CYTOGENETIC CHARACTERIZATION OF MACHERLA BROWN SHEEP

By

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B.V.Sc & A.H

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CERTIFICATE

Mr. NITYANAND.B has satisfactorily prosecuted the course of research and that the thesis entitled "**CYTOGENETIC CHARACTERIZATION OF MACHERLA BROWN SHEEP"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: **(Dr. B. EKAMBARAM)**

Place: Tirupati **Major Advisor**

CERTIFICATE

This is to certify that the thesis entitled "**CYTOGENETIC CHARACTERIZATION OF MACHERLA BROWN SHEEP"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE of Sri Venkateswara Veterinary University, Tirupati** is a record of the bonafide research work carried out by **Mr. NITYANAND.B** under my guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.

No part of the thesis has been submitted for any other degree or diploma. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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LIST OF ABBREVIATIONS

S.No	Symbol	Full Form
1	µl	Microlitre
2	ml	Millilitre
3	µg	Microgram
4	mg	Milligram
5	g	Gram
6	kg	Kilogram
7	mm	Millimeter
8	IU	international unit
9	^{o}C	degree Celsius
10	rpm	revolutions per minute
11	G	Gauze
12	p^{H}	negative logarithm of hydrogen ion concentration
13	lb	Pounds
14	%	percent
15	d.f.	degrees of freedom
16	MSS	Mean sum of squares
17	S.E.	Standard Error
18	ISCNDA	International System for Cytogenetic Nomenculature of Domestic Animals
19	No.	Number
20	PHA	Phytohaemagglutinin
21	BAHS	Basic Animal Husbandry Statistics

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Nityanand.B….

DECLARATION

I, **Mr. NITYANAND.B**, hereby declare that the thesis entitled "**CYTOGENETIC CHARACTERIZATION OF MACHERLA BROWN SHEEP"** submitted to Sri Venkateswara Veterinary University for the degree of **MASTER OF VETERINARY SCIENCE** is the result of the original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier elsewhere in any manner.

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ABSTRACT

A karyological study was conducted on Macherla Brown sheep maintained in four districts namely Prakasam, Guntur, Krishna and Nalgonda districts of Andhra Pradesh. Blood samples were collected from 80 animals (40 males and 40 females) of field flocks to analyse the chromosomal profiles by employing the short term lymphocyte culture technique.

The modal diploid chromosome number was found to be 54 (2n = 54, XY). The first three pairs of the autosomes were found to be submetacentric and the remaining 23 pairs of autosomes were acrocentric. The X-chromosome was the longest acrocentric, while the Y-chromosome was the smallest biarmed chromosome in the karyotype.

The effect of sex on all the morphometric measurements was studied. The analysis of variance based on the arcsin transformed data revealed that sex had no significant effect on the relative length of chromosomes. The mean relative lengths based on raw data for the autosomes varied from 1.80 ± 0.01 to 9.76 ± 0.03. The X-chromosome constituted 5.05 ± 0.01 percent to the total genome, whereas the Y chromosome constituted 1.57 ± 0.01 percent to the total genome.

The overall mean arm ratios for the first three biarmed autosomes based on raw data varied from 1.20 ± 0.02 to 1.23 ± 0.01, while the mean arm ratios based on transformed data varied from 6.30 ± 0.01 to 6.37 ± 0.01 and found that sex had no significant effect on arm ratios of first three biarmed autosomes.

The overall mean centromeric indices (C.I.1) for the first three biarmed autosomes based on raw data are 0.452 ± 0.01, 0.452 ± 0.01 and 0.460 ± 0.01, respectively. Significant differences were observed among the chromosomes but sex had no significant effect on centromeric indices. (C.I.1).

The overall mean centromeric indices (C.I.2) for the first three biarmed autosomes based on raw data are 0.548 ± 0.01, 0.550 ± 0.01 and 0.544 ± 0.01 respectively. The effect of sex was not evident on centromeric indices for the first three biarmed autosomes.

The overall mean morphological indices for the first three biarmed autosomes based on transformed data varied from 14.82 ± 0.05 in third biarmed autosome to 16.65 ± 0.06 in first biarmed autosome and sex had no significant effect on morphological indices, while significant differences exists between chromosomes.

CHAPTER - I
INTRODUCTION

Cytogenetic studies reveal the association of variations in chromosome morphology with gross anatomoical or physiological features of animals as a whole. Certain viruses and mycoplasma, therapeutic agents, feed additives and even fodder exposed to the treatment of herbicides and pesticides were found to be capable of inducing chromosomal aberrations in farm animals (Fechheimer, 1972). It provides explanation for loss of fitness in populations that are continuously inbred. Chromosomal investigation can also help in sexing the embryos at an early stage, which helps in shifting the sex ratio to the desired direction.

Cytogenetic screening of phenotypically normal animals intended for use as breeding stock is a routine standard procedure for high pedigreed animals, since the inherited aberrations can quickly become distributed in the next generations which have direct effects on fertility and reproductive performances. Cytogenetic findings on farm animals had high significance after the discovery of Robertsonian translocation (1; 29) in Swedish red cattle (Gustavsson and Rockborn, 1964) and the demonstration of its deleterious effects on fertility (Gustavsson, 1969). Chromosomal identification of domestic animals is difficult by using conventional methods which hindered the progress in cytogenetics till the advent of banding techniques. This vital step led to development of clear and unambiguous standard karyotypes in domestic animals (Ford *et al.* 1980; ISCNDA 1990).

India is rich in sheep diversity having about 42 recognized sheep breeds with 71.6 million sheep population and ranks third in the world. Andhra Pradesh with sheep population of 25.53 millions ranks first in India and constitutes 34.18 percent of sheep (BAHS, 2010) population of the country.

Sheep with their small body size and rapid growth rates are best suited to production by resource poor farmers especially in areas where crop and dairy farming are not economical and play an important role in the livelihood of a large proportion of small and marginal farmers and landless labourers. Sheep are valued for both meat and wool production and contribute greatly to the Indian economy.

Macherla Brown sheep are not yet recognized as a breed and are found in the villages mainly adjacent to the Krishna River flowing through Prakasam, Guntur, Krishna, Nalgonda districts under Nagarjuna sagar project area. Characterization of this specific group of sheep is necessary for its recognition and further development. The phenotypic characters of Macherla Brown sheep are bicolour of brown and white (61.96%) followed by brown (36.46%) and brown and black (11.44%). Convex head, pendulous ears and black muzzle are predominant (Venugopal, 2013).

However, information on chromosomal profile of Macherla Brown is lacking which is an important prerequisite for characterization of a breed. Therefore, the present investigation is undertaken with the following objectives,

- To standardize the protocol for short term lymphocyte culture for Macherla Brown Sheep
- To develop karyotypes of Macherla Brown Sheep
- To study the different morphometric measurements

CHAPTER - II
REVIEW OF LITERATURE

2.1 DISTRIBUTION AND PHENOTYPE OF MACHERLA BROWN SHEEP

The breeding tract of Macherla Brown sheep located on the banks of Krishna River and Nagarjunasagar project irrigated areas in Prakasam, Guntur, Krishna and Nalgonda districts of Andhra Pradesh.

Macherla Brown sheep are bicolour of brown and white (61.96%) followed by brown (36.46%) and brown and black (14.44%). Convex head, pendulous ears and black muzzle are predominant characteristics.

Venugopal, (2013) reported that the overall least squares means at 2, 4, 6 and 8 tooth stage were 33.79 ± 0.40, 39.74 ± 0.33, 44.52 ± 0.45 and 46.98 ± 0.37 kg for body weights; 65.74 ± 0.51, 68.96 ± 0.37, 70.09 ± 0.35 and 71.53 ± 0.33 cm for body length; 20.22 ± 0.27, 21.91 ± 0.16, 22.80 ± 0.17 and 22.90 ± 0.12 cm for face length; and 15.29 ±0.19, 15.43 ± 0.17, 15.76 ± 0.15 and 15.95 ± 0.11 cm for the ear length, respectively. Males recorded significantly higher means for all the body measurements studied at all the ages.

Among the four districts, the least squares means for age at first mating in Macherla Brown ewes varied from 573.98 ± 6.02 (Nalgonda) to 584.56 ± 5.78 (Guntur) days; 722.95 ± 6.10 (Nalgonda) to 734.31 ± 5.86 (Guntur) days for age at first lambing and 375.33 ± 1.47 (Guntur) to 381.93 ± 2.06 (Prakasam) days for lambing interval.

2.2 EVOLUTION OF SHEEP CHROMOSOMES

Cytogenetic evidence suggested that the caprids (sheep and goats) evolved from a common ancestor possibly the aoudad (*Ammotragus lervia*) and the course of evolution in *Ovis* compared to its wild ancestor occurred due to the chromosomal rearrangement of acrocentric chromosomes.

Borland (1964), in his chromosomal studies revealed that there is no variation in diploid chromosomal number among seven different breeds of sheep and stated that the diploid chromosome number was 54.

Bunch *et al.* (1976) found that the chromosomal number in sheep is reduced than goats due to centric fusion resulting in formation of metacentric chromosomes. Acrocentric chromosomes of 1 and 7 positions of the primitive karyotype fused to form the first and longest pair of metacentric (M1), while the fusion of acrocentric chromosome 4 and 14 or 15 resulted in the third longest metacentric chromosomes (M3). Similarly, fusion of 3rd and 12^{th} or 13^{th} acrocentric chromosomes resulted in the formation of second largest metacentric chromosome.

Nadler *et al.* (1971) analysed the chromosomal profile of seven wild sheep populations in Iran and stated that the eastern population posses a diploid number of 58, including one pair of metacentric and 27 pairs of acrocentric autosomes. The 2n for the three western localities is 54 having three pairs of metacentrics and 23 pairs of acrocentric autosomes and sheep inhabiting Central Iran had shown varied number of diploid chromosomes (2n = 54, 55, 57, 58) and concluded that this chromosomal variation was due to intergradations between two chromosomally homogeneous and distinctive taxa.

Nadler (1971) reported a diploid chromosomal number of 54 with six large metacentrics and 48 acrocentric chromosomes and X-chromosome being the largest acrocentric in Dall sheep chromosomal profile.

Moraes *et al.* (1980) found a frequency of 11.3 percent of hypo or hyper diploidy but the number of aneuploid cells did not vary significantly among Brazilian Rams and breeds. A frequency of 2.5 percent of chromosomal variations was found.

2.3 CHROMOSOME NUMBER IN SHEEP

The diploid (2n) chromosome number in different indigenous and exotic sheep breeds were reported by various authors as mentioned below.

Table 1. Diploid chromosomal number (2n) in different breeds of sheep as cited in literature

Breed	Culture Material	Diploid Number	Author (s)
Domestic sheep (*Ovis aries*)	Amnion	54	Shiwago (1931)
Leicester	Spermatogonial cells	54	Berry (1938, 1941)
Merino, Karakul and Corridale	Testicular germinal epithelium	54	Makino (1943)
Merino, Southdown, Ryeland, Dorset Horn, Border Leicester, Romney Marsh and Chevoit	Bone marrow	54	Borland(1964)
Suffolk ram	Whole blood	54	Mcfee *et al.*(1965)
Ovis dalli dalli	Skin biopsies	54	Nadler *et al.*(1971)
Dorset	Leukocytes	54	Ponce de Leon and Marcum (1975)
Muzaffarnagari, Dorset x Muzaffarnagari	Whole blood	54	Benjamin and Bhat (1978)
Arkhar/Argali (*Ovis ammon*)	Leukocyte culture	56	Bunch (1978)
Mouflon sheep (*Ovis musimon*)	Leukocyte culture	54	
Urial (*Ovis vignei*)	Leukocyte culture	58	

Breed	Culture Material	Diploid Number	Author (s)
Bannur	Leukocytes	54	Langhe *et al.*(1993)
Munjal	Whole Blood	54	Bhatia and Shanker (1994)
Malpura	Whole Blood	54	Gupta and Gupta (1995)
Magra	Whole Blood	54	Bhatia and Shanker (1996)
Mandya	Peripheral Lymphocytes	54	Umrikar and Narayankhedkar (1997)
Severtzov's (*Ovis ammon severtzovi*)	Skin biopsies	56	Bunch *et al.* (1998)
Patanwadi	Whole blood	54	Bhatia and Shanker (1999)
Dwarf blue sheep	Fibroblast culture	54	Bunch *et al.* (2000)
Nellore	Whole Blood	54	Amareswari *et al.* (2005)
Mecheri	Whole Blood	54	Karunanithi *et al.* (2005)
Kari sheep	Whole Blood	54	Ahmad and Khan (2007)
Deccani	Whole Blood	54	Prakash *et al.*(2008)
Coimbatore	Whole Blood	54	Devendran *et al.*(2009)
Konya wild sheep	Peripheral blood culture	54	Arslan and Zima (2011)
Lohi sheep	Whole blood	54	Ahmadali *et al.*(2011)
Vizianagaram sheep	Whole blood	54	Satish *et al.* (2012)
Laticauda sheep	Peripheral blood	54	Ianuzzi *et al*. (2013)

2.4 CHROMOSOME MORPHOLOGY

Berry (1938) observed the metaphase plates prepared from the amnion cells of 30 day old embryos of sheep, goat and sheep x goat hybrids and noticed characteristic of the

chromosome complex of the sheep was the J or U shaped condition of the four largest chromosomes.

