# aJava 

Asian Journal of Animal and Veterinary Advances


# Comparative Cytogenetic Study of Garole and Bonpala Breeds of Sheep 

${ }^{1}$ M.A. Ravichandran, ${ }^{2}$ M. Saminathan, ${ }^{3}$ A. Arun Prince Milton, ${ }^{2}$ K. Dhama, ${ }^{4}$ C. Suresh, ${ }^{5}$ K. Jeeva and ${ }^{6}$ S.K. Misra<br>${ }^{1}$ Veterinary Dispensary, V. Pudur, R.K. Pet-631303, Tamil Nadu, India<br>${ }^{2}$ Division of Pathology,<br>${ }^{3}$ Division of Veterinary Public Health, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttat Pradesh, 243122, India<br>${ }^{4}$ Department of Animal Nutrition, Veterinary College and Research Institute (VCRI), Tirunelveli, 627 001, Tamil Nadu, India<br>${ }^{5}$ Department of Veterinary Parasitology, Madras Veterinary College, Chennai, 600007, Tamil Nadu, India<br>${ }^{6}$ Department of Animal Genetics and Breeding, West Bengal University of Animal and Fishery Sciences (WBUAFS), Belgachia, Kolkata, 700037, West Bengal, India<br>Corresponding Author: M.A. Ravichandran, Veterinary Assistant Surgeon, Veterinary Dispensary, V. Pudur, R.K.Pet, 631303, Tamil Nadu, India


#### Abstract

Cytogenetic studies in domestic animals are gaining more importance because of their genetic implications to breeding programmes. The present study describes the chromosome profile and morphometric characteristics of Garole and Bonpala sheep and comparison of chromosomes between males, females and between breeds. The Karyotype revealed diploid chromosome number of 54 (2n) in both breeds and sexes. The first three pairs of autosomes were bi-armed, submetacentric and remaining 23 pairs were acrocentric. The X-chromosome was acrocentric and largest and Y -chromosome was the smallest, biarmed and metacentric. The morphometric analysis showed significant variation in mean relative length of 13th chromosome pair of Garole and Bonpala males and significant variation in the arm ratio of 2 nd chromosome pair of females and variation also noticed in almost all the pairs of chromosomes but not up to the level of significance. The mean relative length of autosomes of Garole and Bonpala male ranged from $1.39 \pm 0.05$ to $11.45 \pm 0.15$ and $1.48 \pm 0.06$ to $11.69 \pm 0.25$ percentage, respectively. The mean relative length of X-chromosome of the males was $5.66 \pm 0.15$ and $5.83 \pm 0.17$, respectively while the Y -chromosome length was $1.20 \pm 0.02$ and $1.27 \pm 0.06$, respectively. The mean relative length of autosomes of the females ranged from $1.43 \pm 0.06$ to $10.80 \pm 0.20$ and $1.42 \pm 0.04$ to $11.42 \pm 0.36$, respectively. The mean relative length of X-chromosome of Garole and Bonpala female was $5.51 \pm 0.13$ and $5.61 \pm 0.15$, respectively. The mean arm ratio of first 2 pairs of autosomes of Garole male was higher than Bonpala male while the 3rd pair was higher in Bonpala males. The mean arm ratio of first 3 pairs of autosomes of Garole female was higher than Bonpala female. The present study for the first time compares the cytogenetic profile between Garole and Bonpala sheep breeds.


Key words: Garole, bonpala, sheep, chromosome, karyotype, relative length, morphometry, arm ratio

## INTRODUCTION

Domestic sheep (Ovis aries) belongs to phylum Chordata, class Mammalia, order Artiodactyla, family Bovidae, genus Ovis and species aries (Shackleton, 1997). Sheep are the very good economic converter of wastelands, grass, stubbles of cultivated corps, tree topping, farm wastes or weeds into meat, milk and wool (Sahana et al., 2001). India has a livestock population of 71.6 million sheep and 140.5 million goats which play a vital role in improving the socio-economic conditions of rural masses (DAHDF., 2012). Though the number of sheep is only about half that of goats, they form the important species of livestock in India.

