CHROMOSOME PROFILE OF ZEBU X TAURUS CATTLE IN KERALA

By

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THESIS

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[redacted]

DECLARATION

I hereby declare that this thesis entitled "Chromosome profile of Bos x Taurus cattle in Kerala" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Mannuthy,
17-10-1988.

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CERTIFICATE

Certified that this thesis entitled "Chromosome profile of Zebu x Taurus cattle in Kerala" is a record of research work done independently by Sri.K.V. Raghunandanan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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Introduction

INTRODUCTION

Every organism whether it is unicellular or multicellular, needs a set of directives for its creation, growth, reproduction and finally death. In case of multicellular organisms the creation process includes fertilization, differentiation and organogenesis. All these processes involve series of complex biochemical, hormonal, physiological and physical activities which are directly or indirectly controlled by genes. For ease of ordered activity, replication and precise distribution in multiplying cells, the genes are contained in packages known as chromosomes.

Although the interest in cells and chromosomes began almost a century ago it gained momentum, when it was realised that these organelles are repository of genetic information. As a result, of great enthusiasm evinced, thereafter, by the scientific workers, genesis of a new area of cytogenetics, a hybrid between cytology and genetics took place.

The cytogenetics actually correlates the microscopically discernible structures, the chromosomes and its morphology to the phenotype. The genetic material in all animal species is located in specific structures termed chromosomes. All cells of the body possess a complete chromosome set. The number, shape and size of chromosome in the cells of a particular species are usually constant and fairly typical for that species.

Precise quantification of shape, structure or size of chromosome and the determination of model diploid number for a particular species are primary pre-requisites for identification and description of the chromosome and their position in karyotype array. This knowledge later on, led to establish the involvement of chromosomes in various physiological functions. Owing to the fact that chromosomes are the repositories of genetic information and directly involved in the transmission of heredity the elucidation of structural details of chromosomes has been a subject of research for long time.

The intense studies of human chromosomes for the last two decades revealed the importance of cytogenetics in normal as well as abnormal conditions. But only after 1960 the chromosome techniques however, became available to enable accurate and detailed observations of chromosomes of mammals and birds. Since then, catalogue of methods began to lengthen and thereby detailed and refined analysis enabled to understand not only of the gross genome but of the morphological fine structures of individual chromosome. The development of in vitro culture technique of cells opened a new vista for cytogenetic studies. The science of cytogenetics was thereafter applied for finding out association between variations in chromosome number and morphology with gross anatomical or physiological features of animals as a whole.

The identification of chromosome aberrations and its correlation with conception rate in animals established

clinical application of karyotype in veterinary practice. The inclusion of cytogenetic analysis in the diagnostic armamentarium of laboratories working with animal infertility would enable finding primary cause of difficulty in a number of cases which cannot be diagnosed otherwise.

There are three main areas in which cytogenetics can contribute to the theory and practice of animal breeding. Much of animal breeding is based on assumptions for which critical tests heretofore have not been available, cytogenetic techniques can be adopted to test the validity of some of these assumptions. Secondly, new sources of genetic variation may be made available to animal breeders by cytogeneticists. Thirdly, the large number of chromosome markers segregating in animal population enables accurate assessments of the relationship among animals within populations and determination of relationships of populations, one to another.

The problem of anatomical and physiological anomalies or subnormal conditions in interspecific and intraspecific hybrids has attracted attention of animal breeders as well as cytogeneticists. The introduction of refined techniques for chromosome preparation has helped the understanding of possible cytogenetic reasons for reproductive disorders in the mammalian hybrids. There is a considerable variation both between species and within species in chromosome morphology which helped in assessing the genesis of particular breed or association between breeds.

The domestic cattle belong to the order Artiodacytyla and super family Bovidae which has five subfamilies. Though the diploid number may vary between 30 and 60 the nombre fundamentale (number of chromosome arms) was found to be restricted between 58 to 62 with majority of them at 60. This reveals the extent of conservation in the karyotype in this family. Speciation seems to have taken place by fusion or fission of a maximum of 60 chromosomes. This indicates that it is in the gene order more than the nature of genes which has dictated the variations between the species.

As a result of a massive cross-breeding programme involving European (Bos taurus) and indigenous non-descript (Bos indicus) cattle, merely 56 per cent of the cattle population in the State of Kerala is cross-bred. According to the recent report published by Department of Animal Husbandry (1987) it is estimated that 43.9 per cent of the reproductive disorders diagnosed is due to delayed puberty which is suspected to have genetic disposition. The infertility due to repeat breeding, ovarian hypoplasia, defective genitalia, specific and non-specific infection have also been identified.

However, systematic studies on the cytogenetics of cross-bred cattle has not been attempted in this State. Hence the present study is undertaken with the following objectives:

1. Comparative analysis of the karyotype of Zebu x Taurus crosses and purebreds.

2. Assessing the incidence of chromosomal aberrations among cross-bred cattle.

3. Finding out the possible association of chromosomal aberrations, if any, with reproductive problems and other abnormalities.

Review of Literature

REVIEW OF LITERATURE

Human chromosomal study as a prelude to mammalian cytogenetics

Development and application of cytological techniques in man are older than those in animals and birds. The scope of cytogenetics in animals and birds is vast, especially in the areas of breeding, reproduction, management, clinical medicine and various evolutionary studies. In order to reach the level of cytogenetic research in man, animal cytogeneticists have to go a long way, since these techniques have been applied for the last twenty years only. The great deal of information on the different aspects of human genetics has been the direct consequences of the process in sophistication of methodology employed.

A whole succession of events and discoveries relating to cells and genetics were divided by Swanson et al. (1982) into pre-cytogenetic era, classical period and modern period. Pre-cytogenetic era starts with the discovery of cells by Hooke in 1766 and ends in 1896 with the publication of The Cell in Development and Heredity by Wilson. Hybridisation of cytology with genetics was carried out in 1902-1903 by formulations of the Suttan and Boveri chromosomal theory of inheritance. The discovery of Mendel's laws and its recognition in 1900 is taken as the beginning of classical period. McClung correlates the X chromosome in sex determination in 1901. In 1906 distinction was made between heterochromosomes

(X and Y) and autosomes by Montgomery. Blakeslee and Eigsti (1937) discovered the use of colchicine as mitotic inhibitor. Hsu in 1952 employed technique of osmotic shock for the study of mammalian chromosomes. Tijo and Levan (1956) accurately determined the number of chromosomes in a human cell.

Till 1959, the cells were collected from human tissues directly and used as such for chromosomal studies. For the first time, Hungerford et al. (1959) demonstrated peripheral blood leucocyte culture technique and explained chromosome constitution of a human phenotypic intersex. They reported that the functions of Y chromosome are not clear and it does not seem essential in the development of testicular tissue in as much as the majority of true hermaphrodites has only X chromosome.

Lejeune et al. (1959) described the first chromosomal anomaly demonstrable in man when they reported trisomy 21 in a number of mongols. Mongols show a peculiar syndrome called Down's syndrome with peculiarly folded eye lids and facial characteristics, stubby hands and feet, mental retardation, cardiac malformation and peculiar pigmentation of eye. Non-disjunction during maternal gametogenesis may be explained as most likely cause in view of the close relationship between advanced maternal age and mangolism. Subsequent investigations (Carter and Ewans, 1961 and Hamerten et al., 1961) have confirmed the chromosomal anomaly with mangolism. This

finding opened up a new diagnostic method in human medical practice.

Jacobs and Strong (1959) reported XXY sex determining mechanism associated with abnormal male syndrome described in 1942 by H.F. Klinefelter and known as the Klinefelter's syndrome. Characteristic of Klinefelter's syndrome is a primary microarchism with a small firm scrotal testis manifesting tubular dysgenesis histologically, 86 per cents are usually sterile. Eunuchoidism gynaecomastia are frequent but not invariable findings. Subnormal intelligence is also noted.

Ford et al. (1959) reported monosomic condition with a chromosome complement of 44 autosomes and one X chromosome associated with abnormal female phenotype described in 1938 by H.H. Turner and associates and known as Turner's syndrome characterised by short stature exhibiting sexual infantilism, neck webbing, and dysgenesis of the gonads which are represented by streaks of ovarian stroma. These patients are amnoerrhic and are sterile. Bony and soft tissue anomalies are also present and include shield like chest, cubitous vulgus, micrognathia, high arched palate, low set ears, hypertension, peripheral lymphoedema and aortic coartation.

Edwards et al. (1960) observed another syndrome characterised by the variable anomalies of the hands, feet and ears (which are usually low set) micrognathia, short webbed neck duodenal diverticule, mental retardation and congenital heart disease of variable severity and attributed the cause to trisomy involving chromosome 18.

Patau et al. (1960) described trisomy involving one or more of D group chromosome. Because of the difficulty in identifying the individual chromosome involved, the syndrome is referred to as Group D trisomy syndrome. The clinical features include defects of the globe (anophthalmia or microphthalmia), cerebral and mental defects, defects in the falx cerebri, multiple capillary hemangiomata, hyper-extensible thumbs, cleft palate, hare lip, simian palmar creases and occasionally polydactyly and congenital heart diseases.

Book et al. (1960) reported the conclusions originated at the discussion of a meeting held at Denver in the year 1960. In order to avoid confusion arising out of the variations in nomenclature, a standard nomenclature was proposed and criteria for the identification of individual chromosome were established in human beings. The criteria used in the identification of chromosomes include length, relative position of centromere and ratio of long arm to short arm length. He suggested letter designations of the major groups. Thus on the basis of the suggestion of Patau (1961) and recommendations of the meeting at Denver, human chromosomes were grouped into seven major groups which are named after Denver. The groups are, Group A consisting of 1, 2 and 3 large chromosomes with medium or nearly median centromere, Group B with 4 and 5 large chromosomes with sub-median centromeres, Group C with 6 to 12 and X-medium sized chromosomes with sub-median centromere, Group D of 13 to 15 medium-sized

acrocentric chromosomes which may bear satellites on their short arms, Group E with 16 to 18 - rather short chromosomes with medium or sub-median centromeres, Group F with 19 to 20 short chromosomes with median centromeres and Group G with 21 to 22 and Y being very short acrocentric chromosomes which may bear satellites on their short arms. These grouping was done taking the averages of the chromosomes of the several cells. The consistency of the value obtained is found to be good indicating reliable statistical estimate of real length of human chromosome. In the case of human chromosomes, values published by Denver study group are probably still the best one available.

There are instances in which XYY form of trisomy has been considered and studied by Muldal and Ockey (1960), Sandberg et al. (1961). The patient was described as an apparently normal male who sired 3 children, of whom 2 were abnormal (Mongolism, amenorrhea).

Incidence of combined autosomal and sex chromosomal aneuploidy considered as variants of Klinefelter's syndrome and sex chromosome mosaics have been summarised by Eggen (1963).

Many reports (Ashley and Jones, 1958; Witschi et al., 1957) illustrate some of the changing concepts of intersex. Many intersex states are not due to detectable errors in chromosome pattern, some such as adrenal virilism and testicular feminisation seem to have a hormonal basis.

The next conference in London (1963) considered the presence of satellites in some chromosomes as an additional criterion and also took into account the presence of the secondary constriction regions and differential patterns of incorporation of isotopically labelled thymidine in specific chromosomes demonstrated through autoradiography. The third Conference at Chickago (1966) formulated a system of nomenclature for the description of human chromosome complement and its abnormalities utilising designations which can be coded for retrieval. A group of workers concerned with human genetics met in September 1971 (Paris, 1971) on the occasion of the 4th international conference in genetics at Paris to agree upon a uniform systems of human chromosome identification. Their objective was extended by standing committee which met in Edinburgh (1972) and proposed a standardised system of designating not only individual chromosomes but also chromosomal regions and bands.

Nichols et al. (1964) studied and reported extreme chromosome breakage induced by measles virus in different in vitro systems. Three in vitro systems of human cells such as Lu 106 cell line, embryonic diploid cell strains of lung and kidney origin, leucocytes from measles susceptible and measles donors. In Lu 106 and leucocytes from the measles susceptible donor, a striking and extreme chromosome fragmentation, referred to as chromosome pulverization was observed.

Chromosome deletions are usually lethal event as heterozygotes resulting in zygotic loss, still births or infant death (Gardner, 1981). Sometimes infants with small chromosomal deficiency survive long enough to permit observations of some of the abnormal phenotypes expressed. Lujeune and his colleagues (1963) for example discovered a chromosome deficiency in human that has been associated with the cri-du-chat (catcry) syndrome. The name of the syndrome came from a plaintive cat like mewing cry from small weak infants with the disorder. Other characteristics explained are microcephaly, broad face and saddle nose, widely shaped eyes with epicanthic folds, unique facial features and physical and mental retardation. The 126 of cri-du-chat children studied are in the range of 20 to 40 years. The chromosome deficiency is in the short arm of chromosome No.5 and is designated 5 p - (46, XX 5 p-). Cri-du-chat patients die in infancy or early childhood and do not transmit the chromosome deletion to offspring. It was mentioned by Lejeune that this chromosome deficiency has been involved in a reciprocal translocation and thus to be transmitted to offspring. The chromosome No.5 short arm became translocated to chromosome No.15 the heterozygous translocation was carried in the normal healthy parent and some gametes carried only the deficient member of the translocation pair and the children inheriting the 5 p expressed the cri-du-chat syndrome.

Chromosomology and cytogenetics in Domestic Animals

The chromosomal studies pertaining to domestic animals was initiated by Makino (1944) who reported using testicular tissue that the diploid number in cattle was 60. He could not observe any difference between the karyotype of Bos taurus and Bos indicus. The X and Y chromosomes were described to be acrocentric in both subspecies.

Chiarelli et al. (1960) described the growth of renal tissue in cultures and found the diploid chromosome number in cattle as 60 with X chromosome as submetacentric and all the autosomes as acrocentric.

Crossley and Clarke (1962) confirmed this diploid number in cattle as 60 and conducted experiments with peripheral blood leucocyte culture and muscle tissue culture, in both the diploid number was found to be 60. They described the autosomes as acrocentric and X and Y chromosomes as large and small submetacentric, respectively.

Ohno et al. (1962) studied the possible germ cell chimeras among new born dizygotic twin calves (Bos taurus) and showed that the proportion of 60, XX: 60 XY cells varied between different individual freemartins and also between 9 freemartin and its co-twin.

Trujillo et al. (1962) studied the chromosomes of Horse (Equus caballus), Donkey (Equus asinus) and the mule. The diploid chromosome number for horse was 64, donkey 62 and

for mule 63. The chromosome complements of horse and donkey are completely different morphologically. The horse possessed 12 pairs of metacentric autosomes and 18 pairs of acrocentric autosomes, while donkey 19 pairs of metacentric autosomes and 11 pairs of acrocentrics. The morphology of sex chromosome also varies. The disparity of parental chromosome explained as the reason for male sterility in their hybrid, the mule. There were fertile female hybrid which produced several offspring, it was described that morphological disparity between parental chromosomes was only a small part of the relatively complex matter of female gametogenesis.

Gustavssen and Rockborn (1964) described three cases of overt lymphatic leukaemia and the chromosome morphology in these animals. It was described that all the cells (100%) possessed 59 chromosomes, with one diverging morphologically from the normal complement. This chromosome had a subterminally situated centromere and presumably might have arisen by centric fusion or translocation. The real nature was not however, described. The chromosome pairs affected were 1 and 29 and the same aberration was observed in a foetus from one of the cows. The foetus was 8 months of age and looked normal. The mitosis obtained *in vivo* from bone marrow and chromosomes of kidney cells after 8 days of incubation were examined.

Shive *et al.* (1965) made a survey of fifteen dogs with congenital cardiac defects. In one of the male dogs with a median cleft in the upper lip and premaxilla, tetrology of

of fallot and a patent foraman ovale, the chromosome number was 77 with 74 telocentric, 2 large sub-metacentric and one readily visible small sub-metacentric Y chromosome. This dog died and the study of testis revealed normal spermatogenesis. It revealed that the additional large sub-metacentric chromosome was not an X chromosome but formed by a translocation or centric fusion between 2 autosomes. Another dog with cardiac defect which was also a male black cocker spaniel with a ventricular septal defect and persistent left cranial vena cava. There are 79 chromosomes including a minute chromosome. The presence of the extra minute chromosome can be explained on the basis of a non-junction coupled with a deletion or of a non-disjunction coupled with a reciprocal translocation and loss of the large chromosome. In another dog which was a female with cardiac defect and congenital defect such as marked bowing of the fore-limbs and a disproportionate growth of the fore- and hind-limbs and was diagnosed as having achondroplasia revealed 77 chromosomes including 2 large sub-metacentric X chromosomes and an abnormal large sub-metacentric chromosome. The large sub-metacentric chromosome was formed either by translocation or a centric fusion between a large and a small telocentric chromosome.

Gustavsson (1966) described the experiments conducted on Bos taurus in Sweden. Out of the 1134 animals studied, 89% appeared to have 60 chromosome, 122 animals 59 chromosomes with one abnormal autosome and four bulls only 58 chromosomes per cell. All the animals appeared normal and used for

artificial insemination purposes also. The abnormal chromosomes found in these cattle explained as a result of interspecific chromosomal polymorphism. This type of variation either centric fusion or translocation of Robertsonian type is common in invertebrates but not in mammals. This Robertsonian type of chromosome translocation described in cattle was the first example of this type of rearrangement observed in domestic animals.

