CHROMOSOME PROFILE OF CROSS-BRED BULLS IN KERALA

BY

C.P.GOPAKUMAR

THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Animal Breeding and Genetics
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy, Thrissur

1994

DECLARATION

I hereby declare that this thesis entitled **"Chromosome profile of cross-bred bulls 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 of any degree, diploma, associateship, fellowship or other similar title of any other University or society.

Mannuthy,

C.P. GOPAKUMAR

CERTIFICATE

Certified that this thesis entitled " **Chromosome profile of cross-bred bulls in Kerala** " is a record of research work done independently by Dr. C. P. Gopakumar 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.

Dr.K.V. Raghunandanan
(Chairman, Advisory Board)
Associate Professor,
Department of Animal Breeding and Genetics,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur.

CERTIFICATE

We, the undersigned members of the Advisory Committee of Dr. C.P. GOPAKUMAR, a candidate for the degree of Master of Veterinary Science in Animal Breeding and Genetics, agree that the thesis entitled " **Chromosome profile of cross-bred bulls in Kerala**" may be submitted by Dr. C.P. GOPAKUMAR in partial fulfilment of the requirement for the degree.

Dr. K. V. Raghunandanan,
Associate Professor,
Department of Animal Breeding and Genetics,
College of Veterinary and Animal Sciences,
Mannuthy, Thrissur.

Dr. G. Mukundan,
Director,
Centre for Advanced Studies
in Animal Genetics and Breeding,
College of Veterinary
and Animal Sciences, Mannuthy.

Dr. K. Prabhakaran Nair,
Professor,
Dept. of Animal Reproduction,
College of Veterinary
and Animal Sciences,
Mannuthy.

Dr.C.T. Chacko,
Dy. General Manager (AH)
Kerala Livestock Development
Board Limited,
Shasthamangalam,
Thiruvananthapuram.

Dr. R. Madhusoodhanan Pillai,
Associate Professor,
Dept. of Microbiology,
College of Veterinary and
Animal Sciences.
Mannuthy.

EXTERNAL EXAMINER 18/2/95

Dedicated

to My Mother

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to Dr.K.V. Raghunandanan, Associate Professor, Department of Animal Breeding and Genetics and Chairman of Advisory Committee for his valuable guidance, inspiring advice and constructive criticisms at all stages of the study and while preparing this manuscript.

I gratefully acknowledge my indebtedness to Dr. G. Mukundan, Director, Centre for Advanced Studies in Animal Genetics and Breeding and member of Advisory Committee for his invaluable suggestions and encouragement.

I wish to place on record my sincere gratitude to Dr.C.T. Chacko, Deputy General Manager, Kerala Livestock Development Board, Dr.K. Prabhakaran Nair, Professor, Department of Animal Reproduction and Dr. R. Madhusoodhanan Pillai, Associate Professor, Department of Microbiology, members of the Advisory Committee for their timely help and guidance through out the period.

I am grateful to Dr.C.R. Girija, Assistant Professor,

Department of Animal Breeding and Genetics for providing constant inspiration and support.

I am indebted to Dr.Sosamma Iype, Dr.B. Nandakumaran, Dr.P. Nandakumar, Dr.Stephen Mathew, Dr. T.V. Aravindakshan and other staff members of the Department of Animal Breeding and Genetics for extending timely help and suggestions.

I wish to pay a grateful acknowledgement to Dr.A. Rajan Dean, College of Veterinary and Animal Sciences for providing the facilities for this study.

I am thankful to Kerala Livestock Development Board Ltd. (K.L.D.B.) for deputing me to persue this work.

I take great deal of pleasure to thank Dr.T.K. Jose, former Managing Director and Dr.P.J.Joseph, Managing Director, K.L.D.B. for making the animals and their relevant records available for the study.

I wish to thank the staff of K.L.D.B. Farm, Dhoni, particularly Dr.G. Dinesh and Dr.B.N.Kishan for their assistance in collecting the blood samples and relevant data of the animals studied.

I extend my sincere thanks to Dr.K. Muraleedharan, Dr.G. Kishore and Sri. Sunesh Rajan, staff members of K.L.D.B., Muvattupuzha unit for helping me in the statistical analysis of the data collected in the course of this study.

I am particularly indebted to Dr. S.K. Choudhari, Ph.D. student, Department of Pathology for his technical advices and help at various stages of this investigation.

I am grateful to Dr.K.M. Ramachandran, Director in-charge, Centre of Excellence in Pathology for providing the facilities and Sri. Peter for developing and processing the photographs.

The keen interest taken in the progress of this work and the inspiration given by my colleagues are gratefully acknowledged.

The task would not have been completed but for the patience and love of my wife Deepa and daughter Radhika. I am grateful to them for bearing with all the inconvenience.

C.P. GOPAKUMAR

CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

The livestock policy of India, since mid-sixties favoured breeding high quality milch animals, and cross-breeding of indigenous cattle with exotic bulls to achieve a quick break-through in milk production. In Kerala also, cross-breeding was taken up, limiting the exotic inheritance to 50 per cent. The development of frozen semen technology and artificial insemination (A.I.) resulted in a significant increase of cross-breds in the population.

Breeding programme of Kerala envisages creation of a synthetic breed by cross-breeding the local non-descript cattle with exotic breeds like Jersey, Brown-Swiss (B.S.) and Holstein-Friesian (H.F.). Subsequently the cross-breds are interse mated and selection is carried out for milk yield. The frozen semen for A.I. in the state is produced by 200 to 250 cross-bred bulls maintained at the bull stations of Kerala Livestock Development Board (K.L.D.B.). In any cattle development programme utilising A.I., the males kept as gene reserve is small and hence rapid spread of hereditary defects in the population is possible if semen of unscreened bulls are used.

An enthusiasm to observe chromosomes has characterised animal geneticists since it was recognized that chromosomes are the vehicles in which the genes reside. The science of cytogenetics developed in the expectation of finding association between variation of chromosome number, morphology or behaviour and gross anatomical or physiological functions of animals as a whole. In farm animals, much of the pregnancy wastage may be caused by abnormal chromosome complement of afflicted zygote. Sterility and infertility in animals may also be mediated by a chromosomal anomaly. The inclusion of cytogenetic analysis in the diagnostic armamentarium of laboratories working with animal infertility will be of great value.

The Bos taurus x B. indicus bulls are established to be inferior in their semen producing ability compared to pure B. taurus breeds. A substantial number of cross-bred bulls are eliminated in the process of selection for semen production. If in a herd one or more animals are afflicted with an abnormal phenotype with regard to seminal attributes, a comparison of karyotype of these probands with that of other representative normal bulls will be of value. If the karyotypes are found to differ, then grounds will be set for further studies. The results of such studies may be helpful in taking early decision during the routine selection process.

As more animals are karyotyped with various banding techniques, a number of variant banding patterns may be detected in livestock. Banding variations as well as structural polymorphisms of the Y chromosome, and perhaps of the other chromosomes, will become increasingly useful as genetic markers for identification of different breeds of cattle and to study the relationship between them.

Karyotyping of stud bulls and bull mothers was one among the recommendations put forward by the committee constituted to evaluate and formulate livestock breeding programmes and policies in the state of Kerala. However, karyological investigation had been conducted in cattle only on a limited scale, and routine cytogenetic screening had not been done as part of the selection process in the state. Hence, this work was taken up in the breeding bulls with the following objectives.

1. Assess the chromosomal status of cross-bred bulls used for A.I. in Kerala.
2. Analyse the G-banded chromosomes of the bulls.
3. Study the semen quality and related attributes of the bulls subjected to cytogenetic screening.
4. Analysis of the correlation between chromosomal status and semen characteristics.
5. Study the cytogenetic variants of bulls belonging to different genetic groups.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Report of the Fourteenth Quinquennial Livestock Census in Kerala (Government of Kerala, 1989) revealed that about 49 per cent of the cattle in the state were cross-breds. The sex-ratio in cattle expressed as number of males for every 100 females was reported as 17.1. The coverage of the cross-breeding programme had undergone considerable change with the introduction of frozen semen technology. Introduction of this technology, decline in the requirement of work animals and slaughter of cattle shifted the sex-ratio of cattle in favour of females (George and Nair, 1990).

Kovács (1984) had attributed A.I. and the small number of animals kept as gene reserves as the possible source for the rapid distribution of genetic abnormalities. These situations upheld the importance of screening the bulls for chromosome abnormalities. The committee to evaluate and formulate livestock breeding programmes and policies in the state of Kerala (Mukundan, 1992) had recommended karyotyping of the breeding bulls and bull mothers to eliminate the animals carrying chromosomal abnormalities.

1. Characters of bovine chromosomes:

Makino (1944) reported the diploid chromosome number of cattle as 60. All the 58 autosomes appeared to be telocentric or acrocentric. The X chromosome was reported to be sub-metacentric, and Y chromosome sub-metacentric in B.taurus and acrocentric in B. indicus.

Lin et al. (1977) proposed a system for the nomenclature of the G-bands produced by trypsinisation of bovine chromosomes. A total of 310 bands were assigned in the bovine karyotype. Autosomes 3,5,7,9,12,15,16,17,21 and 26 were reported to have remarkable banding features which made identification of these chromosomes easy. The remaining 19 autosomes were also reported to have specific banding characteristics, making individual identification possible.

Potter et al. (1979) studied the G-banding patterns in some breeds of Australian cattle. A similarity of banding patterns in B. taurus and B. indicus cattle, except in Y chromosome was described.

Arruga and Zarazaga (1984 b) calculated the relative length of chromosome pairs in five breeds used in Spain. Variation in relative lengths were found to be more in sex chromosomes compared to the autosomes, variation being highest in Y chromosome.

The second international conference on standardisation of domestic animal karyotypes established a system of nomenclature for G- and R-bands in cattle (ISCNDA, 1989). The numbering of regions and bands in different chromosomes were done based on International System for Human Cytogenetic Nomenclature (ISCN, 1985). In some chromosomes the number of bands observed were not constant, and hence few bands were divided into sub-bands. An equal size was arbitrarily fixed for the centromere of all autosomes. A total of 410 G-bands were described in the karyotype of cattle(ISCNDA,1989).

Raghunandanan and Mukundan (1991) studied the chromosome profile of cattle in Kerala. Cattle belonging to local (Zebu), half-bred Jersey, half-bred H.F. and Jersey were karyotyped. In all the genetic groups, the diploid number was 60, all autosomes were acrocentric and X chromosome was sub-metacentric. A polymorphism was reported in the Y chromosome morphology; Y chromosome was acrocentric in local bulls and sub-metacentric in bulls of other groups. The relative length, arm ratio and centromere index of the chromosomes were described.

2. Chromosome abnormalities in cattle:

The first Robertsonian like translocation in cattle was

described by Gustavsson and Rockborn (1964). The cells of three animals with overt lymphatic leukaemia were found to possess only 59 chromosomes. One of the autosome was described to have a sub-terminally situated centromere, and was presumed to have arisen by the centric fusion or translocation of the first and twenty-nineth autosome.

Herschler et al. (1966) reported both XX and XY cells in the blood of heterosexual bovine co-twins. The first diagnostic test to identify the sex chromosome chimaerism was proposed.

Zartman and Fechheimer (1967) studied the frequencies of peridiploid and polyploid somatic cells in in-bred and line-cross Hereford bulls. The study revealed significant difference in the frequencies of peridiploid and polyploid cells between in-bred lines; peridiploid and polyploid cells were more in highly in-bred lines.

Gustavsson (1969) described a diploid chromosome number of 59 and 58 in some Swedish Red and White cattle. The variation in the diploid number from normal (2n=60) was described to be due to 1;29 translocation in heterozygous (2n=59) or homozygous (2n=58) condition. The overall frequency of 1;29 translocation in Swedish Red and White breed was reported as 14.3 per cent (164 of 1173).

Daughters of bulls heterozygous for 1;29 Robertsonian translocation were found to have lowered fertility (Gustavsson, 1970). The daughters of carrier bulls had lower non-return rates at 56 and 273 days.

Hansen (1970) reported an autosomal tandem-fusion translocation in Red Danish cattle. The chromosome number was 59 instead of 60 and the tandem-fusion chromosome was the largest acrocentric chromosome in the complement. The translocation was reported to be transmitted to the progeny of carrier animals. Bulls and cows carrying the translocation was reported to have reduced fertility.

Dunn et al. (1970) described a hermaphrodite Holstein. The animal had a penis, an empty scrotum, a left abdominal ovary, and a right abdominal ovotestis. Karyotyping of the bone-marrow cells revealed most of the cells to have a chromosome complement of 60, XX, but 1.5 per cent of cells were 61, XXY. Small percentages of triploid cells (90, XXY and 90, XXX) were noted in the blood leucocyte culture.

Gustavsson (1971) compared the culling rate in daughters of bulls heterozygous for 1;29 translocation with daughters of contemporary bulls. The culling rate in

daughters of carrier bulls was significantly higher which reflected a reduced fertility in these females.

The incidence of secondary abnormalities viz, secondary constrictions, gaps, fragile sites and achromatic regions in an infertile bull was found to be 60 per cent (Halnan, 1972). A similar karyotype was also noted in two males which were not fertile and in a third male suspected to be infertile.

Popescu (1972) described a possible pericentric inversion in a phenotypically normal bull of the Normandy breed with reduced fertility. The cells in blood culture had the usual diploid chromosome number (2n=60), but one medium-sized autosome was sub-metacentric. The occurrence of this sub-metacentric autosome was presumed to be due to a pericentric inversion.

In a cytogenetic survey involving 48 cattle of several exotic breeds imported into New Zealand, two translocation carrying bulls were found by Bruere and Chapman (1973). A Simmental bull carried a translocation with chromosome 11 or 12 for the long arm component and 15 or 16 for the short arm component. The other bull, an Aquitaine Blonde was carrier for 1;29 translocation.

Fechheimer (1973) reported chimaerism in young bulls of USA, with sex chromosome complement as XX in some cells and XY in other cells. Three of the chimaeric bulls were not recorded as twins. It was presumed that the female co-twin of these three bulls had suffered an embryonic death. A B.S. bull reported to have born singly had only one per cent cells with Y chromosome. One Ayrshire bull had an elongated Y chromosome but no effect was found on sexual development or function of the bull.

A Robertsonian translocation, 2;4 centric fusion in the heterozygous condition was observed in a phenotypically normal Friesian bull by Pollock and Bowman (1974). This was the first report of a translocation in British Friesian or in other strains of Holstein/Friesian cattle. It was suggested that the translocation had arisen de novo during gametogenesis or embryogenesis. The fertility of the bull was found to be normal.

Eldridge (1974) described a translocation involving chromosome 5 or 6 for the long arm component and 15 or 16 for the short arm component. The translocation was described to be dicentric fusion. The bull carrier for the translocation was reported to be born singly, but was a mosaic with approximately 8 per cent of cells having a chromosome complement of 60, XX.

Eldridge (1975) reported very high frequency of a Robertsonian translocation in British White cattle. The long arm component of the translocated chromosome was identified as the longest chromosome and the short arm was described as chromosome 27 with the loss of centromere. The translocation was assumed to be the same translocation identified as 1; 29 in several breeds of cattle.

Halnan (1975) reported higher frequency of gaps and breaks in chromosomes of eight infertile bulls. However, the frequency of gaps and breaks were not correlated to semen quality. Reviewing the clinical status of chromosome studies it was proposed that, if the effect of any chromosome abnormality had as marked an effect on fertility as the 1;29 translocation, the loss could be significantly reduced by eliminating the stud bulls and cows with that defect.

A cytogenetic survey in Guernsey bulls of Canada by Bongso and Basrur (1976) revealed high frequency of chromatid breaks and achromatic gaps in three bulls which also exhibited lowered fertility. One of the bull was a carrier for 1;29 translocation. In a bull with normal fertility the cells exhibited a 27;29 translocation which was a dicentric fusion.

Halnan (1976) reported a sex chromosome chimaerism in Hereford bull of Australia. Some cells of the bull had a chromosome complement 61, XXY while the other cells were normal (60 XY). The semen of the bull was of poor quality.

Refsdal (1976) compared the fertility of daughters born to bulls carrying 1;29 translocation with daughters of normal bulls. A significant reduction in fertility was noted among the daughters of bulls with 1;29 translocation.

A chromosomal abnormality identified as Robertsonian translocation was reported in New Zealand, Scotland and England by Harvey and Longue (1976). Ancestory of all the carrier bulls were traced back to one Swiss Simmental bull. The translocation was identified as 13;21 Robertsonian translocation and the carriers were found to transmit the abnormality to about 50 per cent of the offspring.

Kovács (1976) described two autosomal translocation in Hungarian Simmental bulls. One was a 1;29 centric fusion and the other a 11; 16 translocation.

Popescu (1977) reported a new type of Robertsonian translocation in a Limousin cow. Studies by R-banding indicated a centric fusion involving chromosomes 3 and 4. The translocated chromosome appeared to be dicentric.

Chromosome investigation conducted in Swiss cattle breeds by Tschundi et al. (1977) revealed some animals to be carriers for translocation or sex chromosome chimaerism. Two types of translocations were identified, one a 1;29 translocation and the other 8;9 translocation. Chimaeric animals had a karyotype 60, XX / 60, XY.

Blazak and Eldridge (1977) reported a Robertsonian like translocation in B.S. cattle. Translocation was presumed to be homologous to the 1;29 translocation reported in other breeds. The length of the translocated chromosome was less than the length of chromosomes 1 and 29 put together. Females heterozygous for the translocation were found to have normal fertility.

Longue and Harvey (1978) noted that offsprings of a bull heterozygous for 1;29 translocation were only normal or balanced translocation carriers, and they occurred in a ratio of 1:1. Semen quality, service behaviour and testicular histology of the translocation carriers were unimpaired. Difference of about 5 per cent in the frequency of non-disjunction in the secondary spermatocytes of normal and heterozygous bulls was noted.

Distribution of 1;29 translocation in 24 countries, in more than 30 breeds of cattle had been described by

Gustavsson (1979). The 1;29 translocation was described as the product of an ancient mutation which got distributed world-wide. Absence of a proven case of *de novo* formation of 1;29 translocation, or its formation *in vitro* was described as evidences for this hypothesis. The monocentric appearance of the translocated chromosome was also considered as an indication of ancient origin.

Dyrendahl and Gustavsson (1979) studied the sexual function, semen characteristics and fertility of bulls carrying the 1;29 chromosome translocation. The libido and serving ability of the translocation carriers were normal. A small, but statistically significant reduction in sperm concentration was noted in the ejaculate collected from carrier bulls. The reduction in fertility of the heterozygous bulls was significant among unselected bulls. In the process of selection for fertility, the reduction in fertility of carrier bulls was found to become insignificant.

