

**SEX CHROMATIN STUDIES
IN GOATS AND BUFFALOES**

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN

DAIRYING

(ANIMAL GENETICS AND BREEDING)

TO THE KURUKSHETRA UNIVERSITY, KURUKSHETRA

By

VINITA KALRA

DIVISION OF DAIRY CATTLE GENETICS & BREEDING

NATIONAL DAIRY RESEARCH INSTITUTE

(Indian Council of Agricultural Research)

KARNAL (Haryana) INDIA

1981

Regd. No. 76-VB-485

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Dedicated to my Parents

**S.K.Bhatia
Scientist-1**

**DIVISION OF DAIRY CATTLE GENETICS
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)**

KARNAL, MAY 30th, 1981.

**This is to certify that the work reported
in this dissertation entitled "SEX CHROMATIN STUDIES IN
GOATS AND BUFFALOES," was carried out by Vinita Kalra,
under my supervision, in partial fulfilment of the
requirements for the Degree of MASTER OF SCIENCE in
DAIRYING (ANIMAL GENETICS & BREEDING) of the Kurukshetra
University, Kurukshetra.**


(S.K.Bhatia)

* * * * *
* A C K N O W L E D G E M E N T S *
* * * * *

I am grateful to my guide, Miss S.K.Bhatia, Scientist-1, Division of Dairy Cattle Genetics, National Dairy Research Institute, Karnal, for her constant encouragement throughout the period of this study.

Sincere thanks are extended to Prof. D.S.Bhatnagar, Head, Division of Dairy Cattle Genetics and Dr.D.Sundaresan, Director, N.D.R.I.,Karnal, for providing necessary facilities to carry out these investigations.

I am grateful to Dr.V.Shanker, Scientist-1 for his kind help in the evaluation of the manuscript.

Thanks are due to Mr.Surinder Singh, Scientist-1(Statistics) and Mr.Murthy, Ph.D.Scholar, for their kind help in statistical analysis. I am also thankful to Mr.Deepak Sehgal, M.Sc.student for his help in the bleeding of the animals.

My thanks are also due to my friends, Alka, Madhu Mohini, Mukesh Mudgal, Ashwani Sharma, Ravinder Bhayane and Ramesh Vij for their interest and constant encouragement.

Lastly, words cannot express my heart feeling gratitude that I owe to my loving brother Capt.D.V.Kalra for his unceasing perservarance and moral support that has made it possible for me to attain this stage of academic achievement in my life.

Vinita Kalra
(VINITA KALRA)

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1. INTRODUCTION

The cell nucleus has been a subject of intensive and exhaustive research ever since it was known that the genetic material was contained in its substance. Cajal (1909) described a paranucleolar mass in dogs, cats and humans which was certainly what we now call the sex chromatin. It was in 1949 that attention was drawn by Barr and Bertram to the fact that the Cajal's paranucleolus was related to the sex and the sex of an individual could be recognised from a simple morphological study of the nucleus by the presence of a distinctly stained body called Barr body or sex chromatin. Sex chromatin body originates from identical sex chromosomes (XX) of the normal female. One of the X chromosomes in the females becomes heteropycnotic, i.e., stains more densely than other chromosomes, and forms the sex chromatin body in interphase nuclei. Existence of a solitary bar of chromatin within the nucleus of a neuron in the female exclusively, have been subsequently confirmed and extended to almost all the tissues of the body. This startling observation provided the first clear basis of sexual dimorphism

at the nuclear level. In placental mammals, the bodies with sex chromatin are also present in the males, but the number of nuclei having the sex chromatin is normally very low. Clinically, the important fact remains that there is a broad range between the upper figures recorded for males and the lower figures recorded for females. Moreover, the body with sex chromatin is much larger in females than in males. Thus the sex chromatin technique has become a simple and rapid method for the determination of sex in man and other mammalian species.

Sexual dimorphism in the neutrophil leucocytes in the peripheral blood in man was first demonstrated by Davidson and Smith (1954). The sex specific structure is a drumstick like nuclear appendage, which for practical purposes is found exclusively in leucocytes of females. The acceptance of the positive role played by the drumstick appendages in the accurate determination of sex has given the assurance that additional diagnostic information may be gained from the study of all neutrophil appendages. To be more specific, accurate interpretation of certain abnormal sex chromosome complexes, those involving structural and numerical deviation of the X chromosome would be impossible without knowing the sex chromatin

pattern of the individual. The preliminary diagnosis of the structural abnormalities of the X-chromosome can be made from the size, shape and frequency of the drumstick appendages. It is not surprising that most of the known chromosomal abnormalities in man involve the aberrations of the sex chromosomes. Recognizable abnormalities of chromosome structure are usually due to loss or gain of chromosomal material. Encouraging results from earlier cytogenetic studies have established that larger than the normal drumstick appendages found in the neutrophils of the peripheral blood are a definite indication of larger than normal X chromosomes. Similarly smaller drumstick appendages may indicate partially deleted X chromosome. Since then, several clinicians and cytologists have become involved rapidly in increasing numbers for applying the sex chromatin test as a practical aid for differential diagnosis in the abnormalities of sex development, which are usually not diagnosed before puberty. All individuals showing structural abnormality of X chromosome appear to be associated with gonadal, ovarian or testicular dysgenesis and primary amenorrhea leading to infertility/impaired fertility in the affected individuals. Sex chromatin studies in the amniotic fluid cells could be adopted for the prenatal

diagnosis of sex. The detection of the sex of unborn foetus can become a very useful method to terminate the pregnancy of undesirable sex. The literature on the sex chromatin of human subjects is thus voluminous primarily due to its clinical significance in the identification of sex chromosomal disorders leading to subfertility/infertility of the affected individual and screening of large populations of both mentally retarded and normal persons.

The phenomenon of nuclear sexual dimorphism of polymorphonuclear leucocytes has also been reported in several other groups of animals and is similar in many representatives of the animal kingdom, but there is a considerable variation in the morphological attributes of sex chromatin patterns from one class to another. In some instances, variation in the sex chromatin patterns exists in the animals of same order and even in the cells of the same animal. Several investigators have attempted to examine the sex chromatin patterns in farm animals. The presence of drumsticks as a female characteristics in the neutrophil leucocytes of the animals belonging to the order artiodactyla (cattle, buffalo, goat, sheep, deer, swine) has been finally established. However, various morphological attributes

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of drumsticks have not yet been fully explored. Moreover, the available findings are beset with controversies. The paucity of information on dimensional characteristics of drumsticks of goats and buffaloes, viz., diameter of drumstick, area of drumsticks, nuclear area (with and without drumsticks), ratio of nuclear area to sex chromatin area and their association with certain biological traits, impelled us to undertake the present investigations. This type of study would be useful to find out the normal size of drumsticks and to enable us to screen those animals showing drumsticks larger or smaller than the normal. Thus young animals possessing such abnormal drumsticks might be removed from the herd soon after their birth which would help to establish superior dairy herd with better fertility and performance.

2. REVIEW OF LITERATURE

The chromosome constitution wields a decisive influence on the development of the phenotype. A simple difference in one chromosome pair brings about the most fundamental normal dimorphism in man and other animals, namely that of sex. A large number of chromosome abnormalities are the cause of various pathological conditions. Here again numerical and structural abnormalities of the sex chromosomes, which may give rise to abnormalities in sexual development, make up a large proportion of all abnormal chromosomal constitutions.

It is one of the difficulties inherent in chromosome analyses that although the same chromosomes are present in every cell of the body, the chromosomes can be individually recognised only in cells which are in the process of cell division. At any one time, the vast majority of the cells in the body are not of course dividing; they are in the resting stage, or in interphase. The chromosomes of non-dividing cells are in a dispersed state and, as a rule, individual chromosomes cannot be made out: together, they are the principal constituents of the cell nucleus. An important exception to this

rule is provided by the giant chromosomes in the salivary glands and other secreting tissues of *Drosophila* and other species of dipteran flies. Although not dividing, these cells contain recognizable chromosomes in a highly enlarged state and these chromosomes have played an important part in cytogenetic research.

In man, unfortunately, there are no giant chromosomes and the recognition of individual chromosomes has had to be based on cells which are either in mitosis or in meiosis. Mitotic cells have been by far the more important for this purpose and, indeed, the bulk of our knowledge on human chromosomes is based on the cells in mitotic metaphase, when the chromosomes are at their most contracted stage. This fact imposes a severe limitation on chromosomal studies and that any possibility of studying chromosomes in non-dividing cells is likely to result in a material advancement of the subject. The discovery of sex chromatin has made this possibility a reality. Each Barr body or sex chromatin represents a single X-chromosome which is present in addition to another X-chromosome, which does not form a sex chromatin body. This means that it is possible to assess the number of X-chromosomes in the karyotype by a mere inspection of non-dividing cells and this in turn has had the following important

results:-

(1) It provides a simple technique in clinical practice when dealing with patients suspected to having an abnormal sex chromosome constitution.

(2) The simplicity of the technique has made it possible to carry out large scale surveys, which have resulted in good estimates of the frequencies of various sex chromosome abnormalities.

(3) Historically, the discovery of sex chromatin patterns in individuals with abnormal sex development has led to a knowledge of mammalian chromosomes, both in normal and in abnormal individuals.

In this chapter, we are concerned with several morphological aspects of sex chromatin in cells which are not actually in the process of division.