Borland (1964) compared the chromosomal profile of Merino, Southdown, Rye land, Dorset Horn, Leicester, Romney Marsh and Cheviot breeds of sheep and found the chromosomal complement of all domestic sheep breeds comprised of 54 chromosomes having six large metacentric chromosomes and 48 acrocentric chromosomes and classified into four main groups based on the arm length namely A (1 to 3), B (4 to 11), C (12 to 17) and D (18 to 26). Of which, Group A includes large chromosomes with median centromere, Group B contains acrocentric chromosomes with chromatid length ranged from 4.2 units to 3.0 units, Group C comprises all the acrocentric chromosomes with chromatid length from 2.8 to 2.0 units and Group D includes very small acrocentric chromosomes with chromatid length of 1.9 to 0.6 units, whereas X-chromosome was one of the very small acrocentric chromosomes with chromatid length of 1.1 units and Y-chromosome appeared as small unpaired dot like structure.

Mc Fee (1965) carried out cytogenetic studies in purebred and crossbred Suffolk sheep and reported that the diploid chromosome number was 54 and 26 pairs of autosomes and one pair of sex chromosomes. First three pairs of autosomes were distinguished well from the remaining 23 pairs. The 23 pairs of autosomes were acrocentric, X-chromosomes were the largest of the acrocentric members and Y-chromosome is very small and submetacentric.

Nadler (1971) studied the chromosomal preparations made from the skin biopsies of female *Ovis dalli dalli* and reported that the diploid number was 54 with six large metacentric and 48 acrocentric chromosomes. The largest acrocentric chromosome was X-chromosome.

Bruere *et al.* (1972) carried out chromosomal studies in Drysdale breed of Newzealand and found diploid chromosomal number as 53 in 26.3 percent of population studied and 52 in remaining population.

Bunch and Foote (1976) examined the chromosome spreads of the 12 native breeds of Iranian sheep namely Bokhtlari, Boluchi, Ghezel, Karakul, Kellakui, Mehreban, Mogani, Sangsari and Shahl and found diploid chromosomes as 54 and the karyotype consist of 3 pairs of metacentric and 23 pairs of acrocentric autosomes. The X-Chromosome was the largest acrocentric, while the Y-chromosome was smallest and biarmed.

Bunch (1978) compared the karyotypes of domestic and wild sheep of Holarctic region and revealed that the chromosome complement contained 29 autosomal acrocentric chromosomes and a small biarmed Y-chromosome and a large acrocentric X-chromosome.

Benjamin and Bhat (1978) studied the karyotypes of Muzaffarnagri ewes and Dorset x Muzaffarnagari cross bred rams by adopting lymphocyte culture technique and revealed that karyotype comprises six metacentric and 46 telocentric autosomes, while the X-chromosome was the largest and Y-chromosome appeared as smallest metacentric.

De Oliveira Filho (1978) examined the karyotype of Pol worth rams and found 6 large metacentric and 46 acrocentric or telocentric autosomes.

Shirinskii *et al. (*1982) carried out Chromosomal studies in Black Karakul ewe and noticed diploid number of 54 having 52 autosomes and 2 allosomes. The first three pairs of autosomes were found to be metacentric and remaining chromosomes were acrocentric including X-chromosome.

Rcheulishvili and Dzhokhadze (1985) found that the first two pairs of chromosomes were sub metacentric and the third pair was metacentric in morphology in Imeritian sheep.

Bhatia and Shanker (1989) reported that the karyotype of Nali sheep comprises of 3 pairs of metacentric and 23 pairs of acrocentric autosomes. The X-chromosome was the largest of the acrocentric and Y-chromosome appeared as a very small bi armed.

Babar *et al.* (1991) studied the chromosome complement of Lohi sheep and noticed 46 acrocentric and 6 biarmed chromosomes. X-chromosome was longest of all acrocentric autosomes and Y-chromosome was smallest and metacentric.

Roy *et al.* (1991) studied the chromosomal profile of native sheep breed of Orissa and reported that the first three pairs of autosomes were biarmed,submetacentric and the remaining 23 pairs of autosomes were acrocentric, while X-Chromosome was acrocentric and Y- chromosome was small dot like structure.

Langhe *et al.* (1993) carried out cytogenetic studies in Bannur sheep and found that the diploid chromosomal number as 54 with 26 pairs of autosomes and one pair of sex chromosomes.

Bhatia and Shanker (1994) analysed the chromosomal profile of Munjal sheep and revealed that a modal diploid chromosome number was 54 and the karyotype consist of 3 pairs of biarmed and 23 pairs of acrocentric autosomes. Among allosomes, X-chromosome appeared as largest acrocentric and Y-chromosome was smallest bi armed.

Gupta and Gupta (1995) carried out cytogenetic studies in Malpura sheep and revealed a diploid chromosomal number of 54 and the karyotype was divided in to three groups. Group A comprises of largest biarmed chromosomes and could be identified distinctly and autosomes were of metacentric type. Group B chromosomes were 23 pairs of acrocentric or telocentric autosomes from number 4 to 26. Group C comprises of sex chromosomes. The X chromosome was the longest and acrocentric while Y-chromosome was very small metacentric.

Umrikar and Narayankhedkar (1997) analysed the chromosomal profile of Mandya sheep and reported a diploid number of 54 and the karyotype contained three pairs of large submetacentric and 23 pairs of acrocentric autosomes. The X-chromosome was the largest acrocentric and the Y-chromosome was the smallest metacentric.

Bunch *et al.* (1998) carried out chromosomal studies in Severtzov's sheep and revealed diploid chromosomal number of 56 and karyotype consisting of two pairs of biarmed and 25 pairs of acrocentric autosomes, a large acrocentric X-chromosome and a minute biarmed Y-chromosome.

Akhuli (1999) examined the metaphase plates of Garole sheep and observed three pairs of sub metacentric autosomes and 23 pairs of acrocentric autosomes. X-chromosome was the largest of all acrocentric chromosomes and Y-chromosome found to be a dot like structure.

Bhatia and Shanker (1999) found that the diploid number was 54 in Patanwadi sheep and consists of three pairs of biarmed and 23 pairs of acrocentric autosomes. The X-chromosome was the longest acrocentric and the Y-chromosome was smallest biarmed.

Intizar *et al.* (1999) noticed that the karyotype of Lohi sheep contained 6 metacentric and 46 acrocentric chromosomes. The X-chromosome was the largest among the acrocentric, while Y-chromosome was the smallest.

Bunch *et al.* (2000) analysed the chromosomal profile of four subspecies of the giant sheep argali (*Ovis ammon Linneaus*) of Asia *viz*., Dalai-lamae, Gobi, Tibetan and Kara Tau argali having a diploid number of 56 chromosomes and the karyotype consist of two pairs of biarmed and 25 pairs of acrocentric autosomes, a minute biarmed Y-chromosome and largest acrocentric X-chromosome.

Mansour *et al.* (2000 a) studied the chromosomal profile of Egyptian male sheep and revealed a diploid number of 54 consisting of three pairs of metacentric and 23 pairs of acrocentric autosomes.The X-chromosome was the largest acrocentric chromosome, whereas, Y-chromosome was a small dot like structure.

Mansour *et al.* (2000 b) noticed that the rams of indigenous breeds of Egypt had a chromosomal complement of 54, XY with three pairs of metacentric and 23 pairs of acrocentric autosomes. The X-chromosome was the largest acrocentric, whereas, the Y-chromosome was identified as a small dot like structure.

Kirkci (2003) reported that the karyotype of Konya wild sheep possessed three pairs of biarmed autosomes which were metacentric and remaining autosomes and X-chromosomes were acrocentric in morphology.

Kaya *et al.* (2004) examined the metaphase plates of Konya wild sheep (*Ovis orientalis anatolica*) and found that three pairs of biarmed and 23 pairs of acrocentric autosomes, X-chromosome was found to be large acrocentric and Y-chromosome was minute biarmed .

Amareswari *et al.* (2005) studied three strains of Nellore sheep and revealed that the diploid chromosomal number was 54. The first three pairs of autosomes were submetacentric in appearance and the remaining 23 pairs of autosomes were acrocentric in nature, the X-chromosome was largest acrocentric and Y-chromosome was smallest bi armed.

Somatic metaphase plates of Mecheri sheep showed diploid number of 54 chromosomes and the karyotype comprises of 3 pairs of metacentric and 23 pairs of acrocentric autosomes. The X-chromosome was the largest of the acrocentric chromosome and Y-chromosome appeared as a small biarmed metacentric chromosome (Karunaunithi *et al.* 2005).

Ahmad and Khan (2007) conducted cytogenetic studies in Kari sheep and revealed a diploid number of 54 including 26 pairs of autosomes and a pair of sex chromosomes. The autosomes were arranged in to four groups according to the size (groups A, B, C, D). A group consists of one to six chromosomes, B group comprises seven to thirteen chromosomes, C group includes fourteen to twenty one chromosomes and D group consists twenty two to twenty seven chromosomes. Group A chromosomes were relatively larger than other chromosomes, followed by remaining groups in decreasing order. Homologous allosome pairs in ram were unequal in size, larger chromosome was considered as X-chromosome and smaller chromosome designated as Y-chromosome. While in ewes, the homologue sex chromosome pair was larger and equal in size.

Prakash *et al.* (2008) carried out Karyological studies in Deccani sheep and reported that the diploid chromosome number was 54 and all autosomes were acrocentric except the first three pairs, which were submetacentric. The X-chromosome was the longest acrocentric, while Y-chromosome was the smallest bi-armed chromosome.

Devendran *et al.* (2008) analysed the chromosome profile of Coimbatore sheep and found that the karyotype comprised 26 pairs of autosomes and one pair of allosomes. The first three pairs of autosomes were biarmed, meta centric and remaining 23 pairs of autosomes were acrocentric. The X-chromosome was the largest acrocentric and the Y-chromosome was smallest metacentric chromosome.

Shaik *et al.* (2009) studied the karyotype of Deccani sheep and revealed diploid number as 54. The chromosomal complement consists of 26 pairs of autosomes and one pair of sex chromosomes. Autosomes consist of three pairs of sub metacentric and 23 pairs of acrocentric chromosomes. Among all acrocentric chromosomes, X-chromosome was longest and Y-chromosome was smallest metacentric.

Ali *et al*. (2011) examined the metaphase slides of Pakistan Lohi sheep and concluded that the first three pairs of autosomes were submetacentric or metacentric and remaining 23 pairs of autosomes including the X- chromosome were acrocentric and Y- chromosome was smallest biarmed chromosome and star like configuration.

Satish *et al.* (2012) reported that the diploid chromosome number was 54 in Vizianagaram sheep. The first three pairs of autosomes were sub metacentric and remaining 23 pairs were acrocentric in nature. The longest acrocentric X-chromosome and smallest biramed Y-chromosome were observed.

Iannuzzi *et al.* (2013) investigated the karyotype in Laticauda sheep and found three pairs of biarmed autosomes and the remaining were acrocentric. X-chromosome was acrocentric and Y-chromosome was the smallest metacentric.

2.5 MORPHOMETRIC MEASUREMENTS

2.5.1 Relative Length

Relative length is expressed as a ratio of length of individual chromosome to the total length of haploid genome including X-chromosome and expressed as percentage. It is useful to compare the length of the chromosomes in different karyotypes.

Langhe *et al.* (1993) reported that the overall mean relative lengths for first, second, third pair of autosomes and Y-chromosome were 14.28, 13.54, 9.84 and 4.48 percent respectively in Bannur sheep. Highest mean relative length was observed for X-chromosome (17.02) and the lowest mean for 26^{th} autosomal pair (2.04).