Succi *et al*. (1979) compared the fertility of normal daughters of two Romagna bulls carrying the 1;29 translocation with daughters carrying the same defect. The number of inseminations per conception, proportion of unsuccessful insemination and the proportion of irregular returns to oestrus were found to be higher in the heterozygous daughters.

In Podolian type cattle, two types of Robertsonian translocation were reported by Di Berardino et al. (1979). One was identified as 1;29 translocation and the other as 14;24. C-banding revealed the presence of two blocks of centromeric heterochromatin in 14;24 translocation.

Giovanni et al. (1979) reported a new autosomal translocation in the Alpine Grey bulls. Out of the 50 bulls studied, five carried translocation which was identified as 25;27 translocation. Two of the translocation carriers had poor libido and never produced semen of acceptable quality.

Longue et al. (1979) described sex chromosome aneuploidy in a British Friesian bull, the 61, XXY syndrome. The bull was azoospermic and had hypoplastic testis. The testosterone level in the bull was below normal.

A higher culling rate among heifers heterozygous for 1;29 translocation was reported by Kovács and Csukly (1980). The culling rate was 3.19 times higher in these heifers compared to normal heifers. The loss of embryos was also reported to be higher in the translocation carriers.

Masuda et al. (1980) in a study of 55 Japanese Black cattle, reported 20 of the animals to be carriers for

Robertsonian translocation. Five of the animals were heterozygous for 1;29 translocation and 13 for 5;21 translocation. Two heifers born from mating a 1;29 sire with 5;21 dam were found to carry both translocations. Females which were translocation carriers were found to have lowered fertility.

Moraes et al. (1980) reported 1;29 centric fusion in Charolais cattle of Brazil. Another structural abnormality was also noted in the same breed characterised by an insertion in the chromosome 16. Two males of Norman breed had a numerical anomaly, 60, XY/61, XY + fragment (F) mosaicism. The structural abnormalities were found to be transmitted from generation to generation.

A translocation involving the chromosome 13 and X chromosome was described by Eldridge (1980). The translocation was detected in a H.F. x B.S. heifer. The sire was a carrier of 1;29 translocation. The fertility of the heifer was normal.

King et al. (1980) reported the presence of 60 chromosomes including 1;29 chromosome in two embryos, retrieved from a cow bred with a bull heterozygous for 1;29 translocation. The embryos were presumed to have arisen

from the fertilization of ova by hyperhaploid spermatozoa containing both the 1;29 chromosome and chromosome 1.

Dunn et al. (1980) described a 61, XXY Hereford bull to have good libido, but it had gross testicular hypoplasia and was azoospermic. The left testis weighed about 10 per cent of the testis weight of normal Hereford.

Succi et al. (1982) studied the influence of 25;27 translocation on fertility and milk production of Grey Alpine cows. The translocation carriers had a lower calving rate to first service. The number of services per conception and service period of the carrier cows were higher compared to normal cows. The normal and carrier cows studied were sired by a bull heterozygous for 25;27 translocation.

According to Ibrahim et al. (1983) no significant variation in the quality, freezability or fertility of the semen was observed in chimaeric (XX/XY) or 1;29 translocation carriers when compared with control bulls. The volume and density of the semen from one 5;18 and one 14;21 translocation bull appeared slightly inferior, but fertility was unaffected.

Swartz and Vogt (1983) in a study of nulliparous

heifers, reported chromosome anomalies in 18.3 per cent. Five heifers were carriers for 1;29 translocation and two were with chromosome complement 61, XXX. Other heifers showed a variety of mosaic conditions viz; 59, X0/60, XX;60, XX/60, XY;59, X0/60, XX/61, XXX;59, X0/60, XX/60, X0 and tetraploid/diploid.

Distribution of the Robertsonian translocation 1;29 in cattle bred in Spain was described by Arruga and Zarazaga (1984 a). The frequency of translocation was higher in primitive and rustic breeds. This finding and the monocentric appearance of 1;29 translocation were mentioned as evidences of ancient origin of the translocation.

Eldridge et al. (1984) in cytogenetic survey of young bulls in U.S.A. found one Brown-Swiss bull to be heterozygous for 1;29 translocation. Fourty-nine per cent of Holstein bulls were XX/XY chimaeras. One of the chimaeric bull was reported to be born single and the chimaerism was explained by the possibility of the bull being twin to a female which had not survived to parturition. Considerable variation among bulls was noted with regard to frequency of gaps and breaks in the chromosomes. No relationship was observed between semen quality and the presence or absence of gaps and breaks. Tetraploid/diploid mosaicism was also noted in

some bulls which was not found to be related to any other characteristics.

Kovács and Gustavsson (1984) described 5;18 translocation in a bull. The somatic tissues showed an uniform mosaic chromosomal picture with cells containing the 5;18 translocation as dominant cell lineage over the 60, XY cells. Analysis of spermatogonial and meiotic cells revealed only the normal karyotype.

In a cytogenetic study on A.I. bulls of Romania, Ciupercescu et. al.(1984) reported sex chromosome chimaerism, mosaicism of diploid autosome and sex chromosome aneuploid cells (60, XX/61, XXY/78, XXY) and centric fusion translocations. Centric fusions involved two different chromosome pairs; 1;29 and 14;20. Breeding data showed a lowered fertility in the 1;29 and 14;20 translocation bulls.

Sysa and Slota (1984) described the effect of sex chromosome trisomy in two bulls of the Black and White breed. One had a karyotype 60, XX/61, XXY and other 61, XXY. Both the bulls had hypoplastic testes.

Tschundi (1984) reported the effect of cytogenetic investigation and culling of A.I. bulls which were carriers

of chromosomal abnormalities, in Switzerland. It was concluded that the exclusion of animals with chromosomal abnormalities was effective in reducing the incidence of the abnormalities in the population.

Describing the role of cytogenetics in veterinary medicine, Rieck (1984) stressed the need for a systematic cytogenetic diagnosis in all clinical fields instead of mere accidental findings of chromosomal anomalies in domestic animals.

Nel et al. (1985) reported 1;29 Robertsonian translocation in South African cattle. This was the first report of 1;29 translocation in Brahman (B. indicus) breeds of cattle.

Long (1985) in a review of centric fusion translocation in cattle, mentioned that the policy to discard all translocation carrying animals may be misguided as they represent genetic variation. It was assumed that a fusion of two non-homologous chromosomes would change the gene linkage which may be desirable in the future with regard to some traits.

Studies of translocation in Podolian and Romagna cattle by Iannuzzi et al. (1987) revealed the identity of the long arm as the longest chromosome. The short arm was identified as chromosome 25 with the loss of constitutive heterochromatin. The translocation was presumed as 1;29 previously reported in many breeds of cattle.

Kovács and Szepeshelyi (1987) reported 1;29, 5;18 and 14;21 translocation in some bulls used for breeding in Hungary. XX/XY sex chromosome chimaerism was found in 51 bulls, and one Holstein-Friesian bull exhibited XX/XXY chimaerism.

In a morphological evaluation of reproductive tract of non-parous cattle, Kasubick et al. (1987) reported no significant differences among the chromosomally normal vs abnormal cattle with regard to number of regressing conceptuses or infection status.

Maurer and Vogt (1988) reported that the female cattle, carriers for 1;29 translocation needed on an average 28 days more for the first conception compared to chromosomally normal cattle. The pregnancy rate as yearling of the carrier females and control females was also reported to differ significantly.

Vezuli (1988) reported a pericentric inversion in fourth chromosome pair of a Jersey bull with reduced fertility. The Y chromosome of the bull was reported to be small and had the size of last pair of autosome. Polyploid cells were also reported in two Friesian bulls and a Simmental bull.

Weber et al. (1989), reviewing low fertility related to 1;29 centric fusion anomaly in cattle, described that the loss of centromeric chromosome substance in the unicentric fusion had no anatomical or physiological effect on the carrier animal. The lack of clear evidence to show that spermatozoa or egg from carrier animals were less capable of fertilization was also mentioned. It was described that the monosomy and trisomy embryos of cattle carrying 1;29 translocation died early during development.

Thiagrajan et al. (1990) reported 1;29 translocation in an anoestrus heifer which was a B. taurus x B. indicus animal. This was the first report of 1;29 translocation in India. The chromosomes of the heifer also showed high frequency of chromatin gaps and achromatic breaks.

Vallenzasca et al. (1990) described Y;17 translocation in a bull. A portion of the long arm of chromosome 17 was

translocated on to Y chromosome. The bull was phenotypically normal, but its semen had reduced sperm concentration and lowered motility.

Raghunandanan and Mukundan (1990) reported tetraploid/ diploid mosaicism in a bullock. The bullock exhibited development of rudimentary teats around the scrotum.

Kovács et al. (1990) studied the chromosomes of a Brown-Swiss bull with low fertility. A long acrocentric chromosome was identified which was the largest and was unpaired. G-banding revealed the chromosome to be 1;16 tandem translocation chromosome. The cells also had a normal pair of chromosome 16, indicating trisomy of chromosome 16 in the bull.

Tambasco (1990) described chromatin gaps in X chromosome, achromatic breaks in X and Y chromosomes, sex chromosome chimaerism and 17; 21-22 translocation in Charolais x Zebu cross cattle. Three of five bulls with chromosomal breaks had abnormal sperm count.

Wilson (1991) studied the embryos sired by bulls heterozygous for 1;29 translocation. Some embryos were carriers for the translocation and few of them had unbalanced karyotype. The embryos with unbalanced karyotype were either monosomic or trisomic.

Poor semen production was reported in chimaeric bulls (60, XY/60, XX) heterozygous for 1;29 translocation by Zhigachev et al. (1991). The daughters of these bulls had a higher rate of return to service when compared to daughters of normal bulls.

Świtoński et al. (1991) reported the proportion of ejaculates accepted for freezing to be lower in chimaeric bulls compared to normal bulls. The proportion of frozen ejaculates discarded was higher in the chimaeric bulls.

Han and Men (1992) recorded the semen volume, number of collections per month, sperm concentration, percentage of live spermatozoa, and sperm viability after freezing of a bull heterozygous for 1;29 centric fusion. These traits in the carrier bull were not significantly different from those of normal bulls.

Iannuzzi et al. (1992) reported a translocation in Borossá cow. The translocation was identified as 15; 25 centric fusion. C-banding revealed the dicentric nature of the translocation.

Gallagher et al. (1992) studied the chromosomes of a normal cow and her albino daughter, both known to be

heterozygous for a X; autosome translocation. A portion between band 12 and 13 of a chromosome 23 was translocated to the p arm of the X chromosome.

The highest incidence of 1;29 translocation was reported in British White cattle by Long (1993). It was found that the frequency of translocation had not reduced following screening and culling of the carriers for a period of six years.

McFeely et al. (1993) identified 14;20 translocation in Simmental cattle of U.S.A. All the animals carrying this anomaly was found to have a common sire which was later confirmed to carry the translocation.

Villagömez et al. (1993) reported a reciprocal translocation in an Ayrshire bull with a history of sub-fertility. The karyotype of the bull was 60, XY, rcp (20;24) (q 17; q 25). Synaptonemal complex analysis of the translocation by electronmicroscopy revealed irregular pairing behaviour of the chromosome axis involved.

Balanced reciprocal translocation was described in a bull by Ansari et al. (1993). The bull with a karyotype 60, XY, rcp (8;13) (q 11; q 24) was azoospermic. Electron-microscopy of the synaptonemal complex revealed high

incidence of terminal asynapsis in the smallest arm of the quadrivalent, leading to complete meiotic arrest at late pachytene.

3. Sex chromosome polymorphism:

Cribiu and Popescu (1974) and Cribiu (1975) studied the relative length of Y chromosomes in various breeds of B. taurus cattle. Charolais and Montbleliard bulls were found to have larger Y-chromosome than other breeds.

Hansen and Elleby (1975) described an elongated Y chromosome in Danish beef bulls which were used for A.I. An elongated Y chromosome was not found to have any effect on the sexual function of the bulls.

Y chromosome variations were studied in 30 breeds of cattle by Halnan and Watson (1982). The Y chromosome, based on relative length and centromere index was found to be distinctive in Africander, Aberdeen-Angus, Charolais, Friesian, Guernsey, Hereford, Jersey, Murray Grey, Shorthorn and Simmental breeds of B. taurus. The Y chromosome was found to be larger in Charolais bulls. Simmental and Charolais bulls were found to have Y chromosomes of similar size, but the centromere index was found to differ in the two breeds. Smallest Y chromosome was found in Romagna breed.

The Y chromosome of B. indicus was described as acrocentric.

Arruga and Zarazaga (1984 b) studied the Y chromosome variation in five breeds used in Spain. The chromosome Y in Spanish fighting bulls was found to be smaller compared to other breeds.

Märki and Robinson (1984) attributed the observed variability in the size and morphology of the Y chromosome in cattle breeds entirely to the addition of constitutive heterochromatin in this chromosome.

Halnan (1989 a) described the use of morphology and morphometry of Y chromosome in checking the paternal line in two breed situations. It was described that in a cross involving Charolais bull, the Y chromosome was so much larger as to be diagnostic. The same was described to hold true when Simmental bulls were used to breed Hereford cows.

4. Cytogenetic technique:

Moorhead et al. (1960) described peripheral blood leucocyte culture technique for studying chromosomes. The venous blood collected in heparinised vial was mixed with phytohemagglutinin and held on ice for 60 minutes. The supernatant plasma obtained by centrifugation was cultured in

medium 199 enriched with plasma. After 70 hours of culturing at 37°C, colchicine was used to arrest the mitosis. The cells were fixed with methanol, glacial acetic acid mixture (3:1), and subsequently slides were prepared, air dried and stained. The application of peripheral leucocyte culture technique in bovine was described by Crossley and Clarke (1962).

Basrur and Gilman (1964) described a modified method for culturing lymphocytes in bovine. Growth medium used was Connaught's H 597 supplemented with in-activated calf serum, sodium bicarbonate, penicillin and phytohemagglutinin. Whole blood collected in heparinised vial was introduced into the culture medium. Colchicine dissolved in phosphate buffered saline was used as mitotic arrester. Hypotonic treatment with distilled water was done prior to fixation with a mixture of methanol and acetic acid. Staining was done with orcein or carbol fuchsin.

Sumner et al. (1971) induced G-bands in chromosomes by incubation in saline solution. Chromosomal preparation was fixed in 3:1 methanol, acetic acid and air dried. The slides were incubated for 1 hour in 2 x SSC (0.3M NaCl, 0.03M trisodium citrate, pH 7.4) at 60°C. Subsequently the slides were rinsed in distilled water and stained with Giemsa stain.

Seabright (1971) used trypsin to produce the G bands. Slides aged at room temperature for seven to 10 days were treated with hydrogen peroxide which was followed by trypsinisation. The staining was done with Giemsa.

Lin et al. (1976) described a modified G-banding technique for bovine chromosomes. Bands were produced by trypsin solution adjusted to pH 8.0 and the slides were subsequently passed through a series of alcohol solutions from 70 per cent to 100 per cent.

Halnan (1977) reported a higher mitotic index in peripheral leucocyte culture of bovine, when the medium 199 was supplemented with glutamine and l cystine. Most suitable solution for hypotonic treatment during harvesting was described as 0.56 per cent KCl. Staining was done using Giemsa stain.

Ibrahim et al. (1983) described a method for producing G-bands in cattle chromosome by incubation in saline solution containing trypsin. The chromosomes, aged for two to three days, were treated with 2 x SSC containing trypsin solution for 40 minutes at 66°C.

Thiagrajan et al. (1989) compared the efficiency of medium 199, medium 199 supplemented with glutamine and RPMI 1640 as culture media for peripheral leucocyte culture technique. RPMI 1640 was reported to yield better growth of leucocytes.

Halnan (1989 b) described the use of medium 199 and Hams F 10 in combination with RPMI 1640 to overcome the deficiency of thymidine in the latter. A combination of pokeweed mitogen with phytohemagglutinin was reported to improve the result of peripheral leucocyte culture in cattle.

5. Semen quality and related attributes:

Knobbed acrosomes, characterised by a localized swelling or bead on the apical ridge, was first observed in the sperm cells of a bull by Teunissen (1946). This defect was reported to be a heriditary defect in Friesian bulls by Hancock (1949). Donald and Hancock (1953) reported that the knobbed acrosome defect in Friesians was an autosomal sex-linked recessive character.

White (1958) described the average volume of semen in one ejaculate of a bull to be four millilitres with a range

of 2-10 ml. The average concentration was 1000 million spermatozoa /ml of semen and the normal range 300 x 10^6 to 2000 x 10^6 in one millilitre of semen.

The appearance of large number of tail-less sperm heads in the ejaculate was described as an early indication of testicular degeneration and in some cases of partial testicular hypoplasia by William (1965).

Hahn et al. (1969) reported good correlation between the semen quality of the youngest group of bull with their semen quality tested a year later. It was postulated that if the relationship persisted, culling for semen producing ability could be done at an earlier age.

Igboeli and Rakha (1971) reported a higher sperm concentration in the semen of Zebu bulls. The mean concentrations for first ejaculation and second ejaculation were reported to be 1,820 x 10^6 and 1650 x 10^6 spermatozoa /ml of semen respectively.

The incidence of proximal cytoplasmic droplets in the ejaculate was observed to be higher in bulls with pathological semen than in normal bulls by Rao (1971). The sperm abnormalities were classified as sperm head abnormalities, mid-piece abnormalities and tail abnormalities.

Blom (1972) proposed a new classification of the bull spermiogram. All sperm defects related to impaired fertility, or to an abnormal condition in the testes or epididymis were classified as major defects. Underdeveloped heads, double forms, knobbed sperm defect, decapitated sperm defect, diadem defect, pear-shaped head, narrow at the base, abnormal contour, small abnormal heads, free abnormal heads corkscrew defect, other mid-piece defects, proximal droplets, pseudodroplet defect and dag defect were classified as major defects. Narrow heads, small, normal heads, giant head and short broad heads, free heads, detached acrosomal caps, abaxial implantation, distal droplets, simple or coiled tail and terminally coiled tail were classified as minor defects. In a normal bull the major defect was expected to be less than five per cent and total abnormalities less than 25 per cent. Minor defect was described to be less important, and only when any minor defect exceeded 10 to 15 per cent, it was described to be of significance.

The semen characteristics of B.S. x Ongole and H.F x Ongole cross-bred bulls were studied by Rao and Rao (1978). The mean values of different semen characteristics were: Volume 4.33 $\pm$ 1.20 ml and 4.17 $\pm$ 1.37 ml; concentration of spermatozoa 984.93 $\pm$ 312.51 and 611.84 $\pm$ 164.26 million per

ml; head abnormalities 9.46 ± 2.96 and 15.86 ± 4.59 per cent; free loose heads 2.97 ± 1.09 and 6.13 ± 1.74 per cent; mid-piece abnormalities 0.74 ± 0.44 and 0.92 ± 0.29 per cent; tail abnormalities 3.02 ± 0.96 and 3.03 ± 0.91 per cent; and proximal protoplasmic droplets 2.86 ± 1.83 and 5.04 ± 2.01 per cent for B.S. x Ongole and H.F. x Ongole cross-bred bulls respectively. All the bulls were two years old.

