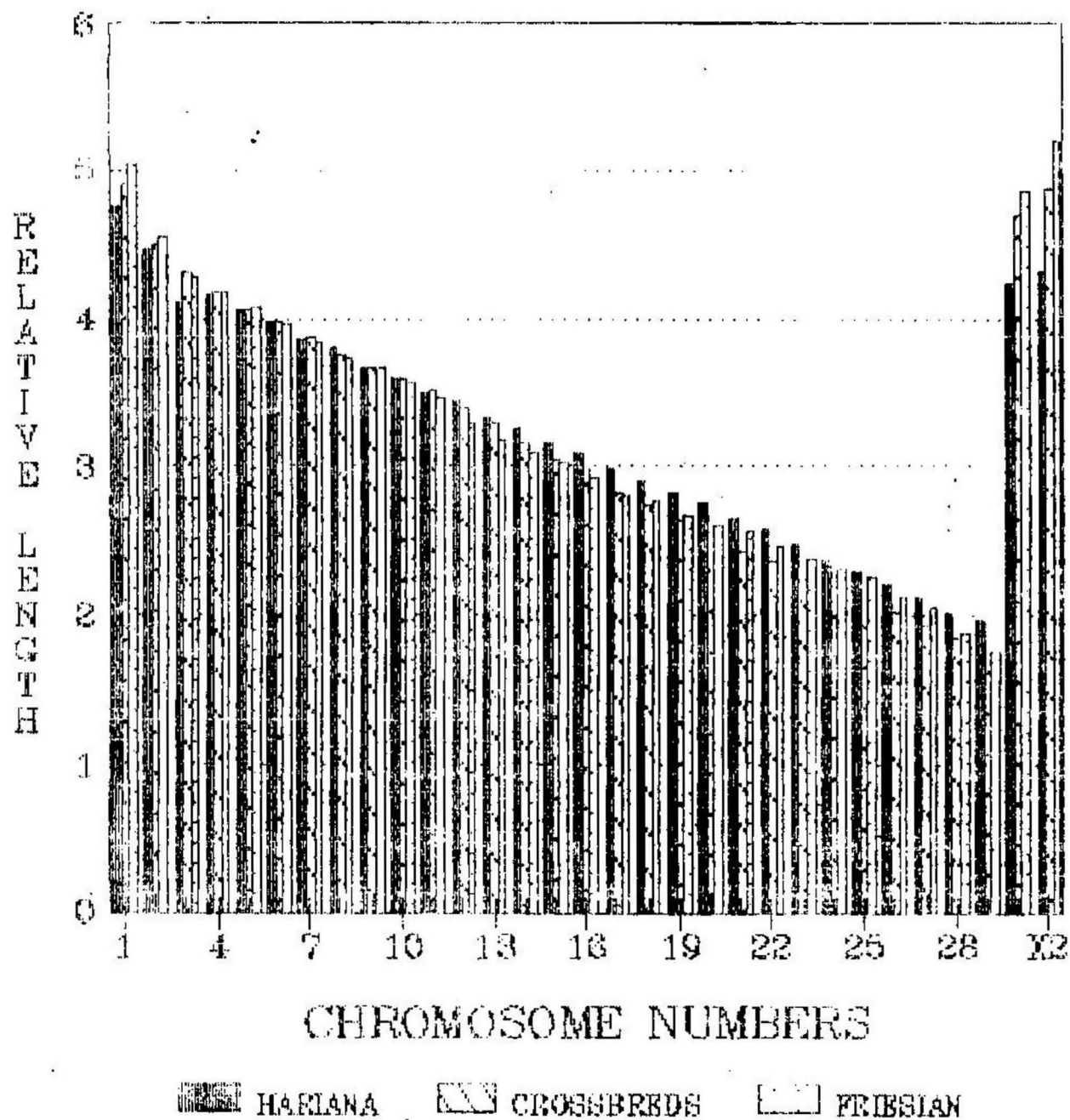


Fig 2. Idiogram in dairy cattle.

exotic and Indian chromosomes. Chromosomal profiles of Haryana Friesian and crossbred animals showed small but non-significant difference in terms of their relative lengths. However, arm ratio and per cent centromeric index of three breeds revealed that 'X' chromosome was submetacentric type (Table 5). The arm ratio was found to be higher in crossbreds in comparison to Haryana and Friesian cattle. This variation in shape might have been due to reciprocal translocations or rearrangement in short arm of the X chromosome during their breed synthesis.

4:4 CHROMOSOME ABERRATIONS IN CATTLE

The inherent stability of karyotype and homology of chromosome is essential for reproductive compatibility among animals of a species. In fact, each species or breed is characterised by its own unique karyotype. Mutations (chromosomal and genic) are responsible for genetic variability. Several types of genetic disorders have been reported which owe their origin due to structural changes of chromosomes (Gustavsson, 1969; Popescu, 1972; Lajde et al., 1976; Eldridge and Balazak, 1977). However, exceedingly low frequencies of aberrations may crop up without serious effect and hence regarded not an unusual feature in normal population.

Table 5. Morphometric parameters of sex chromosome in different breeds of cattle.

Breed	Sex chromosomes 'X'	
	Male	Felae
	Arm Ratio	
Hariana	2.26	2.00
Crossbreds	1.78	1.87
Friesian	1.99	1.77
	Centromeric Index (%)	
Hariana	31.41	33.54
Crossbreds	36.54	35.05
Friesian	33.71	36.22

Well spread and complete 300 metaphases were scored for each sex for the frequency of chromosomal aberrations. Very low levels of chromosomal aberrations have been noted (Table 6) without any adverse effects in three breeds. There was preponderance of chromatid type aberrations (Plate 10 to 15). It was found to be higher among the Haryana animals (5.66 %) in comparison to Friesian (4.0 %) and crossbreds (5%), respectively. It may be assumed that this incidence represent base level of chromosomal aberrations. In fact, there is paucity of literature on the incidence of aberrations in normal animals (Yadav, 1981; Sikka, 1988).

4.5 CHROMOSOME HETEROMORPHISM IN CATTLE

Chromosome heteromorphism depicts variation between homologous pairs of chromosomes of a species. Three different types of banding techniques like G-, C- and NOR's were applied to study the homology of chromosomes in Haryana, crossbreds and Holstein Friesian animals.

Casperson and associates (1971) discovered fluorescent staining technique for identifying zones of varying intensities. These were used as landmarks for the pairing of chromosomes. Arrighi and Hsu (1971) found that denaturation and reassociation followed by Giemsa staining produce characteristic bands along the length of chromosomes.

Table 6. Frequency of chromosomal aberrations (%) in the somatic chromosomes of Hariana, crossbreds and Friesian animals.

Breed	Sex	Total No. of metaphase scored	Total chromosomal aberrations	Type of chromosomal aberrations					
				CG	CB	iCG	ICB	Polyp.	CEB
Hariana	Male	300	5.66 (17)	2.66 (8)	1.66 (5)	0.66 (2)	0.33 (1)	0.33 (1)	-
	Female	300	4.66 (14)	2.00 (6)	1.33 (4)	1.00 (3)	-	-	0.33 (1)
Crossbreds	Male	300	4.33 (13)	1.33 (4)	1.00 (3)	1.00 (3)	0.33 (1)	-	0.66 (2)
	Female	300	5.00 (15)	1.66 (5)	0.66 (2)	2.00 (6)	0.33 (1)	0.33 (1)	-
Friesian	Male	300	3.33 (10)	1.33 (4)	1.33 (4)	0.33 (1)	0.33 (1)	-	-
	Female	300	4.00 (12)	1.00 (3)	2.00 (6)	-	0.66 (2)	0.33 (1)	-

Figures in parentheses indicate the number of observations.