Bhatia and Shanker (1994) reported that the relative length for biarmed autosomes and acrocentric autosomes were varied from 7.59 to 9.48 and 1.81 to 4.71 percent,

respectively in Munjal sheep, while the relative lengths of X and Y-chromosomes were 5.10 and 1.45 percent, respectively.

Gupta and Gupta (1995) found that the relative length percent of first chromosome was 9.377 in males and 9.373 in females of Malpura sheep. The first three chromosomes (group A) constituted about 25.113 percent in males and 24.650 percent in female genome. The mean relative length percent of X-chromosome in male was 4.866 and in females the length being 4.868 with an overall mean of 4.941. The Y-chromosome contributed 1.14 percent of haploid genome in rams.

The relative length of chromosome ranged from 9.15 to 1.98 percent of the haploid genome in Patanwadi sheep. The longest biarmed autosome accounted for about 9.03 % in female and 9.08% in males, while, the smallest autosome contribution varied from 2.04% (female) to 2.10% (male). The mean relative length of X-chromosome was 4.68% in females and 4.97% in males and the mean relative length of Y-chromosome was 1.98%. (Bhatia and Shanker, 1999)

Amareswari *et al.* (2005) reported that the mean relative length of Nellore Palla, Brown and Jodipi strains varied from 1.68 to 9.92, 1.73 to 9.73 and from 1.75 to 9.50 percent, respectively. In males, the X-chromosome of the three varieties contributed 5.08, 5.03 and 5.10 percent to the total genome in males and 5.32, 5.11 and 5.23 percent in females, and Y-chromosome shared 1.63, 1.87 and 1.77% of the total genome in Nellore Palla, Brown and Jodipi, respectively. Sex had significant effect on chromosomes from 1 to 6 and 12 to 25 only.

Karunanithi *et al.* (2005) studied the morphometric measurements of chromosomes of Mecheri sheep and reported that the relative length of chromosomes ranged from 9.62 to 2.13 percent in males and 9.62 to 2.09 percent in females. The first pair of autosomes (longest

biarmed) in males constituted about 9.62 percent and smallest pair of autosomes (26^{th} pair) accounted for about 2.33 percent of the whole genome. Sex had no significant effect on relative lengths of chromosomes.

The mean relative length of autosomes in Deccani sheep was varied from 1.78 to 9.35 per cent and the mean relative lengths of X and Y-chromosomes were 5.05 and 1.70 percent, respectively. Sex had no significant influence on morphometric measurements (Prakash *et al.* 2008).

Devendran *et al*. (2009) observed that the mean relative length of autosomes varied from 1.92 to 9.18%, while the means for X and Y-chromosomes were 5.11 and 1.83%, respectively in Coimbatore sheep. The first three pairs of autosomes contributed for about 24.9 percent of the total genome and X-chromosome contributed highest among the acrocentric chromosomes.

The mean relative length of autosomes varied from 1.50 to 9.71 percent and the means for X and Y-chromosomes were 5.04 and 1.81 percent, respectively in Vizianagaram sheep (Satish *et al*. 2012).

2.5.2 Arm Ratio

Arm ratio is the ratio of length of long arm to the length of short arm of the chromosome.

Bhatia and Shanker (1994) studied the morphological attributes of chromosomes in Munjal sheep and found that arm ratios of the first three pairs of chromosomes were 1.22 ± 0.04, 1.13 ± 0.05 and 1.13 ± 0.09, respectively, while the mean value for Y-chromosome was 1.15 ± 0.07.

The arm ratios of the first three largest biarmed chromosomes in Malpura sheep were found to be 1.231, 1.222 and 1.130 in male and 1.238, 1.198 and 1.125 in females, respectively (Gupta and Gupta 1995).

Bhatia and Shanker (1999) studied cytogenetic parameters in Patanwadi sheep and reported that the arm ratios as 1.29 ± 0.03, 1.18 ± 0.03 and 1.19 ± 0.01 for the first three pairs of biarmed autosomes, respectively.

Amareswari *et al.* (2005) reported the overall mean arm ratios of biarmed autosomes as 1.198 in males and 1.199 in females of Nellore sheep and reported variety and sex had no significant influence on arm ratio.

Biometric characteristics of chromosomes of Mecheri sheep revealed that the arm ratios of first three biarmed autosomes were 1.015, 1.016 and 1.018 in males and 1.011, 1.014 and 1.016 in females, respectively (Karunanithi *et al.* 2005).

Prakash *et al.* (2008) observed that the mean arm ratios of first three biarmed autosomes varied from 1.17 to 1.20 in Deccani sheep.

Morphometric measurements in Coimbatore sheep revealed that the mean arm ratios of the biarmed autosomes ranged between 1.07 and 1.26, while the mean arm ratio for Y – chromosome was 1.07. The arm ratio decreased from first to third autosomes (Devendran *et al*., 2009).

Satish *et al*. (2012) studied the morphometric measurements in Vizianagaram sheep and reported the mean arm ratios as 1.24 to 1.31 in first three autosomes and found that the effect of sex was not evident on the mean arm ratios of first three sub metacentric chromosomes.

2.5.3 Centromeric Index

Centromeric index is the ratio of the short arm of the chromosome to its total length of haploid genome. Centomeric index is an important morphometric measurement which indicates the position of centromere in a chromosome.

Langhe *et al*. (1993) reported that the centromeric indices of the first three pairs of autosomes and Y-chromosome as 41.92, 37.30, 35.68 and 47.32 per cent, respectively in domestic sheep (*Ovis aries*).

Bhatia and Shanker (1994) studied the centromeric index values for the first three biarmed autosomes in Munjal sheep and reported as 0.55 ± 0.04, 0.55 ± 0.01 and 0.52 ± 0.01 and for Y-chromosome as 0.55 ± 0.03.

The centromeric indices of the biarmed autosomes varied from 44.90 (chromosome no1) to 47.03 (chromosome no 3) in males and 44.78 to 47.12 percent in females, respectively in Malpura sheep (Gupta and Gupta, 1995).

The centromeric index values for first three biarmed autosomes in Patanwadi sheep were 0.56 ± 0.01, 0.54 ± 0.01 and 0.54 ± 0.01, respectively, (Bhatia and Shanker, 1999).

Karunanithi *et al*. (2005) investigated the morphmetric characters of chromosomes in Mecheri sheep and found that the centromeric index values for first, second and third autosomes were 0.5039, 0.5042 and 0.5046 in males and 0.5028, 0.5034 and 0.5041 in females, respectively.

Amareswari *et al.* (2005) reported the centromeric index values of Palla, Jodipi and Brown strains of Nellore sheep as 0.522 ± 0.004, 0.546 ± 0.004 and 0.533 ± 0.005 for first; 0.551 ± 0.003, 0.541 ± 0.004 and 0.536 ± 0.004 for second; 0.540 ± 0.004, 0.544 ± 0.003 and 0.541 ± 0.004 for third pair of chromosomes, respectively.

The centromeric index values for the first three biarmed autosomes in Deccani sheep were ranged from 0.54 to 0.55 (Prakash *et al*., 2008).

Devendran *et al*. (2009) observed that the mean values of centromeric indices of the biarmed autosomes of Coimbatore sheep ranged between 0.52 and 0.56.

The centromeric index values for the first three pairs of autosomes in Vizianagaram sheep ranged from 0.545 to 0.565 and sex had no significant effect on centromeric indices (Satish *et al*, 2012).

2.5.4 Morphological Index

The morphological index was calculated by dividing the total chromosome length by arm ratio**.** Morphological index was directly proportional to length of chromosomes.

Langhe *et al.* (1993) computed the morphological index for first three pairs of biarmed autosomes and Y-chromosome as 29.23, 22.83, 15.48 and 11.42, respectively in domesic sheep (*Ovis aries*).

Amareswari *et al.* (2005) reported that the morphological indices of the three sub-metacentric chromosomes varied from 5.13 to 7.41 among the three varieties (Jodipi, Palla, Brown) of Nellore sheep.

Karunanithi *et al.* (2005) estimated the morphological attributes of chromosomes of Mecheri sheep and found that the morphological indices for the first three biarmed autosomes were 12.89, 12.09 and 10.99 for males and 18.10, 14.80 and 13.67 for females, respectively and the morphological index value for Y-chromosome was 3.30.

The mean morphological index values of the first three biarmed autosomes in Deccani sheep varied from 9.40 to 11.47 (Prakash *et al*., 2008).

Devendran *et al.* (2009) reported that the morphological index of the biarmed autosomes ranged between 6.81 and 7.41 in Coimbatore sheep, while the mean index value for Y-chromosome was 1.69.

The mean morphological indices for the first three chromosomes of Vizianagaram sheep ranged from 5.36 to 7.59 and further, sex had no significant effect on morphological indices of first three chromosomes (Satish *et al.*, 2012).

CHAPTER-III

MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The present cytogenetic study is undertaken on Macherla Brown sheep found in adjacent villages namely Singupalli, Thellagatla, Gurrapusala of Prakasam district, Macherla, Rentachintala, Gurajala mandals of Guntur district, Paritala, Moguluru and Eeturu villages of Krishna district and Damaracherla mandal of Nalgonda district of Andhra Pradesh. Blood samples were collected from a total of 80 animals with 20 animals from each district and 10 animals from each sex. The photographs of male and female Macherla Brown sheep included in the present study are shown in Fig. 1 and Fig. 2, respectively. All the animals used in the study were apparently healthy, with no phenotypic or reproductive abnormalities. From each of these animals 5 ml of blood was obtained for karyological studies. The animals were reared under extensive system and during lean season supplementation was made in terms of crop by products, hay and concentrates. Regular deworming, vaccination and health practices are adopted (Venugopal., 2013).

3.2 EXPERIMENTAL METHODS

3.2.1 Aseptic precautions and procedures

All the glassware used in the present study were immersed in potassium dichromate-sulphuric acid mixture (100 g potassium dichromate and 250 ml concentrated sulphuric acid in 1000 ml of distilled water) overnight, rinsed thoroughly in running tap water and left immersed in a mild detergent solution (Labolin) for one hour and were scrubbed thoroughly and washed in running tap water and subsequently rinsed twice with double distilled water and air dried. These were wrapped in aluminium foil and sterilized in hot air oven at 170^0C for two hours. Screw caps, rubber liners, rubber corks and needles were washed and air dried similarly. These along with filter assembly were autoclaved under 15 lb pressure for 20 minutes prior to use. Triple glass distilled water which was used for preparation of reagents was also autoclaved. After sterilization, the external surfaces were sprayed with 70% ethanol and kept under laminar air flow cabinet with ultraviolet lamp. The ultraviolet lamp of the laminar air flow cabinet was switched on one hour prior to setting the cultures after mopping the working area with 70 percent ethanol. Before blood collection and culture operations, hands were thoroughly washed, wiped and rinsed with 70 percent ethanol to prevent contamination.

3.3 PREPARATION OF REAGENTS

3.3.1 Culture medium

The RPMI 1640 (Gibco) sterile liquid was used.

3.3.2 Antibiotic solution

Benzathine penicillin and streptomycin sulphate were used as antibiotics in the culture media. Benzathine penicillin (Pencom-12) was reconstituted with 10 ml of sterile triple glass distilled water, such that 0.1 ml of the solution contained 12,000 IU of benzathine penicillin. Ambistryn-S was also used as an antibiotic with a concentration of 200 mg of streptomycin

sulphate per ml of triple glass distilled water. 16 µl (2000 IU) of benzathine penicillin and 10 µl (2 mg) of streptomycin sulphate were added to each culture.

3.3.3 Phytohaemagglutinin-M (PHA -M)

PHA-M (Gibco) was used in the present study and was stored at -20^{0}C until further use.

3.3.4 Colchicine solution

3.3.4.1 Stock solution

The stock solution was prepared by dissolving 10 mg of colchicine powder (Sigma) in 10 ml sterile triple glass distilled water and stored at -20^{0}C until use.

3.3.4.2 Working solution

One ml of stock solution was added to 24 ml of sterile triple glass distilled water such that 0.2 ml of the above solution contained 8 microgram of colchicine and the working solution was made afresh each time at every setting of culture. 200 µl (8 µg) of colchicine was added to each culture.

3.3.5 Hypotonic solution (0.075 M Kcl solution)

The hypotonic solution was prepared afresh for every setting by dissolving 560 mg of potassium chloride (Sdfcl) in 100 ml sterile triple glass distilled water to make it 0.075M concentration.