Basrur and Moon (1967) described the chromosome complements of domestic cattle (Bos taurus) and American bison (Bos bison) and compared with those of their hybrid cattalo. The diploid chromosome number was 60 and autosomes were acrocentric and X chromosomes were sub-metacentric in both cattle and bison, however the Y chromosome was small metacentric in cattle and acrocentric in bison which is indistinguishable from other autosomes. It was explained that the members of subgroup consisting of general Bibos, Bos, Poephagus and Bison have 60 chromosomes and the crosses between some of members of the subgroup result in viable F_1 hybrids. Mating between different members of the genus Bos were known to be fully fertile. However, the crosses between yak (Poephagus grunniens) and European or Afro Asian domestic cattle or both gave rise to fertile females and sterile males. In these interspecific hybrids as in cattle the observed male sterility was attributed to the rule that the heterogametic sex will express the reproductive barrier between the species through disturbances of

spermatogenesis and resultant failure to produce gametes. The abortive first meiotic division in male hybrids has been attributed to structural inequalities between their parental chromosomes. The only structural differences between the karyotypes of bison and cattle is that concerning the Y chromosome. It is possible that the non-homology between the pairing segment of sex complement may precipitate a distortion of the reduction division in male cattalos, whereas the X chromosomes which are morphologically identical in the parental species will undergo synapsis and facilitate the termination of the primary oocyte division in the female. However, the fact that X and Y chromosomes do not always form sex bivalents in mammals seems to contradict the assumption that meiotic disturbance in male cattalos is causally related solely to structural inequality of the pairing segment in the sex complement. It is conceivable that a general lack of compatibility resulting from genic diversity also exists between the bison and cattle even though the majority of their chromosome elements are similar in gross morphology.

Kieffer and Cartwright (1968) conducted the studies on pure Brahman, Santa Gertrudis or crosses in which the sire was a Brahman and found that the metaphase chromosomes of Bos indicus male possessed subterminal centromeres and the X was submetacentric. The Y chromosome in all the cases was morphologically similar to 58 autosomes in position of centromere. The individuals that were either members of Bos taurus

species or sired by a member of Bos taurus species had Y chromosome with submedian centromeres. The X chromosome of both species had identical morphology. The karyotype construction and chromosome replication patterns indicate the Bos indicus Y to be similar in overall size to Bos taurus Y. The difference in position of centromere of the two species could have originated through a pericentric inversion.

Kanagawa and Basrur (1968) studied 14 Free martins and 9 non-Free martins females belonging to heterosexual twins or triplets. No chimerism was noted in the non-Freemartins, whereas all the 14 Free martins showed varying proportions of male and female cells. The cytological sex of dividing cells in the hemopoietic tissue has been used as a diagnostic feature since all the free martins were chimaeric for male and female cells in a variety of tissues including peripheral blood. Two hypothesis have been advanced to explain the Free martin condition one of which concerns the influence of male hormones from developing male embryo on the female gonad whereas the other involves the presence of cells carrying Y chromosomes in the developing female gonad. It was demonstrated that the non-Free martin females of heterosexual twins are not chimeric for peripheral lymphocytes and this conclusion was supported by the finding that three of the non-Free martin showed non-indication of erythrocyte chimerism when subjected to blood typing. Since all blood cells chimeras have been clinically confirmed to be Free martins the blood culture method would appear to be a most reliable diagnostic aid for

Freemartinism. In the same report the possibility of the successful use of blood for chromosome study also analysed. The successful chromosome analysis was accomplished within 72 hours after sampling. The reduction in mitotic index was less in low temperature than in room temperature. It was recommended to store the samples at low temperature (ice bath preferably) during transit.

Fischer and Ulbrich (1968) reported 52 chromosome complement in African buffalos (Syncerus caffer) and Asiatic water buffalos (Swamp type, Bubalus bubalis) have 48 chromosomes. Fisher and Ulbrich (1968) reported the chromosome number in Murrah buffalos of Indian origin as 50 and also reported cross-breds between Asiatic water Murrah with Swamp type. This was to combine the milk producing ability of Murrah and ability of Swamp type to make better use of more course roughages. The F_1 hybrid was fertile with 49 chromosome and the unpair chromosome was No.24 in the pair.

Gustavsson (1969) studied artificially bred population of Swedish cattle that had three different diploid numbers 60, 59, 58. The variation between individuals was shown to be due to a translocation of Robertsonian type. It was noted, daughters of translocation sires (2n = 58, 59) returned to service more often than daughters of normal sires (2n=60). This difference was attributed to an increased rate of embryonic death in translocation carriers.

Rieck et al. (1969) examined a 22 month old Fleckvich bull with marked hypogonadism which was related to a XXX cow.

The karyotype revealed 56.5% were 61, XXY, 25.6% 60, XX and 17.9% 60, XY.

Dunn et al. (1970) described a Holstein beast with true hermaphrodite characteristic of a penis, an empty scrotum, a left abdominal ovary with corpus luteum and normal uterine horns. The right abdominal gonad was an ovotestes. Diploid cells from bone marrow showed 1.5% 61, XXY. Small percentages of triploid cells from blood lymphocytes culture showed 90, XXY and 90, XXX. The rest of the cells were 60, XX.

Rieck et al. (1970) studied a Fleckvich cow with marked kyphosis and normal reproductive system but familial tendency to disturbances to meiosis. The chromosome analysis revealed 61, XXX complement, trisomy of X chromosome.

Harvey (1971) studied the chromosome complement of breeding bull used for artificial insemination in Great Britain and described autosomes with Robertsonian translocation (1/29) in charlois breed of cattle. The karyotype revealed 59 chromosome with one unpair large submetacentric chromosome.

Nadler et al. (1971) conducted a cytogenetic analysis of wild sheep population in Northern Iran. From the studies on seven wild sheep populations, it was reported that the eastern population revealed 2n=58 with one pair of metacentric and 27 pairs of acrocentric autosomes and the western population showed 2n=54 with 3 pairs of metacentric and 23 pairs of acrocentric autosomes. In the intermediate localities animals revealed 2n=54, 2n=55, 2n=56, 2n=57 and 2n=58. In all these

cases the X chromosome was large acrocentric and Y small acrocentric. The taxonomic status of wild sheep hinges upon from this study, to the interpretation of events that occurred at the hybridization zone between 2n=54 and 2n=58 population. Some systematists would interpret this as to show unrestricted interbreeding within the zone, thus denoting subspecific status for each chromosome population.

Pollock (1972) reported an abnormal chromosome in British Friesian cattle. This revealed only 59 chromosome with an abnormal unpair submetacentric chromosome. The centric fusion of second and fourth chromosome t(2q;4q) was described.

Dunn and Johnson (1972) reported from the studies on cattle, a case of 61, XY cell line in a calf with extreme brachygnathia. This was considered as trisomy of autosomes.

Halnan (1972) reported that in eight animals with a history of infertility, upto 60% of cells possessed autosomes, in the 14 to 26 groups, which had gaps or secondary constrictions. This indicated the animal's impaired breeding potential.

Bruere and Chapman (1973) conducted a study on Simmental bulls in New Zealand. A case of tandem fusion involving 14 and 20 chromosome was explained, t (14q;20q), earlier this was explained as t(13q;21q) but according to the recommendations made at the Reading Conference 1976, it was confirmed as t(14q;20q). The chromosome complement was 59.

Evans et al. (1973) reported a comparative study of three Boveidean species, goat, sheep and ox. A comparison

of G banding patterns between species revealed a remarkable degree of homology of banding patterns. The homology between sheep metacentric and goat acrocentric elements confirms the Robertsonian variation. The close homology in G banding patterns between these related species indicates that the banding patterns are evolutionary conservative and may be a useful guide in assessing interspecific relationship.

Norberg et al. (1976) reported a case of X-trisomy in cattle of Norwegian Red Cattle. Cytogenetic examination of leucocytes from the animal showed the karyotype of 61, XXX. Except for the failure to express oestrus more than one time during 18 months the heifer showed clinically normal. On post-mortem the ovaries were found to be underdeveloped, one of them having a small corpus luteum. The cells of the corpus luteum had the morphological characteristics of active lutein cells but in contrast to this observation the concentration of blood plasma progesterone remained at low levels (0.3 mg/ml) in the period of observation. The rest of the reproductive organs did not show pathological changes. Chromosome analysis of the peripheral blood cells of the mother and the maternal half sisters of the heifer did not demonstrate any genetical disposition in the family to the formation of non-disjunctive gametes and it was concluded that certain chromosomal aberrations of gonosomes are closely associated with specific disturbances of fertility in cattle.

After several different descriptions of banded karyotypes

of domestic animals both normal and aberrant it became desirable for comparative purpose that some form of standardisation of banded karyotype be formulated and an international conference was organised on August 1976 at University of Reading, England with the objective of description of the main G-band patterns of the chromosome with sufficient detail to permit the unequivocal identification of individual chromosomes. In cattle the chromosomes were arranged in order of decreasing length, measurements being made on chromosomes conventionally stained after G-banding. The standard system of nomenclature adopted in adhere to the system developed for the description of human banded chromosomes, laid down in the Paris Conference.

Lin et al. (1977) described a reliable trypsin-Giemsa banding technique for producing clearly differentiated G-bands on bovine chromosomes pairs and identified each chromosome separately. Grouping of chromosomes in the karyotype may have some usefulness in facilitating earlier identification of chromosomes, since it is easier to remember a specific chromosome within a group of five or six individual pairs of chromosomes. However, because of the close morphological similarity between individual bovine chromosomes, grouping will be done on a rather arbitrary basis. Thus it was stressed that a standard system of naming and grouping of chromosomes should be established among laboratories working with bovine chromosomes.

Gustavsson (1979) described the distribution and effects of the 1/29 Robertsonian translocation in cattle. The 1/29 Robertsonian or centric fusion translocation is the result of a fusion in the centromere region of two autosomes, 1 and presumably 29, when the autosomes are arranged in decreasing size and consequently somatic chromosome number is reduced to 59 from 60. It was described that the translocation has world wide distribution and only little is known about the actual geographical distribution. The occurrence of 1/29 in different breeds and countries was explained as a result of recurrent mutation or distribution of an ancient mutation. In spite of some indications to the effect no proven case of de novo formation of 1/29 has been described and in all cases in which ancestors and progenies can be traced, hereditary patterns have been found. Nor has the formation of 1/29 in vitro been described. It is true that at the interphase of mitosis and meiosis, there are centromere association of non-homologous chromosomes. However, there is still no conclusive explanation for these association. It was assumed that they result from the non-random pairing of centromeres with similar DNA sequences and explained that there is no increased cytological risk that chromosome 1 and 29 will undergo centric fusion and this is supported by evolutionary evidence. The phenotypic effect of the translocation is due to the defect in meiosis. In the meiosis of the heterozygous carrier the translocation and its homologous build up a (hetero) trivalent and the segregation, which is dependent on factors such as

the relative size of the chromosomes, the location of centromeres and chiasmata, proceed in a fairly regular way with the production of chromosomally balanced gametes. The 1/29 appears to be inherited in Mendelian fashion. Half of the offspring became normal while the other half are balanced carriers. Nevertheless, it is presumed that to a certain degree the heterobivalent segregates non-disjunctionally at the first meiotic division. There are however proofs of the occurrence of cytologically unbalanced zygotes which give the heterozygous carriers reduced fertility. No correlation of the 1/29 translocation with other characteristics was described and variability in incidence between different populations is probably due to genetic drift. The importance in animal breeding of introducing eradication programmes is emphasized and also stressed that cattle population using artificial insemination should undergo routine cytogenetic investigation.

Balakrishnan et al. (1979) described a typical free martin as a sexually imperfect, usually sterile, female partner of a pair of bovine heterosexual twin. The results obtained on chromosome studies of a Freemartin which was a twin to a male and the dam was a pure-bred Sahiwal and the sire a Brown Swiss. The Freemartin was described to possess underdeveloped reproductive organs and was declared sterile. The cytological studies revealed XY sex chromosome complement though the general external appearance of the animal was unambiguously that of a female. On rectal examination the

animal had shallow vagina which ended blindly. No cervix observed and below this two filiform tubes running for about 10 cm from the blind end of vagina and ending in fibroid masses. It was described that absence of leucocyte with XX chromosomes among all the cells observed is difficult to explain. The possible explanation is that due to some mechanism as yet unknown once the tissue exchange has taken place, there is selective elimination of lymphoid tissue of male origin in the female or vice versa.

Potter et al. (1979) described the cytological study using metaphase chromosomes of cultured lymphocytes on 2 Banteng (Bibos banteng) steers and 218 bulls representing 13 pure-breds (Bos taurus type, Bos indicus type and Sanga) and 7 cross-bred bulls. Studies were carried out on photographic karyotypes of Giemsa stained and C-banded chromosomes of bulls of each breed and of G-banded chromosome from 3 breeds of Bos indicus and one cross-bred (Australian Friesian Sahiwal) cattle. The relative length of chromosomes of Bos taurus and Bos indicus bulls were compared and significant difference in relative lengths of the X chromosomes were reported between these two species. There was a difference in morphology of the Y chromosomes; Sanga, Banteng and Bos taurus type breeds had a small submetacentric Y chromosome except for the Jersey which had a metacentric Y chromosome. All the Bos indicus type bulls had an acrocentric Y chromosome but the Droughtmaster breed had two forms of the Y chromosome (submetacentric

and acrocentric). The C-banding patterns of the autosomes and X chromosomes were similar for all breeds while those of the Y chromosomes of Bos indicus type cattle allowed their accurate identification. G-banding patterns of Bos indicus resembled those of Bos taurus and enabled pairing of homologous chromosomes. Centromeres of the autosomes were unstained but those of the sex chromosomes were darkly stained.

Balakrishnan et al. (1981) reported for the first time sex chromosome chimerism in two calves (male and female) of triplets born to a Murrah buffalo. The metaphase cells revealed 38.7% of male and 42.07% of female calf were XY type and proposed choriovascular anastomosis between male and female during embryonic development.

Sahai (1982) described the relative length of chromosomes in zebu cattle. In Red Sindhi, it ranged between 1.76 to 5.61 and in Hariana ranged from 1.76 to 5.68 between largest and shortest chromosome. In both these breeds the largest chromosome was X-chromosome with 5.61 and 5.68 relative lengths respectively.

Yadav et al. (1984) conducted a screening of buffaloe bulls reserved for breeding purposes, 4.1% of the buffaloes studied revealing cryptorchidism, but the karyotype exhibited normal complement. One buffaloe bull was reported to possess secondary constriction in short.arms of both the homologues of fourth submetacentric chromosome.

Yadav and Balakrishnan (1983) studied the distribution

of certain chromosome in the metaphase. The study described the significance of the deviation of distance between sex chromosomes and first pair of autosomes (I) which can occur due to chance. The study on chromosome pairs X-X, X-Y, X-I and I-I revealed that they were randomly distributed in metaphase plates in cattle.

Yadav et al. (1984) described the report of chromosomal screening conducted on male cattle. The study on 165 male cattle revealed 86.7% with normal chromosome complements such as 29 pairs of acrocentric autosomes and a submetacentric X. The Y chromosome was acrocentric in zebu and metacentric in exotic and cross-bred males. In the study on cryptorchids, azoospermia, poor libido and often physical body defects, no chromosomal abnormalities were detected. In male cattle with chimerism for sex chromosome (XX/XY) ranging between 9 and 85% XY cells, all these were born co-twin to females in whom the proportion of XX/XY cells ranged between 8 and 76%. The gynaecological examination of female twins diagnosed them as freemartin. Three out of 5 male co-twins had attained maturity and yielded poor quality semen. It was also reported and described that no male born co-twin to a freemartin should be reserved for breeding programme. The Y chromosome polymorphism in cross-bred cattle also studied. One cross-bred bull with the sire as exotic found to possess acrocentric Y. In the cytogenic studies conducted on the sire and 17 other male sibs, one of sires sibs also possessed acrocentric Y

whereas the sire and all other 16 male sibs possess metacentric Y chromosomes and physically appeared normal. The hypothesis put forward was the forced meiotic inversion.

Yadav and Balakrishnan (1984) conducted chromosome analysis of all available co-sibs of six sets of isosexual female twins, two sets of isosexual male twins, 16 sets of heterosexual twins and one set each of isosexual and heterosexual triplet cattle, sex chromosome composition of these animals were screened. The isosexual multiple births exhibited XX or XY chromosomes for females and males respectively. All except two heterosexual twin cattle exhibited sex chromosome chimerism, and the per cent of XY cells varying between 2.5 and 86.1. The correlation of per cent XY cells in members in this twins was 0.934. One freemartin had only XY cells while one set of twins exhibited normal sex chromosome. It was stated by the authors that it would probably be prudent not to reserve male calf born of multiple heterosexual births unless it is of extraordinarily good pedigree and rearing when it will be worth the risk.

Yadav and Balakrishnan (1985) studied the possible effect of age on sex chromosome chimerism in cattle. The lymphocyte cultures were set and chromosomes were studied from 12 co-sibships of heterosexual cattle twins at different time intervals. The XX and XY percentages were calculated and three explanations for the proportions at admixture of XX/XY cells in members of multiple heterosexual births (MHB) were

given. The proportion of XX/XY cells were the same within the members of multiple heterosexual births, but it may vary between 0 and 100% in different MHBs. The change that occurred over a period of time was also in the same direction in all the members of a MHB, but the proportion of XX/XY cells may increase, decrease or remain nearly constant in different MHBs. When the data on a large number of MHBs were pooled, the mean number of XX/XY cells remain at 50%.

