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Original Research Article

Conventional Cytogenetics in Black Bengal Goats

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A B S T R A C T

Cytogenetics is the study of the structure and properties of chromosomes, it also includes the study of factors that cause chromosomal changes. Conventional cytogenetics using regular banded chromosomal analysis remains a simple and popular technique to get an overview of the genome of different species. Standardized Karyotype and idiogram of the native Black Bengal goats from Instructional Farm Small ruminants, Birsa agricultural university Ranchi Jharkhand were studied. Blood samples were taken from 20 goats, after short term lymphocyte culture the mitotic chromosome preparation was accomplished. The diploid chromosome no was found to be 60, XY consisting of 29 pairs of acrocentric autosomes and a pair of allosomes. The X chromosome was the longest acrocentric and Y chromosome was suspected to be sub metacentric. The present communication gives emphasis on basic knowledge about cytogenetics parameter taken for chromosome study and application of cytogenetics in goat breeding.

Keywords

Cytogenetics, Karyotyping, Chromosome, Centromere

Introduction

Cytogenetics is the study of the structure and properties of chromosomes, their behavior during somatic cell division during growth and development (mitosis), and germ cell division during reproduction (meiosis), as well as their influence on phenotype. Cytogenetics also includes the study of factors that cause chromosomal changes. In the evaluation chromosomes are arranged in an orderly fashion to produce a karyotype. The karyotype represents the metaphase chromosomes characteristic for an individual, breed or species. The graphical representation of the karyotype is the idiogram, which is a drawing or photograph of the chromosomes of a particular cell. It contains information on the length of the chromosomes, morphological characteristics such as location of the centromere, secondary constriction, etc. Cytogenetics applied to domestic animals is a useful biotechnology to be applied in genetic improvement of livestock. It can be used to select reproducers free from chromosomal aberration responsible for abnormal body conformation (aneuploidy), lowered fertility (balanced chromosome abnormalities) or sterility (sex chromosome abnormalities). Chromosomal study may also be used to applied to assess environmental pollution by studying animals living in hazardous areas and using them as biological indicators. Chromosome also represent optimum biological structures for the study of evolution among related (bovids) and unrelated (bovid-humans) species usually

using comparative FISH mapping which is one of the most important powerful tool to establish the correct order of loci among chromosome (Iannuzzi, L., 2007). Cytogenetics is an useful tool to genetically improvement of the livestock, applied to goats, which represent one of the main important domestic species, cytogenetic studies should receive much more attention because the preventive chromosome analysis are economically very motivated. Since reproductive performance is a very important characteristic of domestic animals, the role of chromosome abnormalities as causes of reproductive failure is very important and very often associated with developmetal anomalies, embryonic death and various levels of infertility. The knowledge of the chromosomal pattern and arrangement and their effects on economic traits is helpful in planning animal breeding strategies. Investigations on the chromosomal profile in livestock provide a useful tool to evaluate the reproductive health and fertility status of the breeding animals even at an early age (Basumatary, 2003). Chromosomal abnormalities, actually, account for a substantial loss in animal production (Yadav, 1996); these economic losses are more severe in seasonal species like the goat, for instance, for which reproduction failures can mean the shift of the pregnancy to the next mating season. Cytogenetic screening of species and breed is also important in Animal genetic resources conservation and management. In in situ' conservation programs it is of fundamental importance, on account of the small number of available subjects, to identify and prevent from breeding those subjects that carry a chromosomal abnormality. For the same reasons, in exsitu' programs, it is important to check that the cryopreserved material (cells, sperms, oocytes and embryos) belongs to animals that show a normal karyotype. In this way the cryopreserved materials could be used in the future to build up the breed or to shift into the future the reproductive capacity of the animals. For this reason, it is necessary to keep breeding animals under cytogenetic control, particularly in goat populations applying artificial inseminations, natural service, embryo transfer etc because the inherited aberrations can quickly become distributed in the next generations. So the chances of spreading defects such as Reciprocal or Robertsonian translocation in the population and causing reduced fertility, early embryonic mortility, abortion, decreased litter size etc. has been increased. Identification of chromosomal defect is possible by studying chromosomal profile which include making standardized karyotype and Idiogram. Based on the above facts it become necessary to screen the breeding animals for finding chromosomal abnormalities and ultimately preventing possible economic losses to farmers. Therefore the present study was conducted to understand the chromosomal complement of Black Bengal goat.