Mathew et al. (1982) studied the semen characteristics of pure-bred European bulls and cross-bred (European x non-descript indigenous) bulls. An increase in total sperm output, initial motility and freezability of semen was noted with an increase in European inheritance.

The semen volume, sperm concentration ($X10^6$/ml) and total ejaculated sperms ($X10^6$) of B.S. x Zebu bulls, following an initial depletion with 10 ejaculates were studied by Nair and Varadarajan (1985). The corresponding values taken at 1,4 and 7 days interval were respectively 1.490 ± 0.205, 208.660 ± 53.644 and 3103.330 ± 1255.083; 1.390 ± 0.123, 313.000 ± 88.191 and 4502.000 ± 1831.131; and 2.280 ± 0.161, 370.000 ± 26.457 and 7556.000 ± 983.823. By linear regression the daily sperm output, when the collections were taken on alternate days, was estimated as 2827.444 x 10^6, which was much lower than the reported values for pure B. taurus bulls.

Barth (1986) reported knobbed acrosome defect in Charolais bulls. The pedigree analysis revealed a genetic predisposition for this sperm defect. The knobbed acrosome defect was found to be associated with infertility.

Belorkar et al. (1991) reported sperm concentration, abnormal sperm percent and other simple seminal estimates to have good correlation with semen quality, freezability and fertility in cross-bred bulls (B.taurus x B. indicus).

Mathew (1991) revealed a correlation between parameters, worked out based on the first 10 ejaculates, and the life time semen production of cross-bred bulls in Kerala. The parameters studied were number of ejaculates accepted for freezing, total sperm output and total acceptable sperm output.

Nair (1994) described the average daily sperm production of cross-bred bulls as 5158.206×10^6 spermatozoa. The bulls were product of a cross involving European breeds (B. taurus) and local non-descript cattle (B. indicus) of Kerala.

MATERIALS AND METHODS

MATERIALS AND METHODS

Cross-bred bulls stationed at K.L.D.B. Farm, Dhoni, formed the material for the study. The bulls included for the study were either those selected for semen producing ability or pre-selected bulls which had started producing semen. Fifty-three bulls were selected which included 30 Jersey cross-breds (C.B.J.), 12 H.F. cross-breds (C.B.H.F.) and 11 B.S. cross-breds (C.B.B.S.).

1. Cytogenetic study:

All the animals selected were subjected to cytogenetic screening. Metaphase spreads of the bulls were obtained by peripheral blood leucocyte culture technique described by Moorhead et al. (1960) with some of the modifications suggested by Halnan (1977, 1989 b) and Thiagrajan et al. (1989).

1.1 Collection and transportation of blood:

Twenty millilitres of venous blood was collected from the jugular vein of the bulls using sterile 16 G needles. The blood was collected in vial containing heparin sodium at a final concentration of 15 IU/ml of blood.

Blood samples were transported in an insulated box packed with ice. The temperature inside the box was maintained between 8°C and 15°C, and care was taken to avoid direct contact between ice and the vials containing blood. Culture of the samples were set within five hours of collection.

1.2 Culture medium:

Culturing of blood was done in a composite tissue culture medium having the following composition.

RPMI 1640 lyophilised powder - Sigma	..	1 g
Benzyl penicillin solution (50,000 IU/ml)	..	0.2 ml
Phytohemagglutinin - M solution (2000 µg/ml)- Sigma	..	0.5 ml
Pokeweed mitogen solution (1000 µg/ml)- Sigma	..	1.0 ml
Sodium bicarbonate solution (3.5%)	..	1.0 ml
Autoclaved, double distilled water	..	97.3ml

The pH of the medium was adjusted to 7.2 with sodium bicarbonate solution or N/10 HCl. The medium was then filtered through membrane filter having a pore size of 0.22 µ. Two hundred millilitres of culture medium was prepared at a time and was divided into five millilitres aliquots in the

culture vials. The culture vials containing the medium were stored at -5°C.

1.3 Culturing:

Whole blood from blood samples were used to inoculate the vials containing culture medium. Two cultures were set up for each sample by inoculating 0.5 ml whole blood into a culture vial. Two millilitres of autologous plasma, obtained by centrifuging the samples at 1500 rpm for 15 minutes, was added to each culture.

The cultures were incubated at 36°C in a B.O.D incubator for a period of 70 h. During the incubation, the cultures were gently mixed three times in a day to avoid sedimentation.

1.4 Harvesting:

The following procedures were done to get the metaphase chromosomes on microscope slides from the cells in the culture.

i. Colchicine (Sigma) solution (10 µg/ml) was added at the rate of 0.05 ml per culture and was incubated for one hour at 36°C.

ii. The cultures were centrifuged at 1500 rpm for 15 minutes.

iii. The supernatant was discarded and the cell button was resuspended in eight millilitres of 0.56 per cent KCl (0.075 M) pre-warmed to 37°C.

iv. The suspension was incubated for 30 minutes at 37°C.

v. The suspension was centrifuged at 1000 rpm for 10 minutes.

vi. The supernatant was discarded and the cell button was resuspended with five millilitres of chilled methyl alcohol, acetic acid fixative (3:1) and left undisturbed for 30 minutes.

vii. The suspension was centrifuged at 1000 rpm for 15 minutes.

viii. The supernatant was discarded and the cell button was resuspended with five millilitres of methyl alcohol, acetic acid fixative.

ix. Procedure (vii) and (viii) were repeated till the supernatant was clear.

x. The supernatant was discarded and a suspension of about one millilitre was made using fresh fixative.

1.5 Preparation of slides and staining:

Clean grease free slides were chilled in cold methyl alcohol. The excess alcohol on the slide was removed by blowing and the slide was kept in a slanting position. Few drops of the cell suspension, obtained from harvesting the culture, was dropped on to the slide from a height of about one meter. If the suspension was thick a gentle blow was given to spread the drops on the slide. The slides were then dried on top of a flame.

Staining of the dried slides were done with Giemsa stain. Working solution of Giemsa was prepared by mixing one millilitre of Giemsa stain (Sigma) with nine millilitres of Sörenson's buffer (pH 6.8). Slides were stained for 10 minutes, and then rinsed with distilled water and air dried.

1.6 Microscopy and photography:

Atleast two slides prepared from each culture were screened under the microscope for metaphase spreads. About 20 to 40 metaphase spreads of each bull were studied for assessing the morphological appearance of the chromosomes.

Good metaphase spreads of all the bulls were photographed using Carl-Zeiss photomicroscope III. The photographs were taken on 100 ASA black and white film.

1.7 Morphometric measurements:

The size of various chromosomes of the bulls were measured. The negative of the photographs of metaphase spreads were projected on a screen with the aid of a slide projector and the measurements of the chromosomes were taken from the projected image.

The size of the chromosomes were represented as relative lengths. Relative length of chromosome was calculated by dividing the length of the chromosome with total length of the haploid set of chromosomes, including X chromosome and expressed as per cent. Centromeric index and arm ratio of the X chromosomes were also calculated using the following formulae.

$$\text{Centromeric index} = \frac{\text{length of short arm}}{\text{Total length}} \times 100; \quad \frac{p}{p+q} \times 100$$

$$\text{Arm ratio} = \frac{\text{length of long arm}}{\text{length of short arm}}; \quad \frac{q}{p}$$

Relative lengths of X and Y chromosomes, and the arm ratio and centromere index of X chromosome of different

genetic groups were compared by doing analysis of variance in one-way classification (Snedecor and Cochran, 1967).

1.8 Preparation of karyotype:

The photograph of good metaphase spread was developed and the chromosomes in the spread were cut and removed. The chromosomes were paired based on their size and morphology. The homologous pairs were pasted on a paper in descending order of their length. The X and Y chromosomes, identified by the presence of both p and q arms, formed the last pair. These homologous pairs were arranged in five rows of six pairs each.

1.9 G-banding:

G-banding was performed using the method described by Ibrahim et al. (1983) with minor modifications. The method was a combination of the methods described by Sumner et al. (1971) and Seabright (1971).

Trypsin solution for banding was prepared by dissolving 0.025 g trypsin (Gibco, 1: 250), 6.0 g $Na_2H\ PO_4$, 1.0 g $KH_2\ PO_4$ and 1.0 g. EDTA in 500 ml of deionised distilled water. The slides prepared by harvesting the cultures were aged for three to seven days at room temperature. The aged slides

were immersed in coupling jars with 50 ml 2 x SSC (0.3 M. NaCl and 0.03 M trisodium citrate) containing 1.0 ml of the trypsin solution, and incubated at 66°C for 45 minutes in a water bath. The slides were treated with 70 per cent methyl alcohol to stop the action of trypsin. The slides were rinsed in distilled water and then in Sörenson's buffer (pH 6.8).

Slides were stained in 10 per cent Giemsa (prepared as mentioned earlier) for five minutes, rinsed in distilled water and air-dried.

1.10 Preparing G-banded karyotype:

Good metaphase spreads which were subjected to Giemsa-banding were identified and photographed using Carl-Zeiss photomicroscope III. The chromosomes were cut out and arranged in pairs based on standard procedures described by ISCNDA (1989).

2. Study of Characters related to semen production :

Reproductive attributes pertaining to semen production of the bulls under study were obtained from the records maintained at Dhoni farm. Age of the bulls when semen was

successfully collected for the first time and the age at which freezable semen was produced were calculated in months.

The data on first 10 collections of semen from the bulls were analysed, and the average volume in an ejaculate, average sperm concentration of semen, total sperm output, number of ejaculates accepted for freezing and the total acceptable sperm output of each bull were calculated.

Smears of semen mixed with eosin / nigrosin stain, prepared at the bull station were studied under a microscope. A total of 200 sperms of each bull were observed for their morphological appearance. Various sperm abnormalities observed were recorded as per cent in each bull. The sperm defects were classified based on the proposal of Blom (1972).

RESULTS

RESULTS

Satisfactory chromosome preparations were obtained for all the bulls, and the G-banding procedure adopted produced characteristic banding patterns on the chromosomes. The metaphase spreads were analysed for assessing the chromosome profile of the bulls used for A.I. in Kerala. The G-banded slides were also analysed so as to establish the banding patterns in cross-bred bulls of the state.

Analysis of the semen quality and related attributes revealed three of the bulls to be sub-normal. The cytogenetic observations made on these three bulls (probands) were compared with the observations made on contemporary bulls.

1. Cytogenetic observations in normal bulls:

The diploid chromosome number in all the metaphase spreads of the normal bulls was 60. There were 29 pairs of acrocentric autosomes and one long sub-metacentric chromosome, the X chromosome. The Y chromosome showed dimorphism; the chromosome was sub-metacentric in C.B.H.F., while the centromere was more towards centre in C.B.J. and C.B.B.S. A representative metaphase spread is depicted in plate 1.

The mean relative length of 29 pairs of autosomes and the sex chromosomes represented as per cent of the total length of haploid complement in which chromosome X was included, are presented in figure 1. The individual variation in the relative lengths, expressed as standard deviations are shown in figure 2 a, and the relative lengths and standard deviations plotted with the same scale are presented in figure 2 b.

The longest chromosome was an acrocentric autosome with a mean relative length of 6.0174 $\pm$ 0.0273. The shortest chromosome, also an autosome, had a mean relative length of 1.6186 $\pm$ 0.0101. The mean relative length of X and Y chromosomes were 5.5918 $\pm$ 0.0401 and 1.9636 $\pm$ 0.0396 respectively. The standard deviation was higher for sex chromosomes, and among the autosomes it was highest for the second longest autosome.

Karyotype was prepared from very distinct metaphase spread (plate 2). The autosomes were not distinctive with regard to morphology, and hence the karyotype was prepared by arranging the 29 pairs of autosomes in descending order of size. The sex chromosome formed the last pair in the karyotype.

A representative G-banded karyotype obtained in the study is presented in plate 3. Homologous chromosomes in the metaphase spread were paired by their characteristic banding patterns. A diagrammatic representation of the G-banded karyotype is shown in figure 3.

The partitioning and numbering of regions and bands in the chromosomes were done based on standard procedures (ISCNDA, 1989). While designating a particular band, the region number and the band number within that region were given in order without spacing or punctuation. Whenever a band was to be sub-divided, a decimal point was placed after the original band followed by the number assigned to each sub-band.

A total of 405 bands were identified in the G-banded karyotype of the bulls studied. The distribution of bands in various chromosomes are as follows.

Chromosome 1 - Four regions. 21 bands; two negative central bands (31 and 33) separated by a positive band (32); dark telomere; a total of 10 positive bands.

Chromosome 2 - Four regions. 20 bands; four positive bands (13,15,22 and 24) in the proximal half and four bands (34, 36,42 and 44) in the distal half; a narrow central positive band (32); negative telomere; a total of nine positive bands.

Chromosome 3 - Three regions. 15 bands; two prominent central positive bands (24 and 32) separated by a negative band (31); negative telomere; a total of seven positive bands.

Chromosome 4 - Three regions. 19 bands; four positive bands (12,14,16 and 18) in the proximal half; a subcentromeric positive band (12); four positive bands (23,31,33 and 35) in the distal half with one of them prominent (31); a centrally placed positive band (21); a total of nine positive bands.

Chromosome 5 - Three regions. 15 bands; two broad negative bands (21 and 31) divides the chromosome into three parts; three positive bands (22,24 and 26) in the central region; negative telomere;a total of seven positive bands.

Chromosome 6 - Three regions. 17 bands; three prominent positive bands (12, 14 and 21) in the proximal half; five positive bands (23,25,31,33 and 35) in the distal half; a total of eight positive bands.

Chromosome 7 - Two regions. 13 bands; three positive bands (12, 14 and 21) in the proximal half; a prominent positive band (25) in distal half; a total of six positive bands.

Chromosome 8 - Two regions. 16 bands; two prominent positive bands (16 and 18) in the proximal half; a prominent central negative band (21); positive telomere; a total of eight positive bands.

Chromosome 9 - Two regions. 17 bands; four positive bands (12,14,16 and 18) in the proximal half and four bands (21,23,25 and 27) in the distal half; a total of eight positive bands.

Chromosome 10 - Three regions. 17 bands; one prominent positive band(12) in the proximal half and two bands (31 and 33) in the distal half; a narrow positive subtelomeric band (35); a total of eight positive bands.

Chromosome 11 - Two regions. 14 bands; two prominent central positive bands (21 and 23); two positive bands (13 and 15) proximal to the prominent bands and two bands (25 and 27) distal to it; negative telomere; a total of six positive bands.

Chromosome 12 - Two regions. 11 bands; a prominent subcentromeric positive band (12); a prominent positive band (23) in the distal half; a total of five positive bands.

Chromosome 13 - Two regions. 11 bands; three positive bands (12, 14 and 16) in the proximal half; two prominent negative bands (17 and 22) in the distal half separated by a prominent positive band (21); a total of five positive bands.

Chromosome 14 - Two regions. 15 bands; four positive bands (12,14,16 and 18) in the proximal half and three bands (21,23 and 25) in the distal half; a total of seven positive bands.

Chromosome 15 - Two regions. 13 bands; prominent subcentromeric positive band (12) and a broad negative band (21) in the proximal half; four positive bands (22,24, 26 and 28) in the distal half; a total of six positive bands.

Chromosome 16 - Two regions. 12 bands; a subcentromeric negative band (12); three positive bands (13,15 and 17) in the proximal half and two bands (22 and 24) in the distal half separated by a broad negative band (21); a total of five positive bands.

Chromosome 17 - Two regions. 11 bands; two prominent central positive bands (21 and 23) separated by a negative band (22); negative telomere; a total of five positive bands.

Chromosome 18 - Two regions. 11 bands; prominent subcentromeric positive band (12); three central positive bands (14,21 and 23); two prominent negative bands (13 and 24) divides the chromosome into three; a total of five positive bands.

Chromosome 19 - Two regions. 10 bands; a subcentromeric prominent positive band (12); positive telomere; a total of five positive bands.

Chromosome 20 - Two regions. 11 bands; three positive bands (12, 14 and 16) in the proximal half and two bands (21 and 23) in the distal half separated by a broad negative band (17); one prominent positive band (21) in the distal half; a total of five positive bands.

Chromosome 21 - Two regions. 11 bands; three positive bands (12,14 and 16) in the proximal half and two prominent positive bands (21 and 23) in the distal half; a total of five positive bands.

Chromosome 22 - Two regions. Nine bands; a prominent positive band (12) in the proximal half and two bands (21 and 23) in the distal half, with a broad negative band (13), separated into sub-bands (13.1 and 13.3) by a positive band (13.2), in between; negative telomere; a total of four positive bands.

Chromosome 23 - Two regions. 10 bands; similar in appearance to chromosome 22; positive telomere; a total of five positive bands.

Chromosome 24 - Two regions. 10 bands; subcentromeric negative band (12) followed by a prominent positive band (13); three positive bands (22, 24 and 26) in the distal half; a total of four positive bands.

Chromosome 25 - Two regions. Nine bands; subcentromeric prominent positive band (12); two central positive bands (14 and 21); a positive band (23) in the distal half; a total of four positive bands.

Chromosome 26 - Two regions. Seven bands; two prominent positive bands; one subcentromeric (12) and one distal (22); negative telomere; a total of three positive bands.

Chromosome 27 - Two regions. Nine bands; prominent subcentromeric positive band (12.1); two central positive bands (13 and 21); a prominent negative band (22) in distal half; a total of four positive bands.

Chromosome 28 - One region. Nine bands; two prominent positive central bands (14 and 16); a subtelomeric positive band (18); a total of four positive bands.

Chromosome 29 - One region. Nine bands; two closely placed positive bands (12 and 14) in proximal half; a prominent central positive band (16); negative telomere; a total of four positive bands.

Chromosome Xp - Two regions. Eight bands; central prominent positive band (21); a total of three positive bands.

Chromosome Xq - Four regions 17 bands; central prominent negative band (31); four positive bands (12,22,24 and 26) in the proximal half and four bands (32,34,36 and 42) in the distal half; a total of eight positive bands.

Chromosome Yp - One region. Three bands; entirely negative with a positive telomere.

Chromosome Yq - One region. Five bands; subcentromeric positive band (12.1) and a prominent subtelomeric positive band (12.3); a total of two positive bands.