CG= Chromatid gap, CB= Chromatid break, ICG= Isochromatid gap,

iCB= Isochromatid break, Polyp.= Polyploidy.

CEG= Cent. gap

The methods for the display of constitutive heterochromatin made chromosome identification more reliable (Hsu, 1973; Schwarzacher and Wolf, 1974; Dutrillaux and Lejeune, 1975). Banding techniques also made it possible to locate structural alterations leading to syndromes in human beings. Evans et al. (1973) reported weak C band staining of sex chromosomes in exotic breeds of cattle. While Gupta et al. (1974) reported all autosomes to be C band positive. No indication of weakly stained centromeric region was reported in Red Sindhi and Sahiwal bulls. The sex chromosome viz., submetacentric X as well as acrocentric Y lacked centromeric heterochromatin.

In metaphase chromosome preparations in Haryana, crossbred and pure Friesian cattle all the chromosomes are found to be weakly stained at the centromeric region (Plate 15). The Y chromosome was small and acrocentric type in Haryana. It was easily distinguishable from other smaller autosomes because of its C-band negative nature. Interstitial or telomeric bands were not detected. Kurnit et al. (1973) reported chemical difference between the sex chromosomal and autosomal centromeric regions using in situ hybridization for the differential C band staining. In the present investigation no such difference was observed.

G bands have been demonstrated in several breeds of Bos taurus cattle (Schnedal, 1972; Evans et al., 1974;

Masuda et al., 1974; Potter et al., 1979; Halnan et al., 1981; Krutzler et al., 1986). Halnan et al. (1981) compared the G band patterns of Sahiwal, Red Sindhi, Brahaman, Santa Gertrudis, Africander and hybrids. They observed few G band differences among Bos indicus and Bos taurus animals. Y chromosome was found to be acrocentric with no or small 'p' arm while the 'q' arm was having two dark bands at proximal region and a third band at distal region. In case of X chromosome it was reported that the central pale region was narrower and bands of distal region were not so prominent.

G bands were successfully developed in three days old slides of Hariana, crossbred and Friesian cattle (Plates 16, 17). All the chromosomes contained bands. Due to the substandard contrast in the coloured photomicrographs precise locations of the diagnostic bands could not be depicted.

The NOR's of chromosomes reveal sites of 18 S and 28 S ribosomal RNA (Matsui and Sasaki, 1973; Howell et al., 1975). Henderson and Bruere (1979) described 10 NOR's in cattle, goat and sheep. The NOR's of cattle Bos taurus were located on chromosome number 2, 3, 4, 11 and 28 (Pinherio et al. 1982). Studies on the inheritance of Ag-NOR's in man

(Marcovie et al., 1978), pigs (Christensen, 1980) and rabbits (Arruga, 1989) suggest that inheritance of Ag-NOR's follows mendelian fashion but there are reports where unusual patterns of Ag-NOR on chromosomes of cattle have been reported (Parkash and Balakrishnan, 1990). They reported the NOR sites on five pairs of autosomes as 2, 3, 4, 5 and 28. Thus, NOR polymorphism has been reported by different workers for identifying genetic markers among different breeds.

In the present investigation metaphase chromosomes stained for Ag-NOR's showed variation in the number of NOR's 5 to 8 pairs (Table 7). These were present on telomeric regions of autosomes. Karyotyping revealed that chromosomes invariably associated with NOR's were 2, 3, 4, 11 and 28 (Plates 18, 19). Similar locations of NOR's in cattle have been reported by Mayr and Gruber (1987), Mayr et al. (1987) and Sashikanth et al. (1991). The number and location of NOR's were similar in three breeds studied. In interphase nuclei the number of nucleoli ranged between 4 to 6 with preponderance of cells with five nucleoli (Table 8). These were found as darkly stained spots in the cells.

Table 7. Frequency of Ag-NOR's (%) in metaphase cells of Hariana (Bos indicus) crossbreds and Holstein Friesian (Bos taurus) cattle.

Breed	Sex	No. of meta- phase scored	No. of metaphase plates with NORs	Total NORs (%)		
				Upto 5 pairs	Upto 6 pairs	Upto 8 pairs
Hariana	Male	200	77.00 (154)	37.00 (74)	21.50 (43)	18.50 (37)
	Female	200	71.50 (143)	40.00 (80)	16.00 (32)	15.50 (31)
Crossbreds	Male	200	74.50 (149)	39.00 (78)	17.50 (35)	18.00 (36)
	Female	200	73.00 (146)	36.50 (73)	21.00 (42)	15.50 (31)
Friesian	Male	200	70.50 (141)	34.50 (69)	19.00 (38)	17.00 (34)
	Female	200	69.50 (139)	31.50 (63)	24.00 (48)	14.00 (28)

Figures in parentheses indicate the number of observations.

Table 8. Incidence of nucleoli (%) in non-metaphase nuclei of Hariana, crossbreds and Friesian cattle.

Breed	Sex	No. of cells scored	Total No. of nucleoli	Cells with No. of nucleoli		
				4	5	6
Hariana	Male	200	76.00 (152)	18.00 (36)	42.00 (84)	16.00 (32)
	Female	200	71.50 (43)	25.50 (51)	36.50 (73)	9.50 (19)
Crossbreds	Male	200	74.50 (149)	24.50 (49)	39.00 (78)	11.00 (22)
	Female	200	77.50 (155)	17.00 (934)	34.50 (69)	26.00 (52)
Friesian	Male	200	69.00 (138)	27.50 (55)	24.00 (48)	17.50 (35)
	Female	200	67.50 (135)	21.00 (42)	28.00 (56)	18.50 (37)

Figures in parentheses indicate number of observations.

4.6 CYTOGENETIC DIVERGENCE

Mahalanobis- D^2 statistic was applied to study the cytogenetic divergence in three breeds. The relative lengths of autosome number 1 as well as sex chromosome X and Y were measured and analysed.

D^2 -values among males for the three traits was found to be non-significant (Table 9). However, it was found to be maximum for the Haryana : crossbreeds group (1.191), while it was 0.047 for Haryana : Friesian and 0.942 for crossbreeds : Friesian group.

D^2 -values for the females in three breeds were also analysed and found to be significant (Table 9). Haryana : Friesian group showed maximum D^2 -value (7.83) while Friesian : crossbreeds group was having intermediate and significant D^2 -value (2.66). This was observed close to the D^2 -value between Haryana : crossbred (2.06). Cluster analysis revealed that Friesians can be grouped under one cluster while Haryana and crossbreeds form a separate cluster on the basis of D^2 -values. Sharkel and Katpatal (1989) also reported the similar pattern of cytogenetic divergence among Bos taurus, Bos indicus and crossbred cattle.

Table 9. D^2 statistics for cytogenetic traits in Hariana, crossbreds and Friesian animals.