The Garole (Garole = stupid) sheep is a small-sized native sheep breed found in the coastal saline belt of West Bengal, Sundarbans, swampy delta region ( $4,226 \mathrm{~km}^{2}$ ) of the Ganges river in West Bengal ( $21-23^{\circ} \mathrm{N}$ latitude and $87-89^{\circ} \mathrm{E}$ longitude) and Bangladesh. Garole sheep are popular meat animals in this area, although average live weight of adult ranges between $10-14 \mathrm{~kg}$ only (Selvakumar, 2003; Thanikaivel, 2004; Banerjee et al., 2010, 2011). However, the breed is best known in the region for its prolificacy with mean litter size of 2.27 (Nimbkar et al., 1998). Owing to their high fecundity and ability to thrive on low quality forages and agro byproducts, these animals constitute a major source of income for their owners (Fig. 1).

Bonpala (Bon = forest, Pala = rearing system) is a short stature meat type sheep breed found in the Sikkim, West Bengal, western parts of Bhutan and eastern regions of Nepal (http://www.cswri.res.in/breed_profiles.asp). The breed is popular for excellent reproductive performance with age at puberty occurring around 6 months and first lambing occurring around 11-12 months. Lambing size varies from single to triplets (Tantia and Vij, 2000). Despite its higher reproductive efficiency, the breed is facing the threat of extinction (Fig. 2).

Chromosome study has contributed significantly to understanding of the evolutionary divergence among sheep taxa and different chromosome numbers were found in individual geographic populations (Arslan and Zima, 2011). Due to breed substitution and indiscriminate crossbreeding, the genetic variability of sheep breeds in India is reducing rapidly. The major consequence is the loss of genetic diversity and degradation of many breeds (Kumar et al., 2009). The Garole and Bonpala are very important breeds of sheep and their population is declining very


Fig. 1: Garole female sheep. Animals are small-sized, polled, white and fawn in coat colour, coarse wool and medium sized ears


Fig. 2: Bonpala male sheep. Animals are medium in size, horned, leggy, well-built, small ears and coarse wool
fast in their breeding tracts. Hence, there is an urgent need to save these valuable breeds from extinction through a well planned conservation programme (Kumar et al., 2006). However, before adoption of such programme, the first step is to evaluate the animals not only at phenotypic level but also at genetic level (Acharya, 2000; Singh, 2000; Dorji et al., 2003; Jeremiah, 2005).

Establishment of standard karyotypes for Garole and Bonpala sheep will be of great use for detecting the numerical and structural chromosome anomalies and establishing the cytotaxonomic relation among Caprini species (Dorji et al., 2003; Jeremiah, 2005). Although several studies were made on the phenotypic characterization of this breed, the information on the cytogenetic characterization is very scanty. Therefore, the present study was conducted to understand the chromosomal complement of Garole and Bonpala sheep and to compare the chromosomes between males, females and between breeds.

## MATERIALS AND METHODS

Experimental animals: Twenty Garole sheep ( 10 male and 10 female) were selected for karyotypic study from the Livestock Farm, West Bengal University of Animal and Fishery Sciences (WBUAFS) located at Haringhata, Nadia and 20 Bonpala sheep ( 10 male and 10 female) were selected from the Ram Sahi farm of WBUAFS located at Jalpaiguri district, North Bengal.

Collection of blood: Aseptically 2 mL of blood was collected from each Garole and Bonpala sheep by external jugular vein puncture in a heparinized vials. The blood samples were transported to the laboratory on ice.

Study of somatic metaphase chromosomes: The study of somatic metaphase chromosomes in Garole and Bonpala sheep were carried out by using the whole blood lymphocyte culture technique with some modification following Eldridge (1985).