The G-bands identified in the chromosomes are summerised in table 1. In the chromosome number 24 of the bulls presently studied, the band 13 was not divided into sub-bands, as described in the standard karyotype (ISCNDA, 1989). One sub-band in the region one of chromosome 27 and two sub-bands in chromosome Yq were also absent in the karyotype of these bulls.

2. The probands:

The three probands which had sub normal semen qualities were designated as bull 'A', bull 'B' and bull 'C'. The age at which an ejaculate was given was found to be comparable among both normal bulls and the probands. But none of the ejaculates produced by the probands were suitable for freezing (table 2).

The reproductive attributes of the bulls based on the first 10 semen collections are presented in table 3. The average sperm concentration and total sperm output of bull 'B' were recorded to be very low. The total sperm out put of bull 'C' was slightly lower than that for all the normal bulls.

The details of sperm abnormalities identified in the semen of bulls are described in table 4. Sperm with proximal cytoplasmic droplet and loose heads observed in semen of bull 'A', and sperms with knobbed acrosome defect identified in the semen of bull 'C' are depicted in plates 4 and 5.

3. Cytogenetic observations in probands:

The metaphase spreads obtained from the blood samples of the probands are presented in plates 6,7 and 8. All the cells of bulls 'A' and 'B' had a chromosome complement of 2n=60. Tetraploid cells were observed in lymphocyte cultures

of bull 'C'; 6.67 per cent of the cells had 120 chromosomes (plate 9). None of the chromosomes in the probands showed an atypical morphology.

The relative length of chromosomes of the probands are described in table 5. The relative length of first autosome showed a tendency to deviate from the range observed in normal bulls. A similar tendency was also observed in chromosomes 19 and 20 of the bull 'A'.

Arm ratio and centromeric index of X chromosome in the probands as well as normal bulls are presented in table 6. The arm ratio and centromere index in the probands were found to be within the range observed in normal bulls.

The G-banded karyotype of the three probands are depicted in plates 10,11 and 12. Analysis of the banding pattern revealed that G-bands in the probands were similar to that in the contemporary bulls.

4. Sex chromosome diversity:

The 53 bulls studied belonged to three genetic groups viz, C.B.J., C.B.H.F. and C.B.B.S. The morphology and mean relative length of X and Y chromosomes, and the arm ratio and centromeric index of X chromosome are presented in table 7.

The X chromosome was sub-metacentric in all the bulls. The centromere was almost centrally placed in the Y chromosome of C.B.J. and C.B.B.S. whereas it was sub-metacentric in C.B.H.F.

The mean relative length of X chromosome was highest in C.B.J. (5.6363), followed by 5.5875 and 5.4873 in C.B.H.F. and C.B.B.S. The mean relative length of Y chromosome was 1.9574, 1.8908 and 2.058 in the three groups respectively (Figure 4).

Centromeric index of X chromosome in the bulls belonging to the three genetic groups are depicted in figure 5. Variation in arm ratio and centromere index between the genetic groups were small.

The relative length of X and Y chromosomes, and centromeric index and arm ratio of the X chromosomes in the different genetic groups were compared by analysis of variance in one-way classification. The test revealed that there existed no significant difference in these parameters, between the genetic groups (table 8).

Plates

Plate 1. Representative metaphase spread

Plate 2. Representative karyotype

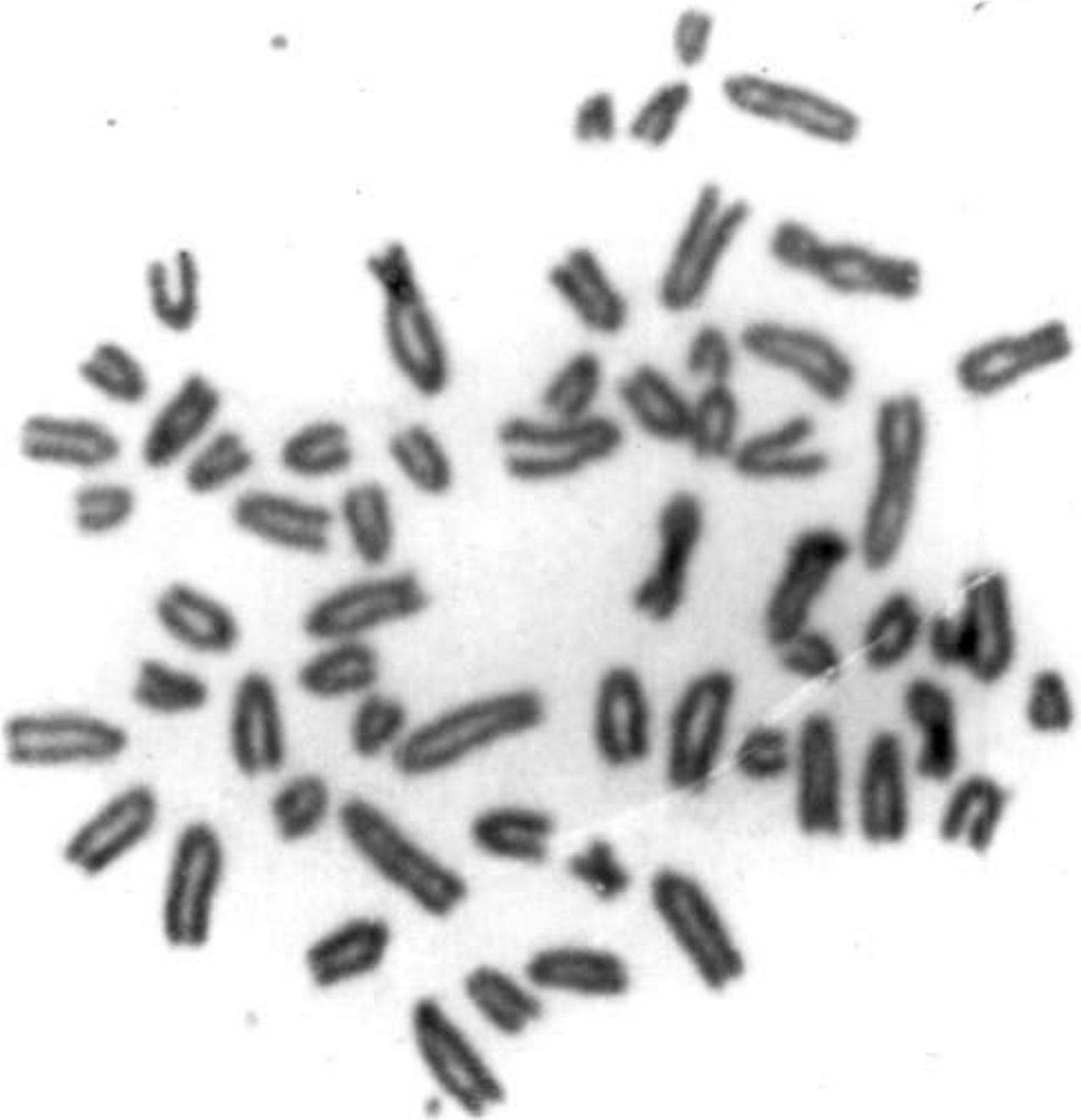


Plate 3. Representative G-banded karyotype

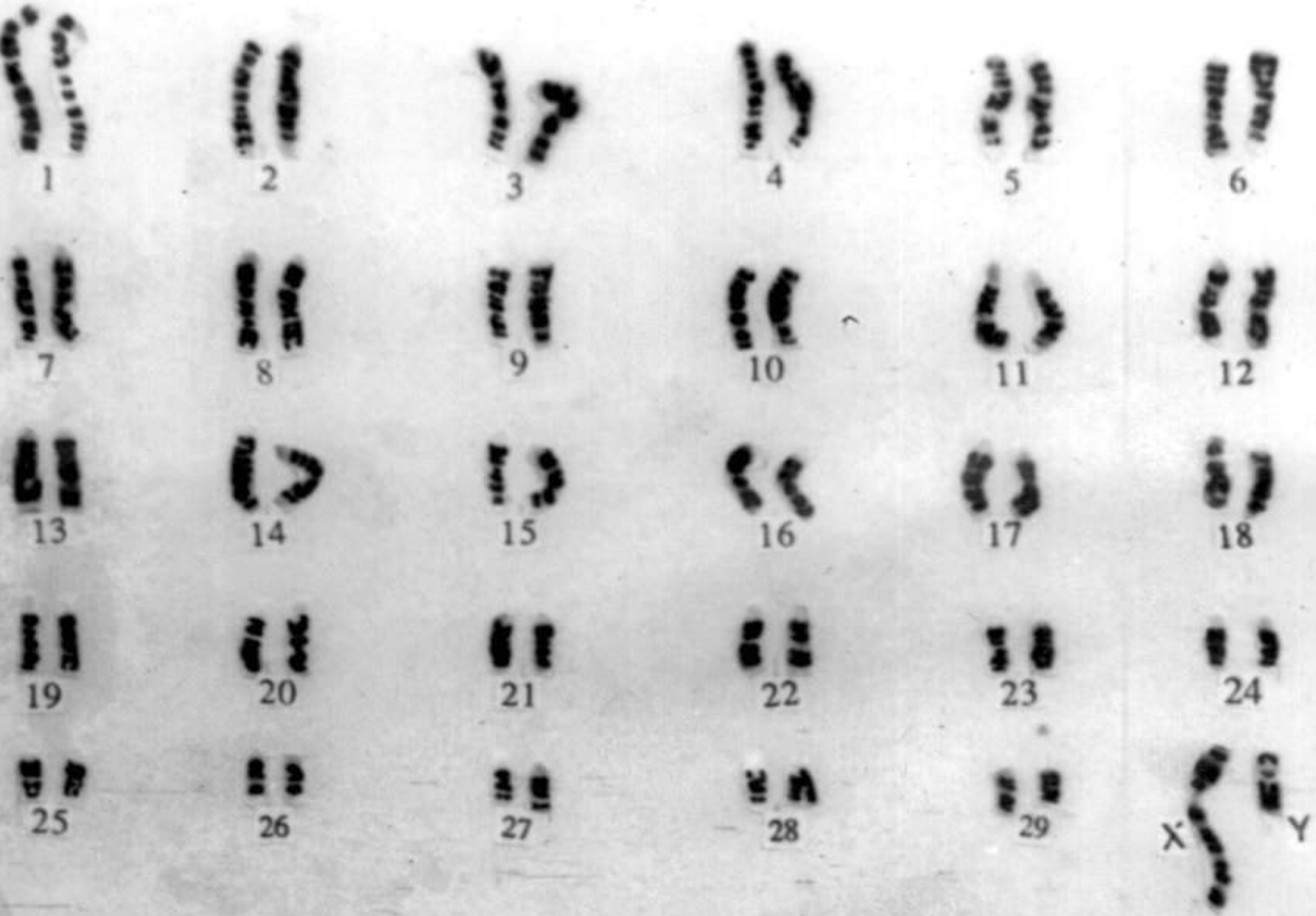

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
X
Y

Plate 4. Abnormal sperms of bull 'A' - Proximal cytoplasmic droplet and loose heads.

Plate 5. Abnormal sperms of bull 'C' - Knobbed acrosome defect.

Plate 6. Metaphase spread of bull 'A'

Plate 7. Metaphase spread of bull 'B'

Plate 8. Diploid metaphase spread of bull 'C'

Plate 9. Tetraploid metaphase spread of bull 'C'

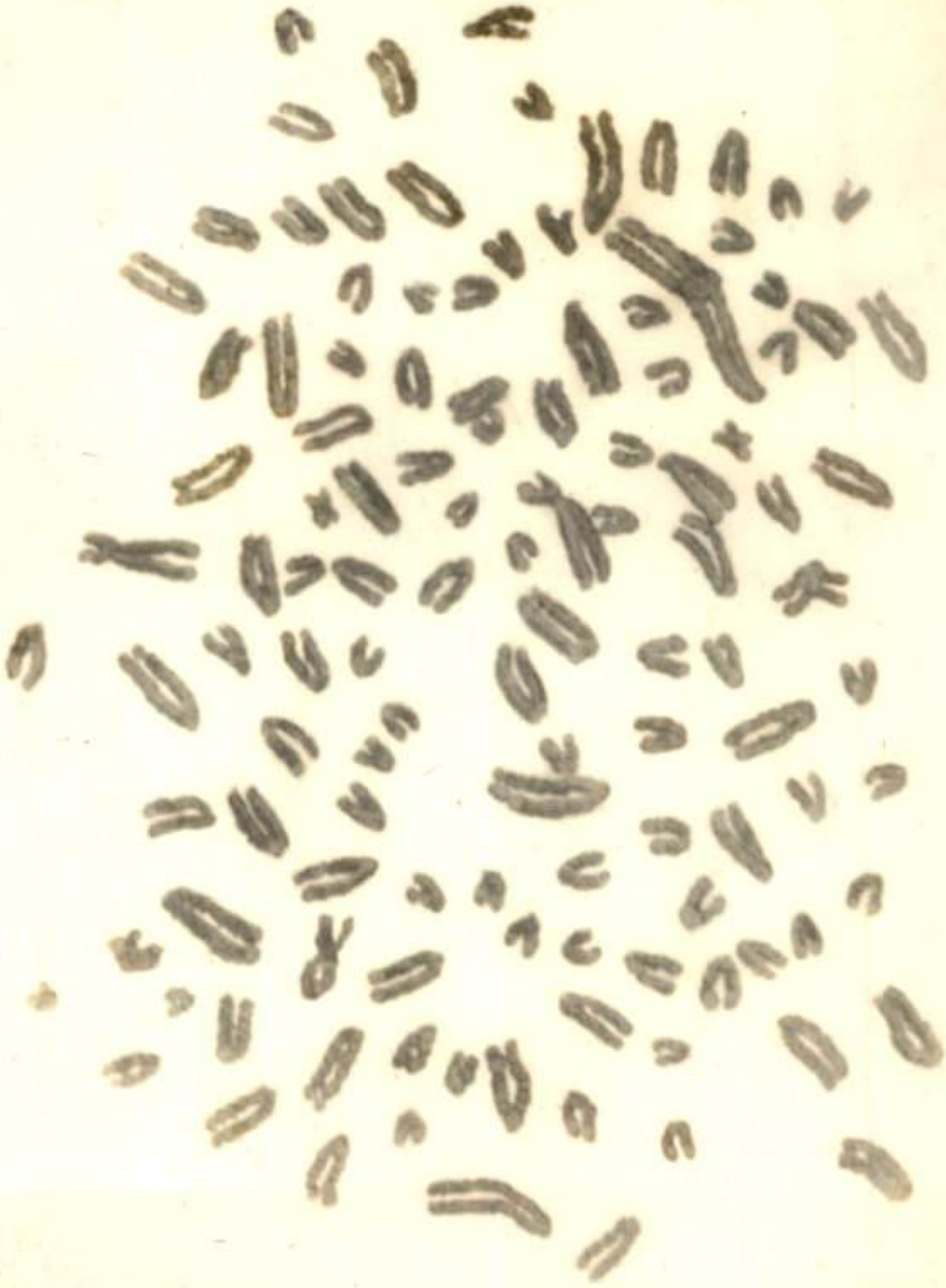
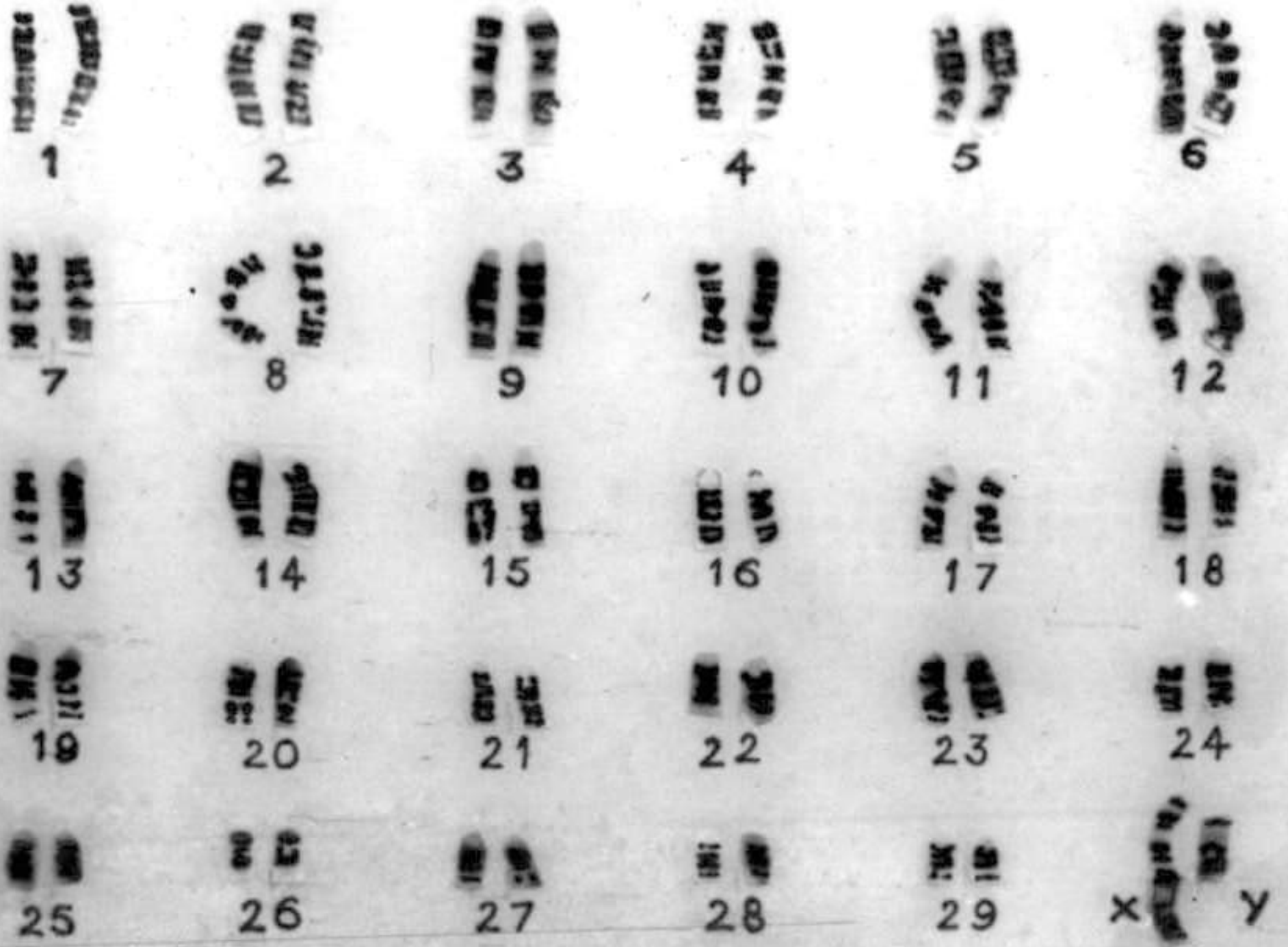


