CYTOGENETIC CHARACTERIZATION OF OSMANABADI GOAT

by

Navnath Baburao Wagh
(Reg.No. 06/072)

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR
MAHARASHTRA, INDIA

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE AND DAIRY SCIENCE

POST GRADUATE INSTITUTE
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APPROVED BY

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MAHARASHTRA, INDIA
2008

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WAG

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part there of has not been submitted by me or other person to any other University or Institute for a Degree or Diploma

Place : M.P.K.V., Rahuri

Dated : 03 / 06 /2008

(N.B. Wagh)

Dr. U.Y. Bhoite
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Department of Animal Science
and Dairy Science
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Rahuri – 413 722, Dist. Ahmednagar,
Maharashtra State, INDIA

CERTIFICATE

This is to certify that the thesis entitled, **"CYTOGENETIC CHARACTERIZATION OF OSMANABADI GOAT"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (M.S.) in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** (AGRICULTURE) in **ANIMAL SCIENCE**, embodies the results of a piece of *bona fide* research work carried out by **MR. NAVNATH BABURAO WAGH,** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or publication in other form.

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

Place : M.P.K.V., Rahuri

Dated : 4 /06/2008

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Research Guide

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Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra State, INDIA

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Place : M.P.K.V., Rahuri
Dated : 9/6 /2008

(R.S. Patil)

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Words are too meagre to express my esteem indebtedness and whole hearted sense of gratitude toward my honourable Research Guide and Chairman of my Advisory Committee Dr. U.Y. Bhoite, Associate Professor, Department of Animal Science and Dairy Science, M.P.K.V., Rahuri. It is my pleasure beyond words to express my deep sense of feeling for his inspiring guidance, generous encouragement, well versed advice. Really, fortunate I am and I feel extremely honoured for the opportunity conferred upon me to work under his perpetual motivation.

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LIST OF ABBREVIATIONS

^0C	:	Degree celsius
et al.	:	Etalia and others
GDW	:	Glass distilled water
g	:	Gram
i.e.	:	Idest, that is
lbs	:	Pounds
mg	:	Milligram
ml	:	Millilitre
mm	:	Millimeter
rpm	:	Revolution per minute
S.E.	:	Standard error
viz.	:	Vide licet, Namely
%	:	Per cent
µ	:	Micron
µg	:	Microgram
/	:	Per
+	:	Plus
-	:	Minus

ABSTRACT

CYTOGENETIC CHARACTERIZATION OF OSMANABADI GOAT

BY

Wagh Navnath Baburao

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

Animal Science

Mahatma Phule Krishi Vidyapeeth,

Rahuri - 413 722

2008

Research Guide	:	Dr. U.Y. Bhoite
Department	:	Animal Science and Dairy Science

The present investigation entitled, "Cytogenetic characterization of Osmanabadi goat" was carried out at Goat Project, M.P.K.V., Rahuri for the study of karyotype, qualitative attributes of chromosomes and effect of sex on the quantitative attributes of chromosomes of Osmanabadi goat.

In the present study the blood samples of apparently healthy Osmanabadi goat were collected in a 10 ml capacity vacutainer tubes. Short term whole blood lymphocyte culture technique was used for chromosomal preparation necessary for staining. Karyotypes were established from the photomicrographs taken from several good metaphase spread of chromosomes of

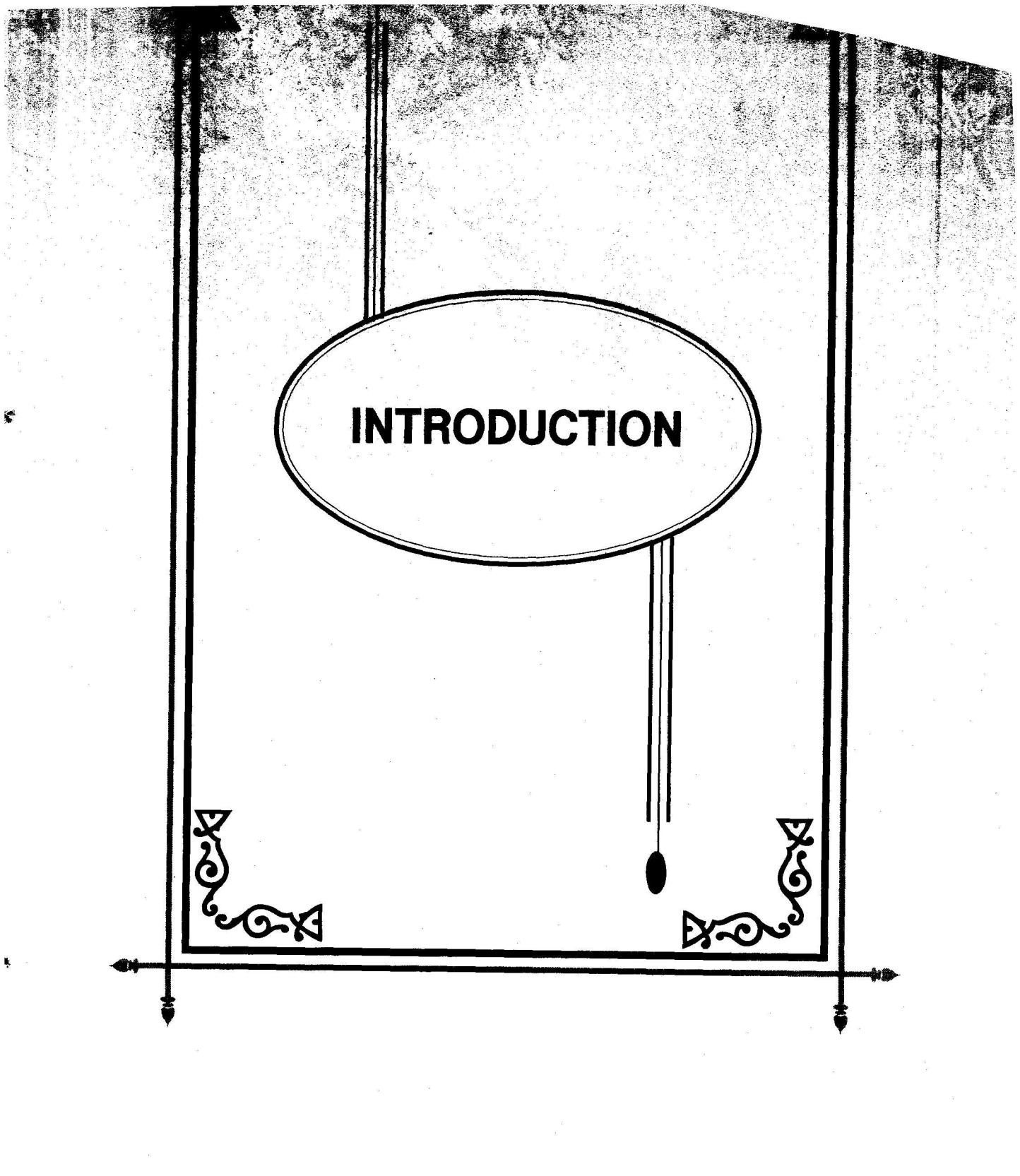
each sex. During the preparation of karyotype of goat, all the chromosome pairs were arranged according to their size. To obtain the idiogram, lengths of chromosomes were measured from karyotypes and relative length of each chromosome pair established as a percentage of total haploid genome.

The chromosome number of Osmanabadi goat was observed as 2n = 60 in both sexes. The normal karyotype was characterized by 29 pairs of autosomes and 1 pair of sex chromosomes. All these autosomes were acrocentric in nature. The male genome differed from the female genome in respect of sex chromosome. The X-chromosome was the largest acrocentric chromosome in both male and female goats and the Y-chromosome was smallest metacentric chromosome in the complement.

The longest autosomes contributed 5.17 and 5.32 per cent and the smallest autosomes 1.88 and 1.76 per cent of the haploid genome in female and male Osmanbadi goats, respectively.

The X-chromosome contributed 5.52 and 5.34 per cent of total haploid genome female and male, respectively, whereas, the Y-chromosome contributed 1.37 per cent only.

There was significant effect of sex on the quantitative attributes of chromosomes of goats.


INTRODUCTION

1. INTRODUCTION

India is one of the largest agricultural country in the world. It is rich in flora and fauna and also in livestock population. Goat (*Capra hircus*) remains an important animal in the national economy and plays a significant role in animal husbandry. India has 1/6th of the world goat population. The total goat population of the world is 807.24 million, out of which India alone possesses 124.36 million goats (FAO, 2005). The total goat population of Maharashtra state is 1.31 million. The goat population has increased at an annual rate of 3.5 per cent.

Goat was the earliest ruminant domesticated around 9000 to 7000 B.C. Presently there are about 102 descript breeds and types of goat in the world and 95 per cent of them are in developing countries. India is having 20 well-defined goat breeds. The flexible integration of goat into dissimilar socio-economic situation resulted in its high economic viability. Goat has been described as a 'Poor mans cow' by father of nation, 'Mahatma Gandhi'. One tenacious ability of goat, which is associated with their wide distribution especially in tropical climate, ability to thrive in harsh environment and ability to live under most adverse conditions of natural habitat, where the rainfall is often below 70 mm. In these situations, they are usually in excellent constitution

compared to other ruminants especially sheep and cattle (Prabhaharan, 2002).