2.1. NATURE AND ORIGIN OF THE SEX CHROMATIN

Several markedly different hypotheses have been proposed from time to time to explain the nature and the derivation of the sex chromatin body. The sex chromosomal origin of the sex chromatin was assumed because of its staining properties and its limitation to the female sex only. The work of Sachs (1953) was very largely responsible for supporting this interpretation by the presence of chromocenters of sex chromosome origin in interphase nuclei of the bank

vole (Microtus agrestes). With some certainty as to the sex chromosome origin of the sex chromatin, the next question was whether it represented a single X-chromosome or had a double-X origin. Early work by Moore and Barr (1954) postulated that the dense pycnotic area in the nucleus of the cell of female was due to the fusion of the heterochromatic portions of the XX chromosome. In the male the XY chromosome apparently was incapable of forming a chromatin mass of comparable size. The bipartite structure of the sex chromatin as seen by several investigators also suggested that two X-chromosomes participated in its formation (Reitalu, 1957; James, 1960a,b). The description of sex chromatin in female heterozygotic fowl, however, raised doubts as to the double-X origin of the sex chromatin in mammals (Kosin and Ishizaki, 1959). The definitive work of Ohno and collaborators then began to appear in 1959. Ohno et al. (1959) observed a difference in behaviour between the two X chromosomes of the female. Ohno and Hauschka (1960) confirmed the presence of a single heteropycnotic chromosome in early prophase figures of female mice, both in normal cells of the liver, ovary and mammary glands.

Studies of DNA and chromosome replication, using tritiated thymidine and autoradiography, also provided valuable evidence of the single-X origin of

the sex chromatin. Various investigators reported the late replicating (hot) chromosome as the heteropycnotic X that constituted the X chromatin of the interphase nuclei (Gilbert et al., 1962; Morishima et al., 1962). Atkins et al. (1962) obtained some evidence that the radioactivity of the late replicating chromosome persisted in the sex chromatin during the next interphase.

Having established the fact of single-X heteropycnosis in female somatic cells the next point was to consider whether the X^M (X maternal) or X^P (X-paternal) was more likely to acquire the property of heteropycnosis. Ohno and Hauschka (1960) suggested that the maternal X is isopycnotic with the autosomes and the paternal X is positively heteropycnotic. The exposition by Lyon (1961a,b; 1962), however, suggested the possible genetic inertness of one X chromosome in female somatic cells, either X^M or X^P in a mosaic fashion. Lyon (1961, 1962) postulated that the inactivation of one of the two X-chromosomes occurs during early stages of development and once it has occurred, remains fixed throughout the further development of that cell line. Davidson et al. (1963) confirmed the validity of Lyon's hypothesis. The erythrocytes of women are composed of two classes, with normal and very low enzyme levels respectively.

The authors succeeded in growing clones from single cells and found that such clones from heterozygous women either had normal enzyme level or were deficient showing random inactivation of paternal or maternal X chromosome. Lyon's hypothesis has proved to be immeasurable value in explaining the nature of sex chromatin and dosage compensation mechanism in mammals.

2.2. OTHER NAMES FOR THE SEX CHROMATIN

A wide array of nomenclatures have been employed from time to time by the various workers to refer to the significantly distinct sex diagnostic body in different somatic tissues. Originally sex chromatin was called "nucleolar satellite" because it was closely associated with the nucleolus in cat neurons (Barr and Bertram, 1949). However, the original term was found to be inappropriate for designating sex specific chromocenter in all the cells as it was observed that the sex chromatin was located against the inner surface of the nuclear membrane (Hamerton et al., 1964). Occasionally the sex chromatin is referred to as "Barr body", owing to its name to the senior discoverer. However, this term has not been widely used because it is neither informative nor descriptive. Moore (1962) designated the name sex chromatin of man as "X-chromatin" indicating its origin from the X chromosome in the cell.

Sex chromatin has been sometimes also described as "Female Sex Chromatin" due to its preponderance in the female subjects, however, this terminology soon became inappropriate as in case of placental mammals, some fairly normal-appearing males have showed sex chromatin bodies similar in all respects to that seen in the females, though the frequency was obtained very low in them.

The sex chromatin of leucocytes nuclei is contained in a sex specific nuclear appendage with its distinct rounded head attached to the nucleus by a thread like neck. It is usually referred to as "drumstick" and is found extruded from the nuclear membrane. The drumsticks occur in a very low frequency as compared to the sex chromatin bodies of other somatic tissues (Tomonaga et al., 1961). Drumsticks and sex chromatin bodies of somatic tissues have been proved to be of identical origin (Colby and Calhoun, 1963).

2.3. QUANTITATIVE CHARACTERISTICS OF SEX CHROMATIN

2.3.1. Size and Area:-

Several morphological aspects of the sex chromatin are of interest especially when one bears in mind that chromocenter is a single X chromosome in a condensed or positively heteropycnotic state. The phenomenon of sexual dimorphism is similar in many

representatives of mammalian species, but there is a considerable variation in their quantitative attributes of the sex specific appendages (frequency, size and area), from one order of mammals to another order.

In most mammalian species, the sex chromatin is generally of the order of 1.0μ in its diameter, but the observed dimensions vary to some extent with the type of cell and the technical nature of the preparation. In a survey of twenty types of cells in human tissues and organs, Moore and Barr (1954) found that the sex chromatin was slightly larger in cells of cartilage, adrenal cortex and thyroid follicles, as compared to the other cell types studied though the measurements were not made. However, in a later study of normal tissues, and benign tumors of man, in which the sex chromatin was measured, a mean value of $0.7 \mu \times 1.2 \mu$ was obtained for the normal cells and $0.8 \mu \times 1.2 \mu$ for cells of benign tumors (Moore and Barr, 1955b). Klinger (1958a) in a study of human cells in whole mounts of membranes, smears and sections (including embryonic material) found that the size of the sex chromatin varied between 0.8μ and 1.4μ with and the average was of 1.1μ .

Rodents have also been investigated thoroughly because a clear identification of sex in their somatic cells could be turned to good use in

experimental work. It was observed that their nuclei were not generally as suitable as those of primates because of their coarse chromatin (Hay and Moore, 1961). Although Hinrichsen and Goethe (1958) found that the sex difference in purkinje cells of female rats was as distinct as it was in man. These workers have also reported a sex difference based on the size and number of nucleolar chromocenters in mouse nuclei. The sex chromatin body in cultured heart, spleen and kidney tissues of rat varied from about 0.5μ to 1.0μ in width and from about 1.0μ to 1.8μ in length (Miles and Koons, 1959).

Nuclear morphology of Aricodactyles appeared to follow a pattern similar to that of the primates. The drumstick (sex chromatin) in polymorphonuclear leucocytes generally measured about $1.0 \mu \times 1.1 \mu$, with a range of $0.8 \mu \times 1.8 \mu$ (Onuma, 1964). Barr (1966) gave 1.4μ , 1.5μ and 1.3μ as the average sizes of the drumsticks in polymorphonuclear leucocytes of cattle, goat and sheep respectively. However, in a later study of blood smears of cattle and buffaloes, a mean value of 0.994μ was found for the drumsticks in cattle and 0.793μ in buffaloes (Mukherjee and Singh, 1974). The study of nervous tissue of swine showed a mean value of $0.8 \mu \times 1.12 \mu$ for sex chromatin of normal animals and $0.82 \mu \times 1.12 \mu$ for intersexes (Cantwell et al., 1958).

The size of sex chromatin may alter due to the cell metabolism. Kayumov and Dmitrieva (1976) found that 15-16 year old woman with contracted diabetes mellitus after carbohydrate metabolism showed an increase in the circular form of sex chromatin bodies which looked like thickenings of the nuclear membrane; sex chromatin body of round shape was also enlarged in woman aged 25-65 year. Oval, triangular and semicircular forms however decreased in all age groups. The enlargement of the area of the sex chromatin body was also reported to be statistically significant by these workers.

Structural abnormalities of the X chromosome have also been reported as affecting the size of the sex chromatin. It was observed by Harden (1961) that the sex chromatin tended to be smaller than usual in females who had one normal and one partially deleted X chromosome. This finding was also confirmed by Jacobs et al. (1960, 1961) and Bamford et al. (1964). Conversely if a normal X chromosome was paired with an isochromosome of the long arm of an X, the isochromosome was likely to produce an enlarged mass of sex chromatin (Bamford et al., 1964).

Sohval and Casselman (1961) found that the sex chromatin in buccal smears became smaller during the administration, either topically or by ingestion of certain antibiotics. Conversely, several cytostatic

drugs could increase the average size of the cytoplasm and nucleus in cells of different tissues (Baserga and Castoldi, 1966). These workers analysed the structural features and distribution of the sex chromatin in the buccal epithelial cells of leukaemic female patients who had received for many months high doses of cytostatic drugs (busulphan and merceptopurine). The cells of oral mucosa renewed rapidly and showed variations in size and structure after cytostatic treatment.

2.4. QUALITATIVE CHARACTERISTICS OF THE SEX CHROMATIN

2.4.1. Shape:-

The shape of the sex chromatin depends to some extent in such details of internal structures as can be made out with conventional microscopy. The shape is also determined by the relationship between the sex chromatin and other components of the nucleus (Barr, 1966). The most common shape of sex chromatin is planoconvex or triangular lying against the inner surface of the nuclear membrane (Burlington, 1959). The sex chromatin is at times irregular, bell shaped or especially in buccal epithelium, flattened into a disc shaped chromatin mass (Barr, 1966). In neurons especially the sex chromatin is often adjacent to the large spherical nucleolus and one surface may be flattened as it fits against the nucleolus (Barr et al.,

1950; Prince et al., 1955). In human sympathetic ganglion cells and occasionally in other cells the sex chromatin may be flattened between the nuclear membrane and an eccentric nucleolus (Mylle and Graham, 1954). When free of nuclear surface the sex chromatin is likely to be spherical or irregular. Graham and Barr (1952) and Moore and Barr (1954) noted a small clear area in the sex chromatin of occasional cells in cat and man, and suggested that this might be indicative of a double structure. The bipartite sex chromatin was also observed in some bovine neurons by Moore et al. (1957). Drumstick (sex chromatin) of blood smear consists of a rounded head which is attached to the rest of the nuclear lobe of a polymorphonuclear leucocyte by a thin filament (Davidson and Smith, 1954). The stalk of drumstick may be of variable length. Carpentier (1957) reported two types of drumsticks delineated on their shape, viz., "Certain drumsticks" which were lying completely free from other nuclear structures and entirely outlined and "probable drumsticks" which were still recognizable but not extremely outlined being sessile or slightly superimposed on rest of the nucleus. Ruhren (1960) designated the drumsticks as a "definite appendage" and the sessile as "possible appendage". The sex chromatin body in polymorphonuclear leucocytes may appear as a "tear drop shaped" body with a rounded

head similar to the drumstick without a distinct stalk (Bhatia et al., 1978).