Plate 10. G-banded karyotype of bull 'A'

Plate 11. G-banded karyotype of bull 'B'


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
X
Y

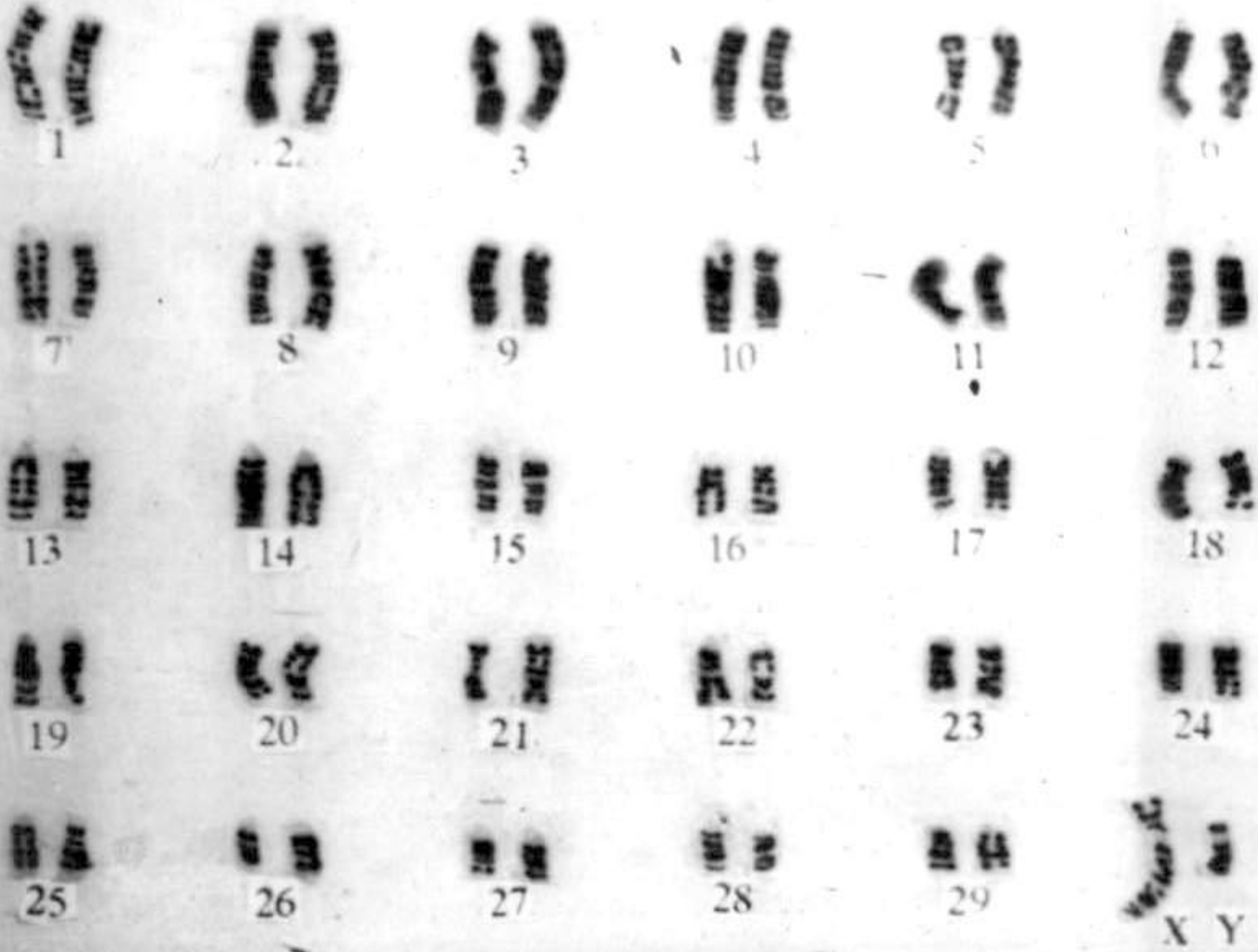
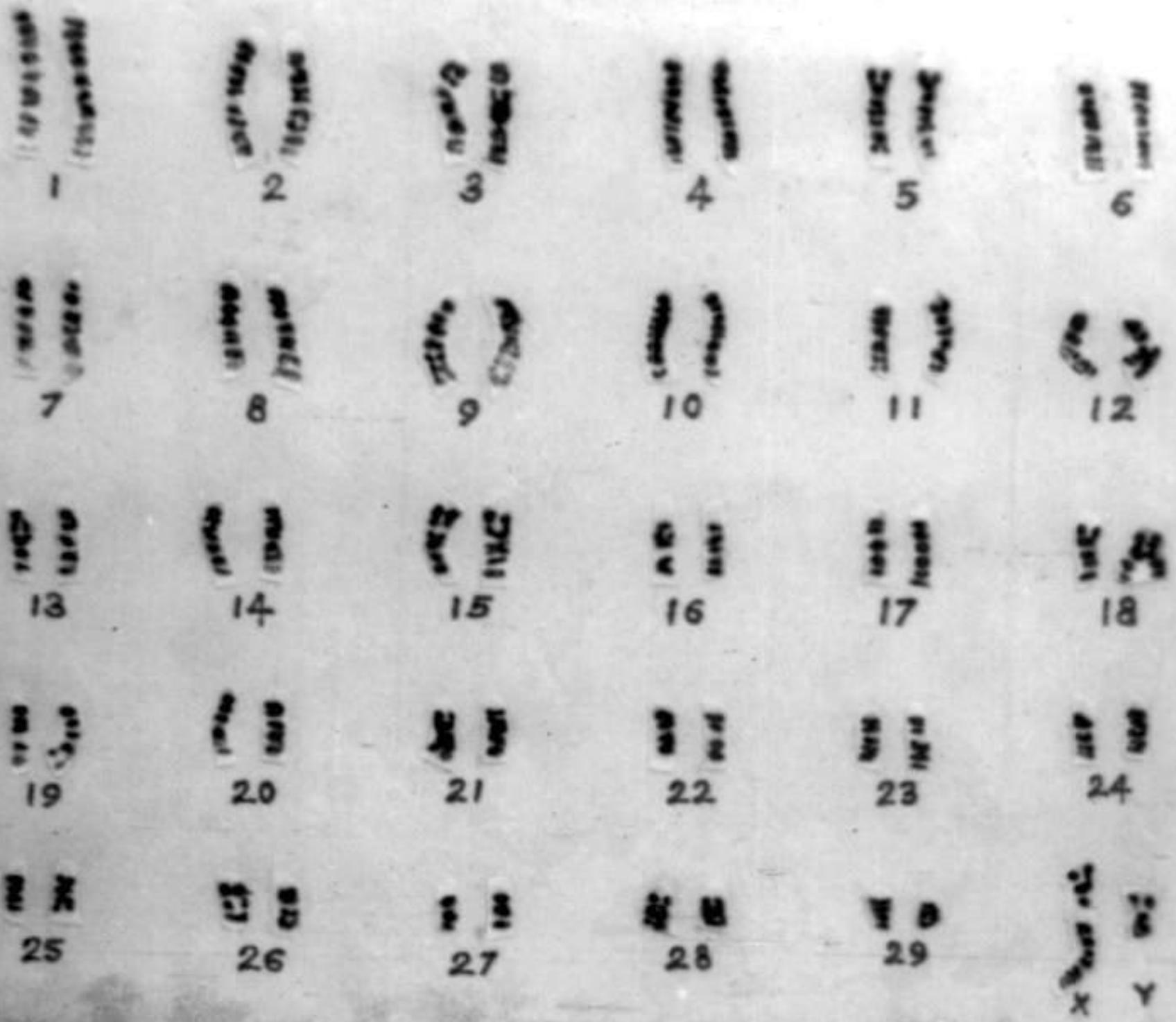

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
X Y

Plate 12. G-banded karyotype of bull 'C'


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
X
Y

Tables

Table 1. G-bands in the chromosomes.

Chr. no.	No.of bands	No.of positive bands	Distal end	Chr. no.	No.of bands	No.of positive bands	Distal end
1.	21	10	+	18.	11	5	-
2.	20	9	-	19.	10	5	-
3.	15	7	-	20.	11	5	-
4.	19	9	-	21.	11	5	-
5.	15	7	-	22.	9	4	-
6.	17	8	-	23.	10	5	+
7.	13	6	-	24.	10	4	-
8.	16	8	+	25.	9	4	-
9.	17	8	-	26.	7	3	-
10.	17	8	-	27.	9	4	-
11.	14	6	-	28.	9	4	-
12.	11	5	-	29.	9	4	-
13.	11	5	-	Xp	8	3	-
14.	15	7	-	Xq	17	8	-
15.	13	6	-	Yp	3	1	+
16.	12	5	-	Yq	5	2	-
17.	11	5	-				

Table 2. Age of bulls at semen production and freezable semen production

	Normal bulls		Probands*		
	Mean ± S.E.	Range	A	B	C
Age at first semen collection (in months)	19.4 ± 0.24	15.4 - 23.0	19.8	19.3	18.3
Age at first freezable semen production (in months)	20.4 ± 0.24	17.2 - 23.7	-	-	-

* None of the probands had produced a freezable ejaculate.

Table 3. Reproductive attributes of bulls based on first 10 semen collections

	Normal bulls		Probands		
	Mean ± S.E.	Range	A	B	C
Average volume (ml)	3.46 ± 0.15	1.55 - 4.95	2.88	2.25	2.08
Average sperm concentration ($x10^9$)	1.39 ± 0.04	0.76 - 1.86	0.88	<0.30	0.83
Total sperm output ($x10^9$)	47.83 ± 2.46	22.40 -70.09	25.23	<6.75	17.28
Number of ejaculates accepted for freezing	3.92 ± 0.36	1.00 -10.00	..	..	..
Total freezable sperm output ($x10^9$)	21.96 ± 2.21	5.31 -61.76	..	..	..

Table 4. Spermiogram: Details of sperm abnormalities

Sperm abnormalities	Normal bulls		Probands			Maximum acceptable for a good bull
	Mean	± S.E	A*	B**	C***	
Major defect (%)	1.79	± 0.17	9.72	1.98	49.50	5
Minor defect (%)	3.70	± 0.39	15.42	59.41	4.95	individual abnormality 10-15
Total defect (%)	5.50	± 0.61	25.14	61.39	54.45	25

* Among major defects, presence of proximal cytoplasmic droplet was predominant.

** More than 15 per cent of the sperms were without a tail.

*** High percentage of sperms with knobbed acrosome defect was present

Table 5. Relative length of chromosomes

Chromosome number	Normal bulls			probands		
	Mean ± S.E		Range	A	B	C
(1)	(2)		(3)	(4)	(5)	(6)
1	6.0174	± 0.0273	5.54 - 6.50	5.8537	6.5217	5.4558
2	5.2074	± 0.0309	4.92 - 5.73	5.0174	5.2372	5.2486
3	4.8520	± 0.0180	4.57 - 5.09	4.7439	4.9901	4.6960
4	4.7302	± 0.0224	4.40 - 4.99	4.4599	4.9407	4.5580
5	4.5886	± 0.0248	4.36 - 4.94	4.3206	4.5455	4.5199
6	4.4654	± 0.0252	4.14 - 4.89	4.1812	4.4165	4.2818
7	4.2816	± 0.0212	4.02 - 4.60	4.1115	4.3478	4.2127
8	4.1367	± 0.0216	3.86 - 4.47	4.0418	4.1502	4.1436
9	3.9706	± 0.0211	3.65 - 4.42	3.9721	3.8538	4.0746
10	3.8178	± 0.0233	3.50 - 4.30	3.7631	3.7055	4.0055
11	3.6554	± 0.0197	3.29 - 3.86	3.6934	3.5573	3.5912
12	3.4578	± 0.0190	3.16 - 3.68	3.5540	3.4585	3.5221
13	3.3020	± 0.0166	3.02 - 3.48	3.3449	3.3597	3.3845
14	3.1680	± 0.0144	2.95 - 3.38	3.2056	3.1621	3.3149
15	3.0646	± 0.0158	2.81 - 3.31	3.1359	3.0632	3.2459

continued

Table 5 continued:

(1)	(2)	(3)	(4)	(5)	(6)
16	2.9382 ± 0.0139	2.73 - 3.17	3.0662	2.9644	2.9001
17	2.8190 ± 0.0165	2.51 - 3.02	2.9965	2.8656	2.6934
18	2.7016 ± 0.0197	2.37 - 2.96	2.9268	2.8162	2.6243
19	2.6080 ± 0.0179	2.33 - 2.83	2.8990	2.7668	2.5552
20	2.5072 ± 0.0157	2.26 - 2.68	2.8153	2.6186	2.4862
21	2.4104 ± 0.0169	2.20 - 2.64	2.5087	2.3715	2.4448
22	2.3132 ± 0.0168	2.04 - 2.57	2.4390	2.1245	2.3895
23	2.2178 ± 0.0163	1.93 - 2.49	2.3693	1.9960	2.3481
24	2.1092 ± 0.0163	1.85 - 2.41	1.9791	1.9566	2.2790
25	2.0182 ± 0.0165	1.77 - 2.32	1.9233	1.9269	2.1409
26	1.9076 ± 0.0162	1.72 - 2.16	1.8815	1.8775	2.0718
27	1.8172 ± 0.0135	1.64 - 2.01	1.7422	1.8281	1.8646
28	1.7036 ± 0.0110	1.52 - 1.87	1.7003	1.6304	1.7956
29	1.6186 ± 0.0101	1.47 - 1.82	1.6446	1.5810	1.7265
X	5.5918 ± 0.0401	5.20 - 6.34	5.7092	5.3360	5.5249
Y	1.9636 ± 0.0396	1.47 - 2.62	1.8118	1.5810	1.5193

Table 6. Arm ratio and centromere index of X chromosome

	Normal bulls		Probands		
	Mean ± S.E	Range	A	B	C
Arm ratio	2.47 ± 0.04	1.97 - 3.18	2.15	2.38	2.33
Centromeric index	28.74 ± 0.33	23.91 -34.29	31.71	29.63	30.00

Table 7. Sex chromosome variants in the genetic groups

Variants	Chromosome	C.B.J.	C.B.H.F.	C.B.B.S
Morphology	X	Sub-metacentric	Sub-metacentric	Sub-metacentric
	Y	Metacentric	Sub-metacentric	Metacentric
Morphometry*	X	5.64 ± 0.07	5.59 ± 0.05	5.49 ± 0.02
	Y	1.96 ± 0.06	1.89 ± 0.06	2.06 ± 0.08
Arm ratio*	X	2.46 ± 0.04	2.49 ± 0.10	2.48 ± 0.08
Centromeric index*	X	28.99 ± 0.37	28.99 ± 0.82	28.00 ± 0.62

* Variants are expressed as mean ± S.E.

Table 8a. Analysis of variance: Effect of genetic group on the relative length of X chromosome

Source	df	SS	MSS	F
Genetic group	2	0.1738	0.0869	1.0826^{NS}
Error	47	3.7728	0.0803	
Total	49	3.9466		

NS - Not significant

Table 8b. Analysis of variance: Effect of genetic group on the relative length of Y chromosome

Source	df	SS	MSS	F
Genetic group	2	0.1630	0.0815	1.0389^{NS}
Error	47	3.6868	0.0784	
Total	49	3.8498		

NS - Not significant

Table 8c. Analysis of variance: Effect of genetic group on arm ratio of X chromosome

Source	df	SS	MSS	F
Genetic group	2	0.0103	0.0051	0.0730^{NS}
Error	47	3.3014	0.0702	
Total	49	3.3117		

NS - Not significant

Table 8d. Analysis of variance: Effect of genetic group on centromeric index of X chromosome

Source	df	SS	MSS	F
Genetic group	2	0.2700	0.1350	0.0282^{NS}
Error	47	225.2852	4.7933	
Total	49	225.5552		