Preparation of the culture medium: The pH of the medium was adjusted to $7.2-7.4$ by adding one or two drops of sodium bicarbonate solution (7.5\%) till the medium turned into a light pink

Asian J. Anim. Vet. Adv., 10 (2): 48-61, 2015
Table 1: Composition of the culture medium:

| Ingredients | Quantity |
| :--- | :--- |
| TC-199 (Sigma) | 1.51 g |
| Preautoclaved triple distilled water | 100 mL |
| Poke weed mitogen Lectin (working solution-Sigma) | $2 \mathrm{~mL}(0.25 \mathrm{~g})$ |
| Benzyl penicillin (working solution) | $2 \mathrm{~mL}(20000 \mathrm{IU})$ |
| Streptomycin sulphate (working solution) | $2 \mathrm{~mL}(20 \mathrm{mg})$ |
| Ovine autologous serum | 30 mL |
| pH | $7.2-7.4$ |

colour (Table 1). The medium was sterilized by filtering through $0.22 \mu \mathrm{~m}$ Millipore filter paper. Sterile ovine autologous serum of 30 mL was added to the medium and mixed by gentle swirling. The culture medium was then distributed in screw cap culture vials of 30 mL capacity in aliquots of 4.5 mL each. The culture vials were stored in deep freeze $\left(-20^{\circ} \mathrm{C}\right)$ until use. Generally they were used within 2-3 weeks.

Setting up of culture: The frozen culture vials were thawed to room temperature by kept in a water bath $\left(37^{\circ} \mathrm{C}\right)$. Fresh blood ( 0.5 mL ) was added to each culture vial containing 4.5 mL of medium. The contents were then mixed by gentle shaking and the culture was incubated at $37^{\circ} \mathrm{C}$ for 72 h . The cultures were gently agitated twice a day to increase the number of cells in mitosis.

Harvesting of culture: Colchicine ( 0.1 mL containing $0.4 \mu \mathrm{~g}$ ) was added 1 h before the harvesting of cultures. At the end of 72 h , the culture vials were removed from the incubator and the contents were transferred to 15 mL centrifuge tubes and centrifuged at 1000 rpm for 10 min . The supernatant was discarded leaving 0.5 mL of the medium above the cell pellet. The cell pellet was then mixed with 7 mL of pre warmed 0.075 M hypotonic potassium chloride ( KCl ) solution for 12 min at $37^{\circ} \mathrm{C}$. The hypotonic treatment was terminated by adding 2 mL of freshly prepared chilled methanol: Acetic acid (3:1) Cornoy's fixative. The contents were again centrifuged for 10 min at 2000 rpm , supernatant discarded and the pellet was re-suspended in 5 mL of chilled fixative and kept at $4^{\circ} \mathrm{C}$ for 20 min . Then the contents were centrifuged at 1000 rpm for 10 min . This procedure was repeated 3-4 times to ensure the removal of traces of cell debris and water. After final centrifugation, the cell button was re-suspended in 0.5 mL of freshly prepared chilled fixative.

Preparation and screening of slides for metaphase chromosomes: Two to three drops of cell suspension was placed on clean glass slides for chromosome preparations and the smears were heat fixed. The slides were mounted with DPX and air dried. The slides were screened for nonoverlapping metaphase spreads by using Leitz microscope fitted with camera under oil immersion. A minimum of 25 metaphase spreads from each slide were examined to ascertain number, morphology and chromosomal abnormalities. Three metaphase chromosome spreads from each coded slide were selected at random for microphotography.

Preparation of karyotypes and morphometric measurements: The microphotographs were properly magnified and photographed for preparing karyotypes and assessing morphometric characteristics. Each chromosome from photo print was cutout separately and leaving sufficient space around, so that the complete outline of an entire photo print chromosome was visible. Then

Asian J. Anim. Vet. Adv., 10 (2): 48-61, 2015
the chromosomes were matched in homologous pairs and arranged according to the decreasing size and keeping the centromere upwards. The sex chromosomes were placed at the last. The length of each chromosome was measured from tip to tip directly from the magnified print by using a dial type digital caliper with an accuracy of 0.05 mm .