Genetic group	D^2 -values	
	Male	Female
Hariana : crossbred	1.1914	2.0608
Hariana : Friesian	0.0470	7.8361**
Crossbred : Friesian	0.9423	2.6682*

* $P/0.05$

** $P/0.01$

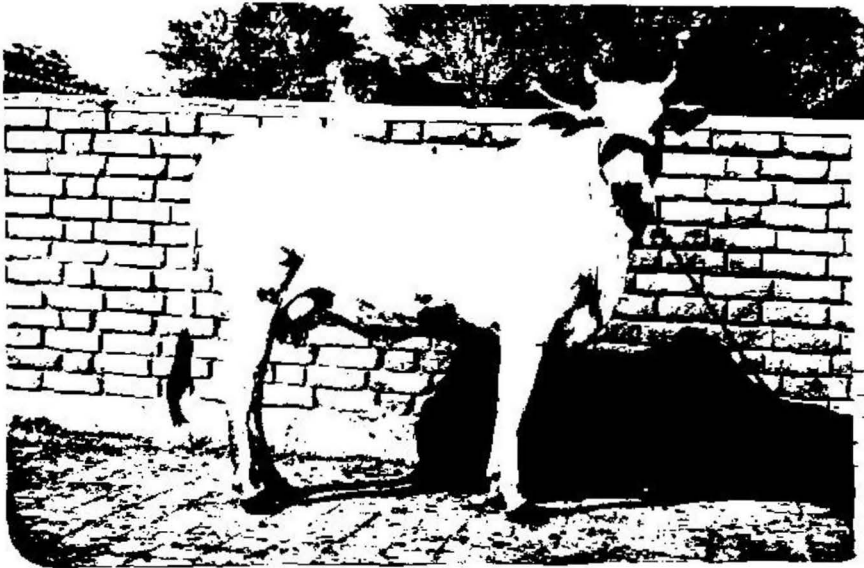
5. SUMMARY AND CONCLUSIONS

- 5.1 Chromosomal heteromorphism was studied in somatic chromosomes of Hariana, pure Holstein Friesian and their crosses on the pattern of morphometric parameters.
- 5.2 Evaluation of somatic metaphase plates revealed preponderance of cells having sixty chromosomes in three dairy groups. The study reaffirms a diploid count of sixty ($2n = 60$) in Indian and exotic breeds. Crossbreds also showed similar diploid count.
- 5.3 All the 58 autosomes were acrocentric. The differences in consecutive pairs were not visibly striking.
- 5.4 The sex chromosomes in three breeds studied were of typical mammalian type being XX and XY in female and male animals, respectively.
- 5.5 The X-chromosome was largest and submetacentric in three breeds and was close to first and second pair of autosomes in size. The Y-chromosome showed heteromorphism. It was small acrocentric type and comparable in length to 25th autosome in Hariana animals, while pure exotic animals and their crosses with Hariana were having small metacentric Y chromosome. However, Y chromosome showed no significant variation in relative lengths for the three breeds, respectively.

- 5.6 On the basis of relative length of the chromosomes, it has been inferred that X chromosome was "original" type as contributing 5% of the total haploid genome in three breeds. However, karyotypes of crossbred females revealed small significant variation for autosomes (16th to 29th) and X-chromosome in relation to Hariana and Friesians. Idiograms of crossbred females depicted them to be intermediate to exotic and native Hariana breed. The sex chromosomes (X and Y) in crossbreds showed higher contribution to the haploid genome.
- 5.7 Chromosomal profiles of Hariana, Friesian and crossbred animals showed small but non-significant differences for relative lengths. However, arm ratio and centrometric indices of three breeds revealed that X chromosome was submetacentric type. Crossbred animals showed higher values of arm ratio in comparison to Hariana and Friesian breeds.
- 5.8 Scoring of metaphases showed the presence of structural aberrations only. However, the incidence was extremely low without any adverse effects in three breeds.
- 5.9 By the application of special staining techniques C bands have been demonstrated in all the autosomes of three breeds. C bands could not be discerned in sex chromosomes (X and Y).

- 5.10 G bands were present in all the chromosomes. These were of varying intensity and can serve as additional marker in identification and pairing of homologous chromosomes.
- 5.11 The nucleolar organizer regions (NORs) were observed in 2, 3, 4, 11 and 28 pairs of chromosomes in three breeds. The metaphase plates of Hariana showed large sized NORs in comparison to Friesian and crossbred animals.
- 5.12 Model karyotypes of Hariana, Friesian and crossbred animals with conventional Giemsa staining, C band, G band and NOR staining have been constructed. Idiograms of chromosomes have also been presented on the basis of relative lengths.
- 5.13 Cytogenetic divergence for the autosome number one and sex chromosomes (X and Y) were measured and analysed in three breeds. Male animals showed no significant divergence. However, cluster analysis in females revealed that Friesians can be grouped under one cluster while Hariana and crossbred form a separate cluster.

PLATE 1



HARIANA COW

PLATE 2 KARYOTYPE OF HARIANA MALE

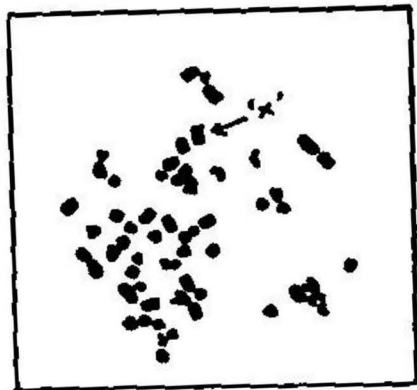
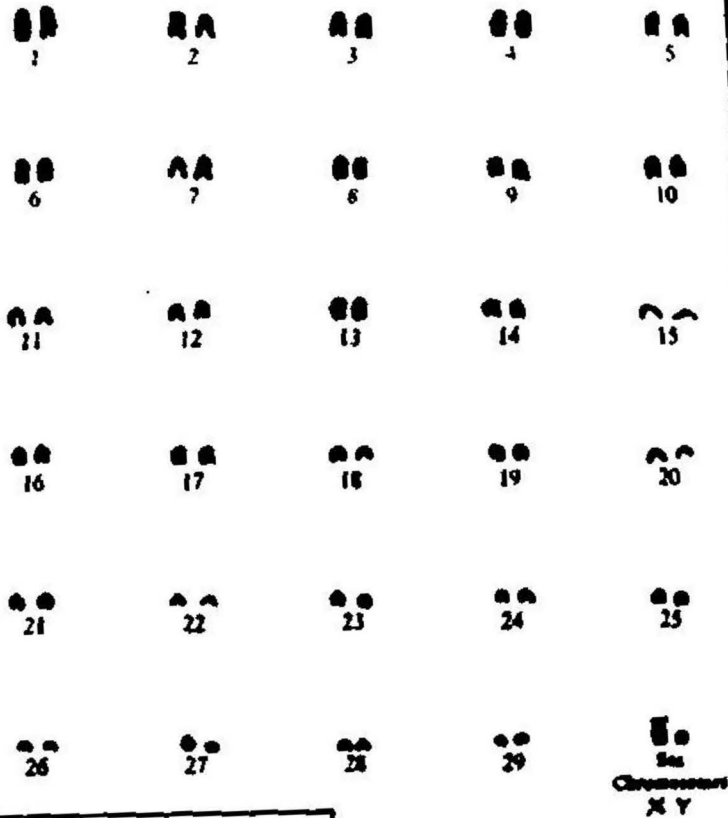