NS - Not significant

Figures

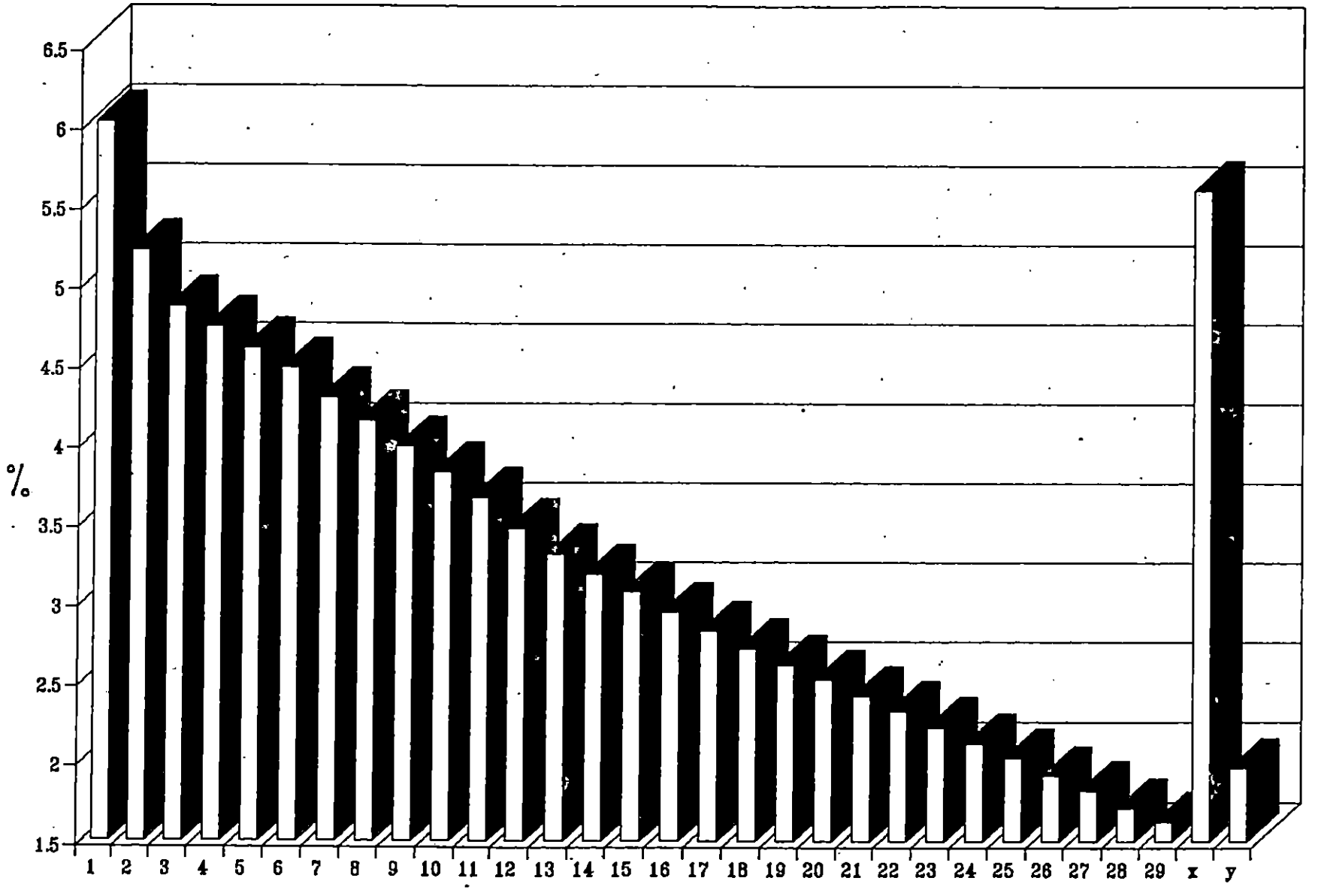


Fig.1. Mean Relative Length of Chromosomes

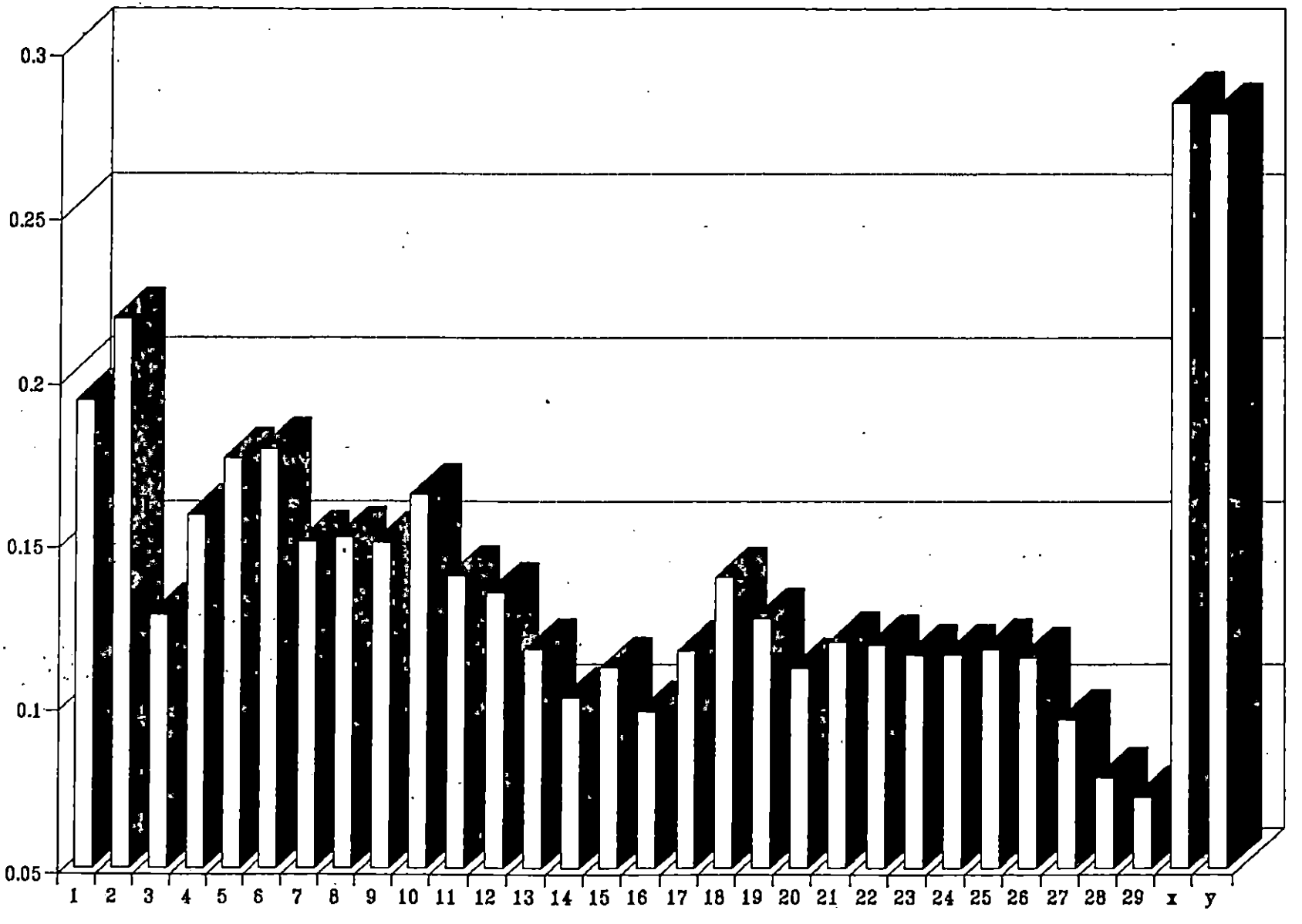


Fig.2a. Standard Deviation of Mean Relative Length of Chromosomes

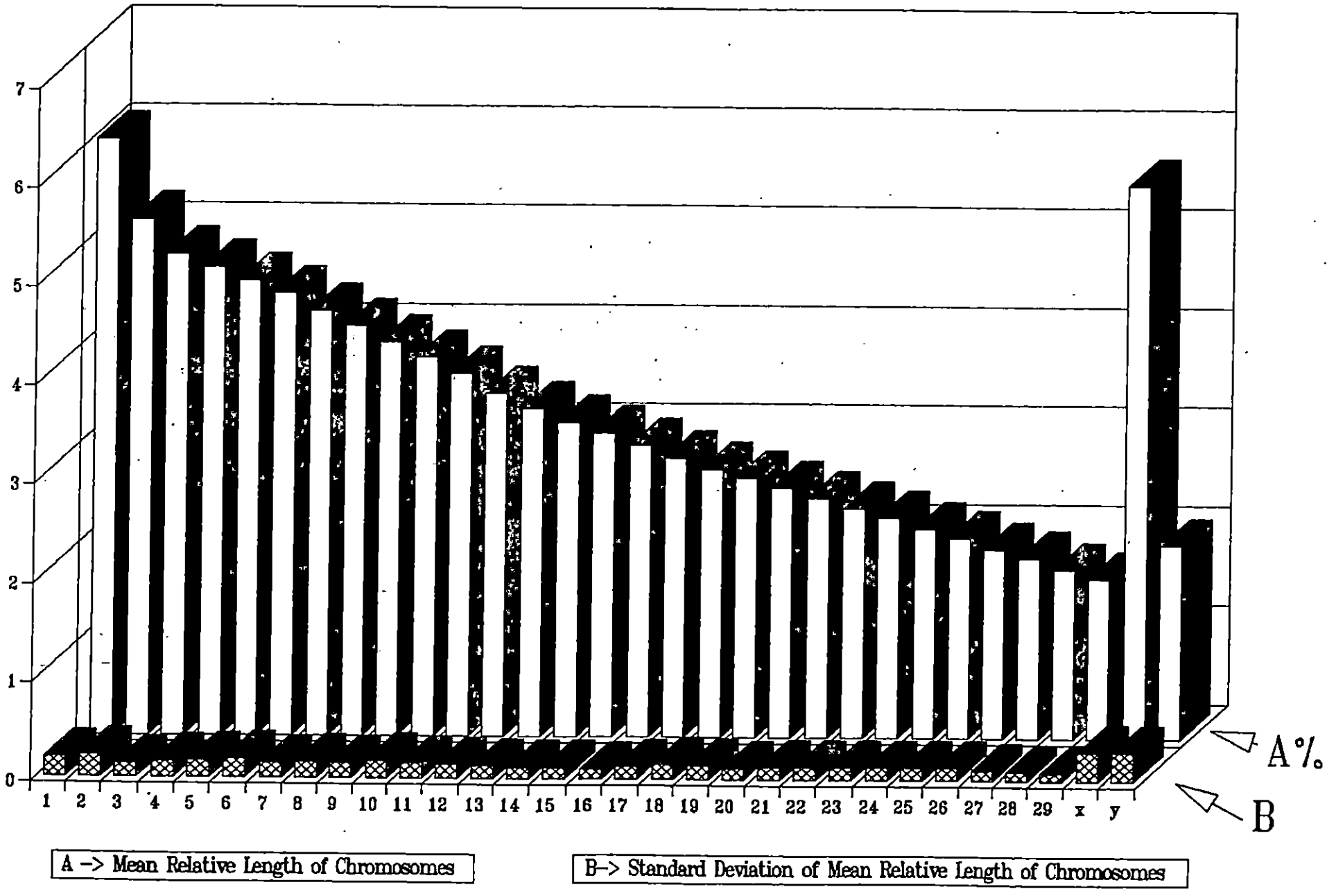


Fig.2b. Mean Relative Length and Standard Deviation of Chromosomes

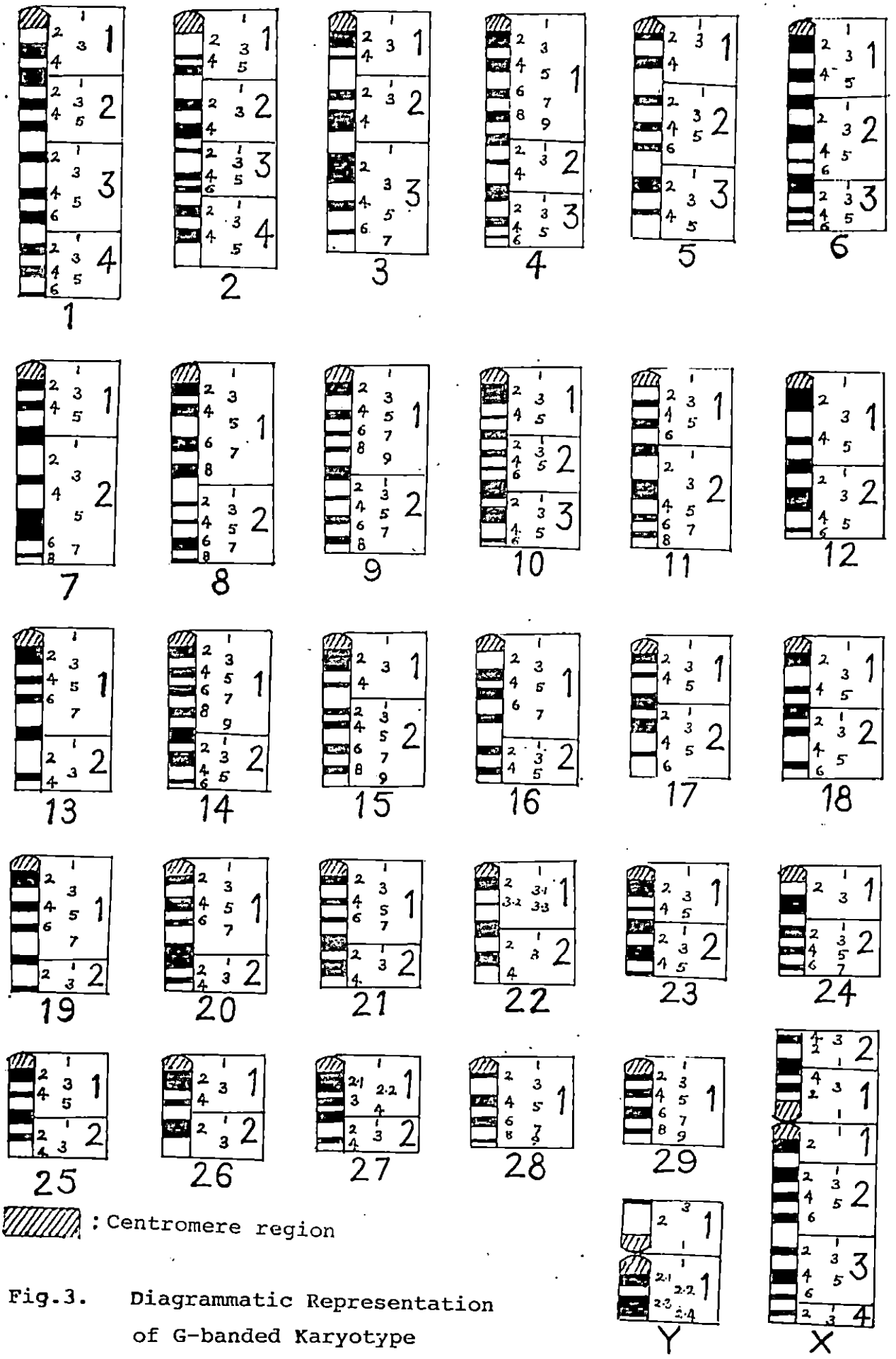


Fig.3. Diagrammatic Representation of G-banded Karyotype

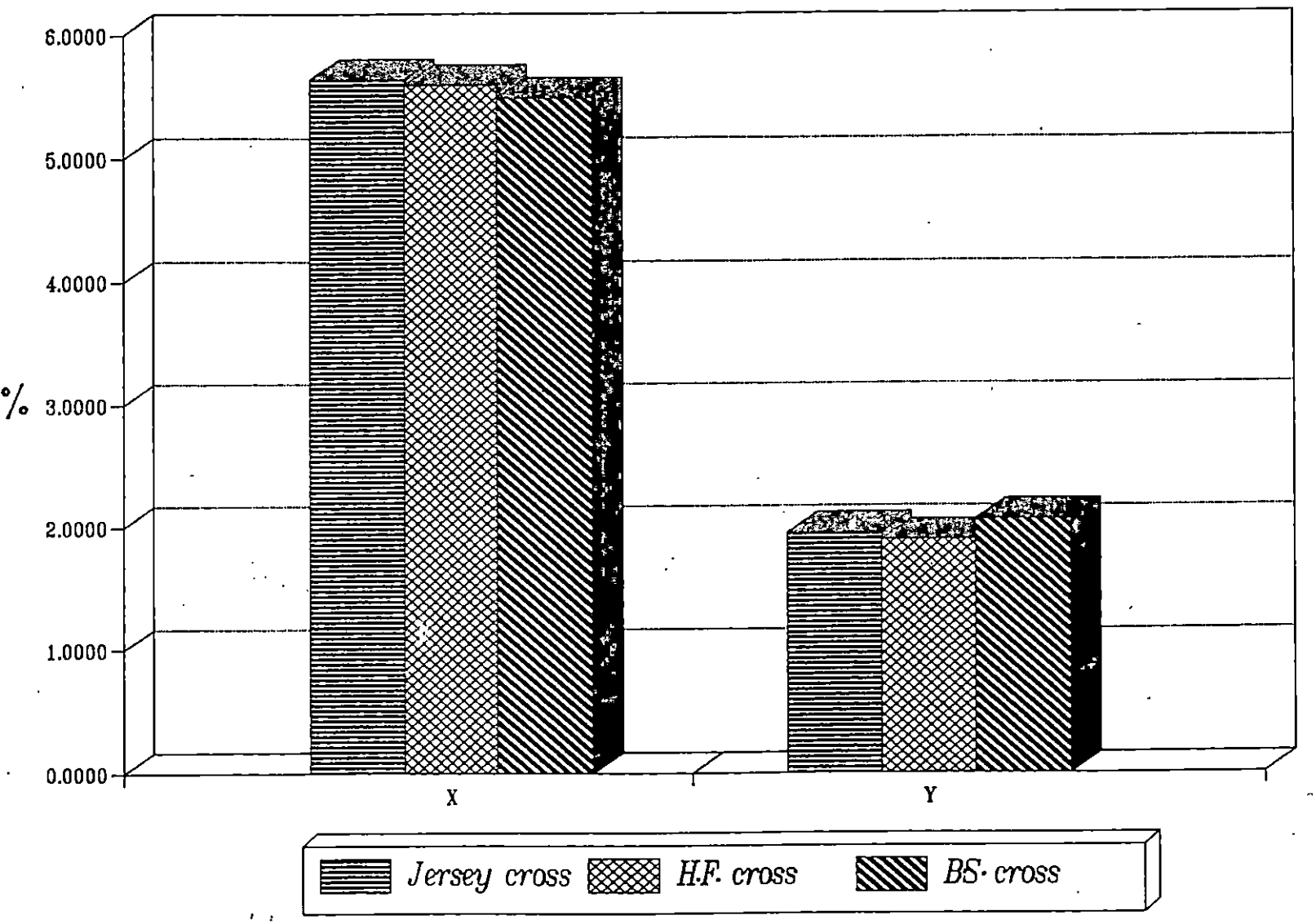
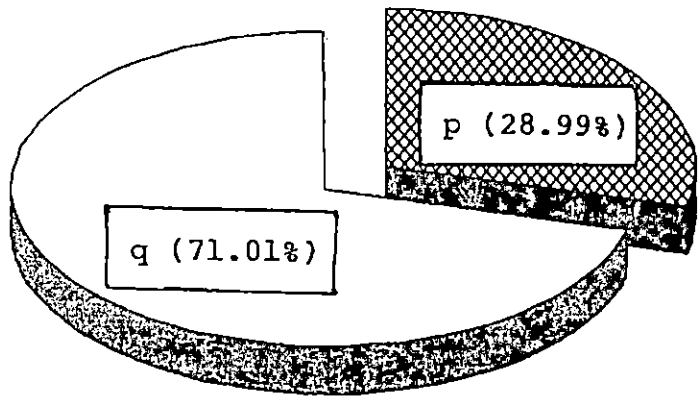
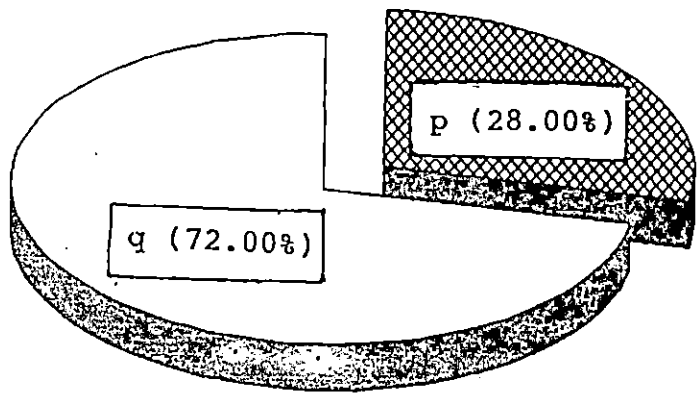


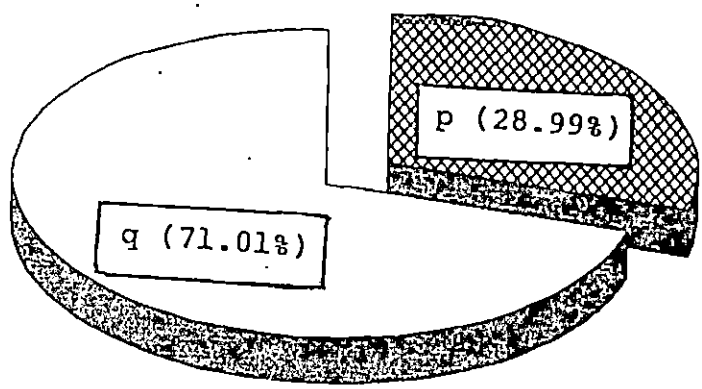
Fig.4. Mean Relative length of Sex Chromosomes of Different Genetic Groups



Jersey Cross



Brown-Swiss Cross



Holstein-Friesian Cross

Centromere index:

Fig.5. Mean Proportions of p and q Arms of X chromosome

DISCUSSION

DISCUSSION

Cytogenetic investigation on cross-bred bulls belonging to different genetic groups, used for A.I. in Kerala was carried out by karyotyping in the present study.