Syed et al. (1986) reported the significance of chromosome studies in a normal breeding in Norway. All the bulls were screened. One bull which sired many calves and excellent otherwise which had a single chromosome translocation (1/29), the frequency of the translocation in 336 calves was 9.2% while the frequency was fallen down to 0.5% due to selection. In cattle certain XXY triasomy was observed in male which are sterile with poorly developed testicles and no spermatozoa formation. In a XXX trisomy heifer though it appeared normal with signs of oestrus, it remained sterile was explained. Testicular feminization was also explained in cattle. The calves with this anomaly appear to be normal and are often raised for breeding purposes. However when they reach the age of sexual maturity they show no signs of oestrus and on examination, revealed bull like heifer with moderately developed udder and teats and with female, although somewhat small external genital organs. The clitoris is normal or slightly enlarged. The vagina is short and non-patent while the uterus

and tubes are either extremely poorly developed or completely lacking. The gonads are situated in the abdominal cavity and have the appearance of small testicles with no spermatozoa formation and underdeveloped seminiferous tubules and ducts. Such animal possessed normal male sex chromosome constitution 2n=60 XY and concluded that the chromosome analysis contributed to an understanding of the casual mechanism involved in the development of congenital defects and thus provided a basis for their control in our domestic animals.

Sethumadhavan et al. (1986) described karyological abnormalities in a leukaemic she-buffalce. The leukaemic animal revealed hypodiploidy, hyperdiploidy leading to immeasurable chromosomal fragmentation and pulverisation and a marked chromatid gap in one of the sex chromosome.

Syed et al. (1986) reported a chromosome abnormality with XO (37 x O) in a pig. The animal showed short, bandy legs, loose shoulders and broad loose hind quarters. These pigs grow slowly and at 7-9 months old has a live weight only 40-70% of the normal. The sexual glands are small, ovary-like or spool-shaped rudiments, so-called rudimentary gonads. The remaining internal and external sexual organs are female in nature though smaller than the normal. The sows exhibited no signs of oestrus.

Stranzinger et al. (1987) screened fifty-two bulls of different breeds and confirmed the distinct morphological difference in the Y chromosome between Bos taurus and

Bos indicus cattle. In indicus the Y chromosome is telocentric whereas in taurus it is metacentric. The Bos taurus breed, Hereford as well as some of the Charbrays and all of the Africanders and AXBX (where Africander was the sire) have shown the metacentric Y chromosome and the reciprocal line BXAX (where the Brahman was the sire) had telocentric Y chromosome. None of the investigated bulls had a chromosomal aberration.

King et al. (1987) described the results of the cytogenetical study of 7-day old bovine embryos of poor morphological quality. Seven day old embryos were collected from Canadian Holstein and Ayrshire heifers after superovulation with pregnant mare's serum gonadotropin (PMSG) or follicle stimulating hormone (FSH). The embryos were classified as normal which is A type and abnormal which is C type. A total of 103 morphologically abnormal (type C) and 23 morphologically normal (type A) embryos were cytogenetically analysed after 4, 20-24, or 44-48 h of culture in enriched phosphate buffered saline or Eagles minimum essential medium. Twentyone of 23 (91.3%) type A and seventyfive of 103 (72.8%) type C embryos had cells in metaphase. Among 21 type C embryoes produced by PMSG stimulation, 17 (80.9%) were analysed; 6 were mixoploid (two 2n/3n, three 2n/4n, one 2n/6n), 2 were aneuploid (61 XXY) and 9 were diploid. Among the 82 type C embryos produced by FSH stimulation, 58 (70.7%) were analysed; 6 were mixoploid (one n/2n, one 2n/3n, three 2n/4n, one 2n/4n/8n),

2 were polyploid (4n) and 50 were diploid. No abnormalities were reported in type A embryos which were normal. This suggests that the mixoploidy is associated with abnormal development at early stage. The difference in frequency of mixoploid type C embryos between the two superovulation treatments (28.6%) for PMSG vs 7.4% for FSH suggest that superovulation especially using PMSG causes an increase in the frequency of mixoploidy at least among abnormal embryos.

Mayr et al. (1987) described a viable female calf with trisomy 22, no other abnormality have been observed since this calf grown into a mature cow. At the age of 2 year this trisomic heifer become pregnant after natural mating with a karyotypically normal, 3 year old Brown Swiss. After a normal pregnancy of 285 days, the cow gave birth to a healthy male calf. The calf proved to be karyotypically normal (60, XY). Eight weeks after calving, the cow showed signs of estrus again and was artificially inseminated and became pregnant. This is the first report on fertile autosomal trisomics in domestic mammals.

Culturing and harvesting of chromosomes in domestic animals

Most mammals possess a high diploid and many chromosomes which show a similar gross morphology in conventional karyotypes. It was not until about 1960 however that techniques became available to enable accurate and detailed observation of chromosomes of mammals.

Until 1960 all the chromosome counts were performed with histologically prepared specimen of testicular tissue but Chiarelli et al. (1960) published the results of their experiments to grow renal tissue in culture. They prepared the culture from male and female calves and confirmed the diploid number as 60 and described the chromosomes as being all acrocentric except the X chromosome which was submetacentric.

Moorhead et al. (1960) described the successful attempt of culturing the peripheral blood leukocytes and air dried slides were studied. The venous blood collected in heparinised tubes was mixed with phytohaemagglutinin at 0.2 ml/ml blood and allowed to stand in ice bath for 60 minutes. It was centrifuged and the supernatant plasma was cultured in TC 199 medium (60-70%) with plasma 30-40% enriched with penicillin and the pH adjusted to 7.2 and incubated at 37°C for 72 hours and at 70 hours, Colchicine was added at a rate of $0.5-1.0 \times 10^{-6}$ M. After 6 hours the culture was centrifuged and resuspended in Hanks BSS solution at pH 7.0 and fixed in 1:3 glacial acetic acid and methanol fixative. Slides were prepared, air dried and stained. It was explained that this combined the cytological and leukocyte culture technique and is the convenient, reliable approach for chromosome studies of human because this involved only small volume of blood and adequate mitotic yield and high numbers of exact count quality metaphase spreads obtained.

Crossley and Clarke (1962) described the application of peripheral blood culture technique to bovine blood and also attempted to grow bovine muscle cells in tissue culture. The peripheral blood was collected in heparinised tubes where the heparin was 0.4 mg/10 ml of blood and the amount of haemagglutinin also increased. The muscle specimen was placed in Glaxo medium 199 and cultures. Both the method yielded 2n=60 in cattle.

Nichols et al. (1962) described modifications in peripheral blood leucocyte culture technique and explained it as the simplest technique for obtaining bovine chromosomes. Ten ml venous blood collected in heparinised tubes (1000 IU/cc) and it was mixed with fibrinogen to accomplish sedimentation and separation of the red blood cells. The white blood cells were separated and cultured in Parker's medium 199 and 30% calf serum. Into this, phytohaemagglutinin at 0.1 cc per 10 ml of medium was added and incubated at 37°C at 5% Co2 gas. On each of days 3 and 4 tubes were taken for chromosome study after the addition of colchicine at 10^{-6} ml for 1 hour. The hypotonic treatment was done with distilled water and fixed in 60% acetic acid 0.1 N HCl and the cells were mixed with orcein. The cells were suspended in orcein and a drop placed on a slide was squashed with cover slip. Good results were reported with this method.

Gustavsson (1963) investigated the five breeds of dog using peripheral blood leucocyte culture technique. Ten ml

blood was taken from venus saphena or vena radialis in heparinised tubes. To each 10 ml of blood 30 ml of dextran was added. After storing in the refrigerator for three hours, the supernahant was pipetted off and centrifuged at 1500 rev. per minute for 5 minutes. The cell deposit was resuspended in the tissue culture medium Parker 199 containing 30% calf serum at a concentration of more than 4 million cells per cc. Before incubation 0.1 ml of PHA was added to the medium and the cultures were incubated at 37°C. On the third day, sufficient colchicine was added to make a concentration of 10^{-7} M. After 30 minutes, the cells were subjected to the hypotonic action of distilled water for 20 minutes, then fixed for 20 minutes in 9 parts, 60% acetic acid and 1 part 0.1 N HCl. Finally, squash preparation was made in aceto-orcein (2%) and chromosomes diploid number was found to be 78.

Stone (1963) reported the diploid chromosome number in pig as 38 using peripheral blood culture technique. 10 ml venous blood was collected in heparinised syringe and allowed to stand for 90 minutes at room temperature to sediment. The supernatant plasma was placed in culture containing Bacto phytohaemagglutinin and TC 199 medium. After incubation, the mitosis was arrested with colcemide and the hypotonic solution used was sodium-citrate. The smears were prepared on slides and stained with Sudan Black B. Distinct mitotic figures showed the diploid chromosome number as thirtyeight.

Ulbrich and Weinhold (1963) cultured the bovine leucocytes. The venous blood collected with heparin at 2.0-4.0 ml/10 ml blood and enriched with 10 mg streptomycin and 10,000 iu of penicillin and veteran or thrombo veteran also added. This was added to a vessel containing 0.02 to 0.24 ml of phytohaemagglutinin P or 0.4 to 0.8 of haemagglutinin M and stored in refrigerator and later centrifuged to separate the leucocyte layer. The leucocyte suspension of 1000 to 1200 cell/mm^2 was mixed with 20% plasma and 80% medium 199. 10 ml of the suspension was incubated at 37°C for 72 hours and 1.0 ml of 0.04 per cent colcemid was added to the culture and after 3 hours the culture was centrifuged and cell suspension prepared and fixed in acetic acid:Methanol fixative (1:3) and the smears were prepared on iced moistured slide air dried and stained in 2% orcein solution and the well spread metaphase spreads obtained and diploid number as 2n=60.

Connolly et al. (1963) described a mini culture method for cytogenetic study. This required small inexpensive, disposible plastic tubes containing culture. The caps are easily loosened and colchicine can be added through the cap. The blood collected directly into the tube. Each tube require only 0.5 ml to 1 ml of plasma and 2 ml of growth medium and it yielded good results in human beings.

Schern and Louro (1963) described the different methods used for chromosome analysis in human being such as the direct examination of cell either tumour cells or effusions without

culturing, long term culture of fibroblasts, bone marrow preparations and short term peripheral blood leucocyte culture. The authors explained the short term culture, of blood leucocytes which is less expensive and does not require sophisticated equipments which can be adopted to fit facilities in even the small hospital laboratory by modifying the culture methods.

Jackson and Kiessling (1963) studied the mitogenic activity of phytohaemagglutinin, a mucoprotein extract of phaseolar vulgaris, which is used commonly to separate leucocyte - rich plasma from whole blood by its ability to agglutinate and sediment erythrocytes. The mitogenic activity of extracts of physically separable elements or dried bean before and after sprouting was assayed in human leucocyte culture. The mitogenic effect had no correlation with specific starch gel electrophoretic pattern and agar gel precipitation reactions with human serum. It was ascertained that the active principle was contained in the bean endosperm rather than the embryo and was reduced by sprouting of the bean, but recovered on ageing. This was used extensively as mitotic inducer.

Basrur and Gilman (1964) described culturing method of lymphocytes in bovine. The modifications were made from the work on lymphosarcoma and normal cattle. The venous blood drawn in 0.2 ml 1% sodium heparin solution. The growth medium used was Connaught's H 597 supplemented with 20% inactivated calf serum, 0.08 per cent sodium bicarbonate and potassium

penicillin G at 100 IU/ml, 9 ml growth medium, 0.5 ml phytohaemagglutinin M and 1.0 ml of whole blood were introduced into culture vials and incubated at 38°C and on third day 1.0 ml of phosphate buffered saline containing 100 ug of colchicine was added and the metaphase cells were mixed with distilled water. The cell suspension was prepared in carnoy solution and slides were prepared air dried and stained with 2% natural orcein or carbol fuchsin. This method of preparation provided well spread metaphase plates from blood samples of cattle, goat, sheep and mink.

Holland and Holland (1965) studied the biological properties such as haemagglutinating, precipitating and lymphocyte stimulating factors of the extracts from certain seeds of Leguminosae, phaseola vulgaris, phytohaemagglutinin (PHA) and reported that the action of PHA in precipitating normal serum proteins was not that of an antigen and thus did not support the concept that PHA stimulate lymphocytes transformation by virtue of being of universal antigen. The haemagglutinating and precipitating activity did not appear to be associated with the lymphocyte stimulating factor in that loss of precipitating ability and marked decrease in haemagglutinin titre are not associated with a change in the ability of PHA to stimulate blood lymphocytes cultured in vitro.

Neu et al. (1965) explained the culturing of peripheral blood with PHA as mitogen and described the possible cytological effect of antibiotics per se on cultured human leucocyte

chromosomes or on the mitotic index. Penicillin G potassium, streptomycin, chloropheniool and tetracycline were the antibiotics tried. Various levels of antibiotics and its effect on mitotic index were explained. It revealed an inhibitory effect on mitosis at higher doses and a stimulating effect at therapeutic range.

Kanagawa and Basrur (1968) studied the clinical application and reliability of leucocyte culture method for the diagnosis of freemartinism and the length of time that the blood samples could be held at room temperature and in the refrigerator prior to culturing, was investigated. The mitotic index in bovine blood after preservation for varying periods was studied on samples from two animals. Blood samples were stored at 5°C for 6 hours in a refrigerator showed mitotic index to be 3.8 and 5.3 per cent which gradually decreased in samples stored for longer than 12 hours. After 72 hours, a very rapid decrease in mitotic index occurred reaching zero in samples stored for 96 and 108 hours. Samples kept at room temperature followed a similar pattern as under refrigeration but with slightly lower values throughout.

Joel et al. (1969) described a rapid and simple procedure to separate lymphocytes from blood of normal calves and goats by means of liquid silicon. In leukocyte suspensions prepared by this method, lymphocytes comprised 96.8% of the leukocytes in calves and 97.7% in goats and the red blood cell contamination was less than 10 red blood cells per leukocyte. Significant loss of cell viability as determined by

dye exclusion method and response to phytohaemagglutinin did not occur. This results indicated that based on cell size there are at least 2 populations of lymphocytes in blood of both species.

Pearson and Bobrow (1970) reported the staining of mitotic chromosomes using fluorescent acridine derivatives and investigated the possibility of positively identifying male nuclei in interphase by virtue of the fluorescent staining property of the Y chromosome. Buccal smears freshly obtained from normal males and from men of 47/XYY individual fixed and stained with aqueous solution of quinacrine dihydrochloride revealed a small brightly fluorescent body on interphase nuclei of cells from male subjects. This rapid method of diagnosing male sex was useful in identifying Y chromosomes.

Lazary et al. (1974) studied the in vitro stimulation of bovine leucocytes by PHA, concavalin and E. coli lipopolysaccharide. Mononuclear cells from bovine peripheral blood were isolated by the silicon gradient centrifugation technique. The cells were cultivated in vitro with and without phytohaemagglutinin P, concavalin A and E. coli lipopolysaccharide. The stimulatory effects of the mitogens was expressed by the incorporation of 3 H-thymidine into the cells. Phytohaemagglutinin P produced a strong stimulation in the dose range of 5 to 20 µg/ml medium in presence of 10 to 30% autologous or adult homologous serum. One to 4 x 10^{-6} mononuclear cells per culture gave the highest values, the incorporation

of radioactivity reached a maximum after 48 h incubation and persisted for several days. Concavalin A at a concentration of 10 to 30 µg/ml medium had a streng stimulatory effect on bovine leucocytes after 3 days of culture. E. coli lipopolysaccharide was less stimulatory than the other 2 mitogens and its possible mode of action is less clear than for phytohaemagglutinin or concavalin A in this system.

Ponce et al. (1975) studied the chromosomes of sheep and explained the G banding as useful tool for individual identification. Samples of venous blood were obtained from sheep. Leucocytes were cultured in 7.5 ml TC 199 medium, 1.5 ml of whole blood and PHA, the cell growth was arrested with colcemid (10 µg/ml) and incubation continued for 2 hours. At the end of 72 hours the cells were treated with 5 ml hypotonic solution (0.075 M KCl) and slides were prepared and flame dired. The slides were stored at 60°C overnight and stained in a mixture of 4.2 ml trypsin solution, 2 ml pH 7 phosphate buffer, 0.8 ml Giemsa staining solution and 43 ml distilled water for 10-12 minutes. It was reported to yield good metaphase spreads with clear G-bands.

Lin et al. (1976) described a rapid and simple method for the isolation and culture of leucocytes for chromosome analysis in domestic animals. Five ml of heparinised blood was mixed with 5 ml of Hank's balanced salt solution (HBSS). The 5 ml of diluted HBSS-blood mixture was then layered slowly over 5 ml of the Ficoll-Hypeque mixture in a sterile 15 ml

conical centrifuge tube. The resulting mixture was then centrifuged at 600 g (2000 rpm) for 40 minutes at 18-20 C. After centrifugation, the leucocytes were located as an opaque coloured band between the Ficoll-Hypaque and HBS solution. The portion of the gradient containing the opaque band was then carefully removed and washed in 9 ml Hank's BSS and centrifuged at 800-1000 rpm for 20 minutes. The supernatant was removed and the pelleted leucocytes resuspended in 10 ml of culture medium. Ham's F10 containing 20% fetal calf serum and 0.1 ml of phytohaemagglutinin. The culture was then incubated at 37°C for 70 h. Colcemid at 0.1 µg/ml medium was added to the leucocyte culture $1\frac{1}{2}$ h prior to harvesting. After the colchimid treatment the media was removed by centrifugation and the cell subjected to hypotonic treatment with 0.075 M KCl for 20 minutes, followed by fixation in two changes at methanol acetic acid (3:1). After fixation two drops of cell suspension was dropped on to a slide covered with cold distilled water. The slides were air dried.