3.3.6 Fixative solution (Carnoy's fluid)

The fixative solution (Carnoy's fluid) was prepared by mixing acetone free anhydrous methanol (Qualigens) and glacial acetic acid (Qualigens) in a ratio of 3:1. The required quantity of fixative solution was prepared afresh for every batch of culture and kept in refrigerator at -20^{o}C between fixations.

3.3.7 Giemsa staining solution

The staining solution was prepared by taking 4 ml Giemsa solution (Sdfcl) and making it upto 100 ml by adding Sorensen's phosphate buffer with pH 6.8. The staining solution was prepared afresh every time.

3.3.8 Sorensen' s phosphate buffer

The working solution of Sorensen's phosphate buffer was made by preparing the stock solutions of A and B.

3.3.8.1 Stock solution A

Solution A was made by dissolving 1.040 g anhydrous monosodium dihydrogen phosphate (0.075M) in 100 sterile triple glass distilled water and stored at 4^{o} C.

3.3.8.2 Stock solution B

Solution B was made by dissolving 1.186 g hydrated disodium monohydrogen phosphate (0.075M) in 100 ml sterile triple glass distilled water and stored at 4^{o} C.

3.3.8.3 Working solution

Solution A (51ml) and Solution B (49ml) were mixed together to give a pH of 6.8 for proper staining with Giemsa stain.

3. 4 COLLECTION AND TRANSPORT OF BLOOD SAMPLES

Blood samples were collected aseptically from the animals by puncturing the jugular vein. The site of blood collection was thoroughly cleaned with spirit and blood was collected into 5 ml vacutainer tubes, each containing 75 usp units of sodium heparin**.**

The blood samples collected from far places were transported to the laboratory in a thermos containing ice. Direct contact of blood samples with ice was prevented by wrapping the vacutainers in a thick polythene cover. Samples were shaken gently to prevent clotting and labled.

3.5 LYMPHOCYTE CULTURE TECHNIQUE

3.5.1 Preparation of Complete Medium and Setting up the Culture

The short term lymphocyte culture method, as described by Moorehead *et al.* (1960) with slight modifications, suitable to our laboratory conditions was followed for the cytogenetic characterization of Macherla Brown sheep.

The ultraviolet lamp of the laminar air flow cabinet was switched on one hour prior to setting the cultures after mopping the working areas with 70 percent ethanol. All the chemicals required i.e., RPMI-1640 with L-glutamine, Phytohaemagglutinin (PHA-M), Antibiotics and Heparin were taken out of refrigerator (4^0C) except PHA-M (-20^0C) and kept at room temperature for thawing. The ultraviolet lamp was switched off and the work area of laminar air flow cabinet was sprayed again with 70 percent ethanol. The thawed culture bottles were taken into the laminar air flow. The caps of the vacutainer tubes containing blood samples were wiped with 70 percent ethanol and mopped with paper towel. Duplicate cultures were set up for each animal.

The complete medium (ready to use) was prepared under sterile conditions in the laminar air flow cabinet, with the following composition per culture.

RPMI 1640 medium	8 ml
Benzathine Penicillin	16 µl
Streptomycin Sulphate	10 µl
Autologous Plasma	1.5ml
Phytohaemagglutinin -M	0.1 ml
Heparin	7.5 IU/ml of culture media
Total	9.626 ml

The cocktail mixture consisting of the complete medium for the total number of culture flasks to be kept was prepared in a sterile glass bottle and the same was kept in incubator maintained at 37^{o}C for one hour with the caps slightly opened so as to adjust the pH of the culture medium at 5 percent carbon-di-oxide level. After one hour, the glass bottle was transferred into laminar air flow cabinet and 9.626 ml of the complete medium was distributed into each culture flask and 0.5 ml of blood sample was added to each culture.

3.5.2 Incubation of cultures

The culture flasks were marked for identification, fitted with caps slightly open and incubated at 37^{o}C with 5 percent carbon-di-oxide levels for one hour for the adjustment of pH of culture medium and then the caps were tightened and kept at 37^{o} C for 72 hours. The culture flasks were shaken gently every 12 hours for proper suspension of cells.

3.5.3 Mitotic arrest

One and half hour (90 minutes) prior to harvest the cells, 200 µl (8 µg) of colchicine was added to each culture flask in laminar air flow cabinet and the cultures were reincubated after thorough mixing.

3.5.4 Harvesting the cultures

At the end of 72 hours of incubation, the culture flasks were taken out from the incubator, mixed gently and the contents were transferred to sterile centrifuge tubes and centrifuged at 1500 rpm for 10 minutes. The supernatant fluid was decanted using a Pasteur pipette, leaving one or two drops of the medium above the cell button. The cell button was re suspended by gently tapping the tube with a finger.

3.5.5 Hypotonic treatment

The cell button was resuspended in prewarmed (37^0C) hypotonic solution (0.075M), by adding drop by drop initially up to one ml and then filled up to seven ml. The centrifuge tubes were incubated in water bath at 37^oC for 20 to 25 minutes, followed by centrifugation at 1000 rpm for 10 minutes. The supernatant fluid was discarded leaving the cell button with 0.5 ml hypotonic solution. The cells were thoroughly mixed in the remaining hypotonic solution.

3.5.6 Fixation

The cell button was resuspended in seven ml of freshly prepared chilled fixative, initially drop wise up to one ml and then filled up to seven ml. The contents were gently mixed by flicking the bottom of the tube with finger and centrifuged again at 1500 rpm for 10 minutes. Supernatant was discarded and five ml of fixative was added to the cell pellet and kept in refrigerator at 4^0C over night for further processing.

On the next day, the tubes were centrifuged at 1500 rpm for 10 minutes and the supernatant discarded. Fixative washes were repeated 3 to 4 times to get rid of methaemoglobin (brown supernatant) and red cell ghosts. After final centrifugation and removal of supernatant, a milky cell suspension of 0.5 ml was left at the bottom of the centrifuge tube for preparation of slides.

3.5.7 Cleaning of slides and preparation of metaphase spreads

The slides were made grease-free by soaking in acid alcohol (99 percent ethanol and 1 percent concentrated hydrochloric acid) solution overnight. Then the slides were thoroughly washed under running tap water, rinsed with distilled water and stored in double distilled water at 4^0 C, until use.

Approximately 20 µl of homogenous cell suspension was drawn into micro pipette and dropped on the chilled wet slides held at 45 degrees angle from a height of about 2 to 3 ft with pressure. The slides were air dried at room temperature, labeled and checked under phase contrast microscope for the quality of metaphases. Care should be taken while dropping the cell suspension on to the slide to avoid underspread or overspread and the cell suspension was gently trickled from one edge of the slide and was allowed to spread evenly.

3.5.8 Staining of Slides and Preparation of Karyotypes

The slides containing good quality metaphase spreads on preliminary screening under phase contrast microscope were stained with freshly diluted Giemsa stain in a glass coplin jar for 20 minutes and rinsed gently with distilled water and air dried. Slides were screened under 10× and 40× magnifications on a binocular phase contrast microscope (Olympus). The best metaphase spreads were identified and captured into the digital camera attached to the microscope using 'Progres'(Olympus, Mumbai) software under 100× magnification under oil immersion, then the digital prints of the metaphases were taken on photo quality glossy paper.

The photo prints of individual chromosomes were cut carefully and homologous pairs pasted on a paper in pre printed format in descending order of their length and also based on the morphology, according to International System for the Cytogenetic Nomenculature of Domestic Animals (ISCNDA, 1989).

3.5.9 Morphometric Measurements

The length of short arm (p), long arm (q) and total length of chromosomes in the karyotypes was measured in millimeters with an accuracy of 0.05 mm, using the digital vernier calipers (Mitutoyo, Japan) and the following morphometric measurements were computed.

3.5.9.1 Relative Length

The relative length of a chromosome was computed as the ratio of the length of a chromosome to the total length of haploid set of chromosomes including the X-chromosome and expressed in percentage, as per the formula given by Bhatia and Shanker (1991).

$$\text{Relative length (\%)} = \frac{\text{Length of individual chromosome}}{\text{Total length of haploid genome including X-chromosome}} \times 100$$

$$\text{Relative length of X} = \frac{\text{Length of X chromosome}}{\text{Total length of haploid genome including X and Y chromosomes}} \times 100$$

$$\text{Relative length of Y} = \frac{\text{Length of Y chromosome}}{\text{Total length of haploid genome including X and Y chromosomes}} \times 100$$

The relative length was computed for all the autosomes and sex chromosomes. The idiogram was drawn by taking the chromosome number on X-axis and the relative length (%) of the respective chromosome on Y- axis.

3.5.9.2 Arm ratio

The arm ratio of the biarmed chromosomes was calculated using the following formula.

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

3.5.9.3 Centromeric Index

Centromeric index was computed using the following formula.

$$\text{Centromeric index } 1 = \frac{\text{Length of short arm (p)}}{\text{Length of the chromosome (p+q)}} \times 100$$

$$\text{Centromeric index } 2 = \frac{\text{Length of long arm (q)}}{\text{Length of the chromosome (p+q)}} \times 100$$

3.5.9.4 Morphological Index

The morphological index was calculated by dividing the total chromosome length by the arm ratio.

$$\text{Morphological index} = \frac{\text{Total chromosome length}}{\text{Arm ratio}}$$

or

$$\text{Morphological index} = \frac{p\,(p+q)}{q}$$

3.6 STATISTICAL ANALYSIS

3.6.1 Morphometric Measurements

The relative length for each of the karyotypes was calculated according to the formula given by Bhatia and Shanker (1991). The data were transformed using arcsin transformation (Snedecor and Cochran, 1989), Since the relative lengths of all the chromosomes were below 30 percent, The transformed data was subjected to t- test to study the effect of sex of the

animal on the morphometric measurements of autosomes and X-chromosome. The data on relative length of all the autosomes and X-chromosome and data on arm ratio, centromeric index and morphological index of first three chromosomes was subjected to one way analysis of variance to know the significant differences among the chromosomes.

Transformation formula =ASIN(SQRT(value/100))*180/PI()

CHAPTER - IV
RESULTS

Blood samples were collected from eighty Macherla Brown sheep (40 males and 40 females) maintained in farmers flocks of Prakasam, Guntur, Krishna and Nalgonda districts for present investigation. Short term lymphocyte culture technique as described by Moorehead *et al*., (1960) was employed with slight modifications. The results obtained in the present study are detailed below.

4.1 MODAL CHROMOSOME NUMBER

The giemsa stained metaphase spreads of male and female Macherla Brown sheep are presented in Fig.4 and Fig.6, respectively. In the present study, microscopic screening of 30 good quality metaphase spreads from each animal revealed a diploid chromosome complement of 54 consisting 26 pairs of autosomes and one pair of allosomes (XX in females and XY in males).

4.2 CHROMOSOME MORPHOLOGY

The karyotypes prepared from the giemsa stained metaphase spreads of male and female Macherla Brown sheep are shown in the Fig.5 and Fig.7, respectively.

Among 26 pairs of autosomes, first three pairs of autosomes were designated as sub metacentric and the remaining 23 pairs of chromosomes are acrocentric with decreasing order of length. The X-chromosome was found to be the longest acrocentric, while the Y-chromosome was smallest biarmed in the karyotype.

4.3 MORPHOMETRIC MEASUREMENTS

4.3.1 Relative Length

The mean relative length (%) of chromosomes of Macherla Brown sheep according to the sexes based on raw data are presented in the Table 2. The idiograms representing the

mean relative lengths of the chromosomes of Macherla Brown rams, ewes and both the sexes based on transformed data are shown in the Fig.8, Fig.9 and Fig.10 respectively. Sex had no significant effect on the relative lengths of all the autosomes and the X-chromosome.

The analysis of variance of relative lengths and the mean relative lengths based on transformed data are detailed in Table 3 and 4, respectively. The analysis of variance based on the transformed data revealed significant differences ($p \leq 0.01$) among the relative lengths of different chromosomes.

The overall mean relative lengths for the autosomes ranged from 1.80 ± 0.01 (chromosome no.26) to 9.76 ± 0.03 (chromosome no.1) percent based on raw data. The overall mean relative lengths of first three submetacentric chromosomes are 9.76 ± 0.03, 8.66 ± 0.02 and 7.69 ± 0.03 percent respectively. The submetacentic biarmed autosomes together constituted 25.49 percent of genome in males and 26.71 per cent in females, whereas the acrocentric chromosomes 4 and 5 constituted 4.36 percent and 4.20 percent in males and the corresponding means in females were 4.36 and 4.17 with an overall means of 4.36 ± 0.01 and 4.18 ± 0.01 respectively. The mean relative length of X-chromosome was 5.01 ± 0.01 in males and 5.10 ± 0.01 in females with an overall mean of 5.05 ± 0.01, whereas, the mean relative length of Y-chromosome was 1.57 ± 0.01 percent (Table 2).