PLATE 2 KARYOTYPE OF HARIANA MALE

PLATE 3 KARYOTYPE OF HARIANA MALE

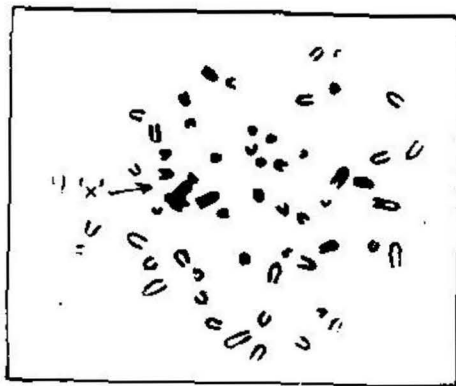
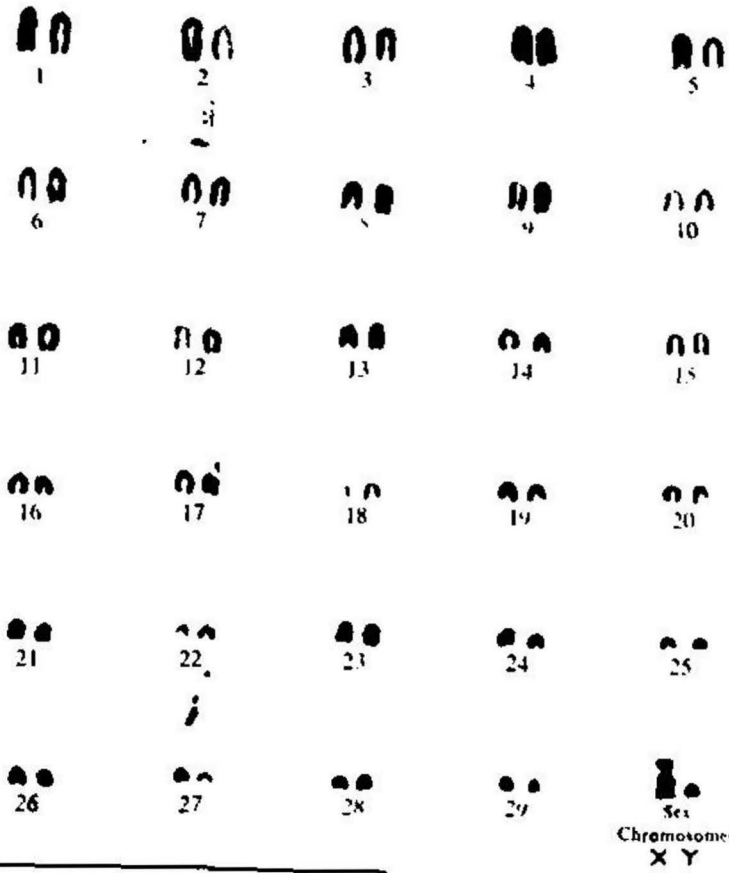
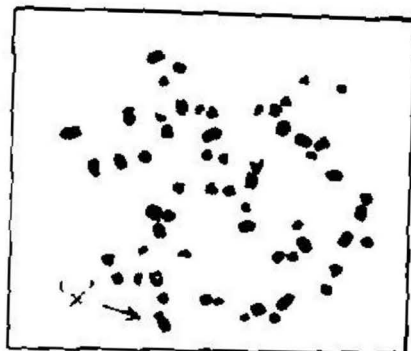
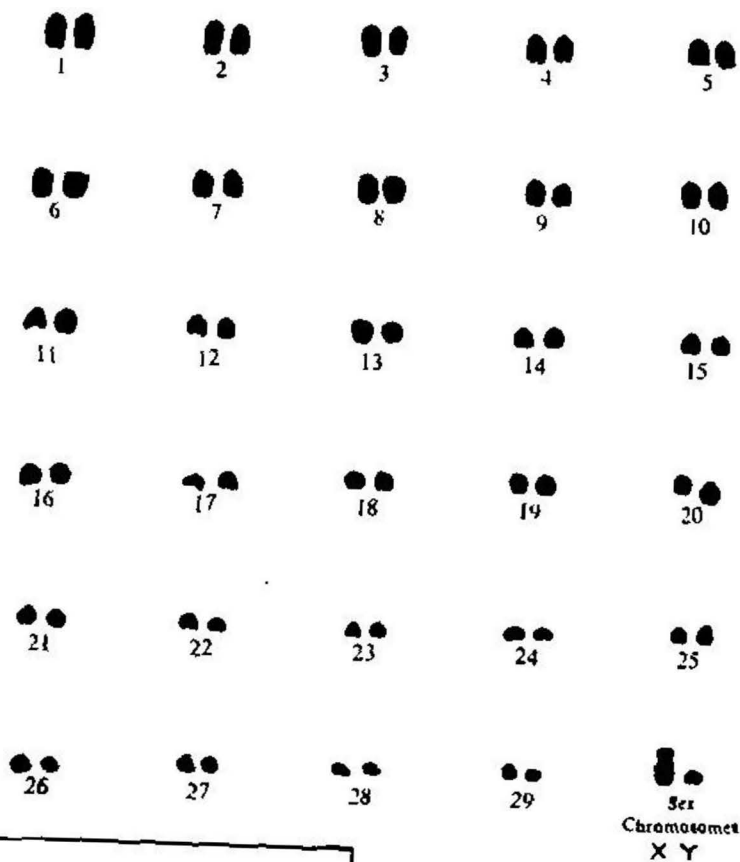