The different Indian breeds of goat *viz.*, Beetal, Black Bengal, Chegu, Ganjam, Jamnapari, Kashmiri, Mehasana, Osmanabadi and Surti are possibly evolved from wild goat (Lall, 1982). Osmanabadi goat is one of the major Indian goat breed found in Osmanabad, Aurangabad, Parbhani and other district of Marathwada region (Chavan *et al.*, 1975). Multiple births, an economically important character is associated in local goats of Maharashtra also. Osmanabadi goats are superior for multiple birth percentage than Angora or cross bred goats (Lawar *et al.*, 1992). In Maharashtra, Osmanabadi and Sangamneri are recognized breeds of goat.

Genetics is the science which deals with heredity and may be studied in various ways. Cytogenetics is one of the branches of genetics which deals with the structure and properties of chromosomes, chromosomal behaviour during somatic cell division (mitosis) and germ cell division (meiosis) in reproduction. Animal cytogenetics is useful for enhancing reproductive performance, genetic capability and management and care in health of all classes of domestic animals. The genetic characterization of native breeds is a major pre-requisite in breed conservation as well as improvement programs.

The chromosomes are the vehicles of genes, which control all biological activities. The length of individual chromosome is one of the criteria used for grouping and identification of chromosomes. The study of chromosome and their abnormalities plays an ever-increasing role in the investigation of disease in man and animal. The number, shape and relative size of chromosome are characteristics of breed or species, but vary between species.

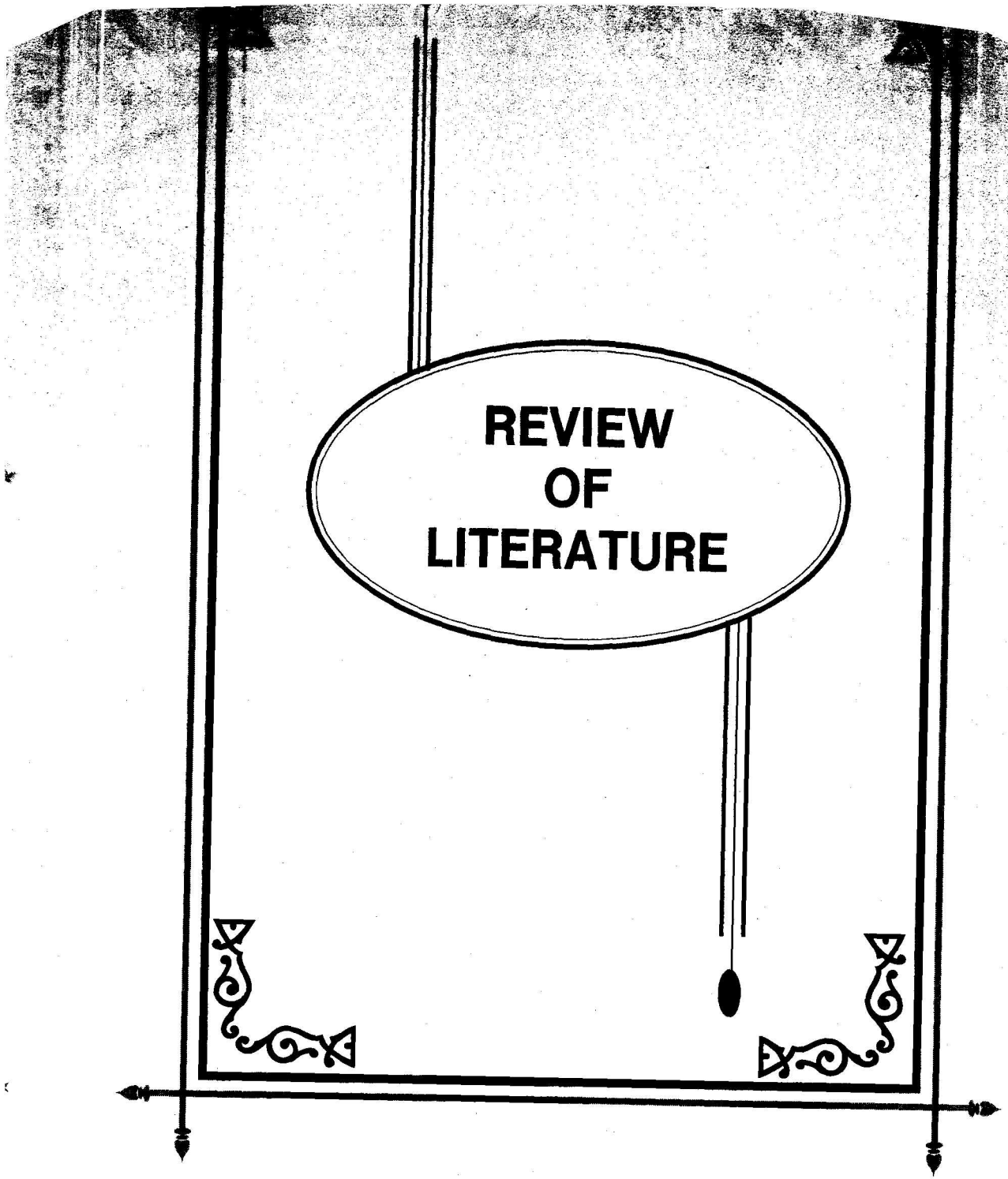
Recent genome mapping programme in human being has evoked the interest in genome mapping in farm animals too. Prerequisite for this is karyotyping, as chromosome are the link between generations. The chromosome complements vary in the length and hence breed identification on the basis of length measurement may be useful. The modern cytological investigations have already found many clinical applications both in medical and veterinary science (Yadav *et al.* 1986).

The diploid chromosome number in goat is 60. The chromosomal profile remains unchanged from generation to generation irrespective of time, provided heredity material is not subjected to the genotoxic effect of environment. The animal improvement programme got momentum in 1960 with the newer approaches of genetics and cytogenetics. Chromosome profile will be identified in most of the species, breeds and types of animals in next decades and subsequently molecular technologies will be added to the armory of scientist

for characterization of animal and breeds, diagnosis of disease and cause of anatomical and physiological defects (Yadav, 2003).

Establishment of standard karyotype will be of great use for detecting numerical and structural chromosomal aberrations and establishing cytotaxonomic relationship among caprini. Variations between chromosomal pattern of different breeds of goats would be useful to carry out karyotypic studies of various available breeds of goats. Genetic evaluation and characterization for establishing standard norms of breed, study on cytogenetic characterization of breeding including model karyotype, relative length of chromosomes and centromeric index of breed is essential. In view of the above, the present study was undertaken to characterize the Osmanabadi breed by cytogenetic parameters with the following objectives :-

1. Karyotyping of Osmanabadi goat.
2. Study of qualitative attributes of chromosomes.
3. Effect of sex on the quantitative attributes of chromosomes.


REVIEW
OF
LITERATURE

2. REVIEW OF LITERATURE

Chromosomes are the nuclear components of special organization individuality and function. They are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species. Chromosome studies have attracted the attention of various research workers. In this chapter an attempt has been made to review some important and related literature available on the chromosome preparation, chromosome number, chromosome morphology and are presented below.

2.1 Procedure for chromosome preparation

Moorhead *et al.* (1960) developed a simple method of culturing lymphocytes from human peripheral blood.

Borland (1964) used bone marrow preparations directly to study sheep chromosome.

Halnan (1977) described an improved technique for the preparation of chromosomes from cattle whole blood.

Benjamin and Bhat (1978) employed cell-culture technique to obtain the karyotypes of sheep chromosome.

Rathnasabapathy and Ganesh (1980) used white blood cell culture technique for display of chromosome in buffalo and found 90 per cent successful results.

Yadav and Balakrishnan (1985) derived the modified medium for lymphocyte culture and investigated the

successful use of adult cattle serum for lymphocyte culture of different mammalian species.

Umrikar *et al.* (1997) analyzed chromosome obtained from the peripheral blood of inter sex goats revealing the presence of 60XX/60XY chromosome complement with predominance of female cells.

Yadav (2003) narrated the methods of chromosome preparation and their identification with the banding patterns by using short-term whole blood culture technique for chromosome preparations.

2.2 Chromosome number

Sokolov (1930) studied the spermatogonial cells and reported the chromosome number in goat as 60.

Berry (1938) recorded the number of chromosomes as 60, 54 and 57 from the cells of amnion of 30 day embryos of the Angora goat, Merino sheep and Angora x Merino hybrid.

Makino (1943) confirmed the diploid count of chromosomes as 60 in *Capra hircus*.

Borland (1964) recorded the diploid number of chromosomes in sheep to be 54 in 40 normal animals of various breeds studied in Australia.

Hauschteck and Meili (1967) reported 60 number of chromosomes in *Capra ibex*.

Makino *et al.* (1967) revised study of the chromosome in goat and sheep and found chromosome number as 60 and 54, respectively.

Benjamin and Bhat (1978) found the 54 diploid number of chromosomes in Muzaffarnagri and Dorset x Muzaffarnagri sheep breed.

Pattnanayak and Patro (1986) conducted cytological investigation in Ganjam, Black Bengal and Ganjam x Black Bengal F_1 crossbreds and found chromosome number as 60.

Prakash (1986) studied the chromosomal complements of Jamunapari and Barbari goats and observed diploid number of chromosomes as 60.

Bhatia and Shanker (1989) confirmed the chromosomes of Nali sheep and exhibited the diploid number of 54 chromosomes in males and females.

Bhatia and Shanker (1989) analyzed the chromosome of peripheral leukocytes of Bengal goat and found the chromosome number as 60.

Bhat and Rawat (1990) analyzed chromosome of Pashmina, Barbari, Jamunapari and Black Bengal goats and demonstrated a common fundamental number as 60.