The mean relative length of first three pairs of submetacentric autosomes in males were 9.85 ± 0.05, 8.40 ± 0.02 and 7.24 ± 0.03, while the corresponding means in females were 9.67 ± 0.02, 8.91 ± 0.02 and 8.13 ± 0.02 percent. The overall mean relative length based on transformed data for the autosomes varied from 7.72 ± 0.02 to 18.19 ± 0.03 percent. The mean relative length of X-chromosome was 12.82 ± 0.04 in

Table 2. Mean relative length (%) of chromosomes in Macherla Brown sheep based on raw data

Chromosome No.	Ram		Ewe		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	9.85	0.05	9.67	0.02	9.76	0.03
2	8.40	0.02	8.91	0.02	8.66	0.02
3	7.24	0.03	8.13	0.02	7.69	0.03
4	4.36	0.01	4.36	0.01	4.36	0.01
5	4.20	0.01	4.17	0.01	4.18	0.01
6	4.06	0.01	3.96	0.01	4.01	0.01
7	3.86	0.01	3.77	0.01	3.82	0.01
8	3.67	0.01	3.65	0.01	3.66	0.01
9	3.56	0.01	3.53	0.01	3.54	0.01
10	3.41	0.01	3.42	0.01	3.42	0.01
11	3.32	0.01	3.32	0.01	3.32	0.01
12	3.24	0.01	3.22	0.01	3.23	0.01
13	3.10	0.01	3.09	0.01	3.10	0.01
14	3.01	0.01	2.98	0.01	2.99	0.01
15	2.87	0.01	2.89	0.01	2.89	0.01
16	2.71	0.01	2.78	0.01	2.75	0.01
17	2.64	0.01	2.69	0.01	2.66	0.01
18	2.55	0.01	2.59	0.01	2.57	0.01
19	2.47	0.01	2.50	0.01	2.49	0.01
20	2.39	0.01	2.44	0.01	2.42	0.01
21	2.31	0.01	2.38	0.01	2.34	0.01
22	2.26	0.01	2.29	0.01	2.27	0.01
23	2.15	0.01	2.21	0.01	2.18	0.01
24	2.04	0.01	2.12	0.01	2.08	0.01
25	1.96	0.01	2.00	0.01	1.98	0.01
26	1.78	0.01	1.82	0.01	1.80	0.01
X	5.01	0.01	5.10	0.01	5.05	0.01
Y	1.57	0.01	-	-	1.57	0.01

Table 3. Analysis of variance of relative length of chromosomes of Macherla Brown sheep based on transformed data

Source of Variation	Df	MSS
Between chromosomes	27	5575.45**
Error	21972	0.128

**Significant ($p \leq 0.01$)

Table 4. Mean relative length (%) of chromosomes in Macherla Brown sheep based on transformed data

Chromosome No.	Ram		Ewe		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	18.26	0.05	18.11	0.03	18.19[a]	0.03
2	16.85	0.02	17.37	0.02	17.11[b]	0.02
3	15.60	0.04	16.56	0.03	16.08[c]	0.03
4	12.05	0.02	12.04	0.02	12.05[d]	0.01
5	11.81	0.02	11.77	0.02	11.79[e]	0.01
6	11.62	0.02	11.47	0.02	11.55[f]	0.01
7	11.33	0.01	11.19	0.01	11.26[g]	0.01
8	11.04	0.01	11.01	0.01	11.03[h]	0.01
9	10.87	0.01	10.83	0.01	10.85[i]	0.01
10	10.65	0.01	10.65	0.01	10.65[j]	0.01
11	10.50	0.01	10.49	0.01	10.50[k]	0.01
12	10.37	0.01	10.34	0.01	10.36[l]	0.01
13	10.14	0.01	10.13	0.01	10.13[m]	0.01
14	9.99	0.01	9.94	0.01	9.96[n]	0.01
15	9.75	0.01	9.79	0.01	9.77[o]	0.01
16	9.47	0.02	9.60	0.01	9.54[p]	0.01
17	9.35	0.02	9.43	0.01	9.39[q]	0.01
18	9.19	0.01	9.25	0.01	9.22[r]	0.01
19	9.04	0.01	9.01	0.01	9.07[s]	0.01
20	8.90	0.01	8.99	0.01	8.95[t]	0.01

21	8.74	0.01	8.88	0.01	8.81^{u}	0.01
22	8.65	0.01	8.70	0.01	8.67^{v}	0.01
23	8.44	0.01	8.54	0.01	8.49^{w}	0.0
24	8.23	0.01	8.37	0.01	8.30^{x}	0.01
25	8.05	0.02	8.13	0.02	8.10^{y}	0.02
26	7.69	0.02	7.75	0.02	7.72^{z}	0.02
X	12.82	0.04	13.04	0.02	12.93^{A}	0.03
Y	7.21	0.02	-	-	7.21^{B}	0.02

Means bearing different superscripts in a column differ significantly

males and 13.04 ± 0.02 percent in females with an overall mean of 12.93 ± 0.02, whereas the relative length of Y-chromosome was 7.21 ± 0.02 percent.

4.3.2 Arm Ratio

The sex had no significant effect on the mean arm ratios of the first three sub metacentric chromosomes. The mean arm ratios varied from 1.22 ± 0.01 in first biarmed autosome to 1.20 ± 0.02 percent in third biarmed autosome based on raw data (Table 5).

The analysis of variance of arm ratios based on transformed data revealed significant differences ($p \leq 0.01$) among the first three biarmed autosomes (Table 6).

The overall mean arm ratios of first three submetacentric autosomes based on transformed data were 6.34 ± 0.01, 6.37 ± 0.01 and 6.30 ± 0.01, respectively. Mean arm ratios for first three submetacentric chromosomes based on transformed data varied from 6.26 ± 0.01 to 6.34 ± 0.01 in males, while in females the means ranged from 6.32 ± 0.01 to 6.39 ± 0.01 (Table 7).

4.3.3 Centromeric Index (C.I.1)

The effect of sex was found to be non significant on the centromeric indices of the first three chromosomes. The sex wise means and overall mean centromeric indices based on raw data are detailed in the Table 8.

The overall mean centromeric indices for first three chromosomes based on raw data are 0.452 ± 0.01, 0.452 ± 0.01 and 0.460 ± 0.01, respectively (Table 8).

The results of analysis of variance for the centromeric indices of the first three submetacentric chromosomes based on transformed data revealed significant differences ($p \leq 0.01$) among the first three chromosomes (Table 9).

Table 5. Mean arm ratios of the first three chromosomes of Macherla Brown sheep based on raw data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	1.24	0.01	1.20	0.01	1.22^{a}	0.01
2	1.24	0.01	1.22	0.01	1.23^{b}	0.01
3	1.21	0.02	1.19	0.01	1.20^{c}	0.02

Means bearing different superscripts in a column differ significantly.

Table 6. Analysis of variance of arm ratios of the first three chromosomes of Macherla Brown sheep based on transformed data

Source of Variation	df	MSS
Between Chromosomes	2	1.149**
Error	2397	0.048

**Significant ($p \leq 0.01$)

Table 7. Mean arm ratios of first three chromosomes of Macherla Brown sheep based on transformed data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	6.29	0.01	6.39	0.01	6.34[a]	0.01
2	6.34	0.01	6.39	0.01	6.37[b]	0.01
3	6.26	0.01	6.32	0.01	6.30[c]	0.01

Means bearing different superscripts in a column differ significantly.

Table 8. Mean centromeric indices (C.I.1) of the first three chromosomes of Macherla Brown sheep based on raw data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	0.46	0.01	0.45	0.01	0.452[a]	0.01
2	0.46	0.01	0.45	0.01	0.452[a]	0.01
3	0.47	0.01	0.45	0.01	0.460[b]	0.01

Means bearing different superscripts in a column differ significantly.

Table 9. Analysis of variance of centromeric indices (C.I.1) of the first three chromosomes of Macherla Brown sheep based on transformed data

Source of Variation	df	MSS
Between chromosomes	2	0.024**
Error	2397	0.006

**Significant ($p \leq 0.01$)

Table 10. Mean centromeric indices (C.I.1) of the first three chromosomes of Macherla Brown sheep based on transformed data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	3.87	0.01	3.83	0.01	3.854^{a}	0.01
2	3.86	0.01	3.83	0.01	3.845^{b}	0.01
3	3.88	0.01	3.86	0.01	3.870^{c}	0.01

Means bearing different superscripts in a column differ significantly

The overall mean centromeric indices based on transformed data for the first three autosomes were 3.854 ± 0.01, 3.845 ± 0.01 and 3.870 ± 0.01, respectively while the corresponding means for rams and ewes are 3.87 ± 0.01, 3.86 ± 0.01 and 3.88 ± 0.01 and 3.83 ± 0.01, 3.83 ± 0.01 and 3.86 ± 0.01 (Table 10).

4.3.4 Centromeric index 2 (C.I.2)

The effect of sex was found to be non significant on the centromeric indices of the first three chromosomes. The sex wise means and overall mean centromeric indices based on raw data are shown in the Table 11.

The overall mean centromeric indices for first three chromosomes based on raw data were 0.548 ± 0.01, 0.550 ± 0.01 and 0.544 ± 0.01, respectively (Table 11).

The results of analysis of variance for the centromeric indices of the first three sub metacentric chromosomes based on transformed data exhibited significant differences among the first three chromosomes (Table 12).

The overall mean centromeric indices based on transformed data for the first three autosomes were 4.244 ± 0.01, 4.253 ± 0.01 and 4.231 ± 0.01, respectively while the

corresponding means were 4.23 ± 0.01, 4.24 ± 0.01and 4.22 ± 0.01 for rams and 4.26 ± 0.01, 4.27 ± 0.01 and 4.24 ± 0.01 for ewes (Table 13).

4.3.5 Morphological Index

Sex had no significant effect on the morphological indices of the first three chromosomes. The sex wise means and overall mean morphological indices based on raw data are shown in the Table 14.

Table 11. Mean centromeric indices (C.I.2) of the first three chromosomes of Macherla Brown sheep based on raw data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	0.54	0.01	0.55	0.01	0.548[a]	0.01
2	0.55	0.01	0.55	0.01	0.550[a]	0.01
3	0.54	0.01	0.54	0.01	0.544[b]	0.01

Means bearing different superscripts in a column differ significantly.

Table 12. Analysis of variance of centromeric indices (C.I.2) of the first three chromosomes of Macherla Brown sheep based on transformed data

Source of Variation	df	MSS
Between chromosomes	2	0.095**
Error	2397	0.006

**Significant ($p \leq 0.01$)

Table 13. Mean centromeric indices (C.I.2) of the first three chromosomes of Macherla Brown sheep based on transformed data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	4.23	0.01	4.26	0.01	4.244[a]	0.01
2	4.24	0.01	4.27	0.01	4.253[b]	0.01
3	4.22	0.01	4.24	0.01	4.231[c]	0.01

Means bearing different superscripts in a column differ significantly.

The mean morphological indices of first three chromosomes in males were 8.54 ± 0.08, 7.13 ± 0.04 and 6.34 ± 0.06, respectively, whereas, the corresponding mean morphological indices in females were 7.96 ± 0.04, 7.31 ± 0.03 and 6.81 ± 0.03 (Table 14).

The results of analysis of variance over the pooled transformed data of morphological indices revealed significant differences ($p \leq 0.01$) among the chromosomes (Table 15).

The overall mean morphological indices based on transformed data for the first three autosomes were 16.65 ± 0.06, 15.57 ± 0.04 and 14.82 ± 0.05, respectively, while the corresponding means for rams were 16.93 ± 0.08, 15.47 ± 0.04 and 14.53 ± 0.06 and 16.36 ± 0.04, 15.67 ± 0.03 and 15.12 ± 0.03 in ewes (Table 16).

Table 14. Mean morphological indices of the first three chromosomes of Macherla Brown sheep based on raw data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	8.54	0.08	7.96	0.04	8.25[a]	0.06
2	7.13	0.04	7.31	0.03	7.22[b]	0.04
3	6.34	0.06	6.81	0.03	6.58[c]	0.05

Means bearing different superscripts in a column differ significantly.