2.4.2. Position of the sex chromatin:-

The data on the position of the sex chromatin in the nucleus may seem to have little basic importance. On the contrary, there are several unexplained phenomenon and continued analysis of variations in the position of the sex chromatin which normally may contribute to the knowledge of forces that operate in the interphase nucleus. The position of the sex chromatin in the nucleus is generally specific to a particular cell, tissue or organ type and is sometimes species specific (Prince et al., 1955). However, in any tissue all bodies will not be situated in the same position. For this reason when sex chromatin counts are made, its position is generally classified into three groups: (1) adjacent to the nucleolus, (2) free in the nucleoplasm, (3) adjacent to the nuclear membrane (Klinger, 1966).

Even if the sex chromatin is at the nuclear membrane consistently this would not always be evident because of variations in the orientation of sex chromatin and nucleus with respect to the optical axis of the microscope (Barr, 1966). James (1960a,b) in a study of the cats adrenal cortex in which nuclei were approximately spherical, found that the sex chromatin was at the

periphery of the optical section through the nucleus in about half of the cells. Leviij and Meulendijk (1960, 1962) subjected James data to mathematical analysis. They showed that the sex chromatin would appear at the periphery of spherical nuclei about half of the time, even if randomly located at the nuclear membrane, because of limitations of the resolving power of the microscope. As a general rule, therefore, the sex chromatin is probably situated at random against the inner surface of the nuclear membrane in most of the cells. Miles (1961) calculated from measurements of nuclear size and the size of the sex chromatin in flat nuclei of buccal smear preparation that if the sex chromatin were situated randomly at the membrane it would appear at the periphery of the nucleus in about 40% of the cells with sex chromatin.

In some cells, sex chromatin position within the nucleus may alter during varying functional or developmental stages of cell. Graham (1954a) found that the sex chromatin of non nervous tissues of the cat was adjacent to the nuclear membrane less frequently in 19 and 24 days embryos than in mature animal. In 52 days old foetus, a still greater change in the position of the sex chromatin was observed during maturation of the cat's nervous system. The sex chromatin was more often adjacent to the nuclear membrane

in early stages of development and came to be next to the nucleolus more frequently with increasing maturity. These changes in the position of sex chromatin might result from altered intranuclear dynamics during metabolic changes in maturity.

Murthy and Haam (1962) suggested that the location of drumsticks anywhere in the curve of the nucleus to the poles, represented their evolution from the simple mass of sex chromatin in non-segmented forms.

2.5. THE SEX CHROMATIN AND DEGREE OF LOBING OF THE POLYMORPHONUCLEAR LEUCOCYTES NUCLEI

The degree of lobulation of the neutrophil nucleus is an important factor in determining drumsticks formation, wherever, quantitative findings on drumsticks are of importance or where the absence of drumsticks requires to be evaluated, the lobe counts must also be carried out. The incidence of drumsticks (sex chromatin) in individual woman varies considerably. One factor that influences the incidence of drumsticks is the degree of lobing of the polymorphonuclear leucocytes nuclei, the more highly lobed the cells are, the more is the incidence of drumsticks (Davidson and Smith, 1954; Luers and Petzel, 1958; Siebner et al., 1963). Thus many women with low drumstick counts also show low nuclear lobe counts (Briggs and Kupperman, 1956). In twelve apparently normal women a detailed

analysis was undertaken by Mittwoch (1964a). The incidence of drumsticks was reported to vary between 1.6 and 7.2% and the correlation coefficient between the incidence of drumsticks and the mean lobe count was found to be 0.56. When cells with constant lobe numbers were considered, it was observed that in all women the drumstick count also increased with the increase in the lobe number from 1 to 4 or 5, but for each given lobe number the drumstick count of the woman with the highest number was about 3 or 4 times that of the woman with the lowest number. Kyung Tai (1969) reported that the incidence of drumstick per each lobe number had the tendency to increase until 3 nuclear lobes but no significant difference above that was shown. He, however, failed to reveal any correlation between the incidence of drumsticks and the nuclear lobe differentiation.

The degree of lobing and the incidence of drumsticks is likely to be below normal in a variety of chromosomal abnormalities and out of the two, the reduction in the frequency of drumsticks is more severe. In mongolism (Down's syndrome) the nuclei of the polymorphonuclear leucocytes showed on an average, fewer lobes than those of unaffected subjects (Turpin and Bernyer, 1947). In Klinefelters syndrome, an incidence of drumsticks considerably lower than that of normal

females was noted by Davidson and Smith (1958). The low incidence of drumsticks in these patients was also found to be associated with decreased lobing of polymorphonuclear leucocytes nuclei (Mittwoch, 1961).

Subject to variations in the lobe count, the drumstick count in any woman tends to remain constant, and it is an individual characteristic which may be genetically determined (Mittwoch, 1961).

2.6. SEX CHROMATIN AND NUCLEAR SIZE IN VARIOUS SOMATIC TISSUES

Several studies have been carried out to investigate the relationship of the sex chromatin with nuclear size in different somatic tissues. Mittwoch (1963) described female donor nuclei with Barr bodies from fibroblast cultures and observed that they were on an average smaller than those in which no Barr bodies could be seen. Most of the cultures, in which this effect was demonstrated, had some abnormality of the karyotype, viz., one had an XXY constitution, another was with XXX chromosomal complement, two had a deletion of a large acrocentric chromosome and only one culture showed an apparently normal female karyotype. No definite conclusion could be reached about the size of cells in which a Barr body appeared to be present in the centre of the nucleus. However, later studies by Mittwoch (1964) revealed that the average size of nuclei

with centrally placed Barr bodies was closely similar to those with peripheral Barr bodies. Accordingly it was assumed that these centrally placed structures were true Barr bodies. She has also reported that these might be least two explanations for the difference in size between nuclei with and without Barr bodies. The smaller size of the Barr body containing nuclei may be incidental, if Barr bodies happened to be present only in those stages of the mitotic cycle, when the nucleus was not at its maximum size. In that case, the population of Barr body containing cells would be present also in XY cells, but would be unrecognizable. On the other hand, the smaller nuclear size may be a direct effect of the condensation of one of the X-chromosomes to form a Barr body. If these were so, this population of cells would be non-existent in XY-cells. It would seem likely that both these causes do in fact operate.

2.7. SEX CHROMATIN AND ITS RELATION TO AGE

Age dependent variations in the incidence of sex chromatin have been encountered in the tissues of various mammalian species. Krauze (1961) showed that the frequency of sex chromatin positive nuclei steadily increased with age. Several investigators have also reported an increase in the frequency of sex

chromatin in new borns during 1st, 2nd and 3rd day which gradually increased to the normal level within the next few days (Lillian et al., 1967; Bulanov, 1968; Curtis, 1969; Diperna and Ricciuti, 1969; Dyshlouayi et al., 1972; Gencik, 1972; Mishvidebadze and Saakashvili, 1975 and Roy et al., 1976). Eidenbenz (1964) reported that cortisone also resulted in statistically significant increase in the percentage of sex chromatin positive nuclei upto 5th day in new borns. Csaba (1974) found that glucose-6-phosphate dehydrogenase activity of the erythrocytes of new borns gradually decreased upto 7th day of life and the process was found to be coincident with the increase of their sex chromatin bodies. Sexual abnormalities also affected the sex chromatin with frequency with the gradual increase in the age of the individuals. Nielson (1969) and Platunova and Lavryashin (1976) noted a direct relationship in the frequency of sex chromatin and age of mentally retarded patients with Turner's mosaic and presumptive triple mosaic. Nielson (1969) further reported a decrease in the sex chromatin frequency with increase in age of Schizophrenic patients. The buccal epithelium was studied by Voitenko (1973) in women of age group between 50-69 years with malignant tumors and healthy females of same age. The frequency of sex chromatin was observed to decrease in menopausal women. This decrease in the

frequency of sex chromatin bodies was, however, less pronounced in patients with mammary, ovarian and uterine cancers. Definite fluctuations in the incidence of sex chromatin bodies have been reported by Paintal and Minina (1974) in normal females. The highest incidence of sex chromatin pattern was on the 14th post menstrual day with a mean occurrence of 33%. Two other peaks were also observed, one on the 22nd day of the cycle when the mean was 28% and another on the 1st day of the next menses when the mean was 26.2%. Platunova and Lavryashin (1976) described that average frequency of sex chromatin bodies was characterised by age, rising during sexual maturation and dropping between the ages of 41-54. Sahai and Goswami (1977) and Bhatia et al. (1978) also showed fluctuation in the frequency of sex chromatin bodies in different age groups of water buffaloes and goats respectively.

2.8. SEX CHROMATIN ABNORMALITIES AND THEIR PHENOTYPIC EFFECTS

The acceptance of the positive role played by the drumstick appendages in the accurate determination of sex originally described by Davidson and Smith (1954) has given the assurance that additional diagnostic information may be gained from a detailed study of all neutrophil appendages. A preliminary diagnosis of gross reproductive disorders involving sex chromosome

abberations can be made within a few hours and with a high degree of accuracy. The value of such a method to the clinicians is readily apparent.

It is not surprising that most of the known chromosomal abnormalities in man involve the sex chromosomes. Abnormal karyotypes, with respect to sex chromosomes and autosomes, consist principally of deviations from the normal number and structure of chromosomes. Recognizable abnormalities of chromosome structure are usually due to loss or gain of chromosomal material. Loss occurs when chromosomal breakage results in (i) deletion of a segment which may or may not be transferred to another chromosome, or (ii) reciprocal translocation with mutual exchange of segments of unequal size. Gain is the result either of (i) duplication due to the addition of a corresponding segment from a homologous chromosome, or (ii) exchange of a small segment for a larger segment in reciprocal translocation (Sohval, 1963).