Bhatia and Shanker (1991) conducted cytogenetic analysis of Gaddi goats and observed diploid number of 60 chromosomes.

Bhatia and Shanker (1992) investigated the chromosomal profile of White Bengal goat and reported diploid number of chromosome as 60.

Bhatia and Shanker (1994) carried out cytogenetic studies in Munjal sheep and observed the diploid number of chromosomes as 54.

Deb and Biswas (1995) reported diploid number of 60 chromosomes in both the sexes of Pashmina goats.

Gupta and Gupta (1995) undertaken study on karyotype of Malpura sheep and found diploid count to be 54 chromosomes.

Bhand *et al.* (1998) confirmed the diploid count of chromosomes as 60 in Surti X Saanen crossbred goats.

Chen and Chen (1999) analyzed cytogenetics of Niutui goat. They observed the diploid number of chromosome as 60.

Sakaram *et al.* (2003) studied cytogenetic character of local nondescript goat of Rohilkhand region and Black Bengal goats and noticed diploid number of chromosome as 60.

Amareswar *et al.* (2005) carried out cytogenetic characterization of Nellore sheep and found diploid chromosome number as 54.

Karunanithi *et al.* (2005) conducted cytogenetic studied in Mecheri sheep of Tamil Nadu and showed that the complement consisted of a diploid count of 54 chromosomes in animals of both the sexes.

Prakash and Singh (2008) found 60 diploid number of chromosomes in the domestic goats (*Capra hircus*).

2.3 Chromosome morphology

Sokolov (1930) differentiated X and Y chromosome in goat. The X chromosome observed was rod shaped while Y chromosome spherical.

Borland (1964) studied the sheep chromosome and observed 6 large metacentric and 48 acrocentric chromosomes. The small dot like chromosome seen only in cells from male sheep was considered to be the Y chromosome.

Basrur and Stoltz (1967) studied the morphological characteristics of the Y chromosome of the male goat and observed that the Y chromosome was smallest (1.0μ) in total length and metacentric among the goat complement.

Makino *et al.* (1967) analyzed the chromosome of goat and found that the autosomes of the goat form a closely graded series of acrocentric pairs. The X chromosome was the largest element of acrocentric nature while, the Y chromosome was the smallest chromosome element of metacentric structure.

Hauschteck and Meili (1967) stated that all chromosomes of *Capra ibex* were acrocentric. The Y chromosome was the only element recognized individually in all mitosis. It was distinctly smaller than any autosome and chromatids were not spread apart as characteristics of all the other chromosomes. In these features *Capra ibex* and *Capra hircus* were identical to each other. The idiograms of the two species showed that the chromosomes 18 to 21 and 4 to 44

were different. The second largest chromosome set of *Capra ibex* was the X chromosome. It was taken into account that the length of the male set was shorter compared with the female set by the difference of X minus Y.

Bunch *et al.* (1977) undertaken the study on karyotype of two Aoudad goat hybrids and found that karyotype comprise of 1 unpaired metacentric, 2 unpaired and 27 paired acrocentric autosomes. They reported that the two unpaired acrocentrics chromosomes were the largest and the X chromosome was the second largest to the acrocentric chromosome. The mean relative lengths of the first four acrocentric autosomes in descending order were 51.18, 44.90, 44.68 and 43.09, respectively. The mean lengths of the unpaired acrocentrics and unpaired metacentric were 95.86 and 93.25 respectively.

Benjamin and Bhat (1978) revealed that the karyotype of Muzaffarnagri sheep consisted of 6 metacentric, and 46 telocentric autosomes and 2 large sex chromosomes. The Dorset x Muzaffarnagri ram had 54 chromosomes in which the Y chromosome was the smallest metacentric chromosome.

Ford *et al.* (1980) found that in banded karyotype of goat Y chromosome was minute metacentric with dark band adjacent to centromere on one arm and X chromosome was minute stained short arm with centromeric region moderately stained.

Pattnanayak and Patro (1986) conducted cytological investigation in Ganjam, Black Bengal and Ganjam x Black Bengal goats and reported that all the 58 autosomes and X chromosomes were telocentric while, Y chromosome was the smallest in the chromosome complement of goats.

Prakash (1986) studied the chromosomal complements of Jamunapari and Barbari goats and reported that all the 29 pairs of autosomes were acrocentric in their morphology. The X chromosome was acrocentric and the Y chromosome was submetacentric. The X chromosome contributed over 5% to the total complement length and the Y chromosome accounted for even less than 1% (0.80%) of complement length of chromosomes.

Berardino *et al.* (1987) analyzed the R-banding pattern of the prometaphase chromosomes of the goat and inferred that the X chromosome was one of the largest acrocentrics of karyotype and Y chromosome was the smallest chromosome of the karyotype and only metacentric one.

Bhatia and Shanker (1989) confirmed the chromosomes of Nali sheep and exhibited that the karyotype comprise 3 pairs of metacentric and 23 pairs of acrocentric autosome. The X chromosome was largest of the acrocentric numbers and Y chromosome appeared as small biarmed chromosome.

Bhat and Rawat (1990) analyzed the chromosomes of Pashmina, Barbari, Jamunapari and Black Bengal goats

and demonstrated that the chromosome complement comprised 29 pairs of acrocentric autosomes. The X chromosome was acrocentric and Y chromosome was very small metacentric.

The results of morphology of Gaddi goat and Bengal goat is given in tabulated form (Table 2.1). In Gaddi goat the largest autosome contributed 4.60 and 4.67 per cent and smallest 2.18 and 2.14 per cent of haploid genome in female and male animals, respectively. The X chromosome accounted for about 5 per cent and Y chromosome about 2 per cent of chromosomal complement length.

In White Bengal goat the longest autosome contributed 4.99% and the smallest 1.87% and the X chromosome accounted for about 5 per cent whereas Y chromosome about 1.63% of the total genome.

Table 1. Chromosomal morphology of goat

Particulars	**Species and Breed**		
	Goat		
	Black Bengal	**Gaddi**	**White Bengal**
Autosome	29 pairs acrocentric	29 pairs acrocentric	29 pairs acrocentric
X chromosome	Largest acrocentric	Largest acrocentric	Largest acrocentric
Y chromosome	Smallest metacentric	Smallest metacentric	Biarmed acrocentric
Total (2n)	60	60	60
Reference	Bhatia and Shanker (1989a)	Bhatia and Shanker (1991)	Bhatia and Shanker (1992)

Prakash and Balaine (1992) analyzed eight Indian goat breeds having 29 pairs of acrocentric autosomes. They reported that the X chromosome was largest acrocentric and Y chromosome was smallest submetacentric in all the goat breeds studied.

Bhatia and Shanker (1994) evaluated chromosome of Munjal sheep and noticed that the largest chromosome was X chromosome and smallest biarmed was Y chromosome. For the four types of chromosome *viz.*, biarmed autosomes acrocentric autosome, X and Y chromosomes the relative lengths were 7.59 to 9.48, 1.81 to 4.71, 5.10 and 1.45 per cent, respectively.

Deb and Biswas (1995) studied the chromosome complements of Pashmina goats and reported that Chegu goat had 58 acrocentric autosomes. The X chromosome was the longest acrocentric chromosome in both male and female goats and Y chromosome was the smallest in the karyotype and looked dot-like structure. They found relative length of autosomes in male and female goats ranged from 5.15 to 1.81 per cent and 4.87 to 1.71 per cent, respectively. The X chromosome contributed 6.30 and 5.34 per cent of the total haploid genome in male and female, respectively. The Y-chromosome contributed 1.59 per cent of the chromosomal complement length.

Umrikar *et al.* (1995) conducted Q and C banding based karyotype in Indian domestic goats and reported that

the X chromosome was one of the largest acrocentric and Y chromosome was the smallest and showed intense fluorescence.

Gupta and Gupta (1995) undertaken study on karyotype of Malpura sheep and found that the arm ratios of largest biarmed chromosome in male and female as 1.231, 1.222, 1.130 and 1.238, 1.198, 1.125, respectively. The centromeric indices of these metacentric autosomes ranged between 44.90 and 47.03 in male and 44.78 to 47.12 in female animals, respectively. The relative length of chromosome no. 1 was 9.377 in male and 9.373 in female. The X chromosome was the longest and acrocentric and relative length in male and female was 4.866 per cent and 4.868 per cent. The Y chromosome was very small metacentric type.

Hyun *et al.* (1999) found that the karyotype of Korean native goat (*Capra hircus*) composed of 58 autosomes and all the autosomes were acrocentric. The X chromosome was submetacentric and Y chromosome was metacentric.

Anna *et al.* (2002) reported the relative length of X chromosome of the haploid set of autosomes in cattle, sheep and goat as 6.11, 5.27 and 5.03 per cent, respectively.