Measurement of relative length: The relative length of each chromosome in relation to the total genomic length was calculated, using the following formula as described by Bhatia and Shanker (1999):

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

Relative lengths of chromosome were calculated based on the contribution of particular chromosome to the total haploid genome length. The relative length of X-chromosome was calculated taking the denominator as total length of autosomes plus X-chromosome. In males, the length of single X-chromosome was taken, whereas in females the average of 2X chromosome was taken. The relative length of Y -chromosome was calculated by taking the denominator as total length of autosomes $+\mathrm{X}+\mathrm{Y}$ chromosomes. The chromosomal lengths thus obtained in magnified values were converted to absolute values in micron ( $\mu$ ).

Arm ratio: The arm ratio was calculated for the biarmed chromosomes using the following formula as described by Bhatia and Shanker (1999).

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

Statistical analysis: The estimates of mean relative length and standard error for each pair of autosomes and sex chromosomes as well as t-test of significance to study the difference of mean relative lengths between males, between females and between breeds of Garole and Bonpala have been estimated (Snedecor and Cochran, 1989) and results are significant at $\mathrm{p}<0.05$.

## RESULTS AND DISCUSSION

For improving production potential of the animal it is important to improve the genetic worth of the animal. Chromosome number and chromosome morphology are the basic requirement for the thorough understanding of the genetics of a species (Dorji et al., 2003; Banerjee et al., 2010). The complete set of chromosomes of an organism is called karyotype and is usually presented as a picture of metaphase chromosomes lined up in descending order of their size. The karyotyping in sheep getting immense importance since its genetic implications to selection and further breeding programmes. Cytogenetics is the hybrid science which attempts to correlate cellular events with genetic phenomena (Acharya, 2000; Jeremiah, 2005). The cytogenetic investigations in domestic animals constitute a matter of both biological and practical importance because of their genetic implications to enhance more economical production of animal products for human consumption. Sheep is having fewer number and smaller size of the chromosomes when compared with cattle and goat (Singh, 2000; Jeremiah, 2005).


Fig. 3: Metaphase chromosome spread of Garole female sheep


Fig. 4: Metaphase chromosome spread of Bonpala female sheep
Modal chromosome number: In the present study, the modal chromosome number of Garole and Bonpala sheep was found to be 54 (2n), comprising of 52 autosomes and 2 sex chromosomes. These findings were in agreement with the reports made in other Indian sheep breeds viz. Nali (Bhatia and Shanker, 1989), Mandya (Langhe et al., 1993; Kumar et al., 2009). Munjal (Bhatia and Shanker, 1994), Malpura (Gupta and Gupta, 1995), Pattanwadi (Bhatia and Shanker, 1999), Garole (Selvakumar, 2003; Thanikaivel, 2004), Mecheri (Karunanithi et al., 2005), Nellore (Amareswar et al., 2005), Coimbatore (Devendran et al., 2009), Marwari, Mandya, Madras Red and Muzaffarnagari (Kumar et al., 2009).

Chromosome morphology: In the present study, based on the position of centromere, the first 3 pairs of autosomes were submetacentric and biarmed in nature. Out of 3 pairs submetacentrics, first pair was the largest followed by 2nd and 3rd pair. The remaining 23 pairs of autosomes were acrocentric and one pair of sex chromosome being homomorphic in females and heteromorphic in males (Fig. 3 and 4). The submetacentric nature of the first three autosomes were in agreement with the reports made in other Indian sheep breeds viz. Mandya (Umrikar and Narayankhedkar, 1997), Lohi (Ali et al., 1999), Nellore (Amareswar et al., 2005), Deccani (Prakash et al., 2008; Arora et al., 2010) and Vizianagaram Sheep (Kumar et al., 2014).