PLATE 3 KARYOTYPE OF HARIANA MALE

PLATE 4 KARYOTYPE OF HARIANA MALE



KARYOTYPE OF HARIANA FEMALE

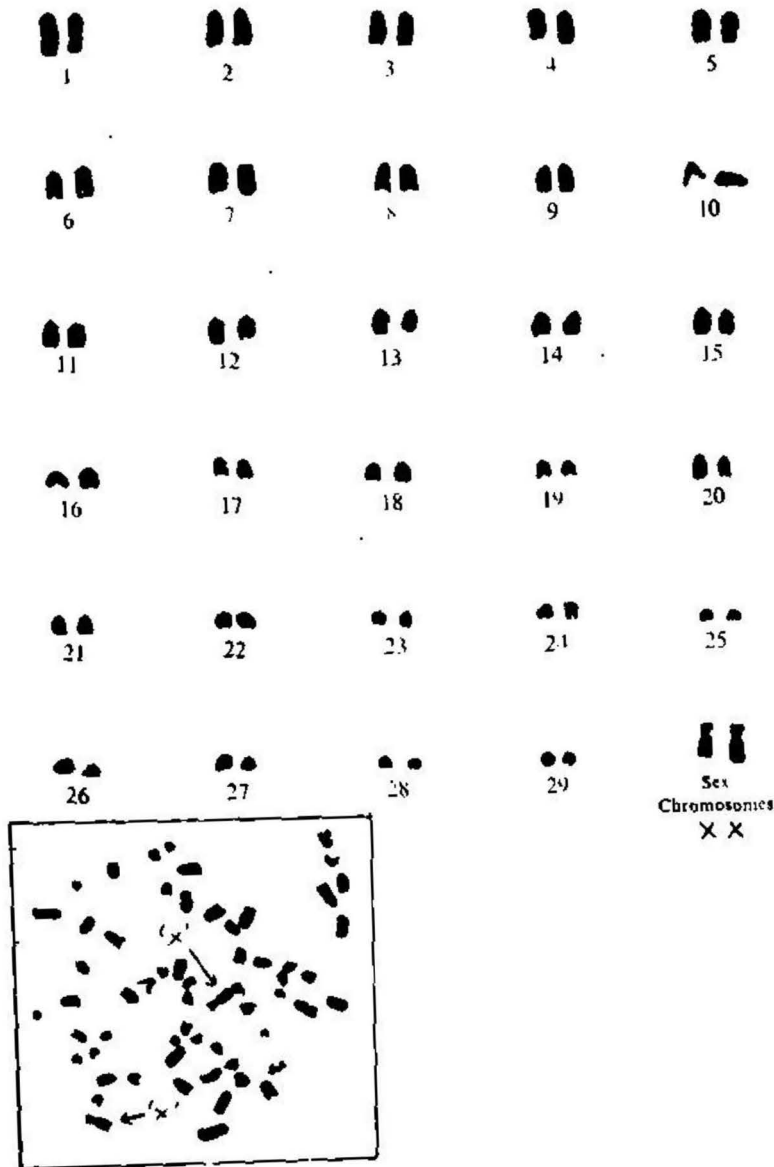


PLATE 5 KARYOTYPE OF HARIANA FEMALE

KARYOTYPE OF FRIESIAN MALE

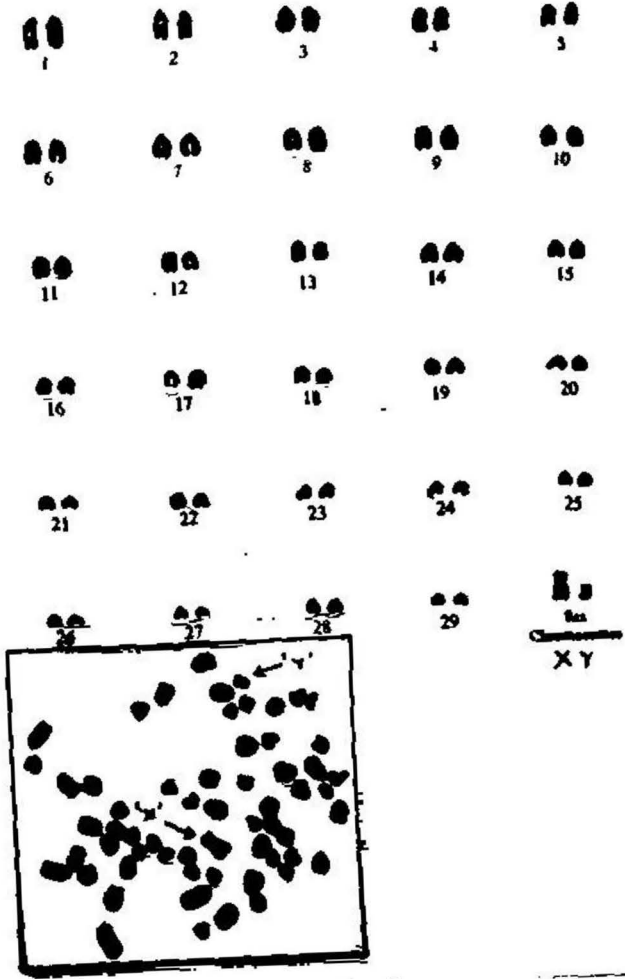


PLATE 6 KARYOTYPE OF FRIESIAN MALE

KARYOTYPE OF FRIESIAN FEMALE

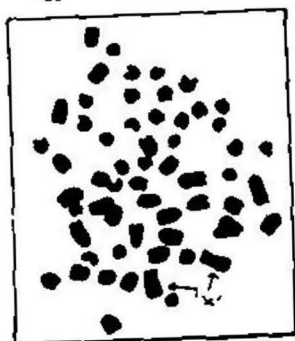
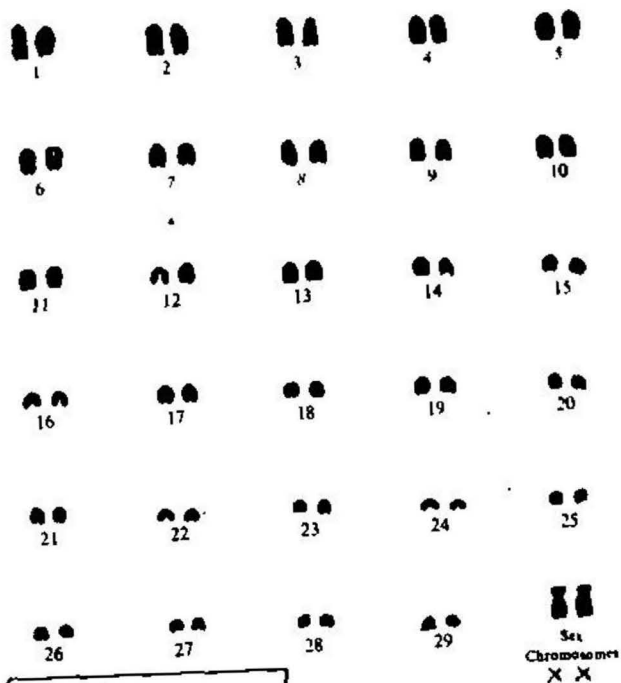