Halnan (1976) recommended a modified method for G-banding in cattle, horse and buffaloes. The peripheral blood lymphocytes were cultured in medium TC 199 containing 0.01 g % glutamine, 0.02 per cent cystine and 750 iu of mucous heparin. After culturing the hypotonic solution used was 0.56 per cent solution of potassium chloride. After the first fixation with methanol and glacial acetic acid at 3:1 the cells were washed with fixative four times. Air

dried spreads were prepared on dry slides cleared with fixative and warmed to 30°C to 35°C. It yielded good spreads. The slides which were of one to seven day old were used for banding. The slides were incubated in 2 x ssc at 69°C in a plastic couplin jar in a liquid paraffin bath using an incubator with an electronic thermostat for 1-3 hrs. The slides were thoroughly rinsed in a 5 ml Gurr's pH 6.8 buffer in 500 ml double glass distilled water. Staining was in Gurr's Giemsa R66 (1 ml:49 ml Gurr's pH 6.8 buffer) for 50 minutes to one hour. The slides were removed from stain, washed and air dried. Steep the slides in Xylene for 5 minutes and mount in DPX. This yielded very good G-bands.

Halnan (1977) described an improved technique for preparation of chromosomes from cattle whole blood. This involves improved media and techniques using TC 199 which yielded large number of analysable cells at 48 h, not only from cattle, but also from horses, sheep, goat and dogs. The whole blood was collected from jugular vein in tubes containing 100 iu heparin/10 ml blood. The blood was transported as early as possible because longer time reduces mitotic index. The culture medium used was TC 199 enriched with calf serum or bovine serum (25%), penicillin (6 mg %) and streptomycin (10 mg %). Sodium bicarbonate at 2.8% level was used to correct the pH to 7.3. The medium can be stored at deep freeze upto 14 days. Extra glutamine was added to improve the keeping quality of medium. An elevated l-cystine level was found to increase the number of mitotic cells. The

culture was set up by the inoculation of 1.5 ml of whole blood into 9 ml of medium using universal bottles of 30 ml capacity and containing 0.5 ml of phytohaemagglutinin. After 24 hrs, the bottles were gently agitated twice a day, a procedure which increases the number of mitosis. Incubation proceeded for 45 to 72 hours at a temperature between 37.5°C and 39.5°C. The colchicine was added to the culture 2 hour before harvesting to achieve a final concentration of 0.05 µg/ml. The culture was poured into 15 ml tubes and centrifuged at 1000 rpm. The supernatant medium removed leaving total volume of cell button and medium of 0.8 ml to which was added 12 ml of 0.56 per cent hypotonic solution which was potassium chloride and allowed to stand for 15 minutes. The tubes were centrifuged, supernatant removed and resuspended and fixed with 4 ml of freshly prepared solution of three absolute methyl alcohol to one part glacial acetic acid. Fixing was repeated four times to ensure the removal of all traces of water. The cells were suspended in 0.75 ml fixative. The suspension was placed on slide and smears prepared and air dried. Giemsa stain was the most satisfactory on aged preparation and orcein was recommended for freshly prepared smears. The good spreads prepared were studied for mitotic figures. The author compared different modification and mitotic index. It was recommended that freshly prepared medium was markedly superior to stored medium. The addition of glutamine at 0.02 g per cent brought significant improvement of mitotic index. The l-cystine addition in the medium had significant effect on increasing the mitotic

index. In the study the bovine serum with calf serum was reported to yield better mitotic index.than the faetal calf serum. The pH at 7.3 was found to be as the optimum for better results.

Balakrishnan (1981) described peripheral leucocyte culturing of cattle blood using Ham's F10 medium. The short-term cultures were set up with 0.5 ml blood in Ham F10 medium supplemented with 20% adult cattle serum and antibiotics. Poke weed mitogen was used as mitogenic agent and incubated at 35.5°C for 60-65 h. The cell mitosis arrested with colchicine 1.3 µg/5 ml of medium for 1 hr and the cells were subjected to hypotonic treatment with 0.075 M KCl. It was fixed in acetic:methanol 1:3. The slides were flame dried and stained with Giemsa. This method was reported to yield good metaphase spreads of chromosome in cattle.

Goswami and Balakrishnan (1985) described the effect of Bud R concentration on sister chromatid exchanges in cattle, buffalo and goat. Sister chromatid exchanges (SCE) are sensitive indicators of the effects of various mutagenic agents on eukaryotic chromosomes. Short-term lymphocyte cultures were set up with 7,5-Bromodeoxyuridine (Bud R) and the overall means of SCE ranged between 6.9 $\pm$ 0.3 and 10.4 $\pm$ 0.4, 8.8 $\pm$ 0.5 and 11.3 $\pm$ 0.6 and 6.1 $\pm$ 0.3 and 9.4 $\pm$ 0.4 in Sahiwal cattle, Murrah buffaloes and Beetal goats respectively. SCE frequencies were similar upto 2 µg/ml and increased linearly thereafter in all the 3 species investigated.

Materials and Methods

MATERIALS AND METHODS

Cattle stationed at various centres of Kerala Agricultural University, Indo Swiss Project and Farmers' premises in various parts of the State and the animals brought to the Veterinary Hospital and Artificial Insemination Centres formed the materials for the present study. The cattle belonged to 4 genetic groups viz., (a) local non-descript, (b) half-bred Jersey, JL (born out of cross between local cows and Jersey bulls), (c) half-bred Holstein Friesian, HL (born out of cross between local cows and Holstein Friesian bulls) and (d) pure Jersey. The animals studied were either normal or abnormal showing various productive and reproductive disorders.

Seventyone animals showing normal phenotype were used for the study. This include 10 local female, 9 local male, 13 JL female, 10 JL male, 9 HL female, 8 HL male, 5 Jersey female and 7 Jersey bulls.

On the basis of clinical examination and history of the abnormal animal, they were classified as (a) sterile, (b) infertile, (c) Freemartin and (d) other abnormalities. The cows with abnormal secondary sexual characters, and those which have not conceived even once during life time and the bulls with abnormal secondary sexual characters lack of ejaculation or semen without live spermatozoa were classified as sterile. Infertile animals included repeat breeders or those exhibiting an increased early returns to service, failure in fertilization, early embryonic death and early abortions,

impaired oestrous and bulls of poor libido, poor quality of the semen. A free martin is a female sterile calf born as a cotwin to male calf. Animals showing various other congenital abnormalities were grouped under 'ther abnormalities'.

One $4\frac{1}{2}$ years old sterile cross-bred (JL) heifer did not conceive in spite of repeated inseminations in oestrus. On examination, the animal was found to have normal external genitalia but rectal examination revealed a small underdeveloped uterus and small spindle shaped ovaries. This animal was sired by a Jersey bull mated to a local cow. In addition, three sterile local cows and one sterile Holstein Friesian cross-bred (HL) cow in the age group of 2-3 years were subjected to cytological investigation.

Infertile class included 5 Jersey cross-bred cows, 8 Holstein Friesian cross-bred and 8 local cows which showed regular heat symptoms and with normal external appearance with a history of repeated insemination. All these animals were inseminated on an average of 3-4 times per conception. On rectal examination all these animals revealed normal sized reproductive organs.

Two Jersey cross-bred bulls and one Holstein Friesian bull with normal external phenotype and libido but showed less motility of spermatozoa were designated as infertile and investigated.

A 2 year old cross-bred heifer born as a heterosexual twin was brought to the veterinary hospital for counselling. This animal was an anoestrus type and sired by a Jersey bull. The general external appearance of the animal was healthy and of a female. On rectal examination it revealed a distinct but shallow vagina which ended blindly. No cervix was apparent. At the blind end of the vagina two lobular structures analogous to seminal vesicle were felt and below this two filiform tubes running for about 10 cm from the blind end and ending in a fibroid mass was palpated. The clitoris was large. This was grouped as free martin and cytological studies conducted. The male co-twin was not available for the study.

A six year old local bullock exhibited development of rudimentary teats and mammary glands. The animal showed the development of 4 teats with a small glandular enlargement at the base of every teat. The teats were seen protruding outwards and around the scrotum. At its 4th year of age onwards the glandular tissue produced a milky fluid. Since the animal was castrated and used as a draught animal, semen studies could not be conducted. The bullock was phenotypically normal and with good stature. This animal was categorised under other abnormalities and chromosome studies were conducted.

All these animals were subjected to cytogenetic study. The karyotype analysis was carried out using peripheral blood leukocyte culture technique as described by Moorhead et al. (1960) in human beings and Halman (1977) in animals with modification.

Blood collection and transportation

Blood was collected from external jugular vein using sterile 16 G needle. The skin was cleaned with spirit and cotton and after discarding 2-3 drops of blood, 10 ml blood was collected in tubes containing heparin (5000 IU/ml blood). The blood was immediately mixed with heparin and covered with aluminium foil. At every step maximum precautions were taken for sterility. The blood samples were transported to the laboratory within one hour. The temperature for transport was maintained between 21° and 32°C.

Medium

The basal medium used was TC 199 (Difco) and the composition of the medium used was as follows:

Triple dist. water	- 47.0 ml
TC 199	- 0.5 g
Tryptose phosphate broth	- 82 mg
l-Glutamine	- 5 mg
Sod. bicarb (3.5% sol.)	- 0.53 ml
Penicillin (5000 IU sol.)	- 0.2 ml
Phytohaemagglutinin-M (Difco)	- 2.5 ml

The distilled water was taken in a sterile conical flask. Tryptose phosphate broth, TC 199, l-Glutamine, Sod. bicarb. sol., Penicillin and PHA were added and the pH was adjusted to 7.2.

This whole medium was filtered through a microfilter and 5 ml of medium was distributed to each screw caped culture

vials of 30 ml capacity. The medium was stored in deep freezer and used for one month.

Culturing

In the laboratory, the samples were brought and cultures were set up within 2 hours after collection.

From each sample 2 ml whole blood was taken in separate syringe and it was used to seed the culture. The rest of the blood was subjected to centrifugation at 1200 rpm for 20 minutes to facilitate sedimentation of erythrocytes and extraction of autologous plasma.

Into each culture vial containing 5 ml medium, 2.5 ml autologous plasma and 0.7 ml whole blood (kept separately in the syringe) were added and mixed gently by rotating the culture between the palms of the hands. The cultures were then incubated at 37° $\pm$ 0.5°C for 72 hours. The cultures were gently agitated twice daily.

At the end of 71 hours of incubation, colchicine solution (0.0001%), as a mitotic arrester was added to the culture, mixed gently and incubated for a further one hour period. The optimum concentration and duration of colchicine treatment were estimated by observing the degree of condensation of metaphase chromosomes. The concentration and duration used were 0.1 ml for one hour, 0.1 ml for two hours, 0.2 ml for one hour and 0.2 ml for two hours.

Harvesting

At the end of 72 hours the culture was removed from the incubator, agitated and gently poured into 15 ml centrifuge tubes. The tubes were centrifuged for 10 minutes at 1200 rpm. The supernatant of medium was removed leaving a total volume of 0.5 ml above the sediment. About 2 ml of 0.075 M potassium chloride hypotonic solution was added and the cells were suspended in the solution. The volume was later made upto 6 ml with hypotonic solution and allowed to stand for ten minutes at room temperature. The contents were then centrifuged at 1200 rpm for 8 minutes and the supernatant removed leaving 0.5 ml above the sediment.

The cells were fixed with freshly prepared fixative containing three parts methanol to one part glacial acetic acid. About 2 ml of freshly prepared fixative was added to each tube and mixed gently to break the cell button by drawing repeatedly into a pasteur pipette. Then the volume was made upto 6 ml and allowed to stand for 10 minutes. The mixture was then centrifuged at 1200 rpm for 8 minutes and supernatant removed leaving about 0.5 ml of the solution above the cell button. The fixative treatment was continued with 5 ml, 4 ml, 3 ml till clear supernatant solution was obtained. Finally, the supernatant was removed upto 0.5 ml above the cell button. A suspension of the cells was prepared in 0.5 ml fixative and allowed to stand for a few minutes for the debris to settle down.

Slide preparation

Corning glass slides were used for chromosome preparation. The fresh, chilled, wet slides were used. Using a pasteur pipette two or three drops of cell suspension were dropped on the slide which was kept at 60° slant, from a height of 30 cm. The slides were waved from side to side as well as to and fro, to form a uniform spread of the material. These slides were air dried.

Staining

The staining was done on the same day using 4% Giemsa (Glaxo) prepared in phosphate buffer (pH 6.8). Freshly prepared staining solution was used. The slides were covered with Giemsa-phosphate buffer mixture and the slides were rocked at every 10 minutes. At the end of 40 minutes the slides were washed with distilled water and kept covered with distilled water for five minutes. The washing was repeated three times. The slides were air dried in slanting position and viewed under microscope.

The efficiency of the medium was tested by counting 300 number of lymphocytes. The lymphoblasts, and the cells in metaphase were counted. The mitotic drive and mitotic index were estimated as follows:

$$\text{Mitotic drive} = \frac{\text{Lymphoblasts + Number of cells in metaphase}}{\text{Total cells counted}} \times 100$$

$$\text{Mitotic index} = \frac{\text{Number of cells in metaphase}}{\text{Total cells counted}} \times 100$$

Effect of storage

The effect of storage of sample at refrigerator temperature (5°C) was determined by estimating mitotic index.

50 ml blood was collected in heparinized tubes and was distributed in 10 ml centrifuge tubes. The cultures were set up at 12 hours intervals and harvested after 72 hours. The chromosome counts were made and mitotic index calculated for each sample.

Morphology and karyotyping

Well spread metaphase spreads without overlapping were identified and photographed, two to three plates were taken using Carl Zeiss photomicroscope III with a combination of research microscope and 35 mm camera with automatic exposure control. The individual chromosomes were identified cut out from one plate and the others were kept for orientation and reference.

The homologous pairs of chromosomes were identified and karyotypes were prepared by pasting them on a bristol board. The chromosomes were grouped and numbered in the karyotype. The morphology of each chromosome was studied and comparative analysis was done.

Morphometric measurements

The chromosomes were classified as metacentric, submetacentric, acrocentric or telocentric depending on the position of centromere as described by Levan et al. (1964).

The morphometric measurements were taken on the basis of the recommendations made in the Denver Conference for describing human chromosomes.

The length of the chromosomes in the karyotypes was measured using a set of calipers with fine points. The morphology of individual chromosome was explained in terms of length and position of centromere. The size of the chromosome was represented as the relative length that is in relation to the length of the haploid set containing the X chromosome. The position of the centromere was indicated by

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

b) Centromeric Index $= \frac{\text{Length of short arm}}{\text{Total length}} = \frac{p}{p+q}$

The relative length, arm ratio and centromeric index were used to identify and compare local and exotic cattle chromosome. The morphological and morphometric differences between these breeds also studied.

The relative length of autosomes and sex chromosomes between genetic groups was compared using analysis of variance technique as described by Snedecor and Cochran (1967). The difference between relative length of X-chromosome and other autosomes in each genetic group was compared using students t-test. The arm ratio and centromere index of different genetic groups were also subjected to the above statistical analysis. In all, 102 animals were subjected to cytogenetic study and the details of which are presented in table 1.

Table 1. Number of animals examined for karyotyping under each genetic group

Animal	Local		Half-bred Jersey		Half-bred Holstein Friesian		Jersey		Total
	M	F	M	F	M	F	M	F	
Normal	9	10	10	13	8	9	7	5	71
Sterile	-	3	-	1	-	1	-	-	5
Infertile	-	8	2	5	1	8	-	-	24
Free martin	-	-	-	1	-	-	-	-	1
Other abnormalities	1	-	-	-	-	-	-	-	1
Total	10	21	12	20	9	18	7	5	102

M = Male

F = Female

Results

RESULTS

In the present study the cytogenetic profile of cattle belonging to local non-descript, half-bred Jersey, half-bred Holstein Friesian and Pure Jersey breeds were investigated and reported.

The efficiency of medium was expressed in terms of mitotic drive and mitotic index as presented in table 2. The mitotic drive and mitotic index were 33.66 per cent and 5.66 per cent in normal and 32.66 per cent and 6.33 per cent in abnormal animals respectively.

Colchicine was used as mitotic arrester. Among the four Colchicine treatments of 0.1 ml for 1 hr, 0.1 ml for 2 hrs, 0.2 ml for 1 hr and 0.2 ml for 2 hrs, it was found that the condensation of chromosomes using 0.1 ml colchicine (0.0001 per cent) for a duration of one hour showed normal size and appearance of chromosomes.

Mitotic indices in blood samples stored for varying periods at refrigerator temperature were presented in table 3. The mitotic index gradually decreased during the first twelve hours but a sudden drop was observed after 12 hours of storage. This phenomenon was observed in both normal and abnormal animals.

Karyotype

The karyotypes of local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey were presented in figures 1 to 8. Karyotypes were prepared for male and female belonging

to each genetic group. The karyotype analysis of all these animals studied revealed that the diploid chromosome number in cattle is 2n=60 comprising of 58 autosomes and 2 sex chromosomes. The chromosomes stained with Giemsa were arranged within the karyotype, in order of descending size from left to right in five rows of six pairs, with the sex chromosome occupying the position of the last pair.

The female karyotype of all the genetic groups revealed similarity in sex chromosome pair while the male sex chromosomes revealed dissimilarity. Thus in cattle the males are XY and females XX.

Chromosome morphology

All the 29 pairs of autosomes were acrocentric (table 4) in appearance and the autosomes showed no distinct morphological variation which would facilitate pairing of chromosomes in the karyotypes and it was therefore arranged, in a descending order based on the size. The autosomes formed a continuous series when arranged according to the decreasing size and individual chromosome could not be identified clearly because of morphological similarity (Fig. 9).

In all the animals the females revealed XX and males XY sex chromosome constitution.

The X chromosome in both sexes revealed a biarmed nature and morphologically revealed a subterminal centromere or submetacentric appearance (Fig. 10).

The Y chromosome of Jersey and Holstein Friesian revealed submetacentric shape whereas in Local non-descript it showed acrocentric morphology as presented in Fig.11. No satellite could be observed in any of the chromosomes.