Materials and Methods

Selection of animals

The present study was undertaken on Black Bengal goats maintained in Instructional Farm Small Ruminants, Ranchi Veterinary College, Birsa agricultural University, Ranchi. Out of the total animals present in this farm, 20 goats (10 Males and 10 females) were taken randomly from the breeding population for karyological study.

Collection of Blood

2 ml of blood was taken from each goat aseptically from jugular vein puncture in a 5 ml disposable syringe containing 40 IU of

sodium heparin. The samples were carried to the laboratory packed in ice. Care was taken to prevent direct contact of the sample and ice. On arrival to the laboratory the blood samples were kept in the refrigerator at 40^0C till the culture was set up.

The study of somatic metaphase chromosome in goats were carried out by whole blood short-term lymphocyte culture method as given by Moorehead *et al.,* (1960) was followed with slight modifications.

Parameters taken for study

Cytogenetic analyses are almost always based on examination of chromosomes fixed during mitotic metaphase. Metaphase chromosomes differ from one another in size and shape, and the absolute length of any one chromosome varies depending on the stage of mitosis in which it was fixed. However, the relative position of the centromere is constant, which means that that the ratio of the lengths of the two arms is constant for each chromosome.

This ratio is an important parameter for chromosome identification, and also, the ratio of lengths of the two arms allows classification of chromosomes into several basic morphologic types like Acrocentric, Metacentric, Sub- metacentric, Telocentric. Centromere position and arm ratios can assist in identifying specific pairs of chromosomes, but inevitably several or many pairs of chromosomes appear identical by these criteria. The ability to identify specific chromosomes with certainty was revolutionized by discovery that certain dyes would produce reproducible patterns of bands when used to stain chromosomes. Chromosome banding has since become a standard and indispensible tool for cytogenetic analysis, and several banding techniques have been developed out of which G banding: produced by staining with Giemsa after digesting the chromosomes with trypsin is routinely practiced in laboratory. Following parameters were studied in routine karyotyping through lymphocyte culture of whole blood:-

Length

The chromosomes in the karyotypes was measured in millimetre with an accuracy of 0.01 mm, using the Software IKRYOS Karyotyping system V5.15

Relative length

Relative length was represented as the ratio of the length of a chromosome to the total length of haploid set of chromosome containing the X- chromosome and the ratio was expressed in percentage.

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

The short-term lymphocyte culture method as given by Moorehead *et al.,* (1960) is followed with slight modifications.