2.8.1. Structural Abnormalities of X Chromosome:-

An X chromosome with an abnormal configuration appears to be associated with gonadal ovarian or testicular dysgenesis. Jacobs et al. (1960) described the first such patient who had Barr bodies smaller than normal. The patient was noted to have an X chromosome from which a part of long arm had been deleted. Small

TABLE - 1

The results of the peripheral blood determination of individuals showing gross reproductive disorders

S.No.	Species/ Variant	Sex(X) chromosome abnormality	Sex chromatin abnormality	Phenotypic effect	Reference
1.	Human beings (female)	Deletion of the long arm of X-chromosome	Abnormally small drum- sticks (sex chromatin) bodies are found with lower frequency	Turner's phenotype, with streak gonad and inferti- lity and webbed neck but with normal stature	Ford (1973)
2.	-do-	Deletions of the short arms of the X- chromosome	-do-	Turner's phenotype, with streak gonad and inferti- lity, but with a short stature	-do-
3.	-do-	Isochromosome for the long arm of the X-chromosome	Abnormally large drum- sticks (sex chromatin)	Secondary amenorrhea and multiple congenital anomalies	Bamford <u>et.al.</u> (1964)
4.	-do-	Isochromosome for the long arm of the X- chromosome	Abnormally large drum- stick (sex chromatin) body.	Full-blown-Turner's syndro- me characterised by gonadal dysgenesis, short stature	Taft <u>et al.</u> (1965)
5.	-do-	Isochromosome for the short arm of the X- chromosome	Abnormally small drum- stick (sex chromatin) body	Turner's syndrome, with streak gonads, but with a normal stature	Casper- soon (1970)
6.	-do-	Partial deletion of upper arm of one X-chromosome	Abnormally small drum- sticks (sex chromatin) bodies	Infertility and secondary amenorrhea	Bamford <u>et.al.</u> (1964)
7.	-do-	Deletion of X- chromosome	Abnormally small drum- sticks (sex chromatin) bodies	Small hypoplastic ovaries with primordial follicles and primary amenorrhea	Grouchy <u>et al.</u> (1961)

Barr bodies were also noted in other patients who had a deletion of the short arm of X-chromosome. Mosaicism in human beings was predicted on the basis of small drumstick appendages indicating partial deletion of an X-chromosome (Bamford et al., 1964). Patients with isochromosome for the long arm of the X-chromosome tend to have a high proportion of the large Barr bodies (Jacobs et al., 1960; Lindsten, 1963). Large drumsticks occurring with lesser frequencies may suggest the presence of mosaicism of the type XO/xX (Bamford et al., 1964).

Table 1 shows the result of the peripheral blood determination of individual showing gross reproductive disorders.

3. MATERIAL AND METHODS

The present investigations were carried out to determine the *morphological* attributes of sex diagnostic appendage (drumstick) in the blood smears of goats and buffaloes.

3.1. EXPERIMENTAL ANIMALS

The animals screened for the sex chromatin studies belonged to the herd of National Dairy Research Institute, Karnal. One hundred and forty goats of different breeds, viz., Beetal, Alpine, Alpine x Beetal crosses, Saanen x Beetal crosses, Saanen x Alpine x Beetal crosses, and also twenty eight buffaloes of Murrah breed were included in the present studies.

Table 2 furnishes the details of the animals in relation to their breeds and sex.

3.2. COLLECTION OF BLOOD

Blood was collected by the following techniques:-

3.2.1. From the Ear Vein:-

The hair over the ear vein of the animal were shaved off with a shaving blade. The cleaned area on the ear was then rubbed after applying a thin layer

TABLE - 2

Distribution of experimental animals in relation to their age and breed

Species/Breed	Age groups				Total
	0-1 year	1-2 years	2-3 years	3 years & above	
A. <u>GOATS</u>					
Beetal	9	-	3	16	28
Alpine	3	5	6	14	28
Saanen x Beetal	6	2	4	16	28
Alpine x Beetal	8	1	3	16	28
SaanenxAlpinexBeetal	3	4	10	11	28
Total for all the goats:	29	12	26	73	140
B. <u>BUFFALOES</u>					
Murrah	16	-	-	12	28
Overall total:	45	12	26	85	168

of xylene. This made the ear vein prominent. Now the cleaned area around the vein was sterilized with a cotton swab dipped in alcohol. The swollen prominent marginal vein was then punctured with a sterilized fine needle and the blood flowed quickly in drops. First few drops were wiped away and then one drop was taken on the clean slide or a cover slip and smear was prepared.

3.2.2. From the Jugular Vein:-

The animals were restrained in a trevis/or at an iron railing. The neck of the animal was turned to one side held with the help of a halter. The jugular vein was located by pressing with the thumb and after applying rectified spirit over and around the vein it was punctured by stabbing with a sterilized hypodermic needle of 15/16 gauge. The blood collected in a test tube containing 2 ml of anticoagulant solution (20 gms sodium citrate, 5 gm sodium chloride dissolved in one litre of water).

3.3. PREPARATION OF BLOOD SMEAR

3.3.1. Cleaning of slides and cover glasses:-

Clean, grease free Blue Star (Super Deluxe) microslides of 1 mm thickness were used for the preparation of blood smears. New slides were dipped initially for 24 hours in dichromate cleaning solution (sodium or

potassium dichromate, 100 gm dissolved in 750 ml of water to which 250 ml of concentrated sulphuric acid was added carefully). The slides were washed well in running tap water for overnight after removal from the dichromate cleaning mixture, and then rinsed in glass distilled water. These slides were stored in 95 or 100% alcohol till used. The slides were dried and polished with a clean soft muslin cloth just before use. The blood smears on the used slides were first washed with water and then cleaned as described above.

Cover glasses required for the preparation of blood smears were also cleaned in the same manner.

3.3.2. Preparation of Blood Films

3.3.2.1. By glass slide method:- One drop of blood was taken directly from the ear vein or from the blood collected from the jugular vein. Occasionally the extra blood was drained off from the slide to help in making of a thin blood smear. The blood drop was placed about one or two centimeters away from one end of the clean glass slide. For the preparation of the smear a "blood spreader" was used. The blood spreader was normally another microslide having an even and unchipped edge and with its corners ground so that the width of the spreading edge was always lesser than the total slide width. This spreading edge of the blood spreader was then held at an angle of about 30 degrees on the

slide having the drop of the blood. The blood spreader was then drawn backwards until it just touched the drop of the blood. The drop of the blood was then immediately, spread smoothly, evenly, quickly along with the edge of the spreader slide maintaining the angle of 30 degrees. Blood smears of modulated thickness were prepared and allowed to dry quickly in the air. The slide having the blood smears were coded with a diamond pencil. Normally two blood smears slides were prepared from each animal.

3.3.2.2. By cover glass method:- A clean, dry, grease free square cover glass (22 mm) was held in each hand by the edge. One drop of blood was placed on the cover glass which was held horizontally. Now the other cover glass was superimposed diagonally on the drop of the blood. The cover glass held above was quickly removed from the contact with the blood drop. This facilitated the preparation of a smear of even thickness. The cover glasses having the blood smears were dried and stained. This technique was discarded after initial experimentation because of difficulties encountered in the safe preservation of cover slip smears.

3.4. FIXATION OF THE BLOOD SMEARS

The blood smears prepared on the slides/ cover glasses were fixed in methanol for five minutes and again dried.

3.5. STAINING OF THE BLOOD SMEARS

The following techniques were employed for the staining of fixed and dried blood smears.

3.5.1. Leishman's Staining Technique:-

British drug house (BDH) Leishman's stain was used for the staining of the slides.

3.5.1.1. Preparation of stain:- The Leishman's staining powder supplied along with the 250 ml staining bottle of BDH was transferred from the packet into the solvent. This was dissolved by occasional shaking and filtered after 48 hours. This staining solution ^{was} used for the staining of the slides and was stored in a refrigerator.

3.5.1.2. Staining procedure:- The dried and fixed smear side of slides were kept upwards on a staining rack (two parallel glass rods 5 cms apart). The smears were then covered with the undiluted stain for 30 minutes. The stain was then diluted approximately two volumes of phosphate buffer (11.758 gms of disodium hydrogen phosphate and 8.981 gms of potassium dihydrogen orthophosphate in one liter of glass distilled water and pH adjusted to 7.34), and the slides were kept for 25 minutes in the diluted stain. The slides were finally washed with the same buffer solution. The lower side of the stained slides were then cleaned and dried with

dry muslin cloth. The stained slides were studied for the quantitative attributes of the sex chromatin (drumstick) appendages. The stained slides were stored in the slide box for the screening.

3.5.2. Wright's Staining Technique:-

3.5.2.1. Preparation of stain:- The working solution of Wright's stain was prepared by mixing dry powder of the stain (BDH) into 250 ml of the solvent in a staining bottle. The staining solution was ripened for about 1-2 weeks. This was filtered and stored in a refrigerator for staining of the blood smears.

3.5.2.2. Staining procedure:- The slides having the dried and fixed smears were placed on a staining rack. The smears were then covered with the undiluted stain for 20 minutes. The stain was diluted with equal volume of phosphate buffer pH 6.58 and kept for 20 minutes. The slides were washed finally in the same buffer. The rear surface of the stained slides were dried cleaned with a clean muslin cloth and stored in slide box for screening.

3.5.3. Giemsa Staining Technique:-

3.5.3.1. Staining solution:- The commercially available BDH staining solution was used for staining the blood smears.

3.5.3.2. Staining procedure:- The air dried blood smears were prefixed for 3 minutes with methanol and then dried and immersed in the diluted Giemsa stain (1 volume stain and 5 to 9 volumes of phosphate buffer pH 7.0) in a couplin jar for 40 minutes. The stained slides were then washed in distilled water and dried in the air. The stained slides were then stored in the slide boxes.