Morphometric measurements

Relative length

The relative length of all the chromosomes in all the genetic groups were presented in table 5. Based on the relative length the autosomes were numbered from 1 to 29.

In local cattle the relative length of the autosomes ranged between 1.3473 per cent and 6.5038 per cent. The largest and smallest autosomes were 6.4735, 1.225 in half-bred Jersey and 6.2190, 1.3388 in half-bred Holstein Friesian cattle respectively whereas they were 6.9125 and 1.3096 in pure Jersey cattle. The continuous nature of the relative length and morphological similarity of autosomes makes individual autosome comparison difficult between different genetic groups.

Comparison of the largest autosomes of all the genetic groups using the analysis of variance (Table 6) revealed no significant difference between the genetic group. The analysis of variance showed that there is no significant difference in the relative length of smallest autosomes between four genetic groups (Table 7). The averages of the relative lengths of autosomes of all the 4 genetic groups are presented in figure 9.

Regarding sex chromosomes, the relative length of X chromosome was 7.2838 per cent, 7.0313 per cent, 6.5138 per cent and 6.3166 per cent in Local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey respectively (Fig. 12).

The relative length of X chromosome was compared between different genetic groups. The analysis of variance (Table 8) revealed that there exists significant difference between genetic groups. Pairwise comparison was made between different genetic groups. It revealed that relative lengths between (a) Local and half-bred Holstein Friesian, (b) Local and pure Jersey, and (c) half-bred Jersey and pure Jersey were significantly different, whereas the difference was not significant between (a) Local and half-bred Jersey, (b) half-bred Jersey and half-bred Holstein Friesian and (c) half-bred Holstein Friesian and pure Jersey.

In the karyological array X chromosomes with the relative lengths of 7.2838, 7.0313, 6.5138 in Local, half-bred Jersey, and half-bred Holstein Friesian respectively occupy the first position whereas in pure Jersey X chromosome with 6.3166 per cent relative length occupies a second position after the first largest autosome pair with relative lengths 6.9125 per cent as shown in table 5.

The Y chromosome of Local non-descript cattle showed an acrocentric morphology and similar to autosomes whereas in pure Jersey and those sired by Jersey and Holstein Friesian sires it revealed a submetacentric morphology. The relative

lengths of Y chromosomes were 2.9415 per cent, 2.5745 per cent and 2.9375 per cent in Local, Jersey and Holstein Friesian respectively (Fig. 13).

The relative length of Y chromosome was compared between different genetic groups and significant difference was observed in analysis of variance study (Table 9). It was observed that the difference between Local and Jersey and Holstein Friesian and Jersey were significant whereas that of Local and Holstein Friesian was not significant.

In the karyotype array the Local Y chromosome with relative length 2.9415 occupies a position between 15th and 16th chromosome. In Jersey and those sired by Jersey the Y chromosome with relative length of 2.5745 occupied a position between 15 to 20. In cross-breds sired by Holstein Friesian the Y chromosome showed a relative length of 2.9375 and occupied a position between 15th and 16th autosome pair in the chromosome array as presented in table 5.

Arm Ratio

Since all autosomes of cattle are of acrocentric nature measurements were available only for one arm and arm ratio for autosomes was not estimated.

The X chromosomes were biarmed and the arm ratio was estimated as 1.739, 2.043, 1.987 and 1.690 in Local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey respectively (Table 10).

The effect on arm ratio by genetic groups was estimated by the analysis of variance (Table 11) and was found to be significant. The pairwise comparison indicated that the arm ratio of X chromosomes between half-bred Jersey and Local, half-bred Jersey and Jersey, half-bred Holstein Friesian and Local, half-bred Holstein Friesian and Jersey were significant whereas that between half-bred Jersey and half-bred Holstein, Local and Jersey were non-significant.

In Y chromosome of the local cattle only one arm was observed and is thus acrocentric.

As regards to Y chromosome in Jersey and Holstein Friesian the arm ratio was 1.210 and 1.663, respectively (Table 12). This difference in arm ratio was found to be statistically significant.

Centromere Index

In autosomes of cattle the centromere index was zero since the short arms were not present and all the chromosomes were acrocentric.

The X chromosome of all the genetic groups had centromere index of 0.365, 0.329, 0.338 and 0.372 in Local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey respectively (Table 10).

The centromere index of different genetic groups was compared (Table 11) and the difference was found to be statistically significant. The pairwise comparison revealed that

the differences between Jersey and half-bred Holstein Friesian, Jersey and half-bred Jersey, Local and half-bred Holstein Friesian, Local and half-bred Jersey were highly significant whereas the differences between Local and Jersey, half-bred Holstein Friesian and half-bred Jersey were non-significant.

The centromere index for Y chromosomes of Jersey and Holstein Friesian were compared and found significant.

The Jersey had a centromere index of 0.463 which indicated close proximity of centromere to the mid point of chromosome whereas in Holstein Friesian it was 0.376 or far away from mid point when compared to Jersey (Table 12).

Abnormality

Among the 5 sterile animals examined, it was found that 4 cows revealed normal chromosome constitution with 60 XX. One Jersey sterile female revealed 59/60 mosaicism (Fig. 14). Four per cent of metaphase cells revealed 59 XX chromosome and 96 per cent 60, XX with a 59/60 mosaicism (Table 13) condition.

The chromosome analysis of infertile animals revealed diploid chromosome number 2n=60 with 58 acrocentric autosome and 2 submetacentric X chromosome (Table 13). The Y chromosome of the animals studied revealed submetacentric nature. All these animals were sired by exotic bulls. The chromosome morphology was similar to that of normal and neither numerical

chromosomal aberrations nor structural aberrations could be identified.

The metaphase spreads of the Free martin revealed that out of the 201 lymphocytes studied one hundred and seventy-three (86%) were of female origin (XX) and twentyeight (14%) were of male origin (XY). This was a case of XX/XY chimaerism (Fig. 15) and the animal was a Free martin.

A local non-descript bullock showing abnormal development of secondary sexual characters such as development of teat and glandular tissue (Fig. 16) with milky fluid. On examination of the metaphase spreads, 4.5 per cent of the cells exhibited a chromosome number 4n=120 (Table 13) showing the incidence of tetraploidy (Fig. 17). The other cells showed normal diploid number of 2n=60. This is a case of diploid/tetraploid chimaerism (mixoploidy).

Table 2. Efficiency of phytohaemagglutinin-M in Medium TC 199 used for bovine lymphocyte culture

Phenotype	Total cells counted	Lympho-cytes	Lympho-blasts	Mitotic spreads	Mitotic drive %	Mitotic index %
Normal	300	228	84	17	33.66	5.66
Abnormal	300	216	79	19	32.66	6.33

Table 3. Mitotic index in culture of blood samples stored for varying periods in the refrigerator (5°C).

Duration of storage (in hrs)	Mitotic index	
	Normal %	Abnormal %
6 hours	3.66(11/300)	3.33 (10/300)
12 hours	2.66 (8/300)	2.33 (7/300)
24 hours	2.66 (8/300)	1.66 (5/300)
36 hours	1.66 (5/300)	1.66 (5/300)
48 hours	1.33 (4/300)	0.66 (2/300)
60 hours	0.66 (2/300)	0.66 (2/300)
72 hours	0.66 (2/300)	0.33 (1/300)

Table 4. Cytogenetic profile of local, half bred Jersey(JL), halfbred Holstein Friesian (HL) and pure Jersey cattle.

Cattle type	Diploid No.	Autosome pair		Sex Chromosome	
		SM/M	A	X	Y
Local	60	-	29	Submetacentric	Acrocentric
JL	60	-	29	Submetacentric	Submetacentric
HL	60	-	29	Submetacentric	Submetacentric
Jersey	60	-	29	Submetacentric	Submetacentric

Table 5. Relative length of chromosomes of Local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey cattle

Chromosome pair	Local	Half-bred Jersey	Half-bred Holstein Friesian	Jersey
1	6.5038	6.4735	6.2190	6.9125
2	5.4290	5.9500	5.8610	5.62261
3	5.1765	5.5140	5.6790	5.5795
4	5.0095	5.1240	4.9850	4.7125
5	4.9371	4.7980	4.7245	4.5575
6	4.7205	4.6065	4.6735	4.5147
7	4.7091	4.2380	4.2985	4.1742
8	4.1575	4.0350	4.0290	4.0885
9	3.8710	3.7250	3.8825	4.0025
10	3.7425	3.6910	3.7715	3.7100
11	3.5645	3.4665	3.6725	3.5810
12	3.3425	3.1305	3.4780	3.4165
13	3.3120	3.0730	3.3090	3.3210
14	3.2620	2.8355	3.2850	2.9835
15	3.1110	2.8350	3.1890	2.8745
16	2.9145	2.5550	2.9510	2.8115
17	2.4855	2.5250	2.8745	2.7530
18	2.4580	2.3780	2.7325	2.6860
19	2.3250	2.3520	2.6495	2.6430
20	2.2030	2.2735	2.4420	2.5540
21	2.1675	2.1730	2.4415	2.4680
22	2.0640	2.0980	2.2590	2.2825
23	2.0640	2.0430	2.1815	2.0540
24	1.8960	2.0190	2.0530	1.9575
25	1.7805	1.9235	1.9210	1.8215
26	1.6240	1.7330	1.7290	1.7010
27	1.4515	1.5595	1.6280	1.4080
28	1.3515	1.4365	1.4640	1.3195
29	1.3473	1.2250	1.3338	1.3096
X	7.2838	7.0313	6.5138	6.3166
Y	2.9415	2.5745	2.9375	2.5745

Table 6. Analysis of variance for the effect of genetic groups on relative length of largest autosome

Source of variation	Degree of Freedom	Sum of squares	Mean square	F
Between Genetic groups	3	1.98	0.66	1.94 NS
Error	28	9.52	0.34	
Total	31			

NS - Not significant

Table 7. Analysis of variance for the effect of genetic groups on relative length of smallest autosome

Source of variation	Degrees of Freedom	Sum of squares	Mean square	F
Between Genetic groups	3	0.07	0.02	0.69 NS
Error	28	0.98	0.03	
Total	31			

NS _ Not significant

Table 8. Analysis of variance table for the effect of genetic groups on relative length of X Chromsome

Source of variation	Degrees of Freedom	Sum of squares	Mean square	F
Between Genetic groups	3	3.61	1.20	5.73*
Error	20	4.21	0.21	
Total	23			

* $P < 0.05$

Table 9. Analysis of variance table for the effect of genetic groups on relative length Y chromosome

Source of variation	Degrees of Freedom	Sum of squares	Mean square	F
Between Genetic groups	2	0.36	0.18	5.98*
Error	9	0.27	0.03	
Total	11			

* $P < 0.05$

Fig 10. Arm ratio and centromere index of X chromosome in Local, half-bred Jersey, half-bred Holstein Friesian and pure Jersey cattle.

	Arm ratio	Centromere Index
Local	1.739[a]	0.365[a]
Half-bred Jersey	2.043[b]	0.329[b]
Half-bred Holstein Friesian	1.987[b]	0.338[b]
Jersey	1.690[a]	0.372[a]

Means with same superscript do not differ significantly

Table 11. Analysis of variance for the effect of genetic groups on arm ratio and centromere index of X chromosome.

Source of variation	Degrees of freedom	Arm ratio			Centromere index		
		SS	MS	F	SS	MS	F
Between genetic groups	3	0.56	0.19	4.88*	0.0082	0.0027	5.9561**
Error	20	0.76	0.04		0.0092	0.0005	
Total	23						

* $P < 0.05$ ** $P < 0.01$

Table 12. Arm ratio and centromere index of Y chromosome in Local, Jersey and Holstein Friesian cattle.

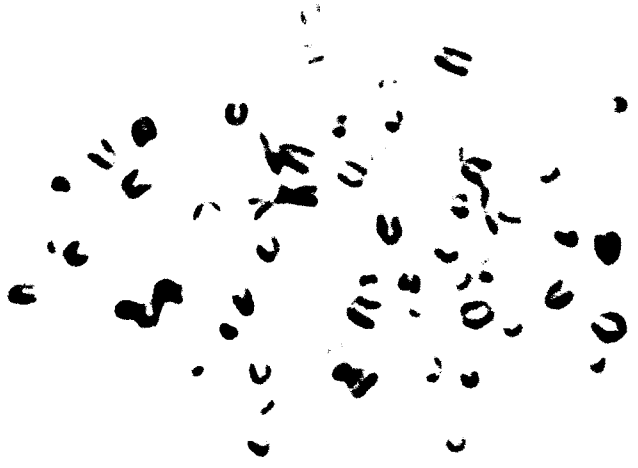
	Arm ratio	Centromere Index
Local	-	-
Jersey	1.210	0.463
Holstein Friesian	1.663	0.376

Table 13. Chromosome profile of reproductive abnormal cattle studied

Phenotype	Sex	Chromosome constitution
Repeat breeder	Female	60, XX
Poor semen quality	Male	60, XX
Sterile with under-developed uterus	Female	59/60 mosaicism
Freemartin	Female	XX/XY chimaerism
Secondary sexual characters	Male	2n/4n mixoploidy

Fig. 1a. Metaphase chromosomes from a Local bull

Fig. 1b. Karyotype of a Local bull



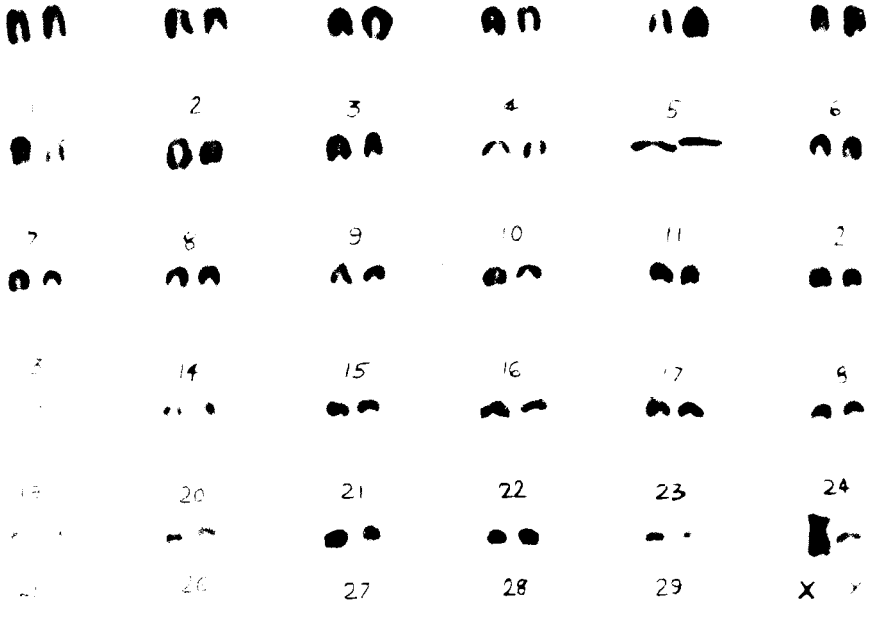

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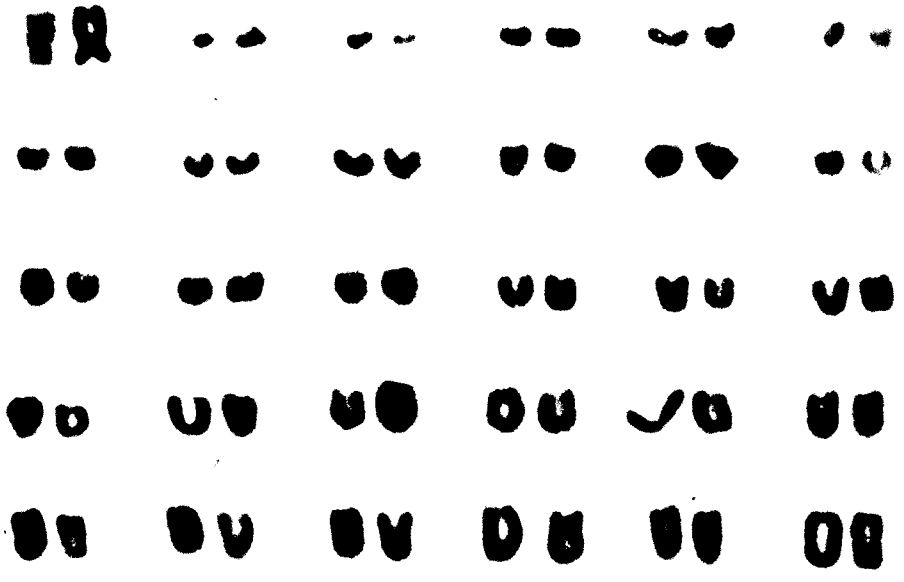
Fig. 2a. Metaphase chromosomes from a Local cow