Results and Discussion

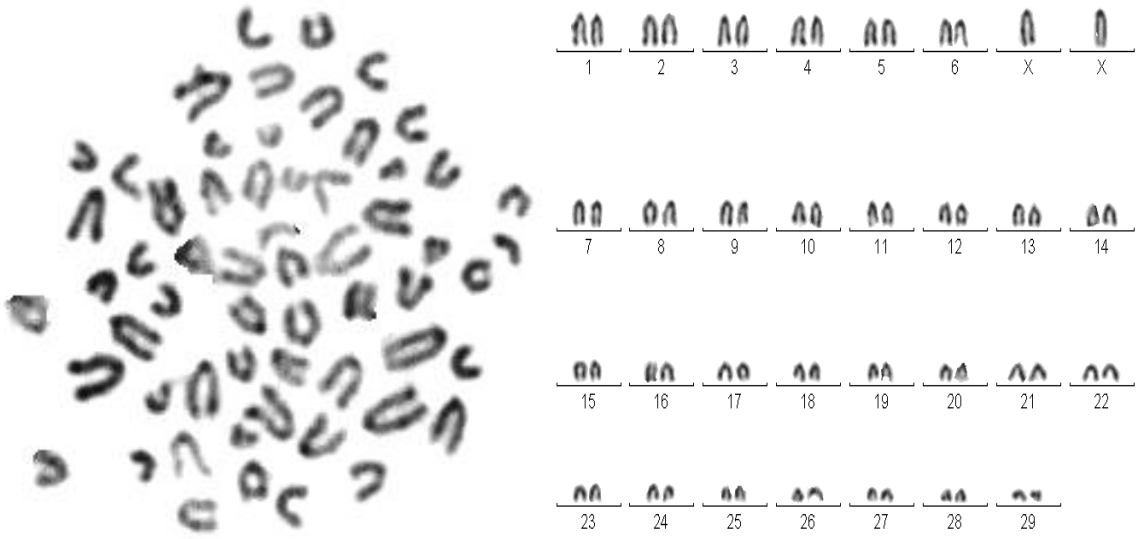
In the present study the diploid chromosome number of Black Bengal goat was found to be 60. Among the total of 60 chromosomes, 58 were autosome and 2 were sex chromosomes. Our findings are in agreement with the observation of Nes *et al.,* (1963), Buttle and Hancock (1966), Gracia Monge (1968), Datta (1970), Evans *et al.,* (1973), Hansen (1973), Khavery (1973), Schnedl and Czaker (1974), Bunch *et al.,* (1977), Ford *et al.,* (1980), Hasanbasic *et al.,* (1984), Cribiu and Matejka (1986,

1987), Das (1990), Bhatia and Shankar (1993, 1994) and Nicodemo *et al.,* (2008). All the 29 pairs of autosome of Black Bengal goat were found to be acrocentric in nature in all the metaphase examined in the present investigation. These finding are in agreement with the findings of Nes *et al.,* (1963) who was the first to describe the autosome in goat as acrocentric. Buttle and Hancock (1966), Evans *et al.,* (1973), Hasanbasic *et al.,* (1984), Berardino *et al.,* (1987), Das(1990), Bhatia and Shankar (1993, 1994) and Kasabe *et al.,* (2009) also reported the acrocentric nature of autosome in different breeds of goat. Pattnanayak and Patre (1986) reported the acrocentric nature of autosome in Ganjam, Black Bengal goat and its crosses. In contrast telocentric autosome has been reported by Khavary (1973). Some of the pioneer scientists like Scaccini (1956), Lopez-Saez and Giamenz-Martin (1963) also reported the autosome in goat as telocentric.

In this study X chromosome was found to be acrocentric and longest one which is in agreement with the observations found by Nes *et al.,* (1963), Buttle and Hancock (1966), Hancock and Jacobs (1966), and Kasabe *et al.,* (2009), though some of the workers posed different views for length of the chromosome. Hauschteck-jungen and Meili (1967) reported the X chromosome to be second longest. Henderson and Bruere (1979) identified X chromosome as third longest and Bhatia and Shankar (1992) as fourth longest chromosome. Bhatia and Shankar (1992) observed the X-chromosome in White Bengal goat as acrocentric as well as biarmed (Polymorphism in X-chromosome).

In this investigation Y-chromosome in Black Bengal goat was found to be smallest, dot like structure and suspected to be submetacentric. This finding is similar to the finding of Bhatia and Shankar (1992) in Marwari goat. This finding also agreed with the observation of Henderson and Bruce (1979), Hasanbasic *et al.,* (1984) and Berardino *et al.,* (1987). However Makino *et al.,* (1967), Datta (1970), Ford *et al.,* (1980), Hasanbasic *et al.,* (1984), Bhatia and Shankar (1992, 1993, 1994), Kasabe (2009) identified the Y-chromosome as minute metacentric.