3.6. MOUNTING OF STAINED BLOOD SMEARS

The blood smear preparation of the stained slides were preserved by mounting them with Distrene-Plastisizer-Xylene (DPX) mountant. Two^{to}three drops of DPX mountant were put on the smear at different places on the slides and a clean coverslip was gently inclined on the slide. The air bubbles, if any, left were removed by slightly pressing the coverslip on the slide with a needle or by slight warming of the mounted preparation over a flame.

3.7. SCREENING OF THE SLIDES

The permanent slides prepared from the individual animals were studied under the oil immersion objective of (X1500) the Meopta binocular microscope. Five leucocytes with distinct drumstick appendages and other five without drumsticks were selected for quantitative measurements, from each of the coded slide.

3.8. MEASUREMENTS OF QUANTITATIVE CHARACTERISTICS

The techniques of quantitative measurements followed were same as given by Levij and Carel (1968) and Mukherjee and Singh (1974).

3.8.1. Diameter of the Drumsticks:-

Distinct non-overlapping drumsticks were selected for quantitative measurements to reduce the error in the measurements. For the diameter of the drumsticks head breadth and lengths were measured with an ocular micrometer. The average of the two measurements were taken as diameter.

3.8.2. Area of the drumsticks:-

The area of the drumstick was calculated from its diameter using πr^2 in which r represented the radius (half of the diameter).

3.8.3. Diameter of the Nucleus:-

The average of the two main axes of the nucleus at right angles to each other give the estimate of its diameter.

3.8.4. Area of the Nucleus:-

The average nuclear area was calculated with the formula πab (Levij and Carel, 1968) where a and b represented 0.5 x average of two main axes respectively.

3.8.5. Ratio of nuclear area to drumstick area:-

The ratio of nuclear area to the sex chromatin (drumstick) was also calculated from their respective areas.

3.9. PHOTOGRAPHY

The micrographs were taken with Leitz microscope at (x640) magnification using ORWO 35mm micro-film. The prints of the microfilm were made at an enlargement of X2784.

3.10. STATISTICAL ANALYSES

Blood samples of one hundred and forty goats and twenty eight Murrah buffaloes were screened to determine the quantitative characteristics of drumsticks (sex chromatin) appendage in their polymorphonuclear leucocytes. The significance of the differences were tested for the averages of the mammalian characteristics namely, nuclear area, area of the drumstick (sex chromatin), the ratio of the nuclear area to the drumstick (sex chromatin) area and the diameter of the drumstick (sex chromatin), employing analysis of variance technique.

The breed means of drumstick area and drumstick diameter of goats were compared with one another using Duncan's Multiple Range Test (1955).

In order to study the effect of age and breeds on size of the drumsticks in goats, least squares

analysis was carried out by fitting the constants for different age groups (0-1 year, 1-2 year, 2-3 year and 3 year and above) and breeds (Beetal, Alpine, Saanen x Alpine x Beetal, Alpine x Beetal, Saanen x Beetal crosses). The model used was:

$$Y_{ijk} = \mu + B_i + A_j + e_{ijk}$$

Where,

Y_{ijk} = k^{th} observation in the i^{th} breed and j^{th} age group,

μ = Overall population mean,

B_i = Effect of i^{th} breed,

A_j = Effect of j^{th} age group,

e_{ijk} = Random error associated with k^{th} individual normally and independently distributed with mean zero and variance σ^2 .

It was assumed that the size of drumstick was normally distributed in this population of goats. The least squares analysis was done on Hindustan Micro-2200 computer.

Standard Error:-

The estimate of standard error of least square means was worked out from the inverse matrix:

$$S.E. = \sqrt{(C^{ui} + C^{ii}) e^2}$$

Where,

C^{ui} = Inverse matrix in u rows,

c^{11} = Corresponding diagonal inverse element
for i^{th} constant,

e^2 = Error mean square

The difference between least squares means of different age groups and breeds were tested for significance by analysis of variance using F-test.

Similarly least squares analysis was done to study the effect of nuclear lobing and different breeds of goats on the size of the drumsticks. The calculated least squares means of these two traits were then tested using F-test.

4. RESULTS AND DISCUSSION

4.1. STAINING OF BLOOD SMEAR AND DRUMSTICK APPENDAGES

The sex chromatin is known to be a heteropycnotic X-chromosome and therefore its staining properties are for the most part self evident. The reactions of the sex chromatin to various dyes helped to establish its chromatin nature and are of some theoretical as well as practical significance (Barr, 1966). Romanowsky's stains, viz., Wright's and Leishman's stains gave soft pink colour to the red blood cells and the nuclei of polymorphonuclear leucocytes alongwith their drumstick bodies which appeared dark purple in their colour. However, staining of blood smears with azure dye (Giemsa stain) resulted in soft pink or yellowish erythrocytes and the nuclei of polymorphonuclear leucocytes and their sex specific appendages appeared from light purple to dark purple coloured. But the staining properties with regard to the colour were observed to be variable, sometimes even two films stained from same bottle of stain differed greatly. Failures to get satisfactory results with these dyes might be attributed to the

incorrect reaction of the staining fluid. In general, a preparation was found satisfactory when both nuclei and neutrophilic granules were distinct, regardless of their colour.

4.2. SHAPE AND POSITION OF DRUMSTICKS

Two types of drumsticks were observed in the present investigations (Figs. 1 and 2). The first type of the drumstick appendage exhibited a well outlined distinct head attached to the nuclear lobe of the polymorphonuclear leucocytes by a single thick stalk. However, the other type appeared as an entirely rounded body lying completely free from the other nuclear structures. It remained attached to the nuclear lobe by a very delicate strand which could not be observed with the resolving power of the light microscope. Murthy and Hamm (1962) have also observed similar types of drumsticks in segmented and non-segmented neutrophilic leucocytes of human beings. Davidson and Smith (1954) reported the presence of stalked drumsticks consisting of filaments of varying lengths. Drumsticks consisting of a well defined solid chromatin nodule joined by a single fine chromatin strand to one lobe of the nucleus have also been shown by Porter (1957) and Carpentier et al. (1957) in bitches and female rabbits respectively. Bamford (1964) classified thick stalked drumsticks as a 'atypical'

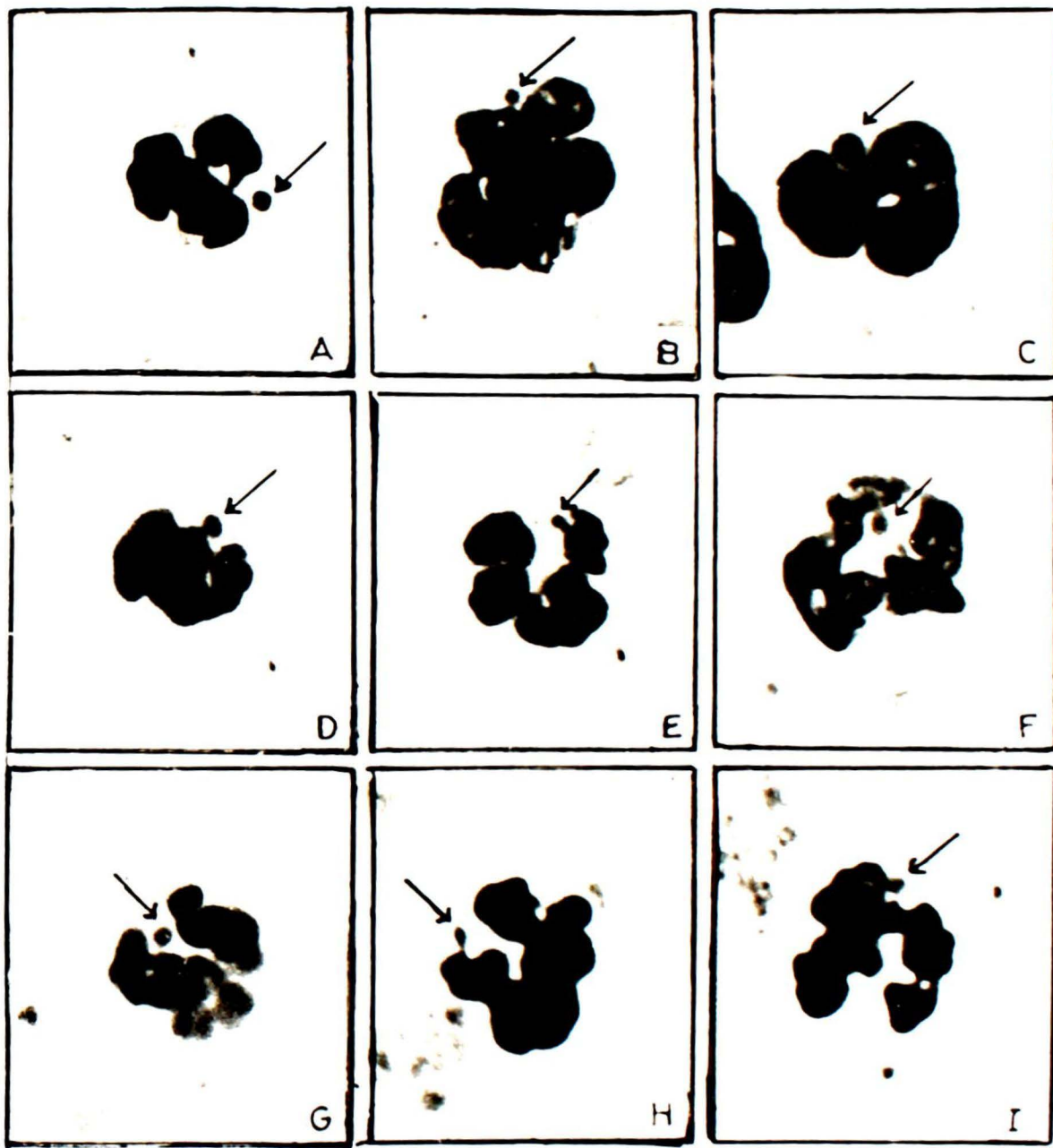


Fig.1. CHARACTERISTIC DRUMSTICKS OF DIFFERENT SIZES IN THE POLYMORPHONUCLEAR LEUCOCYTES OF FEMALE GOATS.(x2784). A-C BEETAL BREED ; D-F ALPINE BREED ; G-I ALPINE X BEETAL CROSSES