Fig. 2b. Karyotype of a Local cow



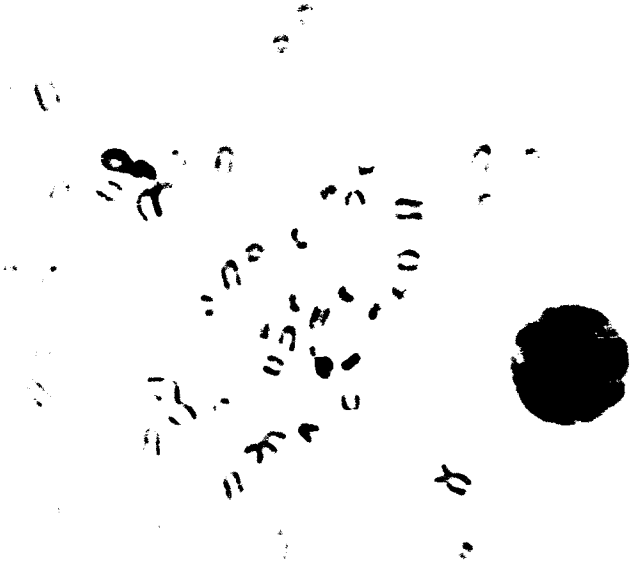


Fig. 3a. Metaphase chromosomes from a half-bred Jersey bull

Fig. 3b. Karyotype of a half-bred Jersey bull

Fig. 4a. Metaphase chromosomes from a half-bred Jersey cow

Fig. 4b. Karyotype of a half-bred Jersey cow

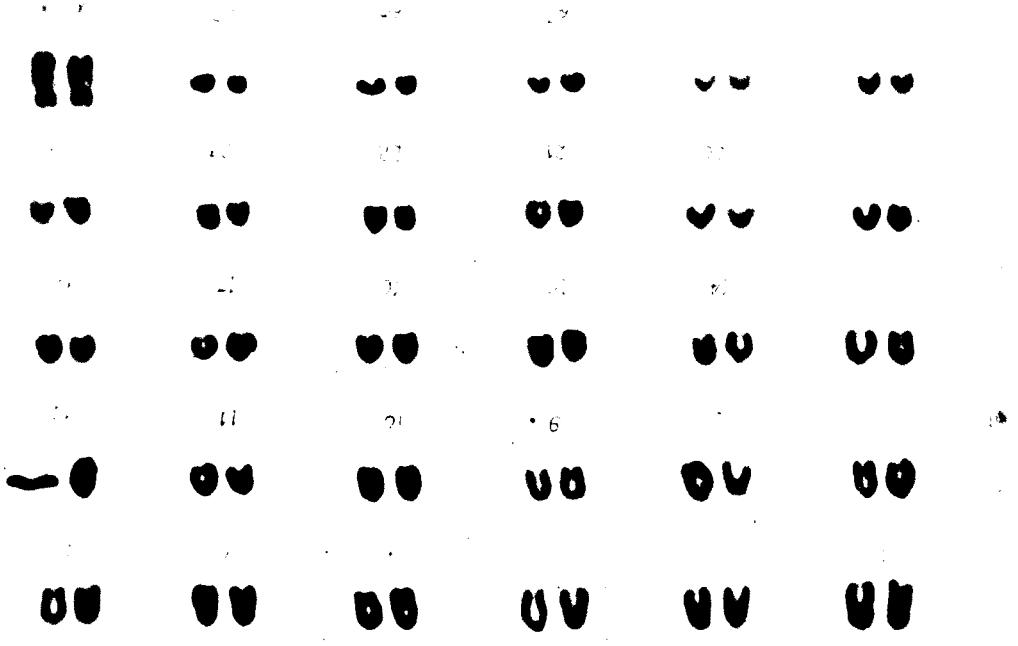
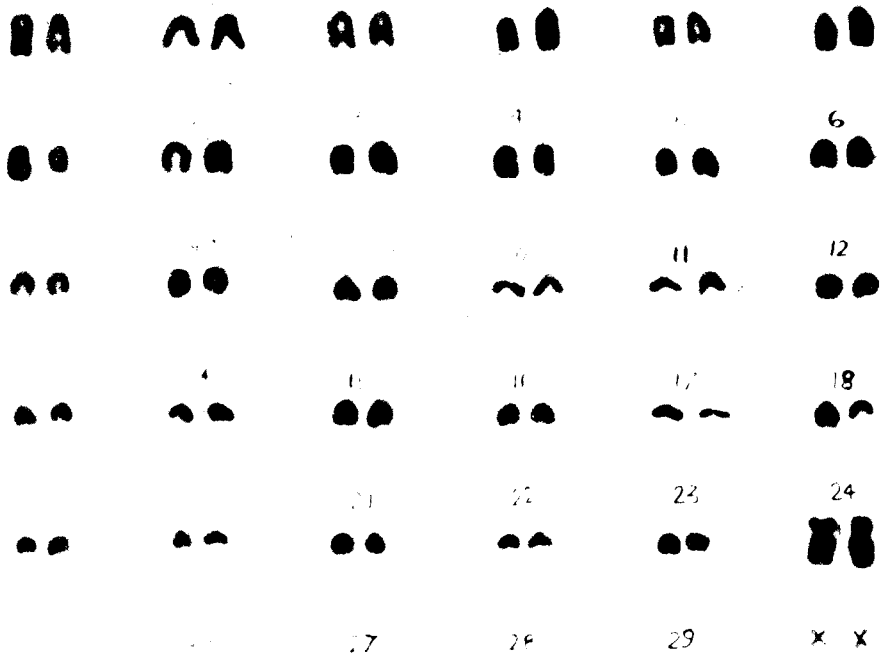


Fig. 5a. Metaphase chromosomes from a half-bred Holstein Friesian bull

Fig. 5b. Karyotype of a half-bred Holstein Friesian bull

Fig. 6a. Metaphase chromosomes from a half-bred Holstein Friesian cow

Fig. 6b. Karyotype of a half-bred Holstein Friesian cow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X X

Fig. 7a. Metaphase chromosomes from a Jersey bull

Fig. 7b. Karyotype of a Jersey bull

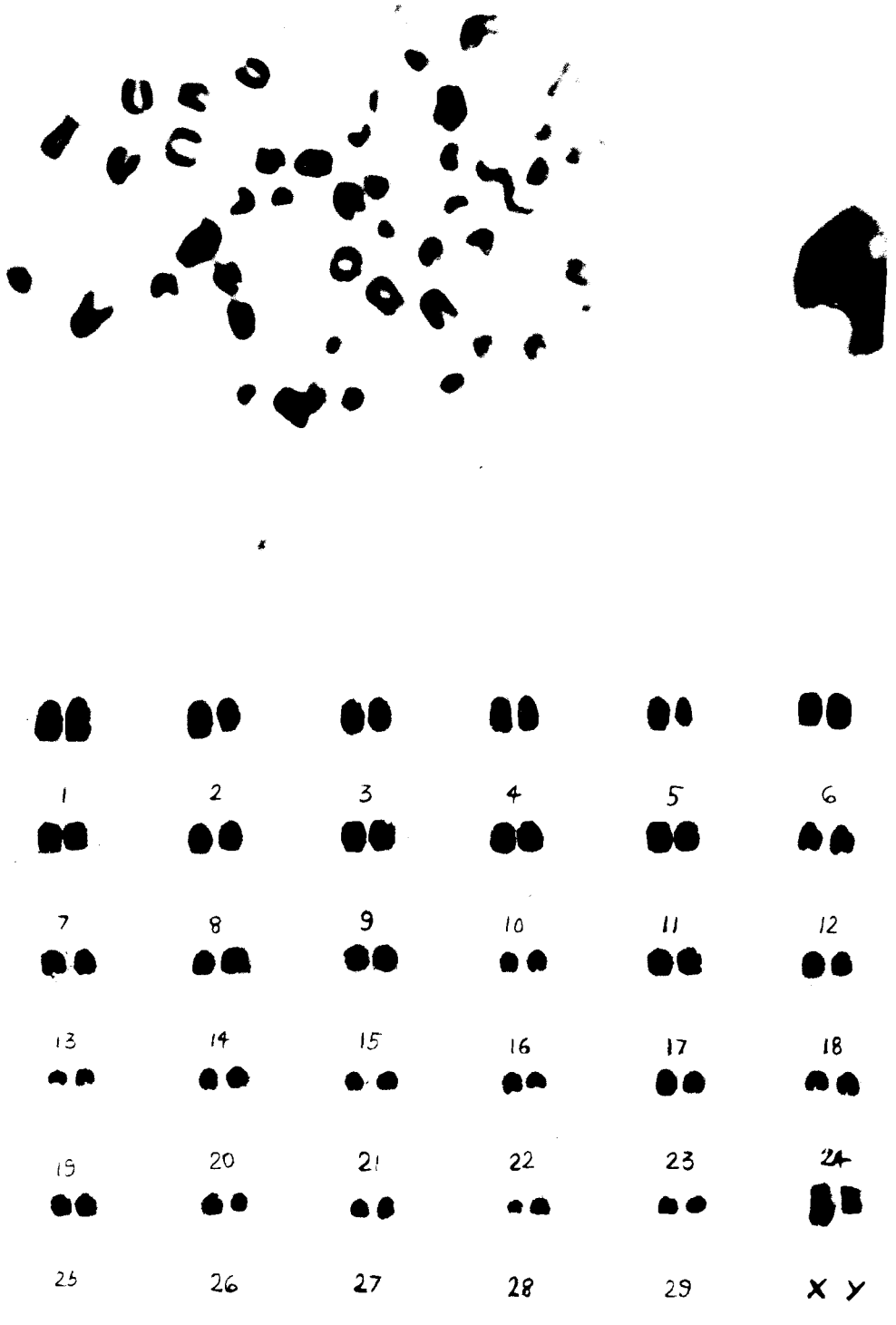
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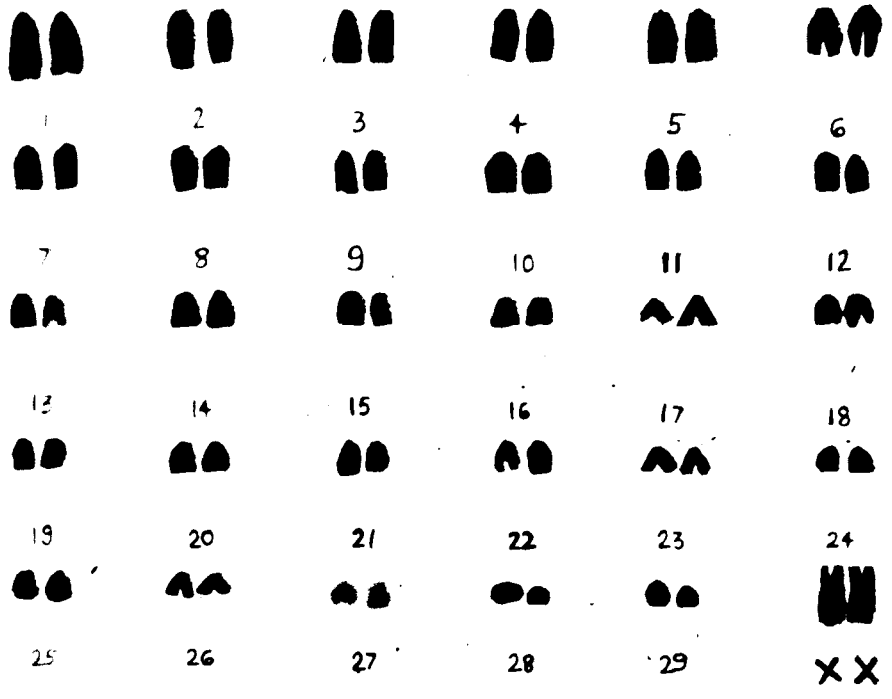
Fig. 8a. Metaphase chromosomes from a Jersey cow

Fig. 8b. Karyotype of a Jersey cow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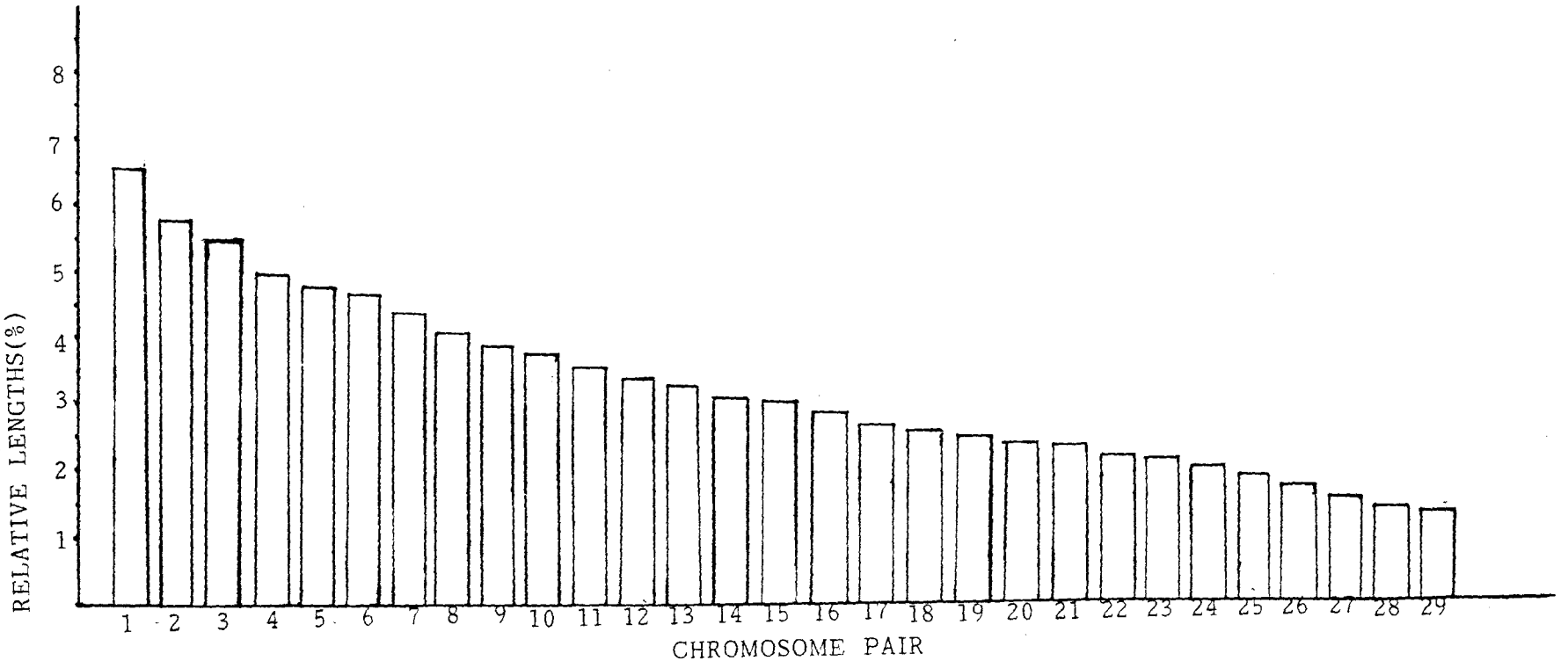

Fig. 9. Idiogram representing the relative lengths of autosomes in cattle

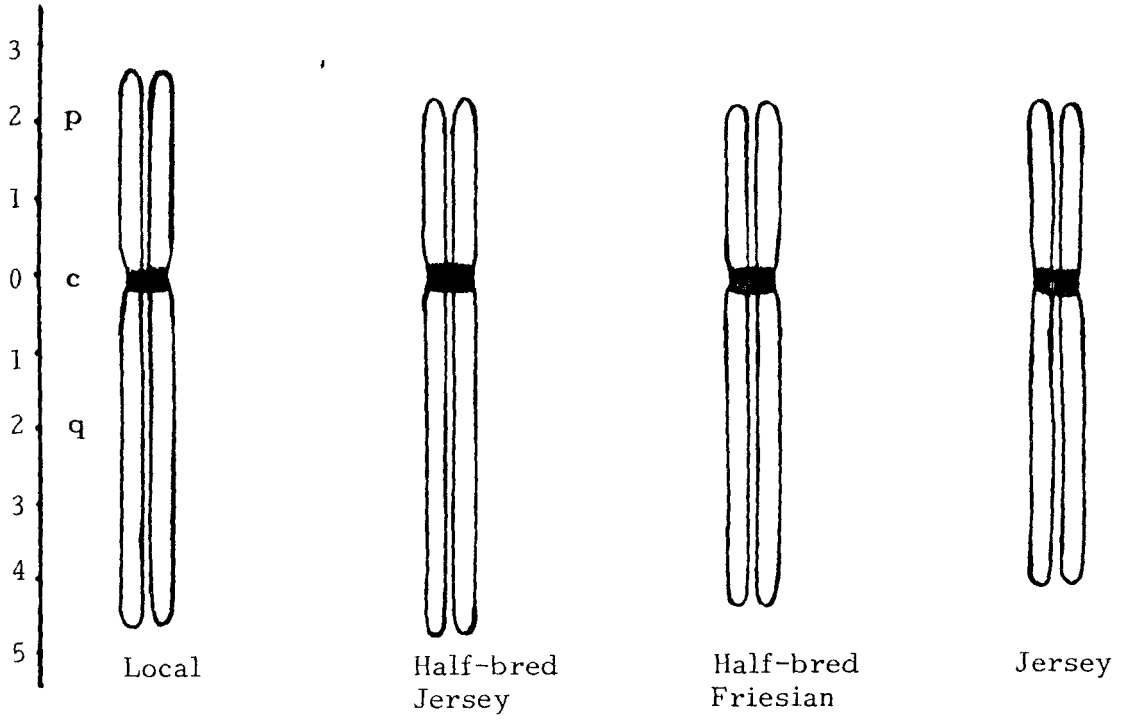

Fig. 10. Diagramatic presentation of p and q arms of the X chromosome in cattle.