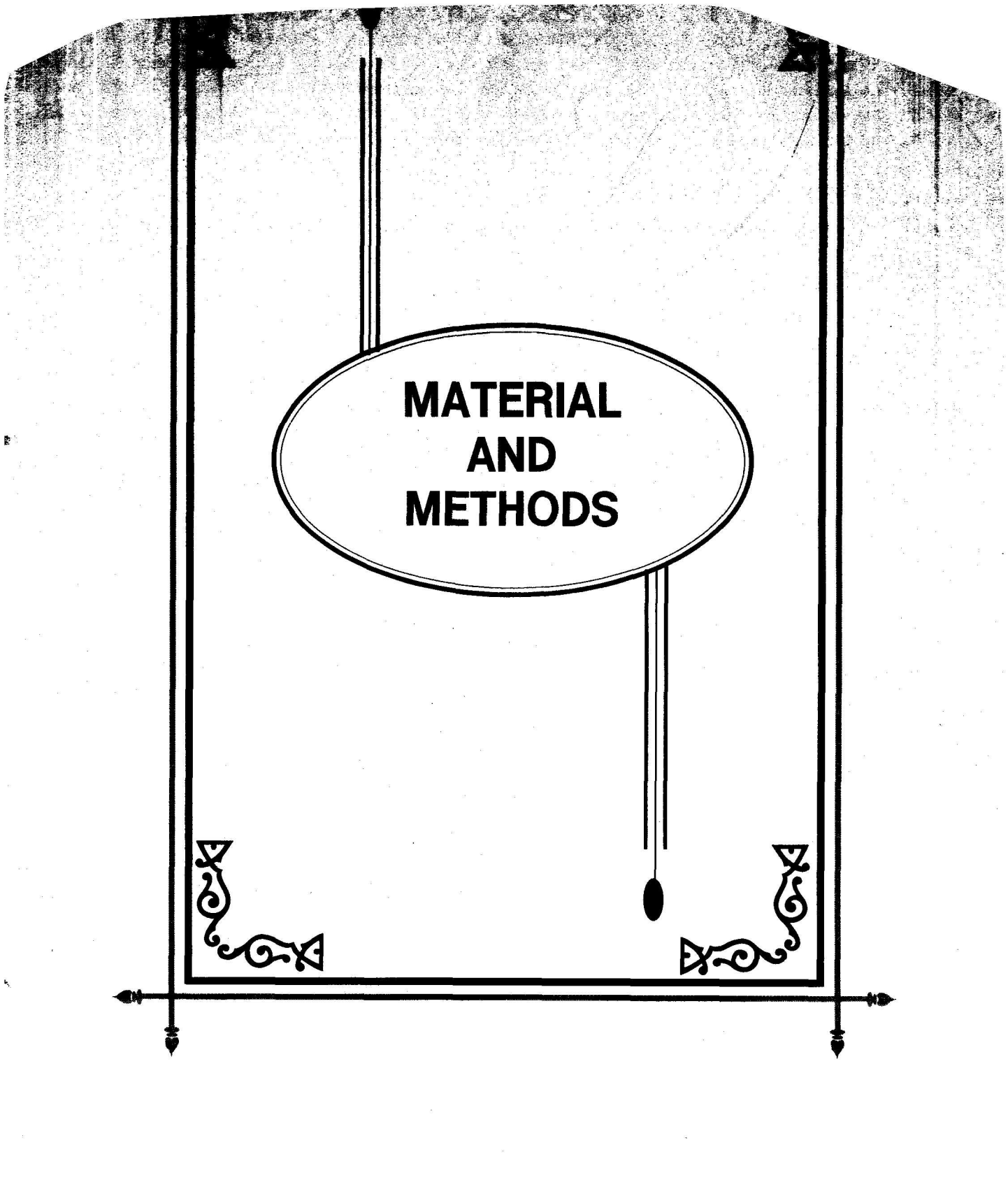
Sakaram *et al.* (2003) revealed that the relative length of chromosomal complement was in the range of 4.96 $\pm$ 0.12 to 2.22 $\pm$ 0.06 in local goats and 5.25 $\pm$ 0.32 to 2.04 $\pm$ 0.12 per cent in Black Bengal goats.

Amareswar *et al.* (2005) conducted cytogenetic characterization of Nellore sheep and reported that the first 3 pairs of autosomes were submetacentric and the remaining 23 pairs as acrocentric in appearance. They noticed the mean relative length of the autosomes in Nellore Palla, Brown and Jodipi varieties in the range of 1.68 to 9.92, 1.73 to 9.73 and 1.75 to 9.50 per cent. The X chromosomes contributed 5.08, 5.03 and 5.10 per cent to total genome in males and 5.32, 5.11 and 5.23 per cent in females, respectively. They observed the X chromosome as the largest acrocentric and the Y chromosome as the smallest biarmed metacentric in appearance.

Karunanithi *et al.* (2005) studied chromosomal profile of Mecheri sheep and reported that the karyotype comprises 3 pairs of metacentric and 23 pairs of acrocentric autosomes. They noticed the length of X chromosome in male and female as 7.10 and 7.90 mm and the length of Y chromosome as 3.30 mm. The relative length of chromosomes ranged between 9.62 to 2.13 per cent in males and 9.62 to 2.09 per cent in females.

Meo *et al.* (2005) performed comparative FISH mapping among Y chromosome of zebu cattle, river buffalo, sheep and goat and found Y chromosome as small metacentric.

Prakash and Singh (2008) evaluated the karyotype of the domestic goat and found 29 pairs of acrocentric autosomes. They found that the X-chromosome was largest acrocentric and Y-chromosome was smallest metacentric in nature.


MATERIAL
AND
METHODS

3. MATERIAL AND METHODS

3.1 Material

The present investigation entitled 'Cytogenetic characterization of Osmanabadi goat' was carried out at 'Goat Project' Mahatma Phule Krishi Vidyapeeth, Rahuri.

3.1.1 Location

Goat Project, Mahatma Phule Krishi Vidyapeeth, Rahuri is located 30 km. North of Ahmednagar on state highway no. 14 and 569 meter above mean sea level on 19°17' to 19°57' North altitude and 74°19' East longitude. The climate is dry and hot with annual average rainfall of 475 mm.

3.1.2 Experimental Animals

For this study apparently healthy 5 bucks and 10 does of Osmanabadi goat were selected randomly.

3.1.3 Blood collection

i. For the study 5-6 ml blood was collected from jugular vein of the animal in a 10 ml capacity vacutainer tube (Becton Dickinson, USA), containing 143 USP units of sodium heparin.

ii. The strict aseptic condition was followed during the blood collection.

iii. The blood was gently mixed with heparin by tilting the vacutainer tube.

iv. The blood samples were transported to the laboratory in a double jacketed ice container at 5^{0}C and kept in the refrigerator at 4^{0}C till culture set up.

The cytogenetic work and analysis was carried out at Biotechnology Research Centre, M.P.K.V., Rahuri.

3.2 Methods

The methodology adopted in this study is being described under the following sections.

3.2.1 Preparation of glasswares

3.2.1.1 Cleaning and rinsing

The extensive and careful preparation of glassware is a key to successful lymphocyte culture technique. The glasswares, bottles etc. were soaked in nontoxic detergent solution (labolene) for overnight and then washed in running tap water and rinsed with GDW and finally dried in hot air oven at 150^{0}C for 2 hours.

3.2.1.2 Sterilization

i. Sterilization was done by autoclaving all the glasswares and Millipore filter assembly at 15lbs/inch pressure for 20 minutes.

ii. After autoclaving spirit was sprayed on the outer surface of the equipments and transferred to laminar flow hood under U.V. light till use.

iii. The materials were autoclaved usually by serial arrangements.

- Millipore filter assembly
- About 30-40 culture vials of 30 ml capacity with plastic cap.
- One conical flask with 200 ml GDW
- About 15-20 glass syringes (2 ml capacity) with needles (gauge no. 23) and
- Two plastic syringe of 50 ml capacity.

3.2.2 Preparation of culture medium

The following constituents were added to the conical flask containing 200 ml autoclaved water.

- TC-199 medium (Hi-media) : 0.218 gm/200 ml
- Poke weed mitogen (Sigma) : 0.5 mg/200 ml
- Penicillin G (sigma) : 2.5 mg/200 ml
- Streptomycin sulphate (Sigma) : 4.0 mg/200 ml

i. The pH of medium was adjusted with 4.4 per cent sterile sodium bicarbonate solution to 7.2 judged by pink colour of the medium.

ii. The medium was filtered through the Millipore filteration assembly by passing it with the help of 50 ml capacity syringe.

iii. The filtered medium was stored at – 20°C till use.

3.2.3 Chromosome preparation

Short-term whole blood lymphocyte culture technique was followed for preparation of chromosome necessary for staining. The technique is briefly described as.

3.2.3.1 Setting up of culture

i. Whole blood culture was established in stored medium (- 20^{0}C).

ii. As the culture medium was in frozen state, the bottles were kept in incubator (37±0.5^{0}C) and brought it to room temperature.

iii. About 4.5 ml medium was disbursed into the culture tubes.

iv. The already collected blood samples in vacutainer tubes were given a mild shaking by inverting the tubes for a few times and the tubecap were cleaned using spirit swab and kept in laminar hood.

v. With the help of sterile glass syringe 0.5 ml blood was taken out from the vacutainer tubes and transferred to the culture tubes containing 4.5 ml of complete culture medium.

vi. The contents were given a gentle swirl for quick mixing.

vii. The cap and mouth of culture tubes were flamed over the spirit lamp before making it airtight to reduce the chances of contamination.

viii. Identification marks like date of inoculation of blood sample, animal number etc. were written on each culture tube and also recorded in the laboratory notebook.

ix. The culture tubes were transferred to an incubator and incubated at 37± 0.5^{0}C for 68-72 hours.

x. The culture tubes were shaken twice a day to improve the dispersion of cells.