The identification and description of chromosomes becomes more profound and perfect by using sophisticated techniques such as banding. Methods most commonly used to produce bands on the chromosomes are G-, R- C- and Q-banding. In G-banding alternative dark stained and light stained areas are produced on the chromosomes. Since the position, width, nature and number of bands are distinct for each pair of chromosome, the identification of individual chromosomes becomes easier by banding. This also aids in identification and characterisation of various structural aberrations in the chromosomes. In the present study chromosomes were subjected to G-banding.

1. Chromosomes of the Cross-bred bulls:

In the present study, all the bulls exhibited a chromosome number of 60 in their cells. This finding was in agreement with the diploid chromosome number established in cattle (Makino, 1944). A similar number (2n=60) of

chromosomes was also described in cattle of Kerala by Raghunandanan and Mukundan (1991).

The karyotype of some lymphocytes of one of the bulls revealed 120 number of chromosomes indicative of tetraploidy. A diploid/tetraploid mosaicism in cattle was described by Zartman and Fechheimer (1967), Swartz and Vogt (1983), and Raghunandanan and Mukundan (1990). Eldridge et al. (1984) observed tetraploid cells in bovine blood culture even when colchicine was not mixed to arrest the spindle fibre formation.

The occurrence of tetraploidy in the lymphocyte culture can be explained to be the result of errors in mitosis like non-disjunction.

1.1 Morphology:

The morphology of chromosomes observed in the present study was in agreement with the previous findings. The Y chromosome of the bulls were either sub-metacentric or apparently metacentric. Polymorphism of Y chromosome in different breeds of cattle was described by several workers (Cribiu, 1975; Halnan and Watson, 1982; Märki and Robinson, 1984). Raghunandanan and Mukundan (1991) reported the Y chromosome to be sub-metacentric in half-bred H.F. and nearly

metacentric in half-bred Jersey. The centromere of Y chromosome in B.S. bulls was observed to be almost centrally located (Blazak and Eldridge, 1977).

The cross-bred cattle of Kerala was developed by breeding the local non-descript females with bulls belonging to one of the three European breeds viz, Jersey, H.F. or B.S. European blood in the paternal ancestory of the synthetic breed, was thought to be the reason for the Y chromosome morphology typical to the B. taurus breeds.

1.2 Morphometry:

The morphometry of chromosomes forms an additional criterion in describing the chromosome morphology and their comparative evaluation. The arrangement and classification of chromosomes according to their decreasing size in itself permits the identification of some of the structural abnormalities where they exist.

The relative length of chromosomes in cross-bred bulls presently studied, ranged between 6.0174 and 1.6186. This was found to agree with the results reported for various breeds of cattle. All the autosomes except the first and second pairs, had a relative length comparable to that observed in Spanish fighting bulls by Arruga and Zarazaga

(1984b);the first two pairs of autosomes were observed to be longer. The relative length of the first and second autosome pairs were found to be lesser than that reported by Raghunandanan and Mukundan (1991) for the cross-bred cattle of the state.

In the present study the X chromosome occupied a second position in the karyotype based on the relative length. Similar results were described in Swedish Red and White (Gustavsson, 1969), and Jersey (Raghunandanan and Mukundan, 1991). The Y chromosome was observed to have a relative length in between the 25th and 26th autosome. This finding was similar to that reported by Lin et al. (1977) in the B. taurus bulls. The present finding was also in general agreement with the observations reported by Potter et al. (1979).

1.3 G-banding patterns:

Cytogenetic study on B. taurus, B. indicus and B. taurus x B. indicus cattle of Kerala was conducted by Raghunandanan and Mukundan (1991), and the morphology and morphometry of chromosomes were described. However, the banding patterns produced by various staining methods were not investigated in the cattle of the state. The chromosomes of cross-bred bulls presently studied, were subjected to G-banding technique and the bands developed were analysed.

The number and clarity of the G-bands were dependent on the degree of contraction of chromosomes and the period of treatment with the saline solution containing trypsin. The various G-bands were better resolved in the chromosomes at the early metaphase stage of the mitotic division.

The G-bands identified in the various chromosomes were in general agreement with those established for cattle in the second international conference on standardisation of domestic animal karyotypes (ISCNDA, 1989). A total of 405 bands were identified in the present study as against approximately 410 bands established in the standardised karyotype. The band 24q 13 was not divided into sub-bands (13.1, 13.2 and 13.3) described in the standard karyotype. Similarly bands 27q 12.3, Yq 12.5 and Yq 12.6 described in the standard were not observed in the present study.

Variation in the number of bands and sub-bands was reported to be possible due to the joint appearance of certain bands (ISCNDA, 1989). Hence the G-banded karyotype presently obtained in the cross-bred bulls was concluded to be a standard karyotype. Identification of a standard G-banded karyotype in the cross-bred bulls presently investigated, supports the finding reported by Potter et al. (1979). In the latter study the B. taurus and B. indicus cattle were observed to have similar G-banding patterns.

The G-banding pattern presently reported in the chromosomes of cross-bred bulls will be of future use in describing various chromosome anomalies, when they exist in the A.I. bulls of the state.

2. Semen quality and related attributes:

Age at maturity of the bulls, expressed as the age at which a successful semen collection was obtained, was found to agree with the previous report on A.I. bulls of Kerala (Mathew, 1991). Three of the bulls presently studied had not produced ejaculates suitable for freezing, but their age when semen was first collected was within the range observed for the remaining bulls.

Among the three sub-normal bulls (probands) the bull 'B' was observed to be oligospermic. The total sperm output obtained from the first 10 semen collections of bull 'C' was found to be lesser than that obtained from the normal contemporary bulls. In bull 'A' the average semen volume, sperm concentration and the total sperm output in the first 10 collections were within the range ascribed for normal bulls. However, the bull like other two probands, did not give a freezable ejaculate.

Large numbers of sperm cells possessing proximal cytoplasmic droplet was found in ejaculated semen of bull

'A'. High frequency of sperms with proximal cytoplasmic droplet in the ejaculate semen was reported to be indicative of abnormal spermiogenesis or epididymal maturation (Rao, 1971; Salisbury et al., 1978). In bull 'B' producing oligospermic semen, the frequency of sperms with minor defects, predominantly loose heads, was very high. The presence of large number of tail-less sperms and oligospermia were described as an indication of partial testicular hypoplasia (William, 1965). A high incidence of sperms with knobbed acrosome defect was detected in bull 'C'. The knobbed sperm defect was reported to be a hereditary defect in Friesian (Donald and Hancock, 1953) and Charolais bulls (Barth, 1986). A high frequency of cells affected with this abnormality was reported to be associated with sterility (Teunissen, 1946; Hancock,1949).

3. Cytogenetic observations in the probands;

In the present study, tetraploid cells were found in the blood samples of bull 'C', to the tune of 6.67 per cent. Mixoploidy was previously reported in cattle with reproductive disorders; small percentages of triploid cells were described in a hermaphrodite (Dunn et al., 1970), and tetraploid cells were reported in a bullock with rudimentary teat (Raghunandanan and Mukundan, 1990) and in anoestrus cow (Swartz and Vogt, 1983). An association between the

incidence of tetraploidy and the degree of in-breeding was reported by Zartman and Fechheimer (1967). However, association between mixoploidy and knobbed acrosome defect was not reported previously.

The cause of tetraploidy in some of the cells of bull 'C' could lie with a defective division apparatus. If the germ cells also carry a similar division apparatus, abnormal gene content may result in some sperms leading to reduced viability, and this may be the cause of poor semen freezability in this bull. Sperms with knobbed acrosome in the semen of this bull may also have influenced the freezability. Since there were no reports of an association between abnormal cell division and knobbed acrosome defect, further series of studies was thought to be essential for drawing a conclusion.

The other two bulls with abnormal seminal attributes revealed no cytogenetic abnormalities. The variations in relative length of chromosomes in these probands were not conclusive of a karyological defect, as the chromosome morphology and the G-banding patterns in these bulls were comparable with those of normal contemporary bulls.

4. Sex chromosome polymorphism:

The cross-bred bulls presently studied were classified

into three genetic groups, based on the paternal line. The usefulness of morphology and morphometry of Y chromosome in detecting the paternal line in two-breed situations was described by Halnan (1989a).

In the present study, variation was noticed in the mean relative length of X and Y chromosome in different genetic groups, but they were statistically insignificant. The diversity in arm ratio and centromeric index was also not significant. The morphological difference in the Y chromosome was the only criterion by which the C.B.H.F. bulls could be cytogenetically differentiated from the other groups.

SUMMARY

SUMMARY

Karyological study was conducted on 53 cross-bred (B. taurus x B. indicus) young bulls stationed at K.L.D.B.Farm, Dhoni. The metaphase chromosomes of the bulls stained by orthodox staining and by G- banding technique were studied to describe their morpholgy, morphometry and banding patterns. The semen quality and related attributes of the bulls were recorded, and the association between these attributes and cytogenetic architecture was analysed. The sex chromosome variants in the different genetic groups were also examined.

In all the bulls, except one the metaphase spreads revealed a chromosome number 2n=60, with sexual dimorphism. In one bull, 6.67 per cent of the cells carried 120 chromosomes indicative of 2n/4n mosaicism. The occurrence of tetraploid cells were thought to be the result of errors in the mitosis.

The metaphase spreads revealed 29 pairs of acrocentric autosomes and one sub-metacentric X chromosome. The Y chromosome was sub-metacentric in C.B.H.F. bulls, while it was nearly metacentric in C.B.J. and C.B.B.S. The Y chromosome morphology of the bulls were diagnostic of the European ancestory in their paternal line.

The mean relative length of autosomes ranged from 1.6186 ± 0.0716 to 6.0174 ± 0.0273, and that of X and Y chromosomes were 5.5918 ± 0.0401 and 1.9636 ± 0.0396 respectively. The arm ratio and centromeric index of the X chromosomes were 2.4706 ± 0.0368 and 28.74 ± 0.33 respectively, indicative of sub-metacentric morphology. All the morphometric parameters obtained in this study were in agreement with previous findings.

A total of 405 G-bands were identified in the karyotype of cross-bred bulls. The characteristic banding pattern of the different chromosomes made their identification easier. The G-banded karyotype prepared from the cross-bred bulls were found to be similar to the standard karyotype established for cattle.

The study revealed three bulls to have sub-normal semen producing ability. One of the bull produced oligospermic semen with high frequency of loose sperm heads, suggestive of a possible partial hypoplasia. Semen of another bull was observed to have high percentage of sperms with knobbed acrosome defect, a defect proved to have hereditary predisposition in some breeds of cattle. The third bull produced semen with normal qualities, but had not given an ejaculate suitable for freezing. The frequency of sperms with proximal cytoplasmic droplet was also high in the semen of this bull.

The bull producing sperms with knobbed acrosome defect exhibited tetraploid/diploid mosaicism. Further study was essential to confirm an association between the mixoploidy and the sperm defect, since the two anomalies were found together only in one bull. No cytogenetic aberrations were detected in the other two bulls with abnormal seminal attributes.

The sex chromosome variants were studied with an objective of detecting the paternal line in the different genetic groups. However, the variation in the morphometric measures like relative length, arm ratio and centromeric index, between the genetic groups was not significant. The Y chromosome morphology was found to be an effective karyological marker for distinguishing C.B.H.F. bulls from rest of the bulls used for breeding in Kerala.

REFERENCES

REFERENCES

Ansari, H.A.; Jung, H.R.; Hediger R.; Fries, R.; König, H. and Stranzinger, G. (1993). A balanced autosomal reciprocal translocation in an azoospermic bull. Cytogenet. Cell Genet. 62 (2-3): 117-123.

Arruga, M.V. and Zarazaga, I. (1984 a). Frequency distribution of the Robertsonian translocation (1/29) in cattle populations bred in Spain. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 43-51.

Arruga, M.V. and Zarazaga, I. (1984 b). Chromosome analysis of cattle breeds currently bred in Spain. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 107-119.

Barth, A.D. (1986). The knobbed acrosome defect in beef bull. Can. Vet. J. 27 (10): 379-384.

Basrur, P.K. and Gilman, J.P. W. (1964). Blood culture method for the study of bovine chromosomes. Nature Lond. 204: 1335-1337.

Belorkar, P.M.; Dhami, A.J. and Kodagali, S.B. (1991). Physiological norms and correlations of seminal characters, biochemical constituents, freezability and fertility in crossbred (K. x J. & K. x H. F.) bulls. Indian Vet. Med. J. 15 (3): 191-197.

Blazak, W.F. and Eldridge, F.E. (1977). A Robertsonian translocation and its effect upon fertility in Brown-Swiss cattle. J. Dairy Sci. 60 (7): 1133-1142.

Blom, E. (1972). The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. Proc. 7th Simposia Internazionale di Zootechnia, Milan. (Cited by Salisbury et al., 1978).

Bongso, A. and Basrur, P.K. (1976). Chromosome anomalies in Canadian Guernsey bulls. Cornell Vet. 66 (4): 476-488.

Bruere, A.N. and Chapman, H.M. (1973). Autosomal translocations in two exotic breeds of cattle in New Zealand. Vet. Rec. 92 (11): 615-618.

Ciupercescu, D.D.; Livescu, B.E. and Lungenau, A. (1984). Cytogenetic studies in artificial insemination bulls in Romania. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 375-382.

Cribiu, E.P. (1975). Variation interraciale de la tailledu chromosome Y chez Bos taurus L. Annls. Génét. sel. Anim. 7: 139-144. (Cited by Halnan, 1989 a).

Cribiu, E.P. and Popescu, C.P. (1974). L'idiogramme de Bos taurus. Annls. Génét. sel. Anim. 6: 291-296. (Cited by Halnan, 1989 a).

Crossley, R. and Clarke, G. (1962). The application of tissue culture techniques to the chromosomal analysis of Bos taurus. Genet. Res. 3: 167-168.

Di Berardino, D.; Inanuzzi, L.; Ferrara, L. and Mastassino, D. (1979). A new case of Robertsonian translocation in cattle. J. Heredity. 70 (6): 436-438.

Donald, H.P. and Hancock, J.L. (1953). Evidence of gene controlled sterility in bulls. J. agric. Sci. 43: 178-181. (Cited by Salisbury et al., 1978).

Dunn, H.O.; Lein, D.H. and Mc Entee, K. (1980). Testicular hypoplasia in a Hereford bull with 61, XXY karyotype: The bovine counterpart of human Klinefelter's syndrome. Cornell Vet. 70 (2): 137-146.

Dunn, H.O.; Mc Entee, K. and Hansel, W. (1970). Diploid-triploid chimaerism in a bovine true hermaphrodite. Cytogenetics. 9: 245-249.

Dyrendahl, I. and Gustavsson, I. (1979). Sexual functions, semen characteristics and fertility of bulls carrying the 1/29 chromosome translocation. Hereditas 90 (2): 281-289.

Eldridge, F.E. (1974). A dicentric Robertsonian translocation in a Dexter cow. J. Heredity. 65 (6): 353-355.

Eldridge, F.E. (1975). High frequency of a Robertsonian translocation in a herd of British White cattle. Vet. Rec. 96 (4): 71-73.

Eldridge, F.E. (1980). X autosome translocation in cattle. Proc. 4th Eur. Colloq. Cytogenet. Domest. Anim., Uppsala : 23-30. (Cited by Halnan, 1989 a).

Eldridge, F.E.; Harris, N.B. and Koenig, J.L.F. (1984). Chromosomes of young A I bulls. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 59-67.

Fechheimer, N.S. (1973). A cytogenetic survey of young bulls in the U.S.A. Vet. Rec. 93 (20): 535-536.

Gallagher, D.S. Jr.; Basrur, P.K. and Womach, J.E. (1992). Identification of autosome to X chromosome translocation in the domestic cow. J. Heredity. 83 (6): 451-453.

George, P.S. and Nair, K.N. (1990). Livestock economy of Kerala. Centre for Development Studies, Trivandrum, Kerala, India: 36-74.

Giovanni, A.M. De.; Succi, G.; Molteni, L. and Castiglioni,M. (1979). A new autosomal translocation in 'Alpine Grey' cattle. Annls. Génét. sel. Anim. 11 (2): 115-120. Abstr. in Anim. Breed. Abstr. (1980) 48 (8): 4559.

Government of Kerala (1989). Report of Fourteenth Quinquennial Livestock census 1987, Department of Animal Husbandry, Trivandrum, Kerala.

Gustavsson, I. (1969). Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. Hereditas. 63: 68-169.

Gustavsson, I. (1970). Economic importance of a translocation in Swedish cattle. Giessener Beitr. Erbpath. Zuchthyg. 63 (Suppl.1): 108-114. Abstr. in Anim. Breed. Abstr. (1971) 39 (3): 3108.

Gustavsson, I. (1971). Culling rates in daughters of sire with a translocation of centric fusion type. Hereditas. 67 (1): 65-73.

Gustavsson, I. (1979). Distribution and effects of the 1/29 Robertsonian translocation in cattle. J. Dairy Sci. 62 (5): 825-835.

Gustavsson, I. and Rockborn, G. (1964). Chromosome abnormality in three cases of lymphatic leukaemia in cattle. Nature Lond. 203: 990.

Hahn, J.; Foote, R.H. and Seidel, G.E. Jr. (1969). Quality and freezability of semen from growing and aged dairy bulls. J. Dairy Sci. 52 (10): 1843-1848.

Halnan, C.R.E. (1972). Autosomal deletion and infertility in cattle. Vet. Rec. 91 (23): 572.

Halnan, C.R.E. (1975). Chromosomes of cattle : Present status and promise. Vet. Rec. **96** (7): 148-151.

Halnan, C.R.E.(1976). A cytogenetic survey of 1101 Australian cattle of 25 different breeds. Annls. Génét. sel. Anim. **8**: 131-139.(Cited by Halnan, 1989 a).

Halnan, C.R.E. (1977). An improved technique for the preparation of chromosome from cattle whole blood. Res. Vet. Sci. **22** (1): 40-43.