Table 15. Analysis of variance of morphological indices of the first three chromosomes of Macherla Brown sheep based on transformed data.

Source of Variation	df	MSS
Between chromosomes	27	671.76**
Error	2397	1.160

**Significant ($p \leq 0.01$)

Table 16. Mean morphological indices of the first three chromosomes of Macherla Brown sheep based on transformed data

Chromosome No.	Rams		Ewes		Overall	
	Mean	S.E	Mean	S.E	Mean	S.E
1	16.93	0.08	16.36	0.04	16.65[a]	0.06
2	15.47	0.04	15.67	0.03	15.57[b]	0.04
3	14.53	0.06	15.12	0.03	14.82[c]	0.05

Means bearing different superscripts in a column differ significantly

CHAPTER-V

DISCUSSION

5.1 MODAL CHROMOSOME NUMBER

The mitotic spreads of Rams and Ewes of Macherla Brown sheep have been presented in the metaphase plates (Fig.4 and Fig.6). The modal diploid chromosomal number was found to be 54 comprising of 52 autosomes and two sex chromosomes. The modal chromosome number of 54 recorded in the present study was in agreement with the findings of earlier research workers in various indigenous sheep breeds viz., Muzaffarnagari (Benjamin and Bhat, 1978), Nali (Bhatia and Shanker, 1989), Bannur (Langhe *et al*., 1993), Malpura (Gupta and Gupta, 1995), Magra (Bhatia and Shanker, 1996), Mandya (Umrikar and Narayankhedkar, 1997), Patanwadi (Bhatia and Shanker, 1999), Nellore (Amareswari *et al.*, 2005), Mecheri (Karunanithi *et al*., 2005), Deccani (Prakash *et al*., 2008), Coimbatore (Devendran *et al*., 2009), Vizianagaram (Satish *et al*., 2012) sheep and exotic breeds of Rambouillet (Berry., 1941), Merino, Southdown, Ryeland, Dorset Horn, Border Leicester, Romney Marsh and Cheviot (Borland, 1964), Lohi (Intizar *et al*., 1999), Kari (Ahmad and Khan, 2007), and Laticauda sheep (Iannuzzi *et al*., 2013).

5.2 CHROMOSOME MORPHOLOGY

Out of 26 pairs of autosomes, first three pairs of autosomes were found to be submetacentric and remaining 23 pairs were acrocentric in nature. Submetacentric nature observed in first three pairs of autosomes in the present study further confirmed the findings of Umrikar and Narayankhedkar (1997) in Mandya, Amareswari *et al*. (2005) in Jodipi, Palla and Brown strains of Nellore sheep and Prakash *et al*. (2008) in Deccani sheep, Shaikh *et al*. (2009) in Deccani, Satish *et al*. (2012) in Vizianagaram sheep, but metacentric morphology was observed by Benjamin and Bhat (1978) in Muzaffarnagari, Bhatia and Shanker (1989,

1994) in Nali and Munjal, Gupta and Gupta (1995) in Malpura, Bhatia and Shanker (1996) in Magra, Bhatia and Shanker (1999) in Patanwadi, Karunanithi *et al*. (2005) in Mecheri, Devendran *et al*. (2009) in Coimbatore sheep, whereas, Evans *et al*. (1973) in Suffolk and Kirikci (2003), Arslan and Zima (2011) in Konya wild sheep noticed the metacentric nature of first three pairs of autosomes.

The remaining 23 pairs of autosomes (chromosome no 4 to 26) observed in the present study were acrocentric in nature (Fig.4, Fig.5, Fig.6 and Fig.7). Similar results were also reported in Nali and Magra (Bhatia and Shanker 1989, 1996), Munjal (Bhatia and Shanker, 1994) Mandya (Umrikar and Narayankhedkar, 1997), Lohi (Intizar *et al*., 1999), Patanwadi (Bhatia and Shanker, 1999), Konya wild sheep (Kirkci, 2003 and Kaya *et al*., 2004), Nellore (Amareswari *et al.,* 2005), Mecheri (Karunanithi *et al*., 2005), Deccani (Prakash *et al*., 2008), Coimbatore (Devendran *et al*., 2009) and Vizianagaram (Satish *et al*., 2012) sheep. In contrast to these findings Benjamin and Bhat (1978) reported that last 23 pairs of autosomes were telocentric nature in Muzaffarnagari sheep. Gupta and Gupta (1995) noticed that these set of autosomes are either acrocentric or telocentric in Malpura sheep.

In the present study the X-chromosome is largest and acrocentric which is corroborated with the findings of earlier research workers [Benjamin and Bhat (1978), Bhatia and Shanker (1989, 1994, 1996 and 1999), Gupta and Gupta (1995), Bunch *et al*. (2000), Mansour *et al*. (2000a), Amareswari *et al*. (2005), Karunanithi *et al*. (2005), Prakash *et al*. (2008), Devendran *et al*. (2009) and Satish *et al*. (2012)] in various breeds of sheep. Borland (1964) also noticed acrocentric nature of X-chromosome in Merino, South Down, Ryeland, Dorset Horn, Border Leicester, Romney Marsh and Cheviot breeds of temperate region.

The Y-chromosome in the present study was found to be smallest biarmed chromosome (Fig.4 and Fig.5). Similar findings were reported in Magra (Bhatia and Shanker, 1996), Patanwadi (Bhatia and Shanker, 1999), Nellore (Amareswari *et al*., 2005), Deccani

(Prakash *et al*., 2008) and Vizianagaram (Satish *et al*., 2012) sheep. However, Benjamin and Bhat (1978) in Muzaffarnagari, Bhatia and Shanker (1989) in Nali, Gupta and Gupta (1995) in Malpura and Karunanithi *et al*., (2005) in Mecheri and Devendran *et al*., (2009) in Coimbatore sheep reported that Y-chromosome was metacentric, whereas, Y-chromosome was reported as small dot like structure in Merino sheep (Borland, 1964), local sheep of Orissa (Roy *et al*., 1991) and Garole sheep (Akhuli, 1999).

5.3 MORPHOMETRIC MEASUREMENTS

5.3.1 RELATIVE LENGTH

The effect of sex on the relative length of all the autosomes was not significant in the present study. The results are in agreement with the findings of Bhatia and Shanker (1996, 1999) in Magra and Patanwadi, Amareswari *et al*., (2005) in Nellore and Prakash *et al*., (2008) in Deccani sheep and (Satish *et al*., 2012) in Vizianagaram sheep.

The mean relative length of autosomes based on raw data ranged from 1.78 to 9.85 percent (Table 2) which were within the range of means reported by different investigators in various breeds (Bhatia and Shanker (1994) in Munjal sheep, Gupta and Gupta (1995) in Malpura sheep, Bhatia and Shanker (1996) in Magra sheep, Bhatia and Shanker (1999) in Patanwadi sheep, Amareswari *et al*. (2005) in Nellore sheep, Prakash *et al*. (2008) in Deccani sheep, Devendran *et al*. (2009) in Coimbatore sheep and Satish *et al*. (2012) in Vizianagaram sheep, whereas, Langhe *et al*. (1993) recorded higher mean relative lengths in Bannur sheep than those recorded in the present study. The first three pairs of biarmed autosomes together contributed 25.49 percent of the genome length in males and 26.71 percent in females. Similar findings were reported in Malpura sheep by Gupta and Gupta (1995), Coimbatore sheep by Devendran *et al*. (2009) and Vizianagaram sheep by Satish *et al*. (2012).

The overall mean relative length of the X-chromosome was 5.05 percent observed in the Macherla Brown sheep. The results are within the limits reported in Munjal (Bhatia and

Shanker, 1994), Magra (Bhatia and Shanker, 1996), Nellore (Amareswari *et al*., 2005), Deccani (Prakash *et al*., 2008), Coimbatore (Devendran *et al*., 2009) and Vizianagaram (Satish *et al*., 2012) breeds, while Gupta and Gupta (1995) in Malpura (4.86 percent) and Karunanithi *et al*., (2005) in Mecheri sheep (4.59 percent) recorded the lower means than the present study.

The overall mean relative length for the Y-chromosome was 1.57 percent which was well in accordance with mean relative lengths recorded in Coimbatore sheep (Devendran *et al*., 2009), Malpura (Gupta and Gupta, 1995), Magra (Bhatia and Shanker, 1996), Nellore (Amareswari *et al*., 2005), Deccani (Prakash *et al*., 2008) and Vizianagaram (Satish *et al*., 2012) sheep. However, Bhatia and Shanker (1994) reported lowest mean (1.45 per cent) in Munjal sheep and Langhe *et al*., (1993) recorded highest mean (4.48 percent) in Bannur sheep,

5.3.2 Arm Ratio

The analysis of variance of arm ratios of first three autosomes revealed significant difference among chromosomes. The effect of sex was not significant on the arm ratios of submetacentric chromosomes in the present study (Table 7) well agreed with the findings of Bhatia and Shanker (1996), Amareswari *et al*. (2005), Prakash *et al*. (2008) and Satish *et al*. (2012) in Magra, Nellore, Deccani and Vizianagaram sheep breeds, respectively.

The overall mean arm ratios for the first three autosomes in Macherla Brown sheep were 1.22, 1.23 and 1.20 respectively. (Table 5). These findings are corrabarated with the means reported by Bhatia and Shanker (1994) in Munjal sheep, Gupta and Gupta (1995) in Malpura sheep, Bhatia and Shanker (1996) in Magra sheep, Bhatia and Shanker (1999) in Patanwadi sheep, Amareswari *et al*. (2005) in Nellore sheep, and Devendran *et al*. (2009) in Coimbatore sheep, but higher means than the present study were recorded by Shaikh *et al*.

(2009) in Deccani sheep and Satish *et al*. (2012) in Vizianagaram sheep, whereas lower means were observed in Mecheri sheep (Karunanithi *et al*., 2005).

The study on arm ratio has revealed nature of first three pairs of autosomes as submetacentric in Macherla Brown sheep.

5.3.3 Centromeric index (C.I .1)

The analysis of variance of centomeric indices of first three biarmed autosomes revealed significant differences among chromosomes. Sex had no significant effect on the centromeric indices of Macherla Brown sheep chromosomes noticed in the present study (Table 10) was well in agreement with the findings of Bhatia and Shanker (1996) and Prakash *et al*. (2008).

The mean centromeric index values for the first three autosomes of Macherla Brown sheep were ranged from 0.46 to 0.47 in males and 0.45 in females (Table 8).

The mean centromeric indices observed in the present study were almost similar to those reported in Malpura sheep (Gupta and Gupta., 1995), Munjal, Magra and Patanwadi sheep (Bhatia and Shanker, 1994; 1996; 1999), Mecheri sheep (Karunanithi *et al*., 2005), Deccani sheep (Shaik *et al*., 2009) and Coimbatore sheep (Devendran *et al*., 2009). While Langhe *et al*. (1993) reported slightly lower values (41.92, 37.30 and 35.68 percent) in Bannur sheep than means reported in the present study in Macherla Brown sheep.

The first three biarmed autosomes of the Macherla Brown sheep chromosomes were confirmed as submetacentric since the centromeric index values of all the first three autosomes deviated from the value of 0.5.

5.3.4 Centromeric index 2 (C.I .2)

The analysis of variance of centomeric indices of first three biarmed autosomes revealed significant differences among the chromosomes. The effect of sex was found to be

non significant (Table 13) on the centromeric indices of the first three chromosomes which were similar with the findings of Bhatia and Shanker (1996), Amareswari *et al*. (2005) and Prakash *et al*. (2008).

The mean centromeric index values for the first three autosomes of Macherla Brown sheep were ranged from 0.54 to 0.55 in both the sexes (Table 11).

The mean centromeric indices observed in the present study were almost similar to the findings of Bhatia and Shanker, 1994, in Munjal and 1999 in Patanwadi sheep, Amareswari., *et al*., 2005 (Nellore sheep), Devendran *et al*., 2009 (Coimbatore sheep), Prakash *et al*., 2008 (Deccani sheep) and Satish *et al*., 2012 (Vizianagarm sheep), whereas, lower means (0.5039, 0.5042 and 0.5046) were observed in Mecheri sheep (Karunanithi *et al*., 2005).

5.3.5 MORPHOLOGICAL INDEX

The effect of the sex of the animal was not evident on the morphological indices of the first three chromosomes of Macherla Brown sheep which was well in accordance with the findings of Prakash *et al*., (2008) in Deccani sheep and contradictory to the findings of Amareswari *et al*., (2005) in Nellore sheep.