PLATE 7

KARYOTYPE OF FRIESIAN FEMALE

PLATE 8 KARYOTYPE OF CROSSBRED MALE

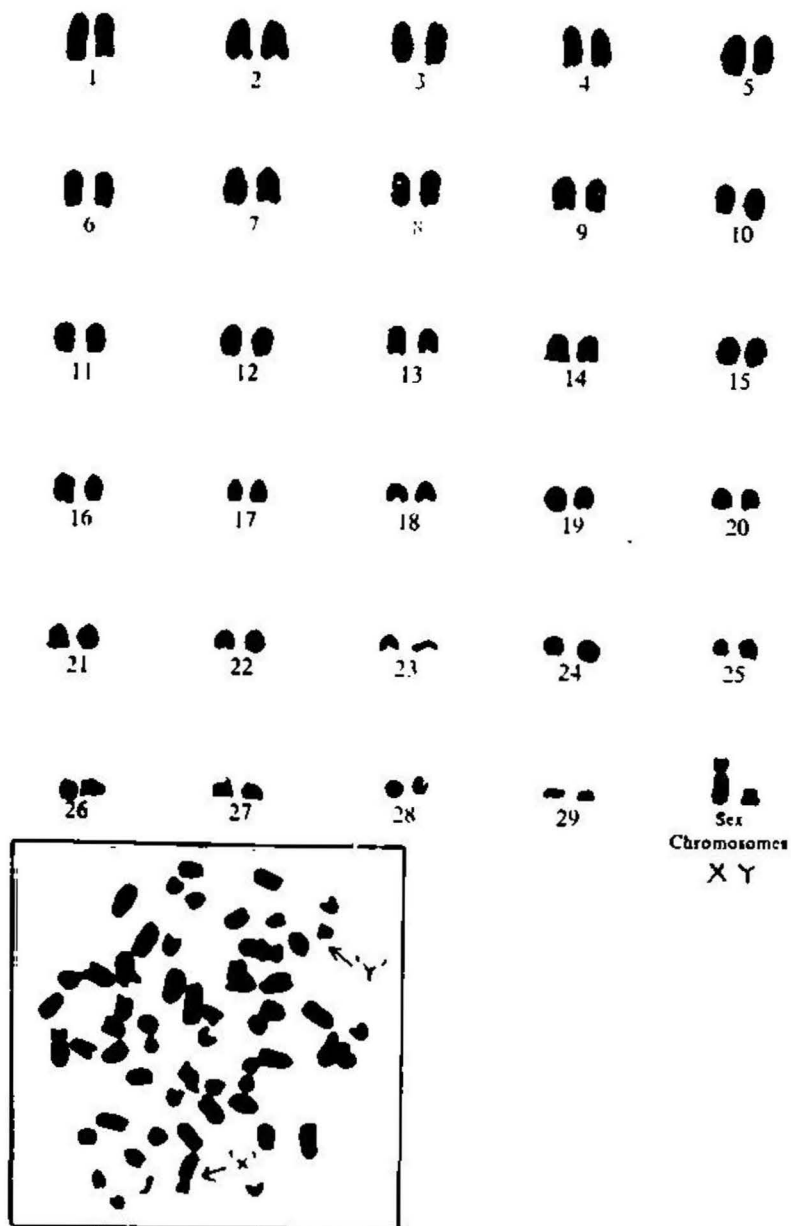


PLATE 8

KARYOTYPE OF CROSSBRED MALE

PLATE 9 KARYOTYPE OF CROSSBRED FEMALE

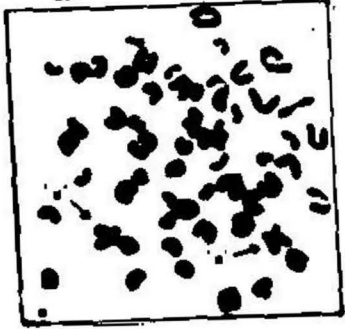
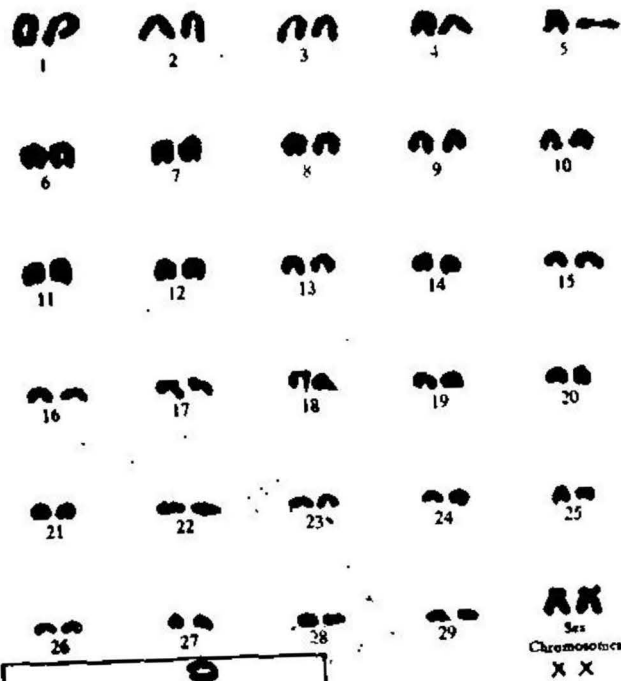
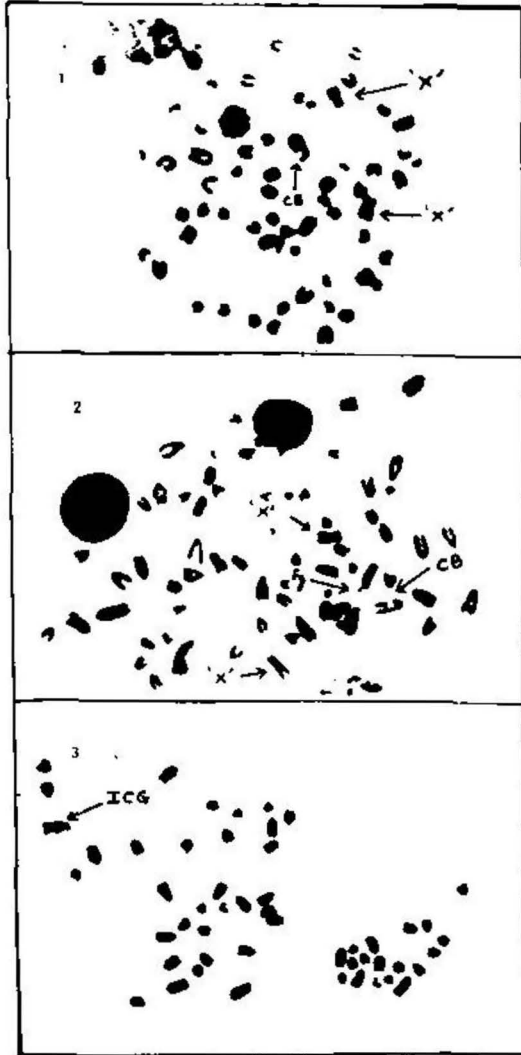


PLATE 9 KARYOTYPE OF CROSSBRED FEMALE.

METAPHASE PLATES SHOWING ABERRATIONS



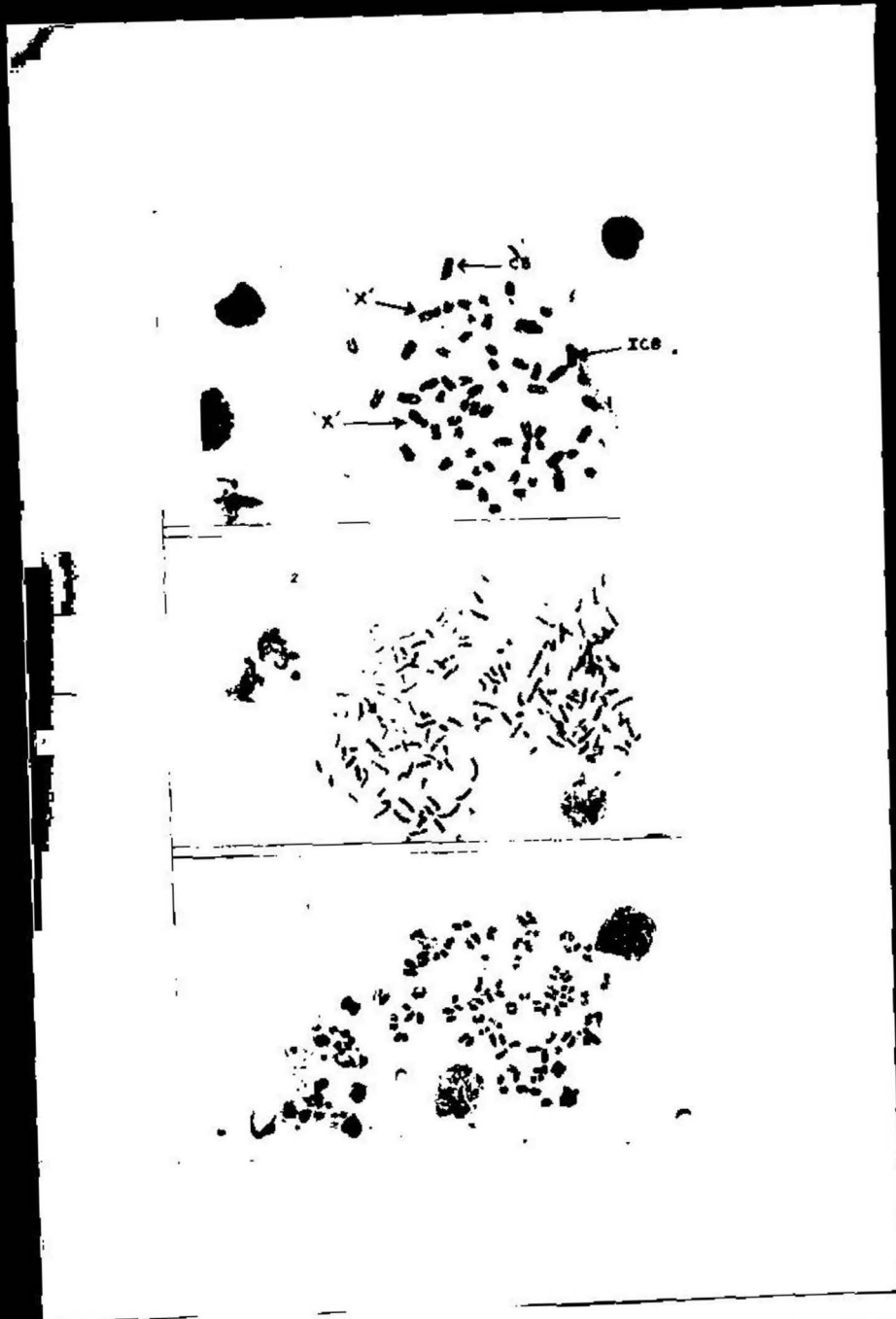
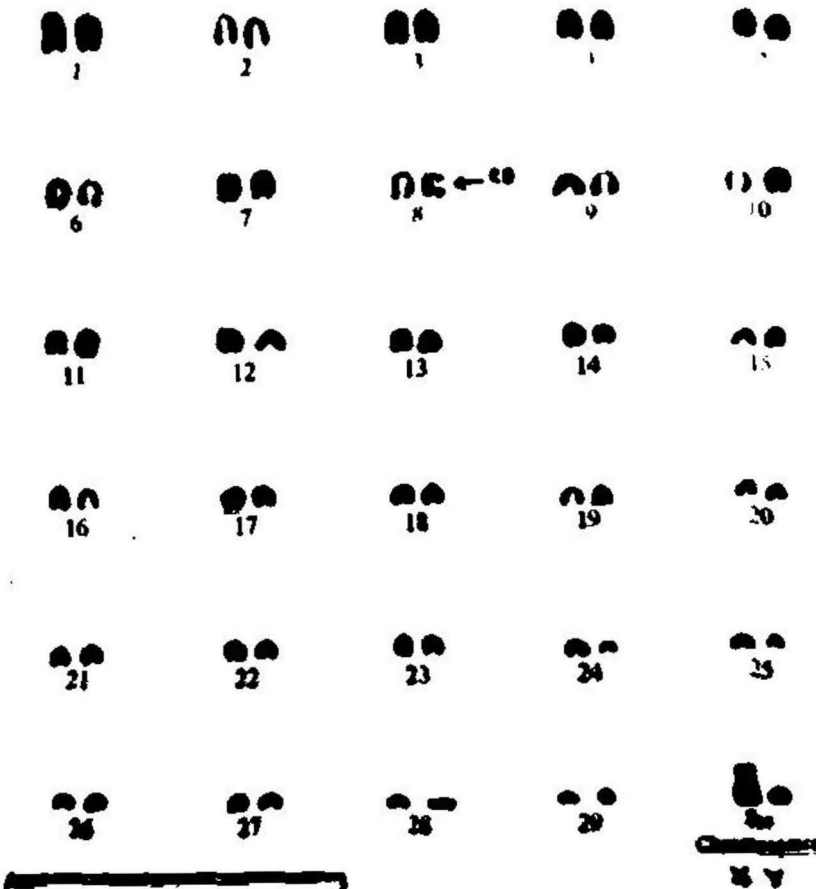