Mitotic metaphase spread and Karyotype of Black Bengal goat



Mean ± SE of the relative length (%) of the chromosomes in Black Bengal Goat pooled over sex

Chr. Pair Number	Pooled
1	$5.22\pm0.03^{z''''}$
2	$4.98\pm0.03^{z'''}$
3	$4.74\pm0.03^{z''}$
4	$4.54\pm0.02^{z'}$
5	4.36 ± 0.02^{z}
6	4.19 ± 0.02^{y}
7	4.04 ± 0.01^{x}
8	3.91 ± 0.01^{w}
9	3.77 ± 0.01^{v}
10	3.68 ± 0.02^{u}
11	3.56 ± 0.01^{t}
12	3.41 ± 0.01^{s}
13	3.33 ± 0.01^{r}
14	3.24 ± 0.01^{q}
15	3.13 ± 0.01^{p}
16	3.03 ± 0.01^{o}
17	2.93 ± 0.01^{n}
18	2.87 ± 0.01^{m}
19	2.78 ± 0.01^{l}
20	2.64 ± 0.02^{k}
21	2.58 ± 0.02^{j}
22	2.49 ± 0.02^{i}
23	2.39 ± 0.02^{h}
24	2.32 ± 0.02^{g}
25	2.26 ± 0.02^{f}
26	2.15 ± 0.02^{e}
27	2.02 ± 0.03^{d}
28	1.92 ± 0.02^{c}
29	1.78 ± 0.02^{b}
X	$5.76\pm0.05^{z''''}$
Y	1.47 ± 0.03^{a}

**P≤0.01, Value having same superscript in column did not differ significantly. N=100

Estimation of relative lengths of corresponding chromosomes of each sex of Black Bengal goats revealed that the longest autosome contributed 5.19%, 5.25% and 5.22% of haploid genome in female, male and pooled over sex, respectively. The relative length of autosome were almost similar to those of Gaddi, White Bengal, Ganjam and Ganjam X Black Bengal goats reported earlier by Pattnanayak and Patro (1986), Bhatia and Shankar (1991) and Bhatia and Shankar (1992) respectively. The relative lengths of longest autosomes in Black Bengal goat was found to be longer than that of observed by Bhatia and Shankar (1993) as 4.87% in male and 5.04% in female Marwari goats and by Bhatia and Shankar (1992) as overall 4.99% in White Bengal goat.

Das (1990) reported that the contribution of largest autosome in male and female of Assam local goat were 5.50% and 5.53% respectively, which were higher than that of observed in our condition. Nicodemo *et al.,* (2008) found higher contribution i.e. 6.18% of longest autosomes to total haploid genome in Angora goat.

The relative length of smallest autosome contributed 1.78% of haploid genome in data pooled over sex in Black Bengal goat. This finding was slightly higher than that of observed by Hansen (1973) in domestic goat as 1.7% and Das (1990) as 1.57% and 1.61% in male and female respectively in Assam local goat. Bhatia and Shankar (1993) also reported the contribution of smallest autosome to be 1.95% and 1.83% of haploid genome in female and male Marwari goats. The longest acrocentric chromosome was found to be X chromosome in this study. This finding was in agreement with the findings of Bunch *et al.,* (1977), Berardino *et al.,* (1987) and Bhatia and Shankar (1992).

The relative length of X chromosome was found to be 5.76±0.05 % in data pooled over sex in the present study. These findings were higher than that of the findings of Bhatia and Shankar, 1992 (5% in White Bengal goat), Bhatia and Shankar, 1993 (5.21% in Marwari goats), Bhatia and Shankar, 1994 (5.33% in Beetal goat), and Nicodemo *et al.,* 2008 (5.30% in Angora goat) but in agreement with the findings of Pattnanyak and Patre (1986) who observed that the relative length of X chromosome varied from 5.1 to 5.8 percent in Ganjam, Black Bengal and Ganjam X Black Bengal goat.

The relative length of Y chromosome was found to be 1.47±0.03% in Black Bengal goat. This finding is almost similar to the findings of Pattnanyak and Patre (1986) in Ganjam, and Ganjam X Black Bengal, Bhatia and Shankar (1993 and 1994) in Marwari and Beetal goat respectively. Hansen (1973), Das (1990) and Nicodemo *et al.,* (2008) reported the relative length of Y chromosome were 1%, 0.73% and 1.08%, respectively which are lower than that of our findings. However Bhatia and Shankar (1992) reported Y chromosome contributed 1.62% in total genome of White Bengal goat which is higher than that of our findings.

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