3.2.3.2 Harvesting of culture

i. One hour prior to harvesting time, two drops (3.2µg/culture) of colchicines solution were added to each culture tube. Colchicines arrest dividing cells at metaphase stage.

ii. After one hour of addition of colchicines, contents of the culture tube were transferred to 15 ml capacity conical graduated centrifuge tube and then centrifuged at 1500 rpm for 15 minutes.

iii. The supernatant was discarded and the cell pellet was suspended into 10-15 ml hypotonic solution (0.075 M KCl) for 7 minutes.

iv. The hypotonic treatment was terminated by adding 1 ml of freshly prepared fixative (3:1; Methanol: Glacial acetic acid) and then mixed thoroughly using Pasteur pipette.

v. The contents of the tubes were again centrifuged for 10 minutes at 1000 rpm at room temperature.

vi. The supernatant was again discarded and cell pellet was resuspended in 5 ml fixative and centrifuged again for 10 minutes.

vii. The process of addition of fixative and centrifugation was repeated thrice so as to get the clear whitish pellet in the bottom of the centrifuge tube.

viii. After third and last washing the supernatant was removed by Pasteur pipette leaving out 0.5 to 1.5 ml fixative over the whitish cell pellet.

ix. The cells were suspended in the fixative by gentle mixing with Pasteur pipette and were ready for preparation of slides.

3.2.3.3 Preparation of slides by air dry method

i. A clean grease free slide was moistened by blowing air from the mouth and was held at an angle of 30^0 with the top of working table.

ii. Three drops of cell suspension were dropped on the slide by Pasteur pipette from a height of above two feet.

iii. The drops were put on a slide one below the other and allowed to flow evenly, by avoiding the overlapping of drops.

iv. The slides were dried by shaking vigorously in the air and after methanol dried, the remaining droplets of acetic acid were flipped away.

3.2.3.4 Staining of slides with Giemsa

i. For this purpose fresh 2% working solution of Giemsa stain was prepared in a conical flask by taking 94 ml of glass distilled water, 4 ml phosphate buffer (pH 6.8) and 2 ml Geimsa stain solution.

ii. The working solution of Giemsa stain was transferred into the staining jar (coupling jar) where slides were stained for 30 minutes at room temperature.

iii. The extra drops of the stain were removed by rinsing the slide gently in GDW and dried in the folds of filter paper.

iv. The slides were then kept in the incubator for overnight at 37°C for drying before mounting.

3.2.4 Mounting of slides

The slides after proper drying were permanently mounted with DPX mountant by using 22 x 50 mm size cover slips. Permanent mounting improves the clarity of chromosomes. This was dissolved in xylene and used for mounting.

3.2.5 Screening of chromosomal plates (slides)

The slides were screened by using Leitz Wetzlar and Leica microscope (Germany). Twenty five well spread metaphase plates of each animal were examined. The plates with excellent spread and chromosome morphology were selected for photography.

3.2.6 Photomicrography

The selected plates were photographed by using Olympus camera on colour (Kodak 400) as well as black and white films (Nova). A green panchromatic filter was used for black and white photography and a blue green filter for coloured photography.

3.2.7 Karyotype preparation

i. For karyotype preparation, photographs were enlarged and the chromosomes were cut and measured with the help of vernier caliper.

ii. These measured chromosomes were then arranged in a descending order of their size and pasted on a karyotype sheet.

iii. These arranged chromosomes were later photographed and developed as "karyotype" of the both male and female goats.

3.2.8 Measurement of relative length of chromosome

The length of each chromosome was measured from tip to tip on the karyotype with the help of vernier caliper.

3.2.9 Calculations

3.2.9.1 Calculation of mean relative length

i. The length of 2 homologous chromosomes within each pair of autosomes were added and the mean was worked out. In males the lengths of X and Y chromosomes were recorded separately.

ii. The mean length was divided by the length of total haploid genome (including mean of X chromosome in the female and one X and one Y chromosome in male) and expressed as percentage.

iii. Relative length of X chromosome was calculated by using the total haploid length excluding the Y in the male.

3.2.9.2 Calculation of centromeric index

The morphology of chromosome depends on its total length and position of centromere.

$$\text{Centromeric index} = \frac{\text{Length of short arm}}{\text{Chromosome length}} \times 100$$

3.2.10 Construction of ideograms

i. Idiograms were constructed from the average relative length of chromosomes of each sex.

ii. Only one chromosome of each homologous pair was represented in an idiogram.

iii. The bars were arranged in descending order of their relative length, the short arm being uppermost.

3.2.11 Statistical analysis

The mean relative lengths of chromosomes between sexes were compared and analyzed by using 2 sample 't' test with equal variance ($P < 0.05$). The data was subjected to further statistical analysis by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The present cytogenetic study entitled "Cytogenetic characterization of Osmanabadi goat" was undertaken at "Goat Project" M.P.K.V., Rahuri on 15 animals of Osmanabadi goat. The results obtained of the study are given below.

4.1 Chromosome study

Giemsa stained metaphase spread of chromosomes of both the sexes of Osmanabadi goats, obtained by short-term whole blood lymphocyte culture technique were screened and analyzed.

4.1.1 Chromosome number

Twenty five well spread chromosomal plates of each goat were identified, out of which 5 best spread plates per animal were selected for photography. The complete metaphase spread plates were considered for counting the chromosome. The photograph of well spread plates of male and female goats are presented in Fig. 1 and 2.

The Osmanabadi goats exhibited a model diploid number of 60 chromosomes in both the sexes in the present investigation. This observation corroborates well with the normal diploid chromosome number for goat species as reported earlier by Sokolov (1930), Berry (1938), Makino (1943), Hauschteck and Meili (1967), Makino *et al.* (1967), Pattnanayak and Patro (1986) in Ganjam, Black Bengal and Ganjam x Black Bengal goats, Prakash (1986) in Jamunapari

and Barbari goats, Bhatia and Shanker (1989a) in Bengal goat, Bhatia and Shanker (1991) in Gaddi goats, Bhat and Rawat (1990), Bhatia and Shanker (1992) in White Bengal goats, Deb and Biswas (1995) in Pashmina goats, Bhand *et al.* (1998), Chen and Chen (1999) in Niutui goats and Sakaram *et al.* (2003) in local goats of Rohilkhand region and Black Bengal goats.

4.1.2 Chromosome morphology

Careful observation of Osmanabadi goat chromosome spreads indicated that, out of 30 pairs of chromosomes in the genome, 29 pairs were autosomes and one pair was sex chromosome (Fig. 1 and 2). All these autosomes were acrocentric (centromere at the end of chromosome) in nature. The male genome differed from the female genome in respect of sex chromosome.

Similar findings were reported by Prakash (1986), Bhatia and Shanker (1989a), Bhat and Rawat (1990), Bhatia and Shanker (1991), Bhatia and Shanker (1992), Prakash and Balaine (1992), Deb and Biswas (1995) and Hyun *et al.* (1999).

The X-chromosome was the longest acrocentric chromosome in both male and female Osmanabadi goats. These results were in agreement with Makino *et al.* (1967), Prakash (1986), Berardino *et al.* (1987), Bhatia and Shanker (1989a), Bhatia and Shanker (1991),Bhatia and Shanker (1992), Prakash and Balain (1992), Deb and Biswas (1995) and Umrikar *et al.* (1995). However, Hauschteck and Meili