The mean morphological indices of the first three submetacentric biarmed autosomes of Macherla Brown sheep were 8.54 ± 0.08, 7.13 ± 0.04 and 6.34 ± 0.06, respectively in males while in females 7.96 ± 0.04, 7.31 ± 0.03 and 6.81 ± 0.03 respectively (Table 14). The mean morphological indices decreased from first to third chromosomes as the morphological index was directly proportional to the length of the chromosomes. Similar trend was also noticed in Bannur (Langhe *et al*., 1993), Nellore (Amareswari *et al*., 2005), Mecheri, (Karunanithi *et al*., 2005), Deccani (Prakash *et al*., 2008) and Coimbatore sheep (Devendran *et al*., 2009).

The overall mean morphological indices of the three biarmed chromosomes 1, 2 and 3 of Macherla Brown sheep were found to be 8.25 ± 0.06, 7.22 ± 0.04 and 6.58 ± 0.05, respectively (Table 14). Higher mean morphological indices were observed in Bannur (29.23, 22.83, 15.48), Mecheri (12.89, 12.09, 10.99) and Deccani sheep (9.40, 10.57, 11.57). (Langhe *et al*., 1993; Karunanithi *et al*., 2005 and Prakash *et al*., 2008) while lower values reported in Nellore sheep by Amareswari *et al*. (2005).

CHAPTER - VI

SUMMARY

A karyological study was carried out on Macherla Brown sheep maintained in four districts namely Prakasam, Guntur, Krishna and Nalgonda districts of Andhra Pradesh. Blood samples were collected from 80 animals (40 males and 40 females) of field flocks to analyse the chromosomal profile. The relative length, arm ratio, centromeric index and morphological index were estimated based on raw data and transformed data.

A total of 2400 metaphase spreads were examined for the chromosome number and karyotypes of Macherla Brown sheep were prepared by employing short term lymphocyte culture technique. The modal diploid chromosome number was found to be 54 (2n = 54, XY). The first three pairs of the autosomes were found to be submetacentric and the remaining 23 pairs of autosomes were acrocentric. The X-chromosome was the longest acrocentric, while the Y-chromosome was the smallest biarmed chromosome in the karyotype.

The relative length, arm ratio, centromeric index and morphological indices were computed. The data on relative length were converted into angles using arcsin transformation for conducting the analysis. The sex has no significant effect on relative length of all the chromosomes.

The mean relative length for the autosomes based on raw data ranged from 1.80 ± 0.01 (chromosome no 26) to 9.76 ± 0.03 (chromosome no 1) percent, while the mean relative length of X and Y-chromosomes were 5.05 ± 0.01 and 1.57 ± 0.01, percent respectively. The biarmed chromosomes together contributed 25.49 percent of genome in males and 26.71 percent in females.

The overall mean relative lengths based on transformed data for the autosomes varied from 7.72 ± 0.02 to 18.19 ± 0.03 percent. The mean relative length of X-

chromosome in males was 12.82 ± 0.04 and 13.04 ± 0.02 percent in females with an overall mean of 12.93 ± 0.03 percent, whereas, the mean relative length of Y-chromosome was 7.21 ± 0.02 percent.

The effect of sex was not evident on the arm ratios of the first three chromosomes. The mean arm ratios based on raw data for the biarmed autosomes varied from 1.20 ± 0.02 to 1.23 ± 0.01, whereas, the overall mean arm ratios based on transformed data ranged between 6.30 ± 0.01 and 6.37 ± 0.01. The second biarmed autosome in males and females exhibited highest arm ratio of 6.34 ± 0.01 and 6.39 ± 0.01, respectively.

The influence of sex on centromeric indices (C.I.1) of all the chromosomes was found to be non significant. Males possess higher centromeric index compared to females. The overall mean centromeric indices (C.I.1) for the first three autosomes were 3.854 ± 0.01, 3.845 ± 0.01 and 3.870 ± 0.01, respectively, while the corresponding means were 3.87 ± 0.01, 3.86 ± 0.01 and 3.88 ± 0.01 for rams and 3.83 ± 0.01, 3.83 ± 0.01 and 3.86 ± 0.01 for ewes.

The effect of sex on centromeric indices (C.I.2) of all the submetacentric chromosomes was found to be non significant. The overall mean centromeric indices for the first three autosomes were 4.244 ± 0.01, 4.253 ± 0.01 and 4.231 ± 0.01, respectively, while the corresponding means for rams were 4.23 ± 0.01, 4.24 ± 0.01 and 4.22 ± 0.01 and the means in ewes are 4.26 ± 0.01, 4.27 ± 0.01 and 4.24 ± 0.01.

Significant differences ($P \leq 0.01$) were observed among the morphological indices of the first three chromosomes. Mean morphological indices based on transformed data in rams were 16.93 ± 0.08, 15.47 ± 0.04 and 14.53 ± 0.06 and 16.36 ± 0.04, 15.67 ± 0.03 and 15.12 ± 0.03 in ewes.

The results of the present study revealed that the modal diploid chromosomal number of Macherla Brown sheep was 54 (2n = 54, XY) comprising of three pairs of submetacentric and 23 pairs of acrocentric autosomes. Macherla Brown sheep with distinct phenotypic pattern

and good productive and reproductive performances, can be differentiated from other breeds. The morphological appearance of the biarmed autosomes was also similar to that of southern indigenous breeds like Nellore, Deccani, Coimbatore and Vizianagaram indigenous sheep, but, slightly differed from some of the north western breeds of India particularly Magra and Muzaffarnagri. The present study is a preliminary step for characterization of Macherla Brown sheep as a recognized breed, However, more studies with large sample size are necessary for further genetic characterization of Macherla Brown sheep.

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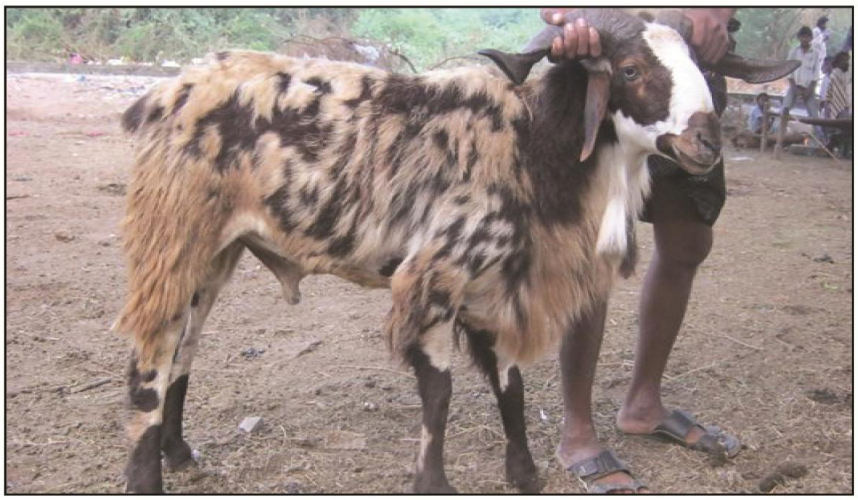

Fig.1. Macherla Brown Rams

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Fig.2. Macherla Brown Ewes

Fig.3. Flock of Macherla Brown Sheep.

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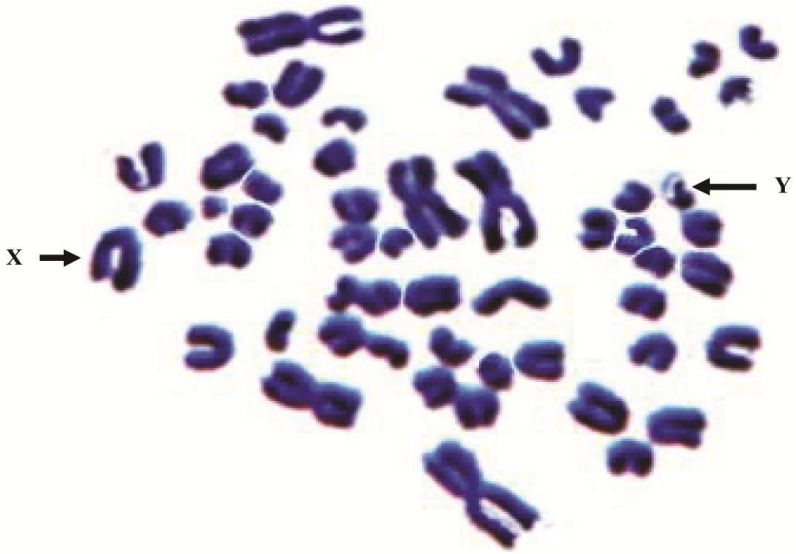

Fig.4. Giemsa stained maetaphase spread of Macherla Brown Ram (2n=54, XY)

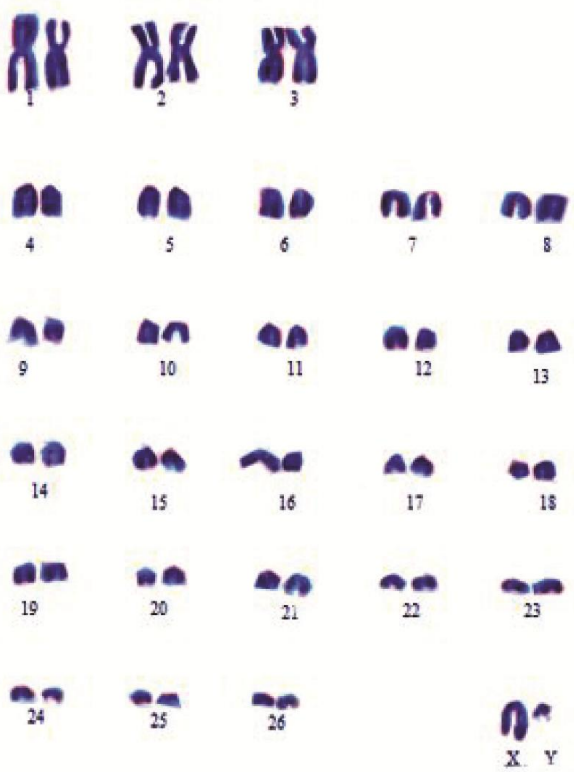

Fig.5. Giemsa stained karyotype of Macherla Brown Ram (2n=54, XY)

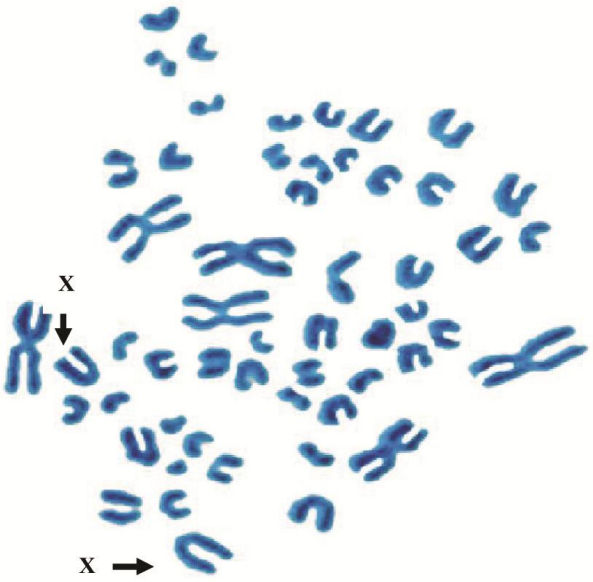

Fig.6. Giemsa stained maetaphase spread of Macherla Brown Ewe (2n=54, XX)

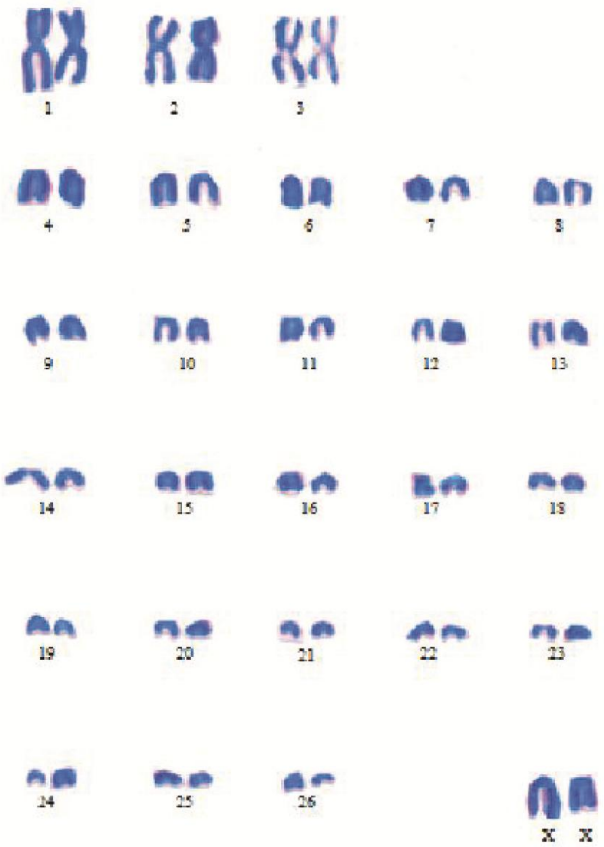

Fig.7. Giemsa stained karyotype of Macherla Brown Ewe (2n=54, XX)