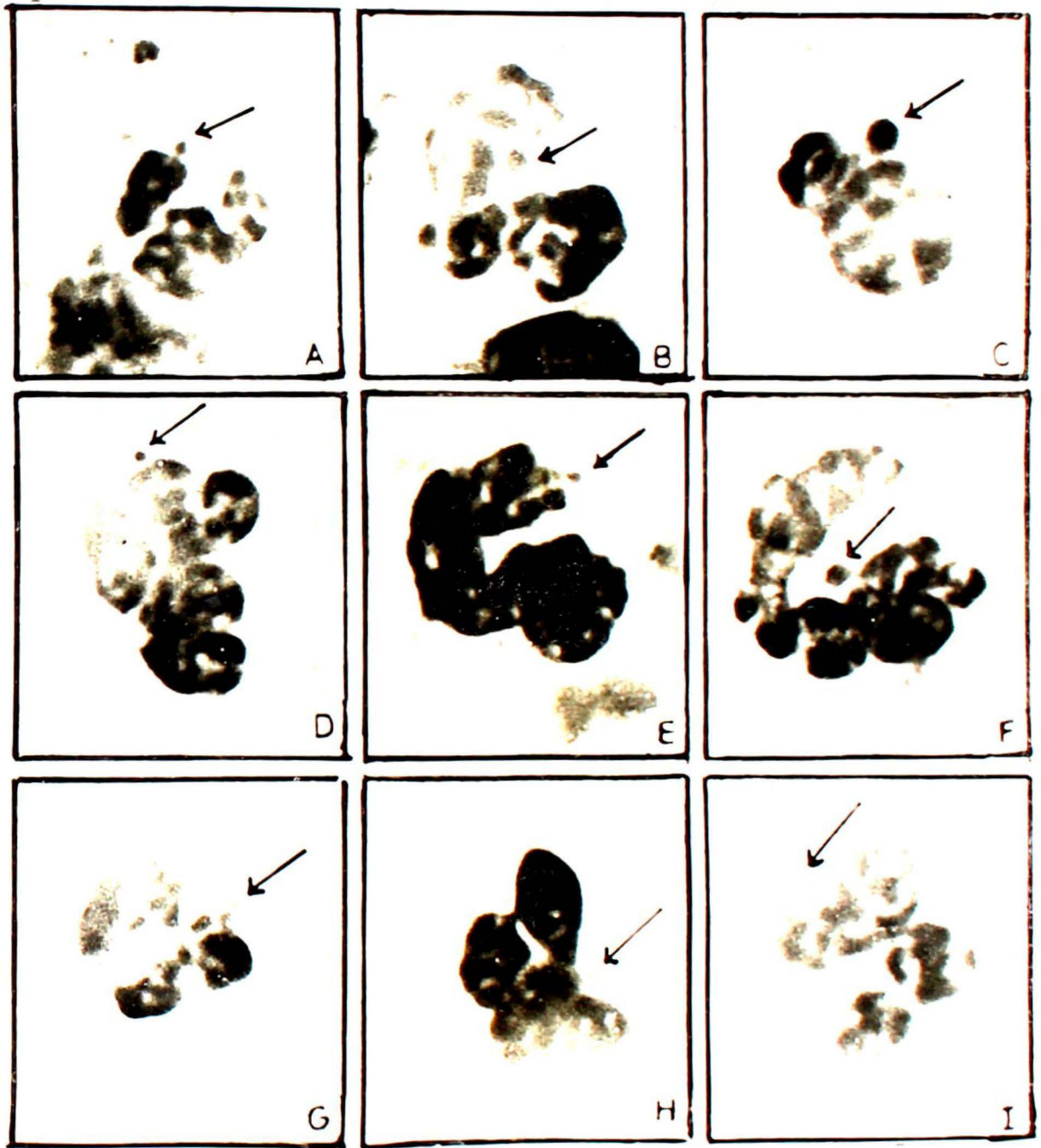


Fig.2. TYPICAL DRUMSTICKS OF DIFFERENT SIZES IN THE POLYMORPHONUCLEAR LEUCOCYTES FROM THE BLOOD FILMS OF FEMALE GOATS AND BUFFALOES. (x2784). A - C SAANEN x BEETAL CROSSES ; D - F SAANEN x ALPINEX BEETAL CROSSES ; G-I MURRAH BUFFALOES.

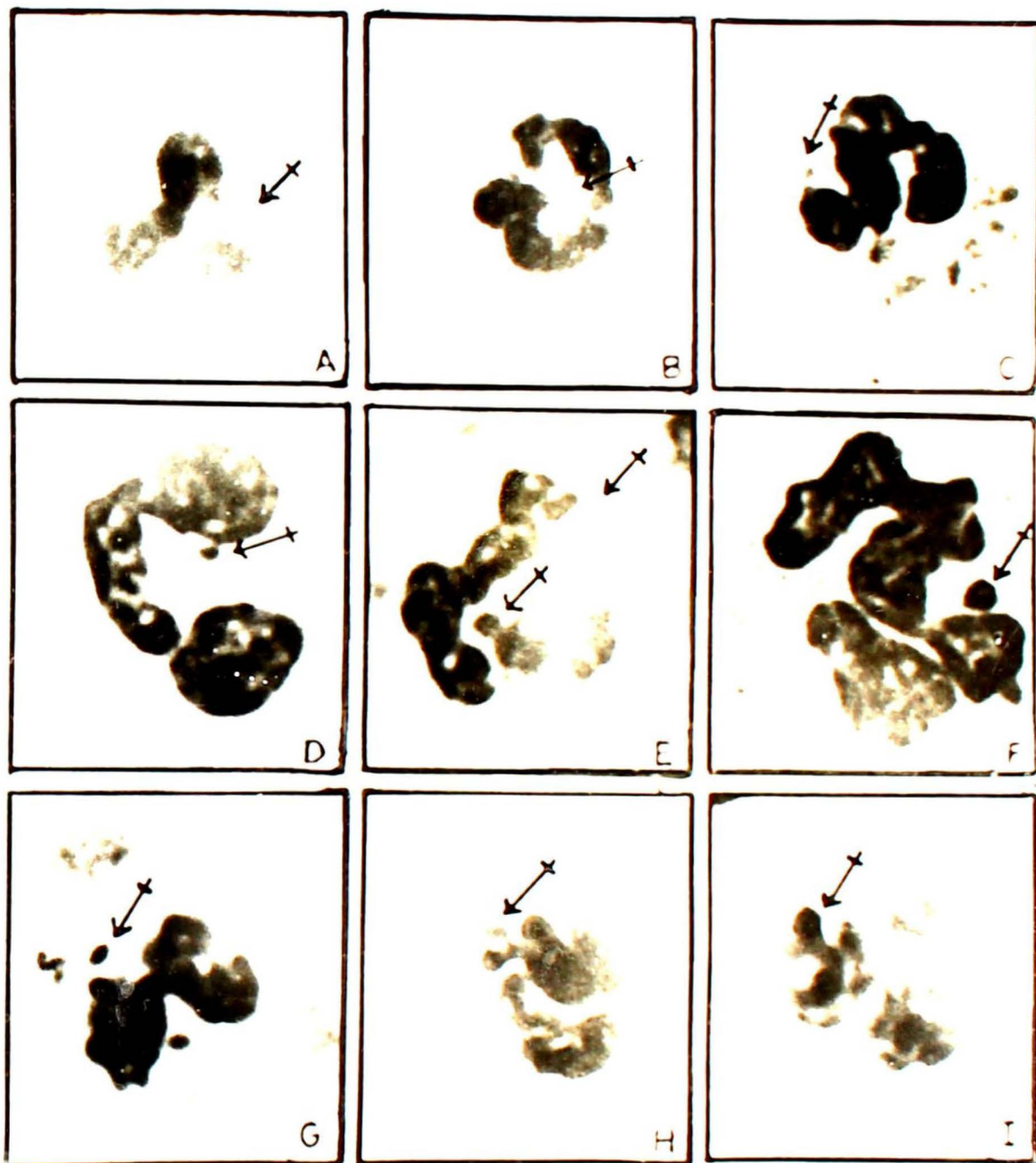


Fig.3. CONFUSING - NON-SPECIFIC APPENDAGES OF THE NUCLEI OF POLYMORPHONUCLEAR LEUCOCYTES. A-C SMALL TAGS AND THREADS, SHAPED LIKE A DRUMSTICK BUT SMALLER & LESS INTENSELY STAINED ; D-I NUCLEAR LOBES, DISTINGUISHING FROM A DRUMSTICK BY IRREGULAR OUTLINES AND CONTOURS.

appendages and thin stalked as 'normal' drumsticks.

Figure 3 shows a variety of non-sex specific appendages observed during the screening of blood films of goats and buffaloes and care was taken to distinguish them from true drumsticks. Similar non-specific appendages have also been reported by Davidson and Smith (1963) and Mittwoch (1964) in blood films of human beings. These appendages were distinguished from drumsticks by their size and shape, and/or their less intense staining (drumsticks were highly condensed deeply staining bodies). Some of such non-specific appendages were observed to be smaller than drumsticks (Fig.3, A-C), others were distinguished from a drumstick by irregular outlines and contours, in addition to their larger size and were termed as 'minor lobes' (Fig.3, D-I). Minor lobes might be connected to the rest of the nucleus by one or two filaments. Davidson and Smith (1954) have also shown similar 'minor lobes' in the blood films of human beings.