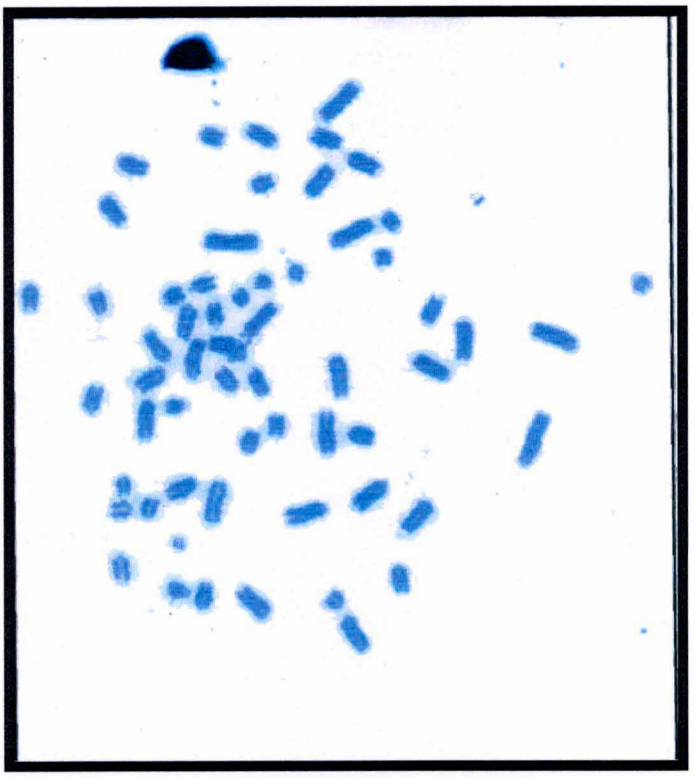


Fig. 1. Conventional stained metaphase plate of Osmanabadi goat - Male

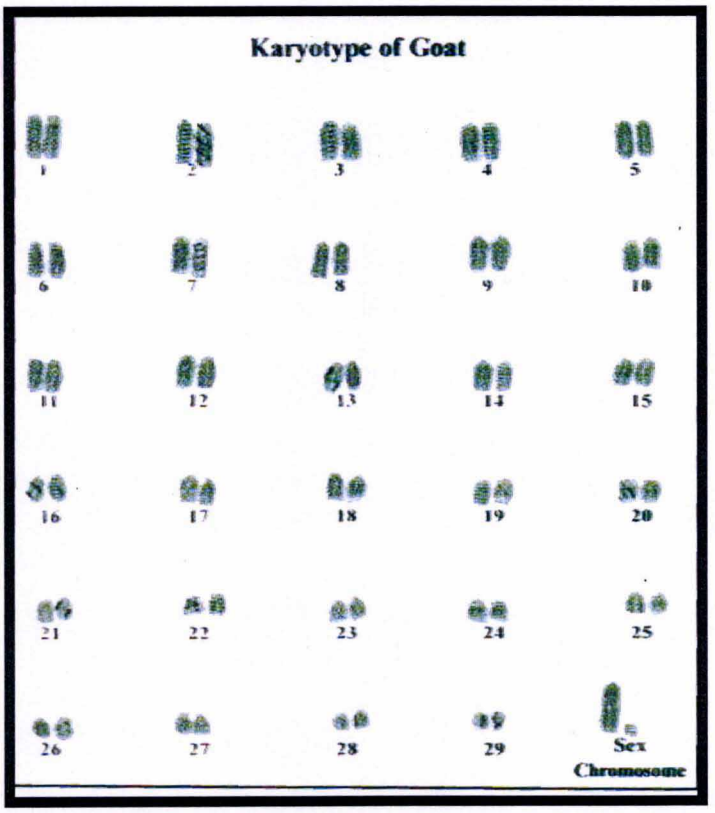


Fig. 3. Karyotype of Osmanabadi goat - Male

Fig. 2. Conventional stained metaphase plate of Osmanabadi goat - Female

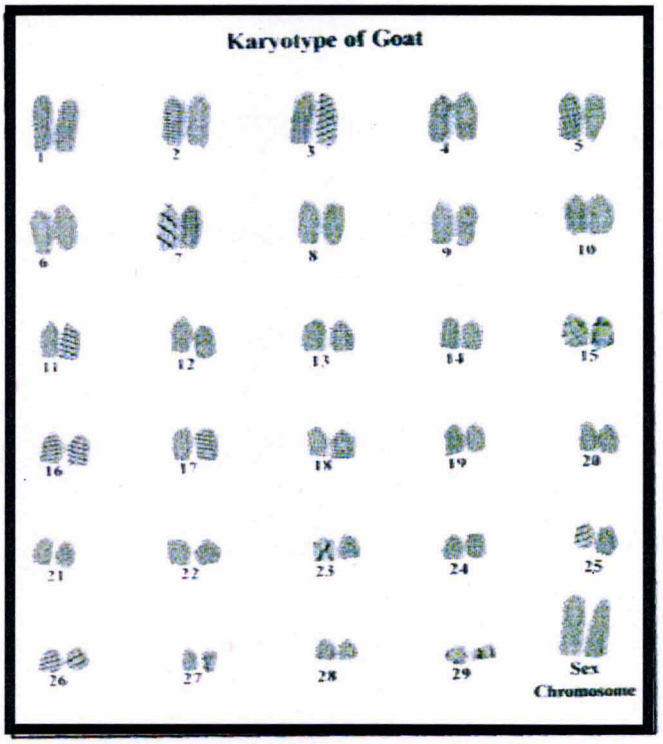


Fig. 4. Karyotype of Osmanabadi goat - Female

(1967) reported X-chromosome as the second largest chromosome. Basrur and Stoltz (1967) failed to distinguish and rank X-chromosome on the basis of length.

In respect of male genome, the Y-chromosome was found to be smallest chromosome and metacentric one. This corroborates with the findings of Basrur and Stoltz (1967), Makino *et al.* (1967), Ford *et al.* (1980), Berardino *et al.* (1987), Bhatia and Shanker (1989a), Bhat and Rawat (1990), Bhatia and Shanker (1991), Hyun *et al.* (1999) and Meo *et al.* (2005).

Prakash (1986) and Prakash and Balaine (1992) observed Y-chromosome to be the smallest submetacentric one. Whereas, Pattanayak and Patro (1986) and Deb and Biswas (1995) found that the Y-chromosome was smallest in the karyotype and dot-like in structure. Bhatia and Shanker (1992) observed polymorphism in the morphology of Y-chromosome of White Bengal animals which exhibited acrocentric as well as biarmed Y-chromosomes.

4.1.3 Karyotyping

During the preparation of karyotype of goat, all the chromosome pairs were arranged according to their size. All autosomes, in both male and female had a gradually decreasing size, however, no difficulty was experienced in placement of autosomes i.e. chromosome pairs from 1 to 29. The karyotypes of male and female are presented in Fig. 3 and 4, respectively. In case of male, the smallest metacentric chromosome was denoted as Y-chromosome, whereas, its

homologue (which being largest and did not paired with any of autosomes) was noted as X-chromosome. The Y-chromosome was found to be biarmed and smaller in size.

4.1.4 Mean relative length (MRL)

The idiograms of male and female Osmanabadi goat chromosomes constructed on the basis of mean relative length of corresponding chromosomes of each sex and are presented in Fig. 5 and 6. The mean relative length of chromosome complements indicated that the longest autosomes contributed 5.17 and 5.32 per cent of haploid genome in female and male goats, respectively (Table 2). These results were in close agreement with Deb and Biswas (1995) reported in Pashmina goats. In the present investigation the mean relative length of the longest autosome obtained in female and male was higher than that reported by Prakash (1986) in Jamunapari and Barbari goats, Bhatia and Shanker (1991) in Gaddi goats and Bhatia and Shanker (1992) in White Bengal goats.

In female and male goats, the mean relative length of the smallest autosomes was 1.88 and 1.76 per cent of total haploid genome. The results obtained in the present study were in close agreement with those reported by Bhatia and Shaker (1992) and Deb and Biswas (1995). However, the values obtained in the present study were lower than that reported by Prakash (1986) and Bhatia and Shanker (1991).

Table 2. Relative lengths of the chromosomes of Osmanabadi goat

Chromosome number	Females		Males	
	M.R.L.	S.E.	M.R.L.	S.E.
1	5.17	0.050	5.32	0.053
2	4.82	0.060	4.75	0.044
3	4.60	0.050	4.48	0.047
4	4.49	0.050	4.35	0.047
5	4.31	0.025	4.25	0.031
6	4.20	0.022	4.18	0.012
7	4.06	0.034	4.02	0.041
8	3.94	0.034	3.94	0.053
9	3.80	0.031	3.87	0.056
10	3.77	0.034	3.58	0.018
11	3.57	0.047	3.51	0.009
12	3.42	0.028	3.31	0.041
13	3.35	0.022	3.27	0.044
14	3.22	0.025	3.21	0.028
15	3.15	0.034	3.14	0.034
16	3.06	0.025	3.05	0.018
17	2.92	0.022	2.91	0.025
18	2.84	0.028	2.82	0.041
19	2.77	0.034	2.76	0.031
20	2.69	0.034	2.68	0.022
21	2.67	0.031	2.57	0.009
22	2.54	0.022	2.49	0.009
23	2.41	0.022	2.35	0.031
24	2.32	0.028	2.30	0.028
25	2.25	0.037	2.22	0.031
26	2.16	0.031	2.15	0.047
27	2.08	0.031	2.01	0.056
28	2.00	0.041	1.92	0.063
29	1.88	0.028	1.76	0.063
X	5.52	0.117	5.34	0.120
Y			1.37	0.088

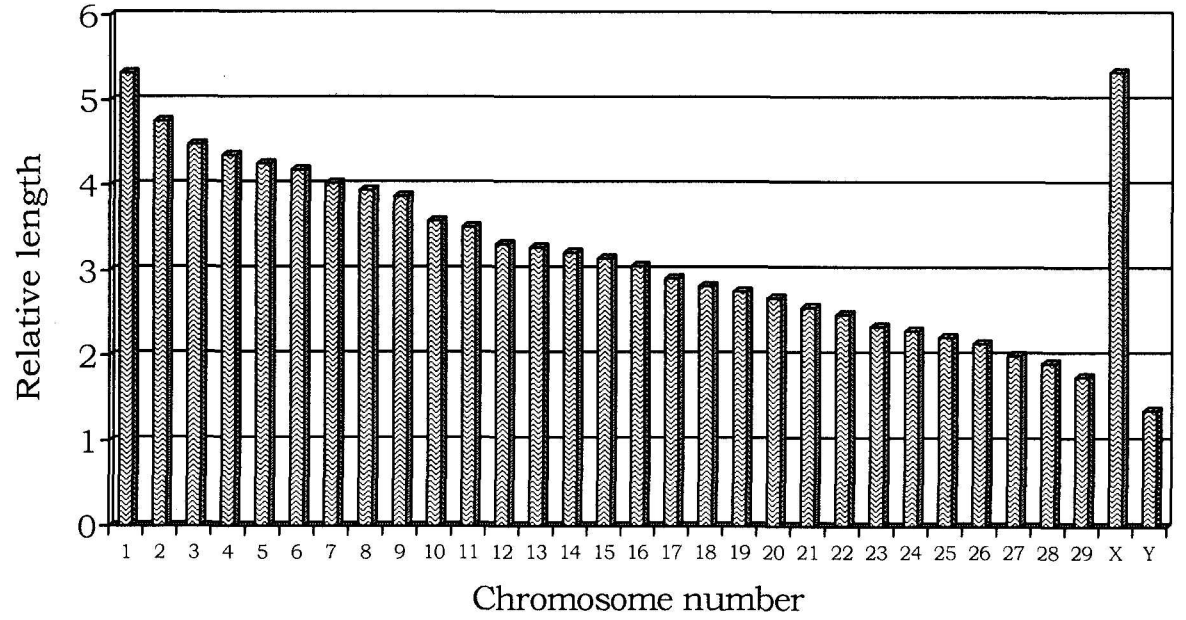


Fig. 5. Idiogram of Osmanabadi goat (male)