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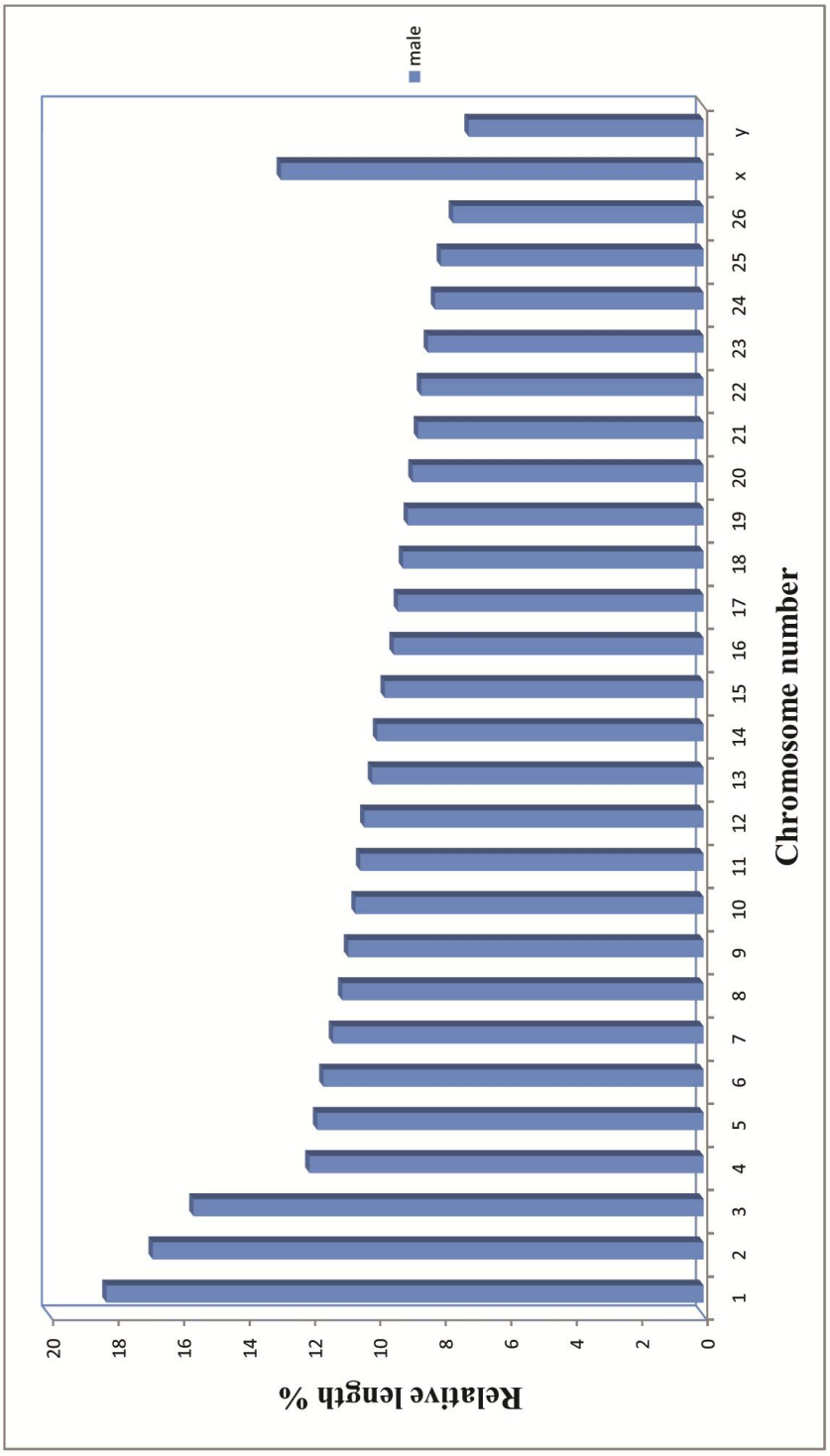

Fig .8. Idiogram of Macherla Brown Ram

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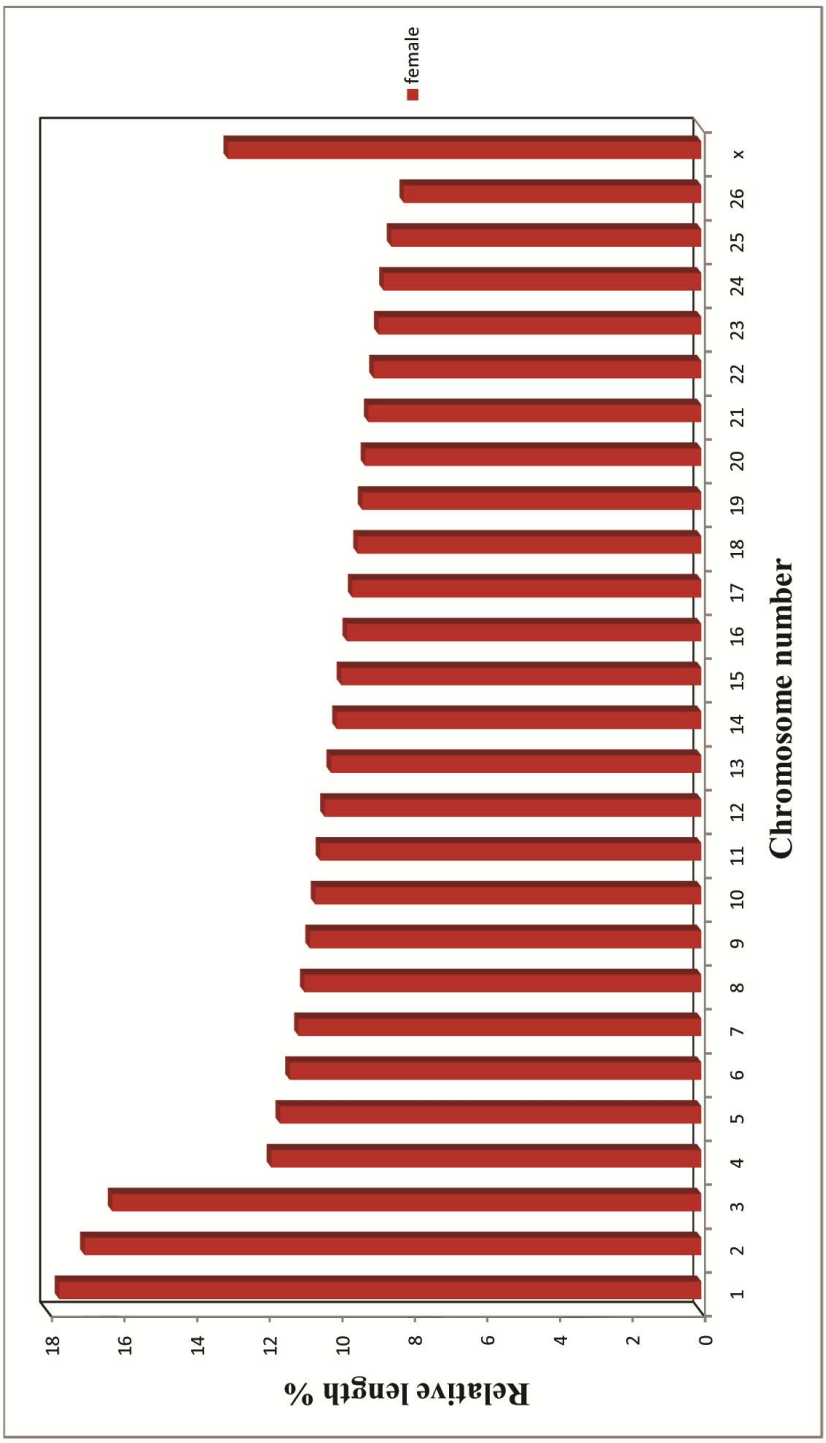

Fig. 9. Idiogram of Macherla Brown Ewe

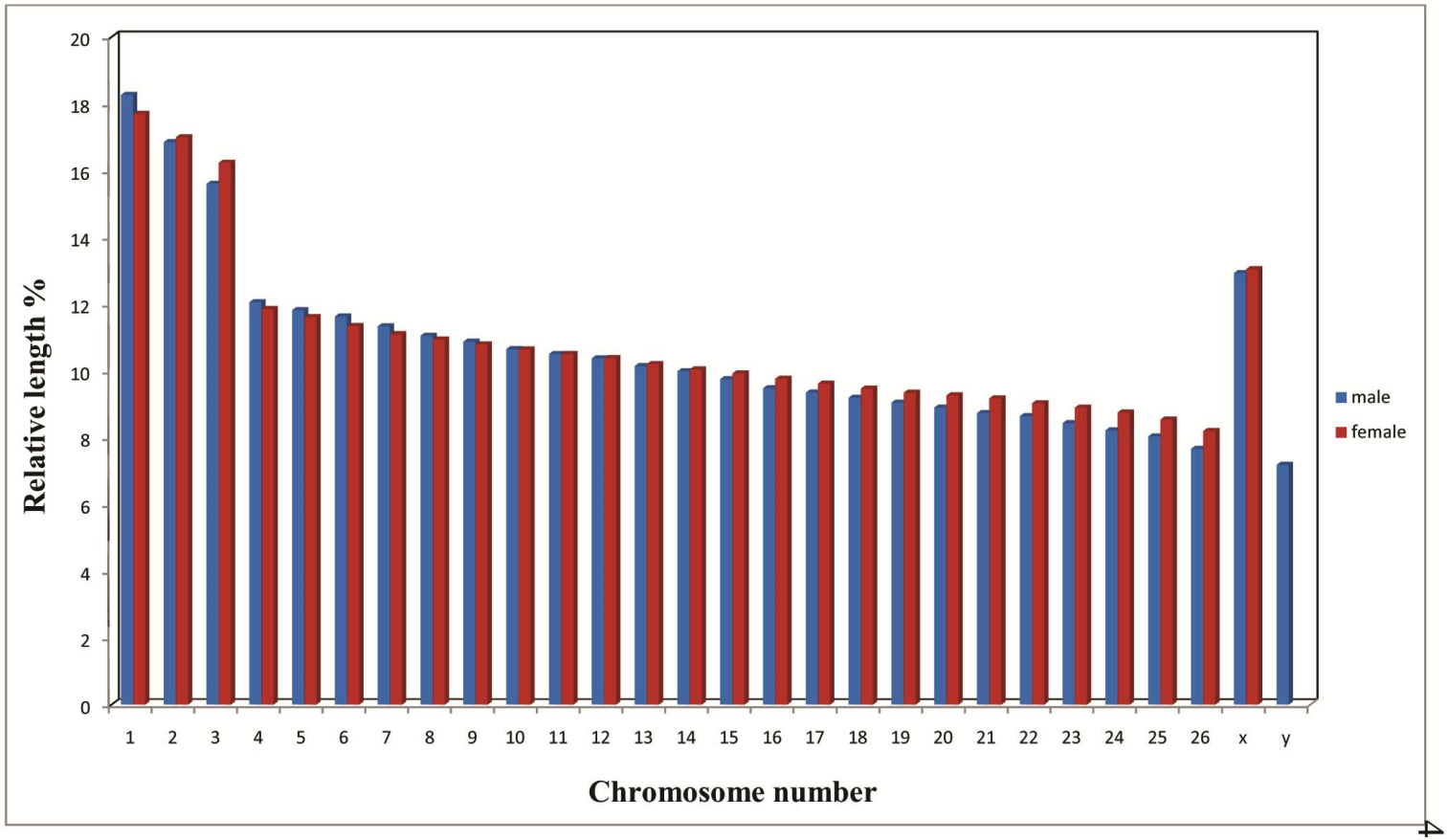

Fig. 10. Idiogram of Macherla Brown Ram and Ewe