PLATE 11

METAPHASE PLATES SHOWING ABERRATIONS

KARYOTYPE OF HARIANA MALE SHOWING ABERRATIONS



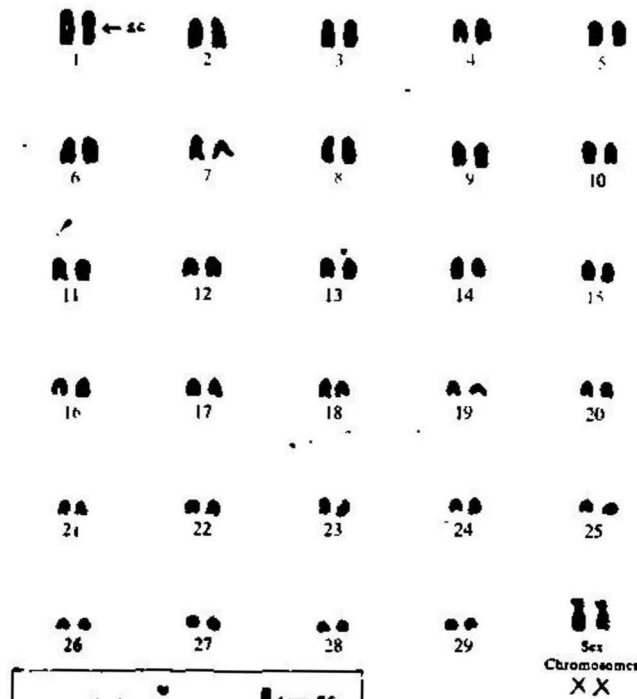


PLATE 13

KARYOTYPE OF HARIANA FEMALE SHOWING ABERRATIONS

PLATE 14 KARYOTYPE SHOWING ABERRATIONS

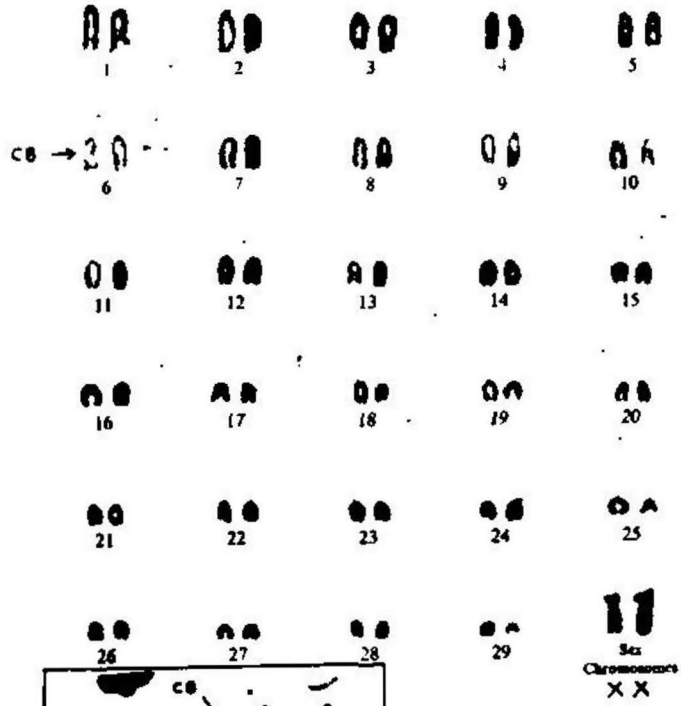
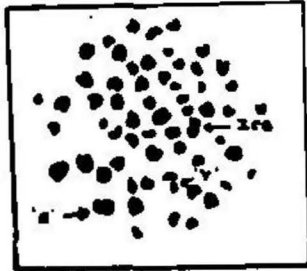
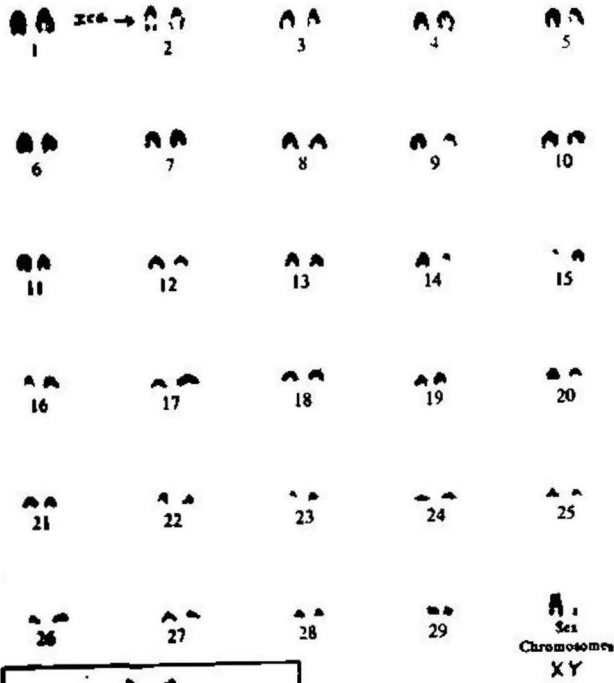


PLATE 14

KARYOTYPE SHOWING ABERRATIONS

PLATE 15 KARYOTYPE SHOWING ABERRATIONS &

(C Banding)