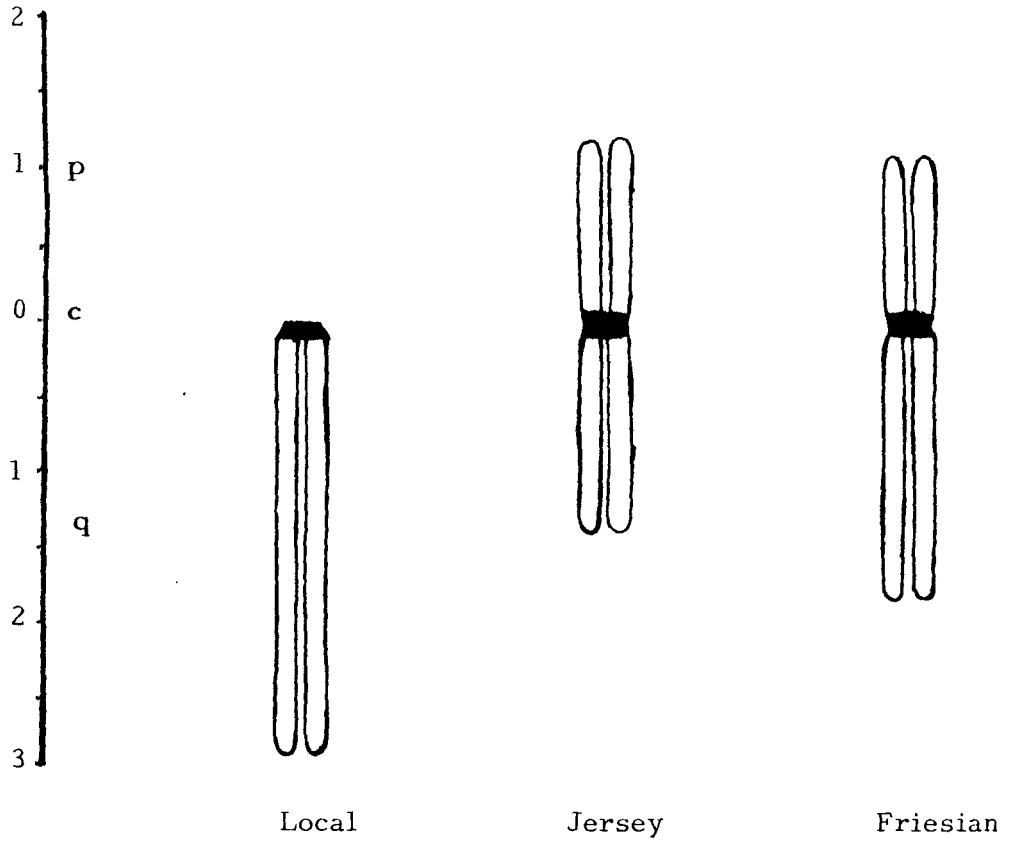

Fig. 11. Diagramatic representation of p and q arms of the Y Chromosome in cattle.

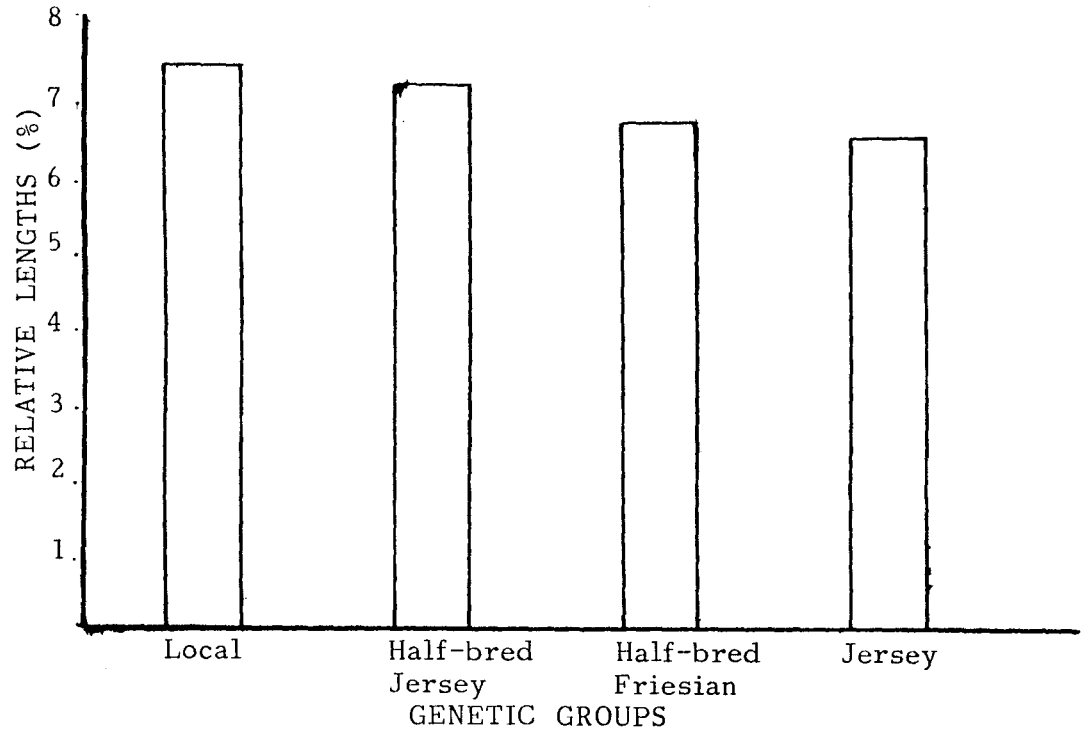

Fig. 12. Idiogram representing the relative lengths of X chromosome

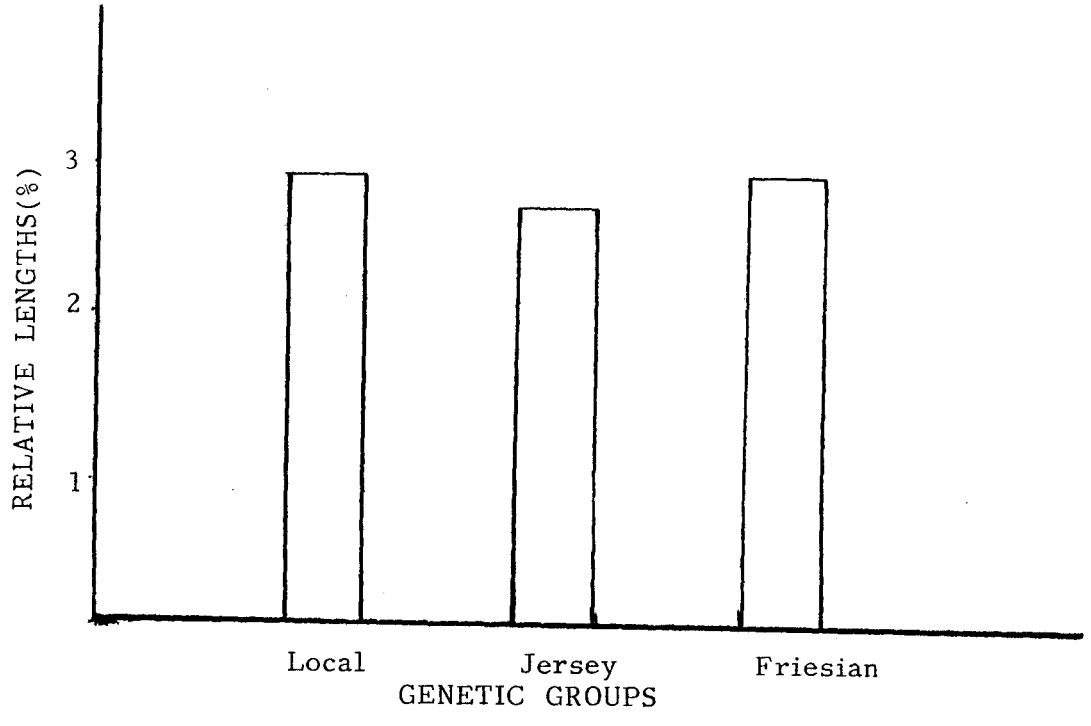

Fig. 13. Idiogram representing the relative lengths of Y chromosome

Fig. 14. 59/60 mosaicism in a sterile heifer

Fig. 15. XX/XY chimaerism in a Freemartin.

Fig. 16. A Local bullock with enlargement of teats

Fig. 17. Metaphase spread of a Local bullock showing tetraploidy

Discussion

DISCUSSION

In the present study cattle chromosome spreads were prepared for different genetic groups using short term peripheral blood lymphocyte culture method with whole blood collected from external jugular vein.

The medium in the present study was TC 199 with extra L-glutamine and which is enriched with autologous plasma and phytohaemagglutinin M at a pH of 7.2. The medium was satisfactory as evidenced by the mitotic drive of 32.6 to 33.6 per cent and mitotic index of 5.6 to 6.3. The use of TC 199 as culture medium and phytohaemagglutinin M as mitotic inducer in cattle was reported by Moorhead et al. (1960), Crossley and Clarke (1962) and Nichols et al. (1962), Ulbrich and Weinhold (1963), Halnan (1977).

The culturing was continued for 72 hrs and one hour before harvesting the mitosis was arrested with 0.1 ml (0.0001 per cent) Colchicine. This yielded proper condensation and the chromosomes were clearly visible.

The possibility of blood samples stored for varying duration in refrigerator temperature at 5°C for lymphocyte culture was evaluated by mitotic index. It was found that the mitotic index suddenly declined after 24 hrs of storage. Similar results were reported by Kanagawa and Basrur (1968). Blood samples stored at refrigerator temperature (5°C) such as in ice bath can therefore be used for lymphocyte culture within 24 hrs after collection.

Basic number

The diploid chromosome number of 2n=60 was observed in Local, half-bred Jersey, half-bred Friesian and pure Jersey cattle. The two haploid sets of chromosomes in female were similar consisting of 29 autosomes and one X chromosome while the two sets were dissimilar with one set consisting of 29 autosomes and one X chromosome and other of 29 autosomes and one Y chromosomes. This observation of homogametic female (XX) and heterogametic male (XY) condition in cattle made in the present study is in agreement with the finding of several workers (Makino, 1944; Chiarelliet, 1960; Basrur and Moon, 1967; Kieffer and Cartwright, 1968; Gupta et al., 1974; Halman, 1975; Sahai and Saxena, 1978; Syed et al., 1986). Thus it can be seen that numerical differences between the four genetic groups could not be noticed in the present study.

Chromosome morphology

The 29 pairs of autosomes of the chromosome set were acrocentric having the centromere almost at the tip. This observation was seen in Local, half-bred Jersey, half-bred Friesian and Jersey cattle studied. This same morphology of acrocentric nature was reported by Krallinger (1927), Makino (1944), Sasaki and Makino (1962), Nichols et al. (1962), Basrur and Gilman (1964). The chromosomes of various breeds by cattle (Bos taurus) have been described as telocentric by Chiarelliet et al. (1960) and Nichols et al. (1962)

implying terminal position of the centromeres. However, various other workers have found these chromosomes as acrocentric (Crossley and Clarke, 1962; Ulbrich and Weinhold, 1963). This controversy may be due to the presence of extremely minute or almost imperceptible second arms in all the autosomes.

The X chromosomes are most conspicuous and easily identifiable element being large and biarmed or a large submetacentric chromosome. The two X-chromosomes in female are isomorphic. Contrary to the present observations, Nichols et al. (1962), Ulbrich and Weinhold (1963) observed the X chromosomes as metacentric. On the basis of the reports of Chiarelliet et al. (1960), Crossley and Clarke (1962), Makino (1962), Basrur and Moon (1967), Sahai (1982), Potter et al. (1979) and the photographic preparations obtained in the study, it can be stated that the X chromosome in both Bos taurus, Bos indicus and their crosses is submetacentric.

The male in all the genetic groups studied revealed a heteromorphic sex chromosome pair with one large submetacentric X chromosome and other small Y chromosome. The Y chromosome of the Local non-descript cattle is revealed a morphology similar to other 58 autosomes in position of centromere or it revealed an acrocentric nature in local cattle. This acrocentric Y chromosome was reported earlier in Bos indicus cattle by Kieffer and Cartwright (1968), Bhatia (1979) and Halman and Watson (1982). The Y chromosomes

of Jersey bulls and cross-breds in which sire is either Jersey or Holstein Friesian, revealed a biarmed nature or submetacentric morphology. Several workers (Melander, 1959; Basrur and Gilman, 1964; Lin et al., 1977; Potter et al., 1979) also reported this submetacentric morphology of Y chromosome in Bos taurus cattle. Stranzinger (1987) confirmed this interbreed difference in morphology of Y chromosome. The present study together with the reports available indicate that there exist a distinct morphological difference in Y chromosomes originated from Bos taurus and Bos indicus cattle. This morphological difference in the two species might have originated through a pericentric inversion. Such an inversion involving breaks at different distances from the centromere could alter the shape of chromosome without a loss or gain of genetic material. In certain species of Drosophila and in members of the orthoptera there is a strong suggestion that pericentric inversions have been influential in producing new karyotypes (Swannson, 1957). Kieffer and Cartwright (1964) observed similar difference in the position of the centromere in Y chromosome of different types of cattle and suggested that the pericentric inversion might be responsible for this anomaly.

Morphometric measurements

Relative length: The relative lengths of autosome in all genetic groups were estimated. The chromosomes were arranged in the form of karyotype after pairing homologs.

In cattle the chromosomes cannot be subgrouped as is done in the case of man owing to their identical morphology. Further the average relative size variation among several pairs makes their identification subjective. In the present study the relative length of largest autosomes was 6.5038, 6.4735, 6.2190 and 6.9135 per cent in Local, half-bred Jersey, half-bred Friesian and Jersey respectively whereas the smallest autosomes were 1.3473, 1.2250, 1.3338 and 1.3096 respectively. The autosomes formed a continuous series and revealed that there is no significant difference in largest and smallest autosomes among the four genetic groups of cattle. This is in agreement with the finding of Potter et al. (1979) who reported that the differences in relative lengths of largest and of smallest autosomes between Bos taurus and Bos indicus cattle were not significant. Sahai (1976) reported the relative length of largest autosomes 5.510 and 5.390 per cent and smallest autosome as 1.760 and 1.760 per cent in Red Sindhi and Hariana breed respectively and concluded that size of the autosomes in both breeds are almost similar.

The relative lengths of X chromosome in Local, half-bred Jersey, half-bred Friesian and Jersey were 7.2838, 7.0313, 6.5138 and 6.3166 per cent respectively. Comparison of different genetic groups revealed that significant differences exist between Local and half-bred Friesian, Local and Pure Jersey, half-bred Jersey and pure Jersey whereas the

differences were not significant between Local and half-bred Jersey, half-bred Jersey and half-bred Friesian, half-bred Friesian and pure Jersey. Among the four genetic groups, the relative lengths of X chromosomes is found to be largest in local cattle and smallest in Jersey.

The X chromosomes in Local, half-bred Jersey and half-bred Friesian cattle occupies the first position in the karyotype array in length of chromosome, whereas it occupies only a position between the 1st and 2nd pair of autosome in pure Jersey cattle. Lin et al. (1977) reported the position of X chromosome as second in the chromosome array in Simmental breed, whereas Sahai (1982) reported a position of first in Sindhi and Hariana breed of cattle. Potter et al. (1979) placed the X chromosome as 2nd in both Bos indicus and Bos taurus cattle. The finding that X is largest chromosome in the chromosome array in the present study may perhaps be the reason for the deviation in findings on comparative size of X chromosomes by Potter et al. (1979).

The relative lengths of Y chromosomes in Local, Jersey and Holstein Friesian were 2.9415, 2.5745 and 2.9375 respectively. The differences in relative length of Y chromosomes between different genetic groups were significant and it was observed that the difference between Local and Jersey, and Holstein Friesian and Jersey were significant whereas that of Local and Holstein Friesian was not significant.

The Y chromosome of cattle was polymorphic in different

species (Halnan, 1976). In the karyological array, the Y chromosome of local cattle occupied a position between 15th and 16th autosome pair. This report is higher than reported by Sahai (1982) who reported the position between 27th and 28th autosome. In Jersey and the cross-breds sired by Jersey the Y chromosomes were submetacentric and occupied a position between 15th to 20th pair in length, whereas it was between 15th and 16th autosomes in cross-bred sired by Holstein Friesian bull. Potter et al. (1979) reported the position of Y chromosome between 45 to 55 chromosomes in Bos taurus cattle. Lin et al. (1977) reported the Y chromosome length between 25th and 26th pair of autosomes in Bos taurus cattle.

Arm ratio: The 58 autosomes of all the groups of cattle studies revealed only single arm or acrocentric nature showing no difference between genetic groups.

The X chromosomes were biarmed or sub-metacentric and the arm ratio was 1.739, 2.043, 1.987 and 1.690 in Local, half-bred Jersey, half-bred Friesian and Jersey respectively. In arm ratio, the differences between different genetic groups was found to be significant. The arm ratio between half-bred Jersey and Local, half-bred Jersey and Jersey, half-bred Friesian and Local, half-bred Friesian and Jersey were significant whereas that between half-bred Jersey and half-bred Friesian, Local and Jersey were non-significant. In all the four groups the centromere is situated in between the mid point and tip. From these values it appears that

centromere in Local cattle is more distant to mid point than those of other genetic groups. Distance between the mid point and centromere was lowest in Jersey.

The Y chromosome of Local cattle revealed only acrocentric nature whereas the arm ratio of Y chromosome was 1.21 in Jersey and it was 1.66 in Holstein Friesian. In the Y chromosome the centromere is nearer to mid point in Jersey cattle than Holstein Friesian.

Centromere Index: The centromere index of X chromosomes in Local, half-bred Jersey, half-bred Holstein Friesian and Jersey were 0.365, 0.329, 0.338 and 0.372 respectively. The difference between genetic groups was significant. The position of centromere was away from the centre in all types of cattle studied indicating sub-metacentric morphology. Comparison of the position of centromere between genetic groups based on centromere index indicates that centromere is nearer to the mid point in Jersey than the other genetic groups and is far away in Local cattle.

The centromere index of Y chromosome in Jersey and Holstein Friesian was 0.463 and 0.376 respectively. This indicates that in Local the Y chromosome is acrocentric. In Jersey the centromere is in close proximity to the mid point. In Holstein Friesian it was 0.376 which is significantly away from centre when compared to Jersey indicating submetacentric morphology.