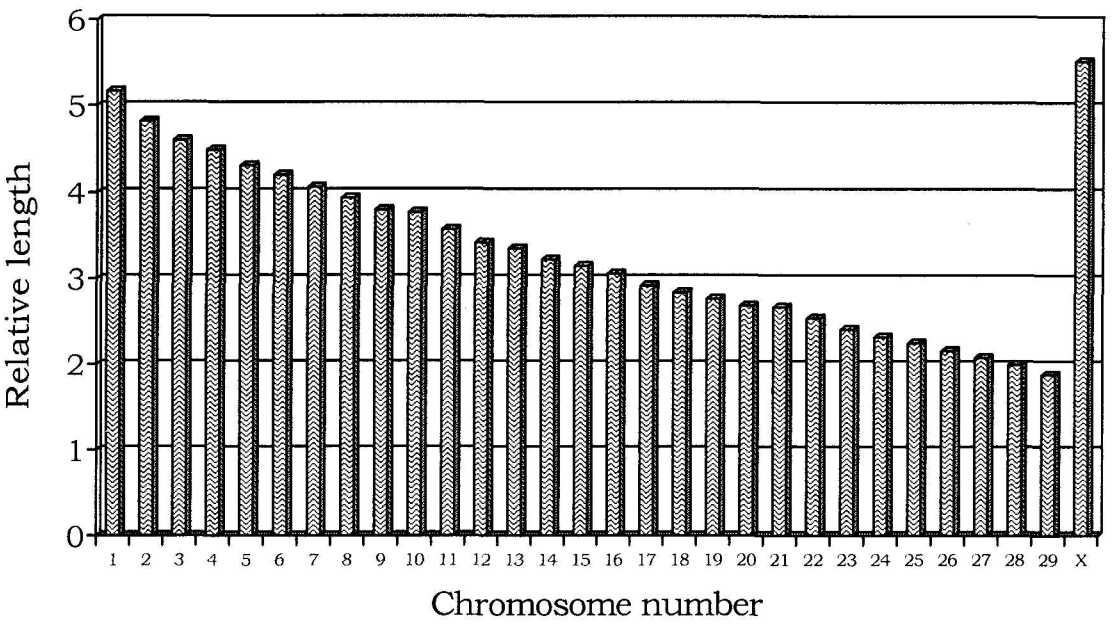


Fig. 6. Idiogram of Osmanabadi goat (Female)

In female and male goats, the mean relative length of X-chromosome contributed 5.52 and 5.34 per cent of the total haploid genome, respectively.

Prakash (1986), Bhatia and Shanker (1991), Bhatia and Shanker (1992) and Prakash and Balain (1992) reported the values of length of X-chromosome were in close agreement with the present findings.

However, these values of length were lower than earlier values reported by Deb and Biswas (1995) in Pashmina goats.

In the present study, the mean relative length of Y-chromosome contributed 1.37 per cent only of the total haploid genome which was in close agreement with Bhatia and Shanker (1991) and Bhatia and Shanker (1992). However, higher value of length of Y-chromosome was observed by Prakash (1986). Whereas, lower values for most goats breeds was reported by Prakash and Balain (1992).

Significant difference ($P < 0.05$) in the mean relative length of chromosome pairs between sexes was observed in Osmanabadi goat except in chromosome no. 8, 14, 15, 16, 17 and 26. Similar significant effect of sex on MRL of chromosomes was reported by Bhatia and Shanker (1992) in White Bengal goats. The non-significant difference in MRL of chromosomes pairs between sexes was observed by Pattnanayak and Patro (1986), Prakash (1986), Bhatia and Shanker (1991) and Deb and Biswas (1995).

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The present investigation entitled, "Cytogenetic characterization of Osmanabadi goat" was undertaken to study the chromosomes of Osmanabadi goat. The results obtained are summarized below.

5.1 Summary

5.1.1 Chromosome study

Giemsa stained metaphase spread of chromosomes of both the sexes of Osmanabadi goats, obtained by short-term whole blood lymphocyte culture technique were screened and analyzed.

5.1.1.1 Chromosome number

Twenty five well spread chromosomal plates of each goat were identified, out of which 5 best spread plates per animal were selected for photography.

The Osmanabadi goats exhibited a model diploid number of 60 chromosomes in both the sexes.

5.1.1.2 Chromosome morphology

The observation of Osmanabadi goat chromosome spreads indicated that, in the genome 29 pairs were autosomes and one pair was sex chromosome. All these autosomes were acrocentric in nature. The male genome differed from female genome in respect of sex chromosome. The Y-chromosome of male was found to be smallest chromosome and metacentric one.

The X-chromosome was the longest acrocentric chromosome in both male and female Osmanabadi goats.

5.1.1.3 Karyotyping

During the preparation of karyotype of goat, all the chromosome pairs were arranged according to their size. All autosomes, both in male and female had a gradually decreasing size, however, no difficulty was experienced in placement of autosomes. In case of male, the smallest metacentric chromosome was denoted as Y-chromosome, whereas, its homologue was noted as X-chromosome. The Y-chromosome was found to be biarmed and smaller in size.

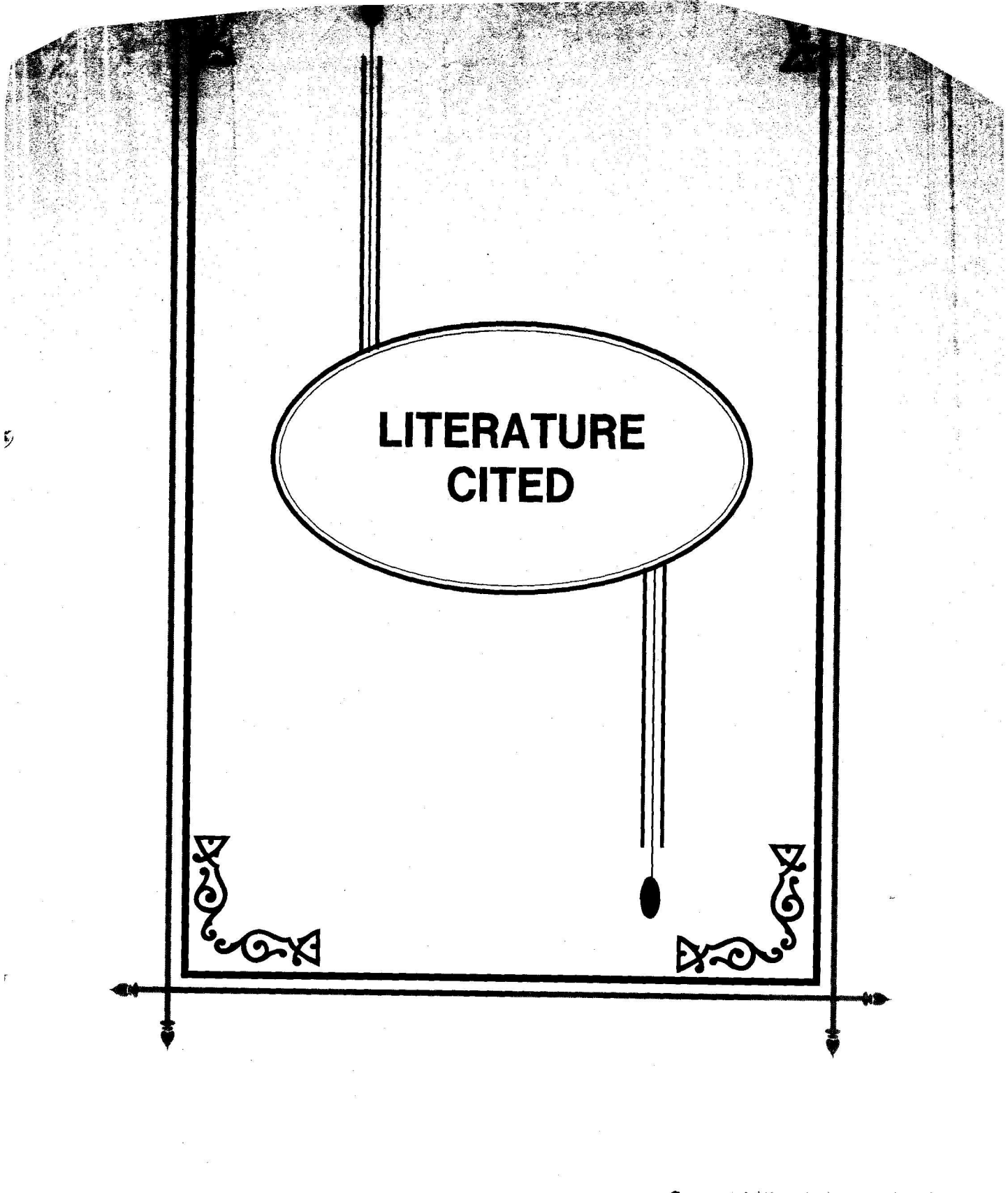
5.1.1.4 Mean relative lengths (MRL)

The idiograms of male and female Osmanabadi goat chromosomes were constructed on the basis of mean relative length of corresponding chromosomes of each sex. The mean relative length of chromosome complements indicated that the longest autosomes contributed 5.17 and 5.32 per cent and the smallest autosomes 1.88 and 1.76 per cent of haploid genome in female and male goats, respectively. The X-chromosome contributed 5.52 and 5.34 per cent of the total haploid genome in female and male, respectively, whereas, the Y-chromosome contributed 1.37 per cent only.