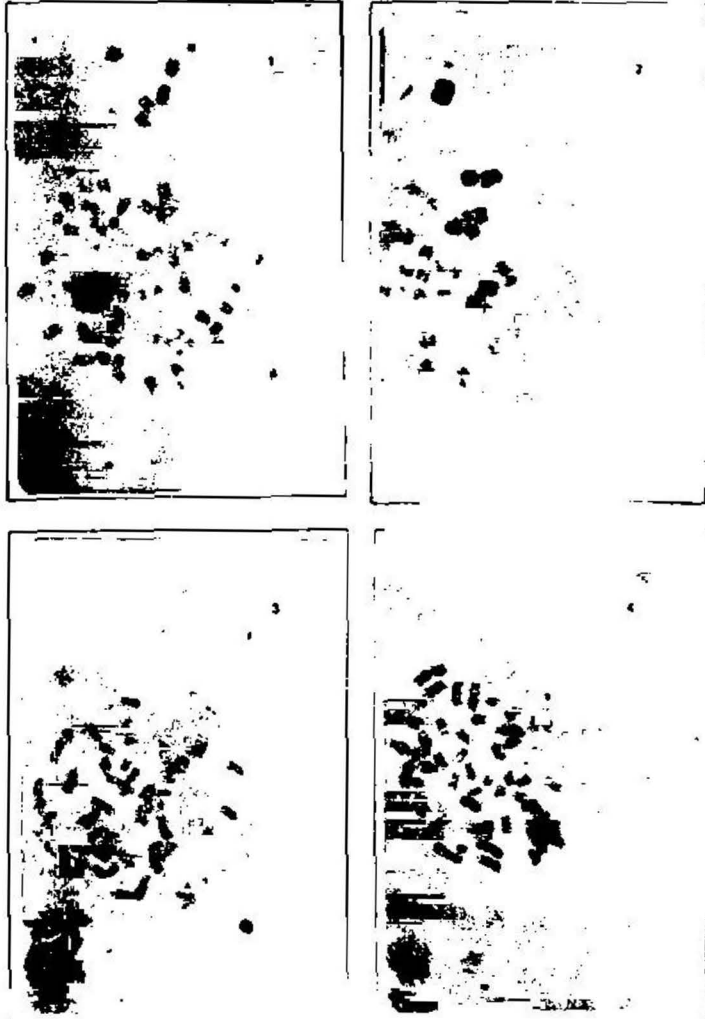


PLATE 16

METAPHASE PLATES SHOWING G-BANDINGS

PLATE 17 KARYOTYPE SHOWING G-BANDINGS

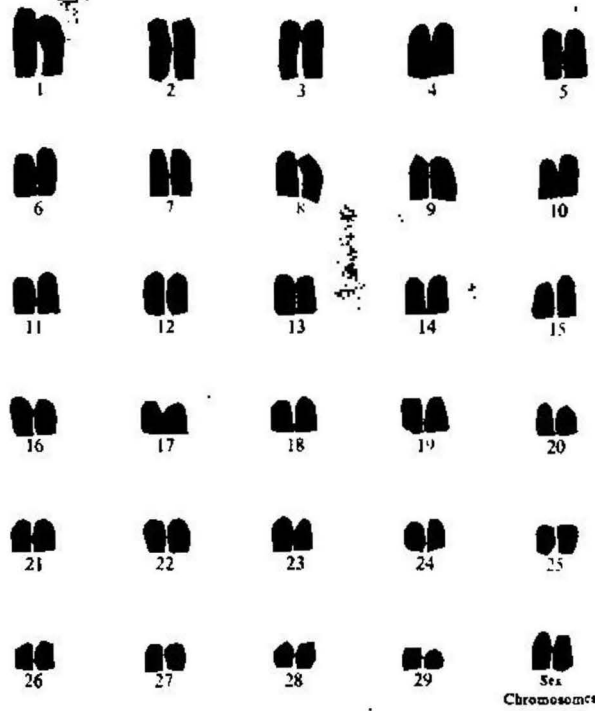


PLATE 17

KARYOTYPE SHOWING G-BANDINGS

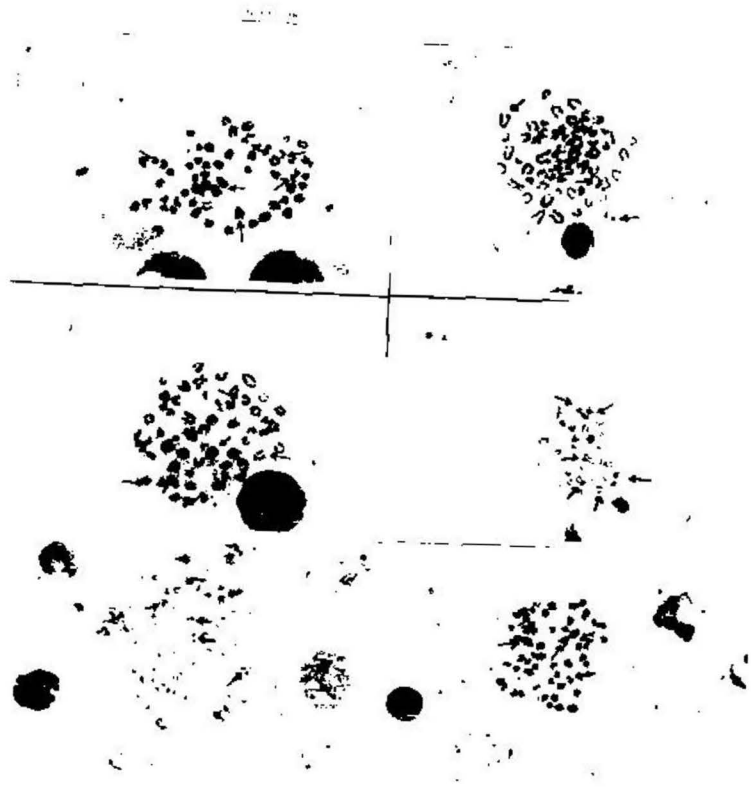


PLATE 18

METAPHASE PLATES SHOWING Ag-NOR'S

PLATE 19 KARYOTYPE SHOWING Ag-NOR'S

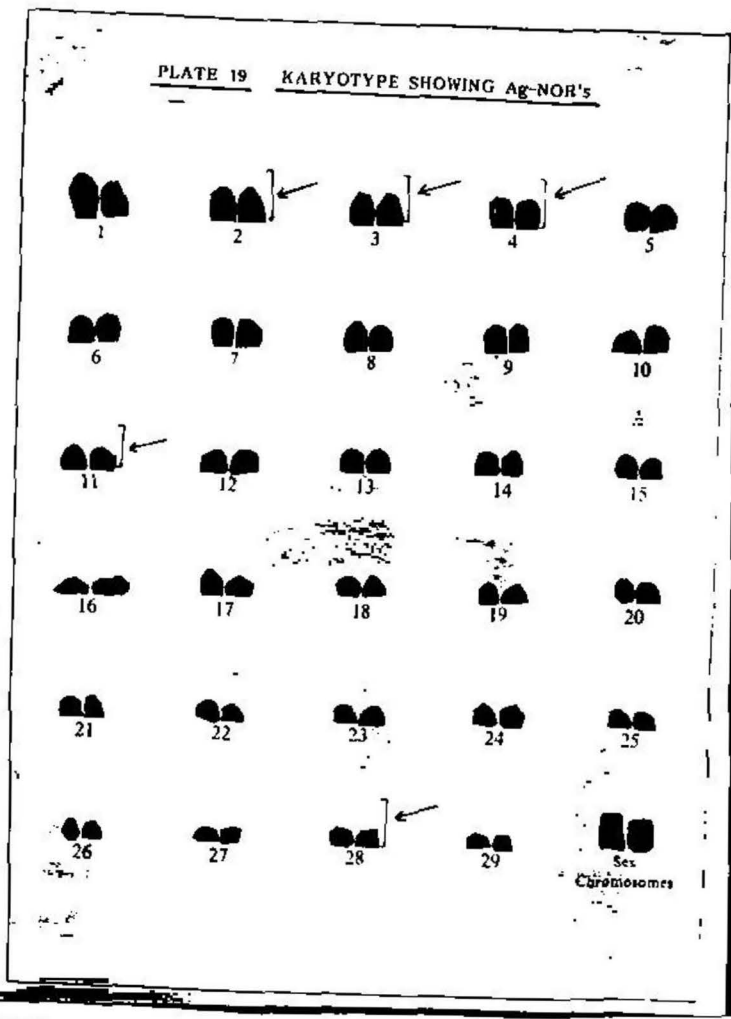


PLATE 19

KARYOTYPE SHOWING Ag-NOR'S

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