Halnan, C.R.E. (1989 a). Karyotype and phenotype in cattle and hybrid of the genus. In: Cytogenetics of animals. Halnan, C.R.E. (ed.) C.A.B. International, Wallingford, U.K.: 235-256.

Halnan, C.R.E. (1989 b). Appendix 3. In: Cytogenetics of animals . Halnan, C.R.E. (ed.) C.A.B. International, Wallingford, U.K: 407-463.

Halnan, C.R.E. and Watson, J.I. (1982). Y chromosome variants in cattle Bos taurus and Bos indicus. Annls. Génét. sel. Anim. **14** (1): 1-16 (Cited by Halnan, 1989 a).

Han, J. and Men, Z. (1992). The effect of the 1/29 Robertsonian translocation on semen traits in a Limousin bull. Gansa Nongye Daxue Xuebao. **27** (3): 185-189. Abstr. in Anim. Breed. Abstr. (1993) **61** (10): 5368.

Hancock, J.L. (1949). Evidence of an inherited seminal character associated with infertility of Friesian bulls Vet. Rec. 61: 308. (Cited by Salisbury, 1978).

Hansen, K.M. (1970). Tendem fusion translocation and infertility in cattle. Giessener Beitr. Erbpath. Zuchthyg. 63 (Suppl.1): 115-118. Abstr. in Anim. Breed. Abstr. (1971) 39 (3): 3110.

Hansen, K.M. and Elleby, F. (1975). Chromosome investigation of Danish A I beef bull. Nord. VetMed. 27: 102-106. (Cited by Halnan, 1989 a).

Harvey, M.J.A. and Longue, D.A. (1976). Studies on the 13/21 Robertsonian translocation in Swiss Simmental cattle. Deutsche Veterinarmedizinische Gesellschaft eV: 155-161. Abstr. in Anim. Breed. Abstr. (1977) 45 (1): 128.

Herschler, M.S.; Fechheimer, N.S. and Gilmore, L.O. (1966). Identification of freemartins by chromosomal analysis. J. Dairy Sci. 49 (1): 113-114.

Iannuzzi, L.; Di Berardino, D.; Gustavsson, I.; Ferrara, L. and Di Meo, G.P. (1987). Centromeric loss in translocations of centric fusion type in cattle and water buffalo. Hereditas. 106 (1): 73-81.

Iannuzzi, L; Rangel-Figueiredo, T.; Meo, G.P. Di and Ferrara, L. (1992). A new Robertsonian translocation in cattle, rob (15;25). Cytogenet. Cell. Genet. 59 (4): 280-283.

Ibrahim, A.R. M.; Rahman, H.A. and Kovács. A. (1983). Quality, freezability and fertility of the semen of pre-selected A I bulls carrying various chromosome aberrations. Anim. Reprod. Sci. 6 (3): 167-175.

Igboeli, G. and Rakha, A.M. (1971). Ejaculation and ejaculate characteristics of Angoni (Short Horn Zebu) bull. J. Reprod. Fert. 26 (1): 183-187.

ISCN (1985). An International System for Human Cytogenetic Nomenclature. Harnden, A.G. and Klinger, H.P. (eds.). (S. Karger, Basel, New York, 1985). (Cited in ISCNDA, 1989).

ISCNDA (1989). International System for Cytogenetic Nomenclature of Domestic Animals. Di Berardino, D.; Hayes, H.; Fries, R. and Long, S.E. (eds.). Cytogenet. Cell. Genet. 53(1): 65-79 (1990).

Kasubick, S.F.; Vogt, D.W. and Maurer, R.R. (1987). Reproductive tract morphology and pregnancy status of chromosomally normal and chromosomally abnormal cattle. J. Dairy Sci. 70 (Suppl. 1): 236.

King, W.A.; Linares, T.; Gustavsson, I. and Bane, A. (1980). Persumptive translocation type trisomy in embryos sired by bulls heterozygous for the 1/29 translocation. Hereditas. 92 (1): 167-169.

Kovács, A. (1976). A new autosomal translocation in Hungarian Simmentals. Deutsche Veterinarmedizinsche Gesellschaft eV: 162-167. Abstr in Anim. Breed. Abstr. (1977) 45 (1): 130.

Kovács, A. (1984). Progress in eradication of the 1; 29 translocation of cattle in Hungary. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich : 52-58.

Kovács, A. (1989). Application of cytogenetics to cattle breeding improvement. In: cytogenetics of animals. Halnan, C.R.E. (ed.) C.A.B. International, Wallingford, U.K.: 221-233.

Kovács, A. and Csukly, S. (1980). Effect of the 1/29 translocation upon fertility in Hungarian Simmental cattle. Proc. 4th Eur. Colloq. Cytogenet. Domest. Anim., Uppsala: 35-43. (Cited by Kovacs, 1989).

Kovács, A.; Foote, R.H. and Lein, D.H. (1990). 1; 16 tandom translocation with trisomy 16 in a Brown-Swiss bull. Vet. Rec. 127 (8) : 205.

Kovács, A. and Gustavsson, I. (1984). Absence of translocation in the germinal cells of a 60, XY/59, 5; 18 Rob, T+, XY mosaic bull. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 267-274.

Kovács, A. and Szepeshelyi, F. (1987). Chromosomal screening of breeding bulls in Hungary. J. Dairy Sci. 70 (Suppl. 1): 236.

Lin, C.C.; Newton, D.R. and Church, R.B. (1977). Identification and nomenclature for G-banded bovine chromosomes. Can J. Genet. Cytol. 19: 271-282.

Lin, C.C.; Newton, D.R.; Smink, W.R. and Church, R.B. (1976). A rapid method for the isolation and culture of leukocytes for chromosome analysis in domestic animals. Can. J. Anim. Sci. 56: 27-31 (Cited by Halnan, 1989 b).

Long, S.E. (1985). Centric fusion translocations in cattle: A review. Vet. Rec. 116 (19): 516-518.

Long, S.E. (1993). Incidence of rob.t. (1;29) centric fusion translocation in British White cattle in Britain. Vet. Rec. 132 (7): 165-166.

Longue, D.N. and Harvey, M.J.A. (1978). Meiosis and spermatogenesis in bulls heterozygous for a persumptive 1/29 Robertsonian translocation. J. Reprod. Fert. 54 (1): 159-165.

Longue, D.N.; Harvey, M.J.A.; Munro, C.D. and Lennox, B. (1979). Hormonal histological studies in a 61, XXY bull. Vet. Rec. 104 (22): 500-502.

Makino, S. (1994). Karyotype of domestic cattle, zebu and domestic water buffaloes. Cytologia 13: 247-264.

Märki, U. and Robinson, T.J. (1984). Y chromosome dimorphism Afri Kaner bulls. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 87-95.

Masuda, H.; Shioya, Y. and Fukuhara, R. (1980). Robertsonian translocation in Japanese Black cattle. Jpn. J. Zootech. Sci. 51 (1) 26-32.

Mathew, A. (1991). Breeding operations vis-a-vis cross-breeding situations in tropics. Compendium of papers presented at National Seminar on Progeny Testing in Bulls in Tropics. Trivandrum, Kerala, India : 9-18.

Mathew, A.; Joseph, P.J. and Jose, T.K. (1982). Semen characteristics of purebred and cross-bred bulls. Indian Vet. J. 59 (5): 364-367.

Maurer, R.R. and Vogt, D.W. (1988). Decreased fertility in related females heterozygous for the 1/29 chromosome translocation. Theriogenology. 30 (6): 1149-1157.

Mc Feely, A. R.; Klunder, R.L. and Reed, A.J. (1993). A 14/20 chromosome translocation in Simmental cattle. J. Am. Vet. Med. Ass. 202 (4): 619-621.

Moorhead, P.S.; Nowell, P.C.; Mellman, W.J.; Battips, D.M. and Hungerford, D.A. (1960). Chromosome preparation of leucocytes cultured from human peripheral blood. Exp. Cell. Res. 20: 612-616.

Moraes, J.C.F.; Mattevi, M.S.; Salzani, F.M.; Poli, J.L.E.H. and Erdtmann, B. (1980). A cytogenetic survey of five breeds of cattle from Brazil. J. Heredity 71 (2): 146-148.

Mukundan, G. (1992). Report of the Committee to Evaluate and Formulate Livestock Breeding Programmes and Policies in the State of Kerala.

Nair, K.P. (1994). Personal Communication.

Nair, K.P. and Varadarajan, M. (1985). Daily sperm production of Brown-Swiss cross-bred bulls. Kerala J. Vet. Sci. 16 (1): 21-26.

Nel, N.D.; Harris, E.J.; Weiermans, J.E. and Meyer, E.H.H. (1985). The 1/29 translocation in South African cattle. J. Dairy Sci. 68 (Suppl.1): 247.

Pollock, D.L. and Bowman, J.C. (1974). A Robertsonian translocation in British Friesian cattle. J. Reprod. Fert. 40 (3): 423-432.

Popescu, C.P. (1972). A possible case of pericentric inversion in cattle. Annls. Genet. 15(3): 197-199. Abstr. in Anim. Breed. Abstr. (1973) 41 (3): 1060.

Popescu, C.P. (1977). A new type of Robertsonian translocation in cattle. J. Heredity. 68 (2): 138-142.

Potter, W.L.; Upton, P.C.; Cooper, J. and Blackshaw, A.W. (1979). C- and G-banding patterns and chromosomal morphology of some breeds of Australian cattle. Aust. Vet. J. 55 (12): 560-667.

Raghunandanan, K.V. and Mukundan, G. (1990). Chromosomal status of a cross-bred bullock with abnormal mammary development. J. Vet. Anim. Sci. 21 (2): 157-158.

Raghunandanan, K. V. and Mukundan, G. (1991). Polymorphism in somatic chromosomes in taurus x zebu cattle. Indian J. Anim. Sci. 61 (3): 301-304.

Rao, A.R. (1971). Changes in the morphology of sperm during their passage through the genital tract in bulls with normal and impaired spermatogenesis. Ph D Thesis. Royal Veterinary College, Stockholm, Sweden.

Rao, T.L.N. and Rao, A.R. (1978). Semen characteristics of cross-bred bulls. Indian Vet. J. 55 (9): 692-700.

Refsdal, A.O . (1976). Low fertility in daughters of bull with 1/29 translocation. Acta Vet. Scand. 17(2): 190-195. (Cited by Kovács, 1989).

Rieck, G.W. (1984). Veterinary medicine and cytogenetics. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 20-34.

Salisbury, G.W.; Van Demark, N.L. and Lodge, J.R. (1978). Physiology of reproduction and artificial insemination of cattle (2nd edn.). W.H. Freeman and Company, San Francisco: 286-328.

Seabright, M.A. (1971). A rapid banding technique for human chromosomes. Lancet II: 971-972.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical methods. (3rd edn.). Oxford and IBH Publishing Co., New Delhi.

Succi, G; Giovanni, A.M. De. and Molteni, L. (1982). Preliminary observations on the influence of the 25/27 translocation on fertility and milk production of Grey Alpine cows. Proc. 5th Eur. Colloq. Cytogenet. Domest. Anim., Milano: 142-147. Abstr. in Anim. Breed. Abstr. (1983) 51 (11): 6435.

Succi, G.; Molteni, L. and Giovanni, A.M. De. (1979). Preliminary observations on the fertility of daughters of bulls carrying the 1/29 translocation. Attidella Societá Italiana della Scienze Veterinarie: 215. Abstr. in Anim. Breed. Abstr. (1982) 50 (4): 1893.

Sumner, A.T.; Evans, H.J. and Buckland, R.A. (1971). New technique for distinguishing between human chromosomes. Nature Lond. 232: 31-32.

Swartz, H.A. and Vogt, D.W. (1983). Chromosome abnormalities as a cause of reproductive inefficiency in heifers. J. Heredity. 74 (5): 320-324.

Świtoński, M.; Lechniak, D. and Landzwojczak, D.(1991). Cytogenetic survey of bulls used in artificial insemination: Reproductive performance of XY/XX chimaeric bull. Genetica Polonica 32(4): 227-232. Abstr. in Anim. Breed. Abstr. (1992) 60 (9): 5669.

Sysa, P.S. and Slota, E. (1984). The XXY syndrome in cattle. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 120-128.

Tambasco, A.J. (1990). Karyotype study of a Canchin cattle herd. Revista Brasileira de Genetica. 13 (2): 283-292. Abstr. in Anim. Breed. Abstr. (1991) 59 (1): 167.

Teunissen, G.H.B. (1946). A deviation of the acrosome in the spermatozoa of a bull. Tijdschr. Diergeneeskunde. **71**: 292-303 (Cited by Salisbury et al., 1978).

Thiagrajan, V.; Rajasekaran, J. and Natarajan, N. (1990). Robertsonian translocation in a anoestrus heifer. Cheiron, 19(1): 7-9.

Thiagrajan, V.; Sethumadhavan, V. and Krishnan, A.R. (1989). Comparative assessment of media and mitogen for karyological studies on buffaloes. Indian Vet. J. **66**(12): 1120-1123.

Tschundi, P. (1984). 12 years of cytogenetic investigation in A.I. bulls in Switzerland. Proc. 6th Eur. Colloq. Cytogenet. Domest. Anim., Zurich: 40-42.

Tschundi, P.; Zahner, B., kupfer, U. and Stamfli, G. (1977). Chromosome investigations in Swiss Cattle breeds. Schweizer Archiv. Fur. Tierheilkunde. 119 (8): 329-336. Abstr. in Anim. Breed. Abstr. (1979) **47** (3): 1216.

Vallenzasca, C.; Martignoni, M. and Galli, A. (1990). Findings of a bull with Y; 17 translocation. Hereditas, 113 (1): 63-67.

Vezuli, A. (1988). Results of a cytogenetic study of bulls (Bos taurus L.) used in artificial insemination. Bulletini i Shkencave Zooteknike e Veterinare. 6 (2): 13-23. Abstr. in Anim. Breed. Abstr. (1991) 59 (4): 2330.

Villagömez, D.A.F., Andersson, M., Gustavsson, I. and Plöen, L. (1993). Synaptonemal complex analysis of a reciprocal translocation rcp. (20; 24) (q 17; q 25) in a subfertile bull. Cytogenet. Cell Genet. 62 (2-3): 124-130.

Weber, A.F.; Buoen, L.C.; Terhaar, B.L.; Ruth, G.R. and Momont, H.W. (1989). Low fertility related to 1/29 centric fusion anomaly in cattle. J. Am. Vet. Med. Ass. 195 (5): 643-646.

White, I.G. (1958). Biochemical aspects of mammalian semen. Anim. Breed. Abstr. 26 (2): 109-123.

William, G. (1965). An abnormality of the spermatozoa of some Hereford bulls. Vet. Rec. 77 (41): 1204-1206.

Wilson, T.D. (1991). Monosomy and trisomy in bovine embryos sired by bull heterozygous for the 1/29 Robertsonian translocation chromosome. Theriogenology . 36 (5): 789-793.

Zartman, D.L. and Fechheimer, N.S. (1967). Somatic aneuploidy and polyploidy in in-bred and line-cross cattle. J. Anim. Sci. 26:678-682.

Zhigachev, A.I.; Kurchanov, N.A.; Cherkasov, V.V.; Bogacheva, T.V.; Konovalova, N.N. and Cheredeeva, M.N. (1991). Cytogentic monitring of reproduction in livestock. Referativnyl Zh. 11. 58: 354. Abstr. in Anim. Breed. Abstr. (1992) 60 (2): 827.

ABSTRACT

Cytogenetic study was conducted on 53 young cross-bred (Bos taurus x B. indicus) bulls stationed at the farm at Dhoni, belonging to Kerala Livestock Development Board. Young bulls included those selected for superior semen quality and others just started producing semen. The bulls were classified into Jersey cross, Holstein-Friesian cross and Brown-Swiss cross based on the paternal line.

The semen quality and related attributes of the bulls were recorded, and the association between these traits and the karyological parameters were determined. Comparative chromosome study were performed in the three genetic groups.

Metaphase spreads for staining and G-banding were obtained by peripheral leucocyte culture technique. The basal medium used for culturing was RPMI 1640 and mitosis was initiated in lymphocytes by a combination of phytohemagglutinin and pokeweed mitogen. The G-banding was done by incubating the chromosome spreads in 2 x SSC containing trypsin solution for 45 minutes at 66°C.

Karyological parameters such as chromosome number, morphology, relative length, arm ratio and centromeric index were studied. The nature, number and position of G bands

were also examined.

The reproductive attributes recorded included age at first semen collection age at freezable semen production, volume of semen, sperm concentration, total sperm output in first ten collections, number of ejaculates accepted for freezing and total freezable sperm output in first ten collections, and the morphological abnormalities of sperms.

All the bulls except one, exhibited a diploid chromosome complement (2n=60, XY) in their cells. There were 29 pairs of acrocentric autosomes and a sub-metacentric X chromosome. The Y chromosome was sub-metacentric in Holstein-Friesian cross, and apparently metacentric in other two genetic groups. In one bull diploid/tetraploid mosaicism was observed with 6.67 per cent of lymphocytes carrying 120 chromosomes. The mean relative length of longest and shortest autosomes were 6.0174 $\pm$ 0.0273 and 1.6186 $\pm$ 0.0101 respectively. The X and Y chromosomes had a mean relative length of 5.5918 $\pm$ 0.0401 and 1.9636 $\pm$ 0.0396 respectively. In the X chromosome the arm ratio was 2.47 $\pm$ 0.04 and the centromeric index was 28.74 $\pm$ 0.33.

A total of 405 bands were identified in the karyotype of the bulls. The G-banding pattern of cross-bred bulls in Kerala was not previously investigated, and hence the banding

pattern observed in the study would be useful for cytogenetic screening of bulls in the state.

On Analysing the semen quality and related attributes of the bulls it was found that one of the bull was oligospermic. The semen of this bull exhibited a high frequency of loose sperm heads. Semen of another bull was found to contain abnormal percentage of sperms with persistant proximal cytoplasmic droplet. A third bull produced semen in which the frequency of sperms with knobbed acrosome defect was very high. All the three bulls had produced ejaculates which were found unsuitable for freezing.

The incidence of diploid/tetraploid mosaicism was detected in the bull producing sperms with knobbed acrosome defect. None of the ejaculates of this bull was suitable for freezing. However, further study was essential to conclude on the association between mixoploidy and knobbed acrosome defect or its influence on semen freezability. The other two bulls with seminal abnormalities exhibited cytogenetic profile similar as that of bulls producing normal semen.

The effect of genetic group on the morphometry of sex chromosomes was found to be insignificant. However, the Y

chromosome morphology was observed to be a suitable marker for identifying Holstein-Friesian crosses among the cross-bred bulls used for breeding in Kerala.