In the present study, the drumsticks were observed to be located anywhere in the curve of the nucleus (Fig.1, C-D,F; Fig.2, B-D,I) to the poles, in some cells it was seen capping close to the pole of the nucleus (Fig.1, E,F; Fig.2; B,F) or jutting slightly from the tip (Fig.1, H,F; Fig.2, A,E,G). Similar locations of the drumsticks have also been

reported by Murthy and Haan (1962) in normal and pathological white blood cells of humans.

4.3. SIZE

Figures 1 and 2 show drumsticks of various sizes in different breeds of goats and Murrah buffaloes used in the present studies. The average size of the drumsticks was observed to be $1.0 \mu \times 1.15 \mu$ and $1.0 \mu \times 1.0 \mu$ in goats and buffaloes respectively (Table 3). Goats of Beetal, Alpine and Saanen x Beetal crosses showed an average size of $1.0 \mu \times 1.25 \mu$. The drumsticks were noted to be of slightly smaller in their size in goats of Alpine x Beetal crosses and Saanen x Alpine x Beetal crosses with a mean value of $1.0 \mu \times 1.0 \mu$. Klinger (1966) reported that the most mammalian species generally exhibited drumsticks of remarkably constant size which measured about $0.8 \mu \times 1.1 \mu$ with a range of $0.7 \mu \times 1.10 \mu$ to $1.0 \mu \times 1.4 \mu$. In the present study the size of drumsticks was observed to lie within the same range, i.e., from $1.0 \mu \times 1.0 \mu$ to $1.0 \mu \times 1.25 \mu$. Cantwell et al. (1958) also recorded drumsticks of same order of size in normal swine ($0.8 \mu \times 1.12 \mu$) and in inter sexes ($0.82 \mu \times 1.12 \mu$). The drumsticks of approximately same size in various animal species have also been recorded by Onuma (1964). He reported the average size of drumsticks to be $1.0 \mu \times 1.4 \mu$ in the

TABLE - 3

Size of drumsticks in neutrophil leucocytes
of goats and buffaloes

Species/Breed	Number of drumsticks observed	Size of drumsticks (μ)
A. GOATS		
Beetal	140	1.00 x 1.25
Alpine	140	1.00 x 1.25
Saanen x Beetal	140	1.00 x 1.25
Alpine x Beetal	140	1.00 x 1.00
Saanen x Alpine x Beetal	140	1.00 x 1.00
Total for Goats:	700	1.00 x 1.15
B. BUFFALOES		
Murrah	140	1.00 x 1.00

females of cattle, horses, goats and rabbits and $1.0 \mu \times 1.3 \mu$ in the females of swine. Barr (1966) assumed a fairly constant degree of condensation of heteropycnotic X-chromosome in most mammalian species.

4.4. DIMENSIONAL ATTRIBUTES OF DRUMSTICKS AND POLYMORPHONUCLEAR LEUCOCYTES NUCLEI

The means of the quantitative attributes of drumsticks and polymorphonuclear leucocytes nuclei have been given in Table 4. In goats the mean area ($1.016 \mu^2$) and mean diameter of the drumsticks (1.090μ) were observed to be slightly more than the buffaloes. The mean area of drumsticks and mean diameter of drumsticks measured $1.006 \mu^2$ and 1.065μ respectively in buffaloes of Murrah breed. Mukherjee and Singh (1974) measured same dimensional attributes in various breeds of cattle and Murrah buffaloes and reported that the mean area ($0.959 \mu^2$) and mean diameter (0.994μ) of drumsticks in cattle was more than the mean area ($0.688 \mu^2$) and diameter (0.793μ) of drumsticks in buffaloes. In the present investigations the ratio of the nuclear area to the drumstick area was observed to be more in buffaloes (78.492) than in goats (61.70) because of the considerable variations in the nuclear area of the buffaloes ($45.386 \mu^2$) and goats ($42.022 \mu^2$). The ratio of nuclear area to drumstick area was also observed to be high in buffaloes (64.299) as compared to cattle (49.210) by Mukherjee and

TABLE - 4

Means of the drumsticks and polymorphonuclear leucocytes
nuclear dimensions of goats and buffaloes

Species/Breed	Nuclear area (μ^2) A	Drumstick area (μ^2) B	Ratio A/B	Diameter of drumstick (μ)
A. GOATS				
Beetal	41.731 \pm 1.338	1.070 \pm 0.043	56.022 \pm 4.726	1.123 \pm 0.025
Alpine	44.388 \pm 1.380	1.155 \pm 0.050	54.834 \pm 4.177	1.171 \pm 0.027
Saanen x Beetal	43.784 \pm 1.322	1.024 \pm 0.045	58.819 \pm 4.095	1.105 \pm 0.025
Alpine x Beetal	37.185 \pm 0.944	0.912 \pm 0.045	64.568 \pm 5.114	1.023 \pm 0.028
SaanenxAlpinexBeetal	43.021 \pm 1.406	0.920 \pm 0.044	74.261 \pm 6.846	1.028 \pm 0.027
Mean for Goats:	42.022 \pm 1.288	1.016 \pm 0.046	61.701 \pm 3.562	1.090 \pm 0.028
B. BUFFALOES				
Murrah	45.386 \pm 1.397	1.006 \pm 0.058	78.492 \pm 5.974	1.065 \pm 0.033

Singh (1974). The present findings indicate that the drumstick has an identity of its own and is least influenced by the rest of the nuclear material from which it is formed. It was expected that in species and breeds with a larger drumsticks, there would be a smaller nucleus than those with a smaller sex chromatin and consequently larger nucleus because the drumstick appendage is the squeezed out part of nucleus of polymorphonuclear leucocytes nuclei. This expectation was, however, not confirmed in the present studies as goats with nuclear area ($42.022 \mu^2$), smaller than the nuclear area of buffaloes ($45.386 \mu^2$) showed slightly larger drumsticks (1.090μ) than the drumsticks of buffaloes (1.065μ). Table 5 gives the analysis of variance of the mensuration characteristics. In the analysis of variance, the variations between Beetal, Alpine, Saanen x Beetal crosses, Alpine x Beetal crosses and Saanen x Alpine x Beetal crosses were considered to be as variations between breeds within a species, and variations between goats and buffaloes as variations between species. The mean sum of square between breeds and species (at 5 d.f.) was partitioned into mean sum of square between species (on 1 d.f.) and between breeds within a species (on 4 d.f.). Except nuclear area, area of drumstick and drumstick diameter between species and the nuclear area between breeds within a species,

TABLE - 5

Analysis of variance of the polymorphonuclear leucocytes nucleus and the drumsticks dimensions

Source of Variation	d.f.	Nuclear area (μ^2)	Area of drumsticks (μ^2)	\sqrt{B}	Diameter of drumsticks (μ)
		A (MSS)	B (MSS)	(MSS)	(MSS)
Between species	1	1320.60	0.01	17191.65	0.085
Between breeds within a species	4	1161.625	1.48*	9673.92	0.617*
Between animals within a breed of goat and buffalo	162	575.53*	0.426*	5790.83*	0.152*
Between slides within animals (Error)	672	158.49	0.290	3340.79	0.097

*Significant at 5% level

the rest of the characteristics varied between breeds within a species and between animals within a breed of goat and buffalo. Comparison of breed means by Duncan's (1955) multiple range test between drumstick area and drumstick diameter (Table 6) shows that the means in Alpine x Beetal crosses and Saanen x Alpine x Beetal crosses are significantly less than Alpine, Beetal and Saanen x Beetal crosses. Out of the latter three breeds, however, none showed significant difference in their parameters from the other. These variations observed in the drumstick dimensions between different breeds of goats might be attributed to the differences in size of their X-chromosomes (Prakash, 1977) which are involved in the formation of drumsticks in the polymorphonuclear leucocytes of mammalian female.

TABLE-6

List of all significant comparisons among the breed means

Charac- teristics	Mean each larger than and significantly different (P/0.05) from those in (b) (a)	Mean each smaller than and significantly different (P/0.05) from those in (a) (b)
Drumstick area (B)	Beetal, Alpine, Saanen x Beetal	Alpine x Beetal, Saanen x Alpine x Beetal
Diameter of drum- stick (u)	Beetal, Alpine, Saanen x Beetal	Alpine x Beetal, Saanen x Alpine x Beetal

4.5. DRUMSTICKS AND NUCLEAR SIZE

Measurements were carried out on polymorpho-nuclear leucocytes of goats of different breeds and Murrah buffaloes. Table 7 shows the differences in sizes between nuclei containing drumsticks and those without them. The drumstick containing nuclei measured 42.022μ in goats and 45.386μ in buffaloes. However, the nuclei lacking the drumsticks were noted to be on an average larger and their sizes were observed to be 44.003μ and 47.687μ in goats and buffaloes respectively. Similar observations have also been reported by Mittwoch (1963). Measurements which were carried out on cultured fibroblast in humans showed sex chromatin containing nuclei on an average, somewhat smaller than nuclei without them. Similarly, the nuclei containing a centrally placed sex chromatin body were also reported to be significantly smaller than those nuclei without such sex chromatin bodies by Mittwoch (1964) in fibroblast cultures from skin biopsies. This difference in the sizes between nuclei with and without sex chromatin bodies may be ascribed to the direct effect of the condensation of one of the X-chromosomes to form a sex chromatin (drumstick) body, which results in the reduction in nuclear volume. This difference could also be associated to an indirect effect of the



TABLE - 7

Nuclear sizes in polymorphonuclear leucocytes
of goats and buffaloes

Species/Breed	No. of cells examined	NUCLEAR AREA (μ^2)			
		Drumstick Zero		Drumstick One	
		Mean	S.D.	Mean	S.D.
A. GOATS					
Beetal	280	44.642	15.843	41.731	15.832
Alpine	280	45.612	16.258	44.388	16.334
Saanen x Beetal	280	45.710	14.701	43.784	15.637
Alpine x Beetal	280	37.432	13.400	37.185	11.174
Saanen x Alpine x Beetal	280	46.619	17.176	43.021	16.632
Total for Goats:	1400	44.003	15.476	42.022	15.122
B. BUFFALOES					
Murrah	280	47.687	15.403	45.386	16.530

presence of sex chromatin bodies only during certain stages of the cell cycle when the nucleus may not be at its maximum size. Mittwoch (1964) also gave a similar explanation, and opined that the Barr body may become unrecognizable during DNA replication, when the nucleus may be largest in its size. Thus the nuclei without Barr bodies appear larger in their size than the nuclei without them. It seems likely that both the factors outlined above operate simultaneously for the differences in the size of the nuclei with and without sex chromatin bodies in them.