Significant difference in the relative length of chromosome pairs between sexes was observed in Osmanabadi goats.

5.2 Conclusion

1. The Osmanabadi goats exhibited model diploid number of 60 chromosomes in both the sexes.
2. Out of 30 pairs of chromosomes, 29 autosomes pairs were acrocentic in nature.
3. The X-chromosome was the longest acrocentric chromosome in both male and female Osmanabadi goats.
4. The Y-chromosome was smallest metacentric one in nature.
5. There was significant effect of sex on the quantitative attributes of chromosomes of goats.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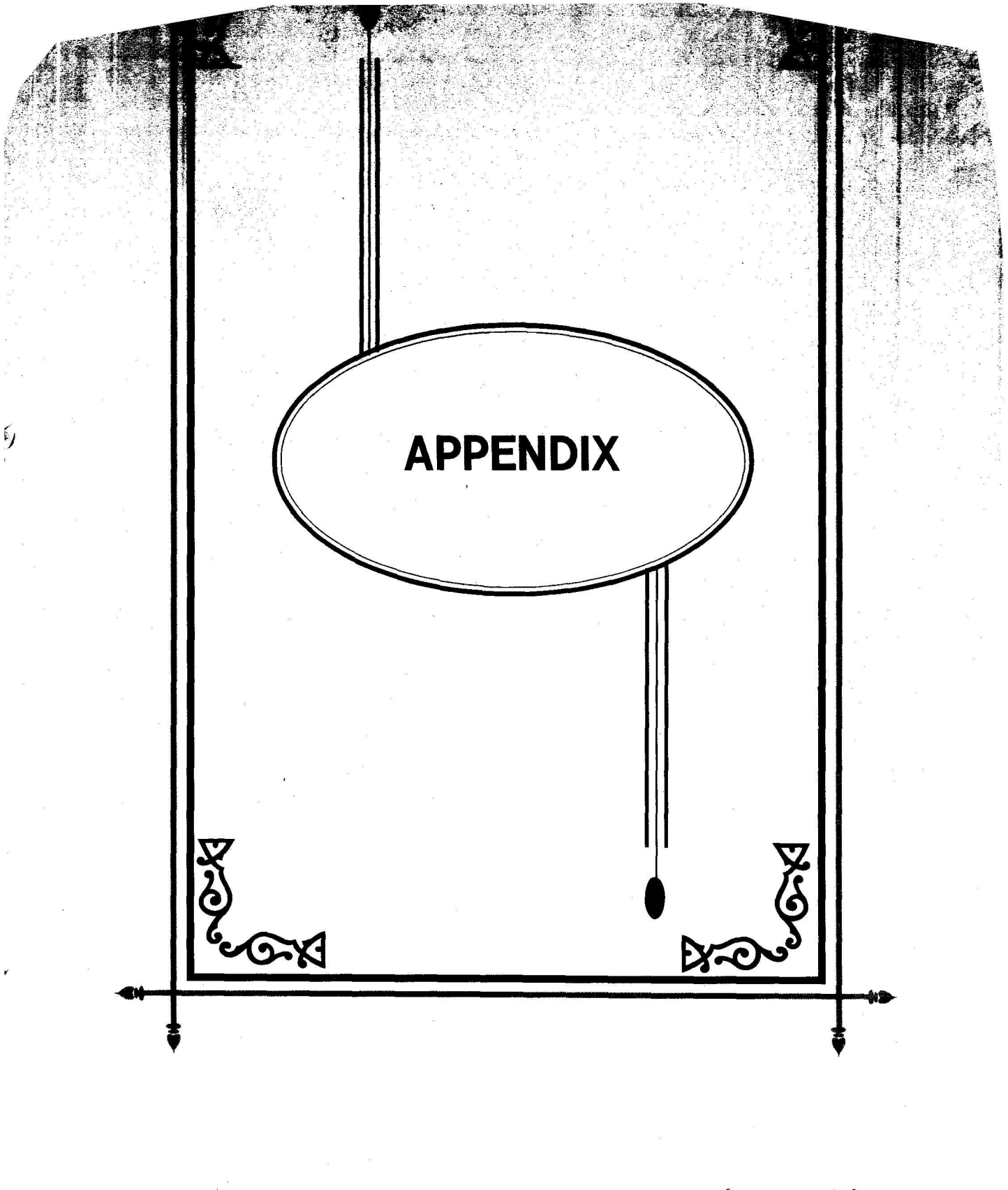

APPENDIX

7. APPENDIX

Reagents / stock solutions

- **Giemsa stain**

Giemsa powder	:	3.8 g
Glycerin	:	250 ml
Methanol	:	250 ml

- **Phosphate buffer**

Solution A

Sodium dihydrogen orthophosphate	:	3.12 g
Glass distilled water	:	500 ml

Solution B

Disodium hydrogen orthophosphate	:	3.56 g
Glass distilled water	:	500 ml

Use 74 ml solution A + 26 ml of solution B + 50 ml GDW

- **Colchicine solution**

Colchicine powder	:	10 mg
Glass distilled water	:	10 ml

Use 1.0 ml stock solution in 24 ml distilled water

- **Sodium bicarbonate solution (4.4 %)**

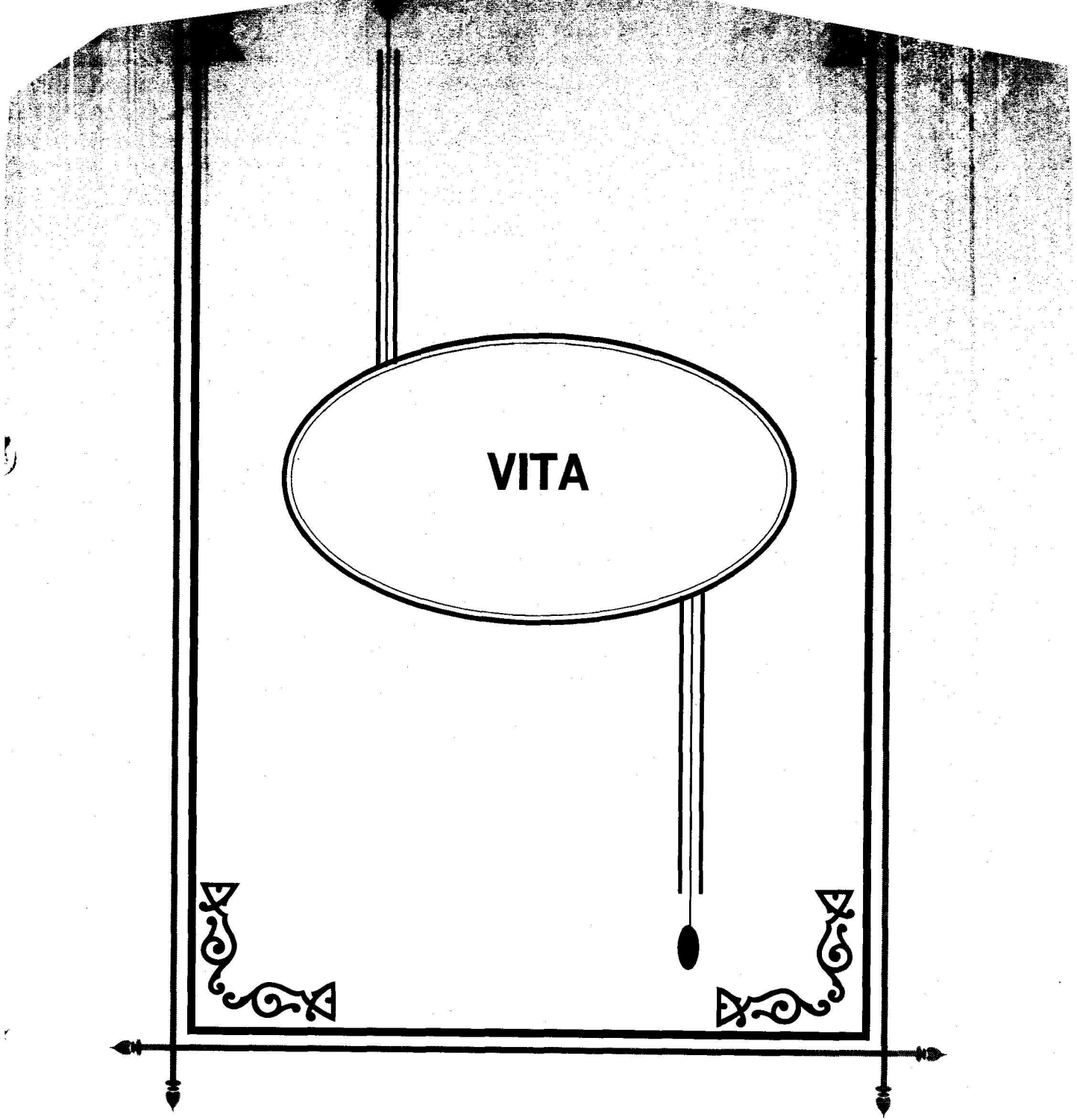
Sodium bicarbonate powder	:	0.88 g
Glass distilled water	:	20 ml

- **Fixative**

Methanol absolute (3 parts)	:	75 ml
Acetic acid-glacial (1 part)	:	25 ml

- **Hypotonic solution**

Potassium chloride	:	0.559 g
Glass distilled water	:	100 ml


VITA

8. VITA

NAVNATH BABURAO WAGH

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

Title of thesis : Cytogenetic characterization of Osmanabadi goat.

Major field : Animal Science

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