Potter et al. (1979) explained the morphology of Y chromosome in Brahman (Bos indicus) as acrocentric and in Friesian

as submetacentric whereas in Jersey it was metacentric. The present findings do not disagree with those of Lin (1977), Potter et al. (1979) and Kieffer and Cartwright (1986) as regards to the position of centromere and distance of the centromere from the midpoint. Since the values of the arm ratio and centromere index were not seen reported in the paper, the metacentric nature of the Y chromosome could not be verified. However tendency to show the metacentric condition in Jersey is observed in the present study also.

No satellite could be identified in any of the chromosomes studied which facilitate grouping and identification of chromosomes. Grouping of chromosomes in the karyotype may have some usefulness in facilitating easier identification of chromosomes, since it is easier to remember a specific chromosome within a group of five or six individual pairs of chromosomes. However because of the close morphological similarity between individual bovine chromosomes, grouping is done on a rather arbitrary basis. Thus a standard system of naming and grouping of chromosomes should be established among laboratories working with bovine chromosomes.

The observed Y chromosome polymorphism is not a rare observation because such variation was reported in human beings. Whether this in any way lead to problems of infertility, as reported in cattalo by Basrur and Moon (1967) could not be established in the present study.

In the present study there is no apparent difference in

the chromosome make up such as basic number except that of relative length and Y chromosome morphology between Local, half-bred Jersey, half-bred Friesian and Jersey. Their karyotype similarity may be the reason for fertile hybrids. No chromosomal aberration such as aneuploidy, euploidy or other numerical aberrations could be identified among the Bos indicus, Bos taurus and their hybrids.

Abnormality

The study of chromosomes from lymphocytes is a convenient technique for clinical survey and population studies. It was established that the chromosomal abnormalities revealed in blood cells are often, although not always, mirrored in cells from other somatic tissue.

Five cows classified as sterile were subjected to chromosome analysis. Four of the sterile cows revealed 60 chromosomes with 29 pairs of acrocentric autosomes and one pair of submetacentric sex chromosomes. No numerical abnormality could be identified in these animals.

One sterile half-bred Jersey heifer showed normal heat symptoms but did not conceive even after attaining 4½ years of age. The chromosome analysis revealed that the animal is 59 XX/60 XX mosaic with normal sex chromosome complement. The normal oestrual signs indicated that anatomical and physiological development have taken place to some extent though not fully. The 59/60 mosaicism may give rise to errors in meiosis resulting in defective oogenesis. Failure in normal

oogenesis may be responsible for the impaired conception. Similar case of 59 chromosome which was arisen as a result of Robertsonian translocation of 1st and 29th autosomes and the impairment in meiosis was described in detail by Gustavsson (1979). Pullock (1972) reported the incidence of abnormal chromosome constitution with 59 chromosome in British Friesians. Bruere and Chapman (1973) reported a case of 59 chromosomes due to tandem fusion of 14th and 20th chromosome in simmental cows. The sterility either as a result of lack of oogenesis or early embryonic death was attributed to this abnormal chromosome constitution. The embryos resulting from errors of meiosis, fertilization or early cleavage division, most often do not survive and are a contributory factor to the relatively large number of early zygotes that suffer embryonic death.

On karyological examination of twenty-four infertile cattle including 21 females and 3 males, it was found that all animals exhibited normal chromosome constitution (2n=60) with 29 pairs of acrocentric autosomes and XX in female and XY in male. No numerical aberration could be detected in any of the animals. It seems that the reason for infertility may be due to the causes other than ploidy condition.

One Free martin which was sired by a Jersey bull was examined. The metaphase spreads revealed that 86 per cent were of female origin (XX) whereas 14 per cent male origin (XY) and the animals showed poor development of sexual organs.

Free martinism in cattle was reported by Ohno et al. (1962), Fechheimer et al. (1963), Kanagawa (1965), Yadav et al. (1984), Syed et al. (1986) with varying proportion of XX and XY cells in blood. According to Lillie's hypothesis (1967) when twins were of different sexes, the anastomosis of blood vessels would allow male testicular hormone from the earlier differentiating foetal testicle to retard normal development of the heifer calf's sex organs and in fact stimulate partial male development in the process. It is no longer quite so certain that this explanation is the correct one. In any case, it cannot just be hormones from the bull foetus which are responsible for the development of the free martin state, as abnormal development of the heifer foetus has been shown to arise as early as the 30 to 40th day of pregnancy not before any differentiation of ovaries and testicles has taken place, and before the time at which it has been possible to demonstrate any production of testicular hormone. Male and female foetuses with a common blood system may, however, also exchange blood cells, thus allowing male XY cells to enter the blood vessels of the heifer foetus, and XX cells enter those of bull foetus. The later event has no demonstrable effect on the sexual development of the bull foetus. On the other hand, the Y chromosome from the bull foetus will divert the sexual development of the female foetus in the male direction with a free martin calf as the result (Fechheimer et al., 1963). It is however, quite possible that hormonal influences from

the bull calf will play a role at a later stage of foetal development.

On examination of metaphase spreads of lymphocytes collected from a Local non-descript bullock which showed abnormal development of rudimentary teat and secreting a milky fluid, it was found that 4.5 per cent of the cells had the chromosome number 4n=120 showing the incidence of tetraploidy. The other cells were of normal type with 2n=60. The animal was a diploid/tetraploid chimaera or mixoploid. 2n/3n mixoploidy was explained by Dunn (1970) in Holstein true hermaphroditism. The animal was explained as having penis with an empty scrotum and a left abdominal ovary but the animal was sterile.

Summary

SUMMARY

Investigations to locate and vacate the problems of chromosomal aberrations leading to physiological disorders of cattle, are inevitable to achieve the progress envisaged in the various developmental programmes under Animal Husbandry sector. An attempt to assess the cytogenetic profile of cattle of the State of Kerala through karyological studies of Zebu, Taurus and their crosses and also to identify the incidence of chromosomal aberrations associated with productive and reproductive efficiency has been made.

Cattle stationed at various centres of Kerala Agricultural University, Indo-Swiss Project and those maintained by the farmers of the State formed the materials for the present study. Cattle of Local non-descript, half-bred Jersey, half-bred Holstein Friesian and pure Jersey breeds were screened to obtain their cytogenetic profile. On the basis of clinical examination and history, the cattle were classified as normal, sterile, infertile, free martin and other abnormal. In all, 102 animals were subjected to karyological study using peripheral blood leukocyte culture.

Blood (15 ml) was collected from external jugular vein into heparinised tubes and brought to the laboratory within 2 hours after collection. One ml of blood was kept separate for seeding the culture and the rest was centrifuged to separate the plasma. The medium used was TC 199 containing l-Glutamine and penicillin. Phytohaemagglutinin-M (Difco)

was used as mitotic inducer. Into 5 ml of culture medium with a pH 7.2, 0.7 ml of whole blood and 2.5 ml of autologous plasma were added and incubated at 37 ± 0.5°C for 72 hours. The mitosis was arrested at seventieth hour with 0.1 ml of colchicine (0.0001 per cent) and incubation continued for one hour. The cells in metaphase were harvested and spreads prepared and stained with Giemsa.

In normal animals the mitotic drive and mitotic index were estimated as 33.66 per cent and 5.66 per cent respectively and in abnormal animals they were 32.66 per cent and 6.33 per cent respectively. The medium used was therefore found to be efficient for cattle lymphocyte culture. The colchicine treatment of 0.1 ml (0.0001 per cent) for one hour yielded distinct chromosome picture and facilitated easy identification. The mitotic index of lymphocytes showed rapid decline 12 hours after the storage in ice bath or refrigerator at 5°C. It is therefore recommended that the duration between the collection of samples and initiation of culturing in the laboratory should not exceed 12 hours.

The karyotypes revealed a diploid chromosome number of 60 (2n=60) in all normal cattle irrespective of genetic groups. The chromosome complement consisted of 58 autosomes and one pair of sex chromosome in all the genetic groups. The sex chromosomes were similar (XX) in female and dissimilar (XY) in male.

In Bos taurus and Bos indicus and their crosses, autosomes

revealed a single arm acrocentric morphology with imperceptible short arm. The genetic groups did not show any difference in the position of the centromere in autosomes.

The X chromosomes were of biarmed nature and showed a large sub-metacentric morphology in all the genetic groups.

The Y chromosome was polymorphic in different breeds. In local it revealed single arm or acrocentric similar to autosomes whereas Y chromosomes of Jersey and Holstein Friesian were biarmed exhibiting submetacentric morphology.

The relative lengths of autosomes ranged from 1.3473 to 6.5080 per cent in Local, 1.2250 to 6.4735 per cent in half-bred Jersey, 1.3788 to 6.2190 per cent in half-bred Friesian and 1.3096 to 6.9125 per cent in pure Jersey cattle.

Comparison of autosomes individually was impossible as a result of continuous nature of relative length and morphological similarity. The largest and smallest autosomes were however, compared. Although, difference in relative length of largest autosome between the 4 genetic groups was observed, the difference was not significant. Similar observation was made as regards to the smallest autosome of 4 genetic groups.

The relative length of X chromosome was 7.2838 per cent, 7.0313 per cent, 6.5138 per cent and 6.3166 per cent in Local, half-bred Jersey, half-bred Friesian and Jersey cattles respectively. The comparison of relative lengths of X chromosomes revealed significant difference between genetic groups.

Relative length of X chromosome of Local cattle was significantly larger than that of half-bred Friesian and pure Jersey cattle. Tendency to be shortest in relative length was noticed in pure Jersey among the genetic groups, but the difference was not significant, between half-bred Friesian and pure Jersey. Half-bred Friesian did not differ significantly from half-bred Jersey.

In the karyotypic array based on relative length, the X-chromosome occupied a first position in Local, half-bred Jersey and half-bred Friesian whereas it was seconded to the largest pair of autosomes in pure Jersey.

The relative length of Y chromosome was 2.9415 per cent, 2.5745 per cent and 2.9375 per cent in Local, Jersey and Holstein Friesian respectively. The difference between Local and Jersey and Holstein Friesian and Jersey were significant whereas that of Local and Holstein Friesian was not significant.

In the karyotypic array, the Y chromosome of Local and Holstein Friesian occupied a position between 15th and 16th pair of autosomes. In Jersey it was in between 15th and 20th pair.

Arm ratio was not relevant to autosomes as they were acrocentric.

With regard to X chromosomes, the arm ratio was 2.043, 1.986, 1.739 and 1.690 in Local, half-bred Jersey, half-bred Friesian and Jersey respectively. On the basis of arm ratio

position of centromere between the tip and mid point of chromosome was described. The comparison of genetic groups based on arm ratio revealed that centromere was located far away from the mid point in Local cattle. The centromere was closer to mid point in pure Jersey cattle.

The arm ratio of Y chromosome in Jersey and Holstein Friesian was 1.21 and 1.66 respectively. The difference in arm ratio between Jersey and Holstein Friesian was significant showing that the location of centromere was more towards the mid point in Jersey than that observed in Holstein Friesian.

The position of centromere of X chromosome in each genetic group determined by arm ratio was further tested using centromere index. The index was 0.365 in Local, 0.329 in half-bred Jersey, 0.338 in half-bred Friesian and 0.372 in Jersey. It was found that the centromere was nearer to the mid point in Jersey than in other genetic groups and is far away in Local cattle. The results obtained through the estimation of arm ratio and centromere index were in agreement with each other.

The centromere index of Y chromosome of Jersey and Holstein Friesian was 0.463 and 0.376 respectively and the difference was found to be significant. In Jersey centromere was more towards the centre and in Holstein Friesian it is in between the mid point and tip, as observed based on arm ratio.

No satellite could be identified in any of the chromosomes.

The cattle showing various conditions of physiological disorders were subjected to chromosome analysis. Out of 5 sterile heifers screened, 4 revealed normal chromosome constitution. One $4\frac{1}{2}$ year old half-bred sterile Jersey heifer showed 59 XX/60 XX mosaicism with 4 per cent of the cells containing only 59 chromosomes. It is assumed that the errors in meiosis consequent to mosaicism may be responsible for sterility.

Twenty-four infertile cattle with various reproductive disorders such as repeat breeding and poor semen quality revealed normal chromosome constitution. Reason for infertility in these animals may be the causes other than changes in the chromosomal number.

Metaphase spreads of one Jersey Free martin with poor development of sex organs revealed that 14 per cent cells were of male origin (XY) and 86 per cent female origin (XX) exhibiting a XX/XY chimaerism. The exchange of XX and XY cells in heterosexual twins by vascular anastomosis during early embryonic development may be the reason for reproductive disorders. The male cells (XY) in female embryo at very early stage will divert the development of foetal embryo towards the male direction leading to free martinism.

One Local bullock showed the development of teats with a small glandular tissue secreting a milky fluid. The teats were protruding out around the scrotum. This animal revealed that 4.5 per cent of cells were of tetraploid nature (4n=120)

and other cells diploid (2n=60) or a diploid tetraploid chimaerism or mixoploidy.

The present study brought out findings that relative length, position in the karyotype array, arm ratio and centromere index of sex chromosomes shall serve as tool for identification of interbreed differences and that the occurrence of tetraploidy stimulate the activity of the female secondary sexual characteristics in male cattle.

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CHROMOSOME PROFILE OF ZEBU X TAURUS CATTLE IN KERALA

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ABSTRACT OF A THESIS

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ABSTRACT

A study was undertaken to evaluate and compare the chromosomal status of Local non-descript, half-bred Jersey, half-bred Holstein Friesian and Jersey cattle and to assess the incidence of chromosomal aberrations causing physiological disorders. The cattle owned by Kerala Agricultural University, Indo-Swiss Project and farmers formed the material for the study. In all, 102 animals consisting of 71 normal and 31 abnormal, were subjected to the study.

Peripheral blood leukocyte culture technique with heparinised whole blood was used for chromosome analysis. The medium was TC 199 with phytohaemagglutinin M as mitotic inducer and colchicine as mitotic arrester. The cells in metaphase were harvested and air dried smears stained with Giemsa. Good spreads were photographed and karyotypes prepared. The efficiency of medium was tested using mitotic drive and mitotic index, which were between 32 to 33 per cent and 5 to 6 per cent respectively. Colchicine treatment of 0.1 ml (0.0001%) for 1 hour yielded clear visible chromosome spreads. The blood samples stored for varying time at 5°C indicated that the whole blood transported in ice bath (5°C) is to be used within 12 hours after collection for lymphocyte culture.

In normal cattle, diploid chromosome number was found to be 2n=60 with 29 pairs of autosomes and one pair sex chromosome. The males were heterogametic. All the autosomes were

acrocentric in Local, half-bred Jersey, half-bred Friesian and Jersey whereas the X chromosome was biarmed and a large submetacentric in all the genetic groups. The Y chromosome was polymorphic being acrocentric in Local and submetacentric in exotic bulls. No satellite was observed in any of the chromosomes.

The relative length of largest and smallest autosomes were 6.5080 and 1.3473 per cent in Local, 6.4735 and 1.2250 per cent in half-bred Jersey, 6.2190 and 1.3788 per cent in half-bred Friesian and 6.9125 and 1.3096 per cent in Jersey respectively. The difference in relative length of autosomes between different genetic groups was not found to be significant.

The relative length of X chromosome was 7.2838 per cent, 7.0313 per cent, 6.5138 per cent and 6.3166 per cent in Local, half-bred Jersey, half-bred Friesian and pure Jersey respectively. The differences between genetic groups were significant. In the karyotypic array based on relative length, the X chromosome occupied a first position in Local, half-bred Jersey, half-bred Friesian whereas in Jersey it was in between first and second pair of autosomes.

The relative length of Y chromosome was 2.9415 per cent, 2.5745 per cent and 2.9375 per cent in Local, Jersey and Holstein Friesian respectively. The difference Local and Holstein Friesian was not significant. In karyological array the Y chromosome occupied a position between 15th and 16th

pair of autosomes in Local and Holstein Friesian whereas in Jersey it was between 15th and 20th pair.

The arm ratio of X chromosome was 2.043, 1.986, 1.739 and 1.690 in Local, half-bred Jersey, half-bred Friesian and Jersey respectively. In Local cattle the centromere was located away from mid point compared to other genetic groups. The distance between mid point and centromere was lowest in Jersey. The arm ratio of Y chromosome of Jersey and Holstein Friesian was 1.21 and 1.66 respectively. The location of centromere in Y chromosome of Jersey was more towards centre than that of Holstein Friesian.

The centromere index of X chromosome was 0.365, 0.329, 0.338 and 0.372 in Local, half-bred Jersey, half-bred Holstein Friesian and Jersey respectively. The values for the centromere index confirm the findings obtained for arm ratio with regard to the proximity of centromere to the mid point of the chromosome.

Among the 31 abnormal cattle, chromosomal aberration were observed in one $4\frac{1}{2}$ years old sterile Jersey heifer, one Free martin and one Local bullock with abnormally developed secondary sexual characters. Infertile cattle showing repeat breeding, poor semen quality and poor libido did not exhibit any aberration.

In the sterile Jersey heifer, 59/60 mosaicism was observed. The Free martin exhibited 60 XX/60 XY chimaerism having 14 per cent of the cells with XY type and others with XX type.

The local bullock revealed abnormal development of teats and secretion of milky fluid. The mitotic spreads were of tetraploid nature (4n=120) in 4.5 per cent cells and the diploid (2n=60) in others. This animal was diploid tetraploid chimaera or mixoploid.

The present study brought out findings that relative length, position in the karyotypic array, arm ratio and centromere index of sex chromosomes shall serve as tool for identification of inter-breed differences and that the occurrence of tetraploidy stimulate the activity of the female secondary sexual characteristics in male cattle.