4.6. DIAMETER OF DRUMSTICKS AND SEGMENTATION OF POLYMORPHONUCLEAR LEUCOCYTES NUCLEI

The diameter of the drumsticks and the number of nuclear lobes as observed among various breeds of goats and Murrah buffaloes have been shown in Table 8. The diameter of the drumsticks showed an abrupt increase or decrease (fluctuations) in different breed groups as the number of nuclear lobes per polymorphonuclear leucocyte increased from one to six. Significant variations in drumstick diameters were observed between lobes and between different breeds of goats (Table 9). There are no such reports available in the literature so far pertaining to similar observations on the diameter of drumsticks and nuclear lobing. However, several investigators have reported the influence of the

TABLE 3

Drumsticks diameter and mean lobe count of polymorphonuclear
leucocytes in goats and buffaloes

Species/Breed	Diameter of drumsticks for lobes (μ)						Mean diameter of drumsticks for all lobe numbers (μ)	Mean lobe counts
	1	2	3	4	5	6		
A. GOATS								
Beetal	1.050 ± 0.121 (10)	1.142 ± 0.051 (29)	1.157 ± 0.038 (62)	1.118 ± 0.047 (35)	1.000 ± 0.125 (3)	0.875 ± 0.000 (1)	1.123 ± 0.025	2.964 ± 0.125
Alpine	1.229 ± 0.123 (6)	1.063 ± 0.062 (19)	1.164 ± 0.037 (57)	1.257 ± 0.055 (36)	1.113 ± 0.088 (21)	1.375 ± 0.000 (1)	1.171 ± 0.027	3.357 ± 0.109
Saanen x Beetal	0.917 ± 0.273 (3)	0.971 ± 0.078 (17)	1.121 ± 0.035 (67)	1.171 ± 0.048 (35)	1.017 ± 0.069 (15)	1.083 ± 0.182 (3)	1.105 ± 0.025	3.343 ± 0.127
Alpine x Beetal	0.800 ± 0.000 (0)	0.991 ± 0.064 (27)	1.014 ± 0.038 (69)	1.024 ± 0.059 (36)	1.250 ± 0.094 (7)	0.750 ± 0.000 (1)	1.023 ± 0.028	3.186 ± 0.082
Saanen x Alpine x Beetal	1.375 ± 0.000 (1)	0.969 ± 0.056 (28)	1.042 ± 0.044 (59)	1.019 ± 0.053 (40)	1.114 ± 0.088 (11)	0.875 ± 0.000 (1)	1.028 ± 0.027	3.250 ± 0.101
Mean for Goats	1.143 ± 0.100 (20)	1.027 ± 0.033 (120)	1.100 ± 0.030 (314)	1.118 ± 0.045 (182)	1.099 ± 0.045 (57)	0.992 ± 0.110 (7)	1.090 ± 0.029	3.220 ± 0.071
B. BUFFALOES								
Murrah	1.200 ± 0.101 (10)	1.055 ± 0.071 (34)	1.053 ± 0.056 (61)	1.009 ± 0.065 (28)	1.031 ± 0.252 (4)	0.792 ± 0.042 (3)	1.065 ± 0.033	2.936 ± 0.118

Figures in parentheses indicate the number of observations

degree of nuclear lobing on the incidence of drumsticks (Davidson and Smith, 1954; Luers and Petzel, 1958; Siebner et al., 1963). These workers have observed an increase in the frequency of drumsticks with the increase in the lobe number from 1 to 5. However, specific and detailed investigations are needed before any conclusive explanation for these variations in the size of drumsticks with the increase in the lobe number of polymorphonuclear leucocytes nuclei may be ascribed.

TABLE - 9

Analysis of variance for the effect of nuclear lobes on the diameter of drumsticks of different breeds of goats

Source of variation	d.f.	SS	MSS
Between breeds	4	6.439	1.61**
Between lobes	5	4.836	0.967*
Error	690	249.404	0.361

*Significant at 5% level

**Significant at 1% level

d.f. = Degree of freedom

4.7. DRUMSTICKS DIAMETER AND AGE DIFFERENCES

Table 10 shows the variations in the diameter of drumsticks observed in the different age groups of goats and buffaloes. The average of the diameter of the drumsticks in the goats from birth to one year age group measured 1.042 μ ; 1 to 2 year 1.095 μ ; 2 to 3 year 1.082 μ and in age group of 3 years and above measured 1.143 μ . However, in buffaloes the measurements of drumsticks were carried out only in the two age groups in which 0 to 1 year age group showed drumsticks of 1.134 μ and 3 years and above measured 0.990 μ . In the goats of different age groups and breeds, the variations in the drumstick diameter were observed to be statistically significant (Table 11). Similar observations have also been made by Kayumov and Dmitrieva (1973). These workers have studied quantitative characteristics of sex chromatin in women of different age groups (30 years, 30-45 years and over 45 years). The sex chromatin areas were reported to vary significantly in each age group, maximum in the middle age group (30-45 years) and minimum in the oldest (over 45 years). Significant enlargement in the area of the sex chromatin bodies in women of different age groups suffering from

TABLE - 10

Drumsticks diameter in different age groups of goats and buffaloes

Species/Breed	Diameter of drumsticks for age groups (u)			
	0-1 year	1-2 years	2-3 years	3 years & above
A. GOATS				
Beetal	1.067 ± 0.055 (45)	-	1.142 ± 0.070 (15)	1.173 ± 0.026 (80)
Alpine	1.192 ± 0.086 (15)	1.240 ± 0.073 (25)	1.142 ± 0.053 (30)	1.155 ± 0.036 (70)
Saanen x Beetal	0.967 ± 0.057 (30)	1.100 ± 0.910 (10)	1.081 ± 0.055 (20)	1.175 ± 0.034 (80)
Beetal AlpinexBeetal	0.909 ± 0.064 (40)	1.025 ± 0.092 (5)	1.150 ± 0.078 (15)	1.064 ± 0.031 (80)
Saanen x Alpine x Beetal	1.075 ± 0.073 (15)	1.013 ± 0.076 (20)	0.893 ± 0.042 (50)	1.150 ± 0.040 (55)
Mean for Goats	1.042 ± 0.049 (145)	1.095 ± 0.052 (60)	1.082 ± 0.049 (130)	1.143 ± 0.020 (365)
B. BUFFALOES				
Murrah	1.134 ± 0.042 (80)	-	-	0.990 ± 0.049 (60)

Figures in the parentheses indicate the number of observations

diabetes mellitus have also been reported by these workers. The reports for similar work are limited and warrant further investigations in detail before any reasons could be ascribed for such variations in the diameter of drumsticks in different age groups. However, drumstick diameter differences observed between different breeds of goats might be due to the differences in their X-chromosome morphology.

TABLE - 11

Analysis of variance of drumsticks diameter and age groups of goats

Source of variation	d.f.	SS	MSS
Between breeds	4	6.131	1.533**
Between age groups	3	4.687	1.56**
Error	692	234.865	0.339

** Significant at 1% level

* Significant at 5% level

5. S U M M A R Y

1) Some qualitative and quantitative attributes of drumstick (sex chromatin) appendages were examined in the polymorphonuclear leucocytes of goats of different breeds, viz., Beetal, Alpine, Saanen x Beetal crosses, Alpine x Beetal crosses, Saanen x Alpine x Beetal crosses and Murrah buffaloes maintained at National Dairy Research Institute, Karnal.

2) The staining properties of the blood smears and drumstick bodies were studied using Romanowsky's stains and azure dyes. The preparations having distinct nuclei and neutrophilic granules were found to be satisfactory, regardless of their colour.

3) The present study revealed two different forms of drumstick appendages in the polymorphonuclear leucocytes of goats and buffaloes. These were drumsticks having distinct visible stalk and the drumstick which did not reveal a clearly visible stalk. The later category of the drumsticks appeared to lie completely free from the rest of the nuclear lobes. The drumsticks were noted to be located anywhere in the curve of the nucleus.

4) The average size of drumstick was found to be $1.0\mu \times 1.15\mu$ in females of different breeds of

goats and $1.0 \mu \times 1.0 \mu$ in female Murrah buffaloes being approximately similar to the size of the drumsticks noted in most of the mammalian species.

5) A study of the quantitative characteristics of the drumsticks in the leucocytes of goats and buffaloes showed the mean area and mean diameter to be slightly more in goats than the buffaloes. The ratio of the nuclear area to the drumstick area was noted to be more in buffaloes than in goats because of the considerable variations in their nuclear areas.

6) The measurements of the polymorphonuclear leucocytes with and without drumstick showed differences in their sizes among different breeds of goats and in the Murrah buffaloes. The nuclei having the drumstick appendages were noted to be on an average smaller than the nuclei without them.

7) The diameter of the drumsticks was also studied in relation to the number of nuclear lobes of polymorphonuclear leucocytes in various breeds of goats and Murrah buffaloes. Fluctuations in the drumsticks diameter were observed with the increase in the number of nuclear lobes.

8) The diameter of the drumsticks was also studied in relation to the age of the animals. Varia-

tions have been observed in the diameters for the animals belonging to different age groups (0-1 year, 1-2 years, 2-3 years, 3 years and above) of goats and buffaloes. These differences in the diameters of drumsticks were found to be statistically significant among the different breeds of goats.



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* **B I B L I O G R A P H Y** *
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VERIFIED
Manjeet
Singh
Signature