Fig. 5: Karyotype of chromosomes of Garole female sheep
However, these findings were in disagreement with metacentric nature of the first three autosomes reported in other Indian sheep breeds viz. Nali (Bhatia and Shanker, 1989), Munjal (Bhatia and Shanker, 1994), Malpura (Gupta and Gupta, 1995), Pattanwadi (Bhatia and Shanker, 1999), Mecheri (Karunanithi et al., 2005) and Coimbatore sheep (Devendran et al., 2009). Furthermore, Rcheulishvili and Dzhokhadze (1985) reported that the first two autosomes were submetacentric and the third autosome was metacentric in the exotic sheep, Imeritian. The variation in the shape of biarmed chromosomes among the different sheep breeds might possibly be attributed to shift or reciprocal translocation in the arms of chromosomes during the process of evolution (Devendran et al., 2009).

In the present study, X-chromosome of female was found to be acrocentric and largest (Fig. 5). The Y-chromosome of male was metacentric, biarmed, very small in size and its morphology was not clear in the prepared sections (Fig. 6). These findings were similar with the reports made in other Indian sheep breeds viz. Malpura (Gupta and Gupta, 1995), Mecheri (Karunanithi et al., 2005), Deccani (Prakash et al., 2008) and Coimbatore sheep (Devendran et al., 2009). However, these findings were in disagreement with Di Meo et al. (2005) who observed that the comparative Fluorescent in situ Hybridization (FISH) mapping is suitable for better understanding of the morphology of Y-chromosome and reported Y-chromosome was a small submetacentric structure in sheep.

Morphometric measurements: In the present study, the morphometric analysis showed that significant variation in mean relative length of 13th pair of chromosome of Garole and Bonpala males and significant variation noticed in the arm ratio of 2nd pair of chromosome of Garole and Bonpala females and variation also noticed in almost all the pairs of chromosomes but not up to the level of significance.


Fig. 6: Karyotype of chromosomes of Bonpala male sheep


Fig. 7: Comparative idiogram for chromosomes of Garole and Bonpala males
The mean relative length of first 26 pairs of autosomes and one pair of sex chromosome ( X and Y ) of Garole and Bonpala males were presented in Table 2 and Fig. 7. For Garole male the mean relative length of autosomes ranged from $1.39 \pm 0.05$ to $11.45 \pm 0.15$ whereas, in Bonpala male the mean relative length of autosomes ranged from $1.48 \pm 0.06$ to $11.69 \pm 0.25$. These findings were in disagreement with Thanikaivel (2004) who reported that lower relative length of autosomes. In the present study, the mean relative length of X-chromosome of Garole male was estimated to be $5.66 \pm 0.15$ and that of Bonpala it was $5.83 \pm 0.17$. The Y-chromosome length was $1.20 \pm 0.02$ in Garole and $1.27 \pm 0.06$ in Bonpala males.

Table 2: Relative length (expressed in percentage) of chromosomes of Garole and Bonpala male sheep (Mean $\pm$ SEM)

| Chromosome pair | Males |  |
| :---: | :---: | :---: |
|  | Garole | Bonpala |
| 1 | $11.45 \pm 0.15$ | $11.69 \pm 0.25$ |
| 2 | $10.12 \pm 0.15$ | $10.08 \pm 0.25$ |
| 3 | $8.73 \pm 0.26$ | $8.57 \pm 0.29$ |
| 4 | $4.96 \pm 0.13$ | $5.04 \pm 0.09$ |
| 5 | $4.38 \pm 0.07$ | $4.32 \pm 0.08$ |
| 6 | $4.05 \pm 0.09$ | $3.96 \pm 0.07$ |
| 7 | $3.81 \pm 0.09$ | $3.72 \pm 0.06$ |
| 8 | $3.57 \pm 0.07$ | $3.46 \pm 0.07$ |
| 9 | $3.36 \pm 0.08$ | $3.28 \pm 0.06$ |
| 10 | $3.20 \pm 0.07$ | $3.17 \pm 0.05$ |
| 11 | $3.07 \pm 0.06$ | $3.03 \pm 0.05$ |
| 12 | $2.97 \pm 0.05$ | $2.87 \pm 0.06$ |
| 13 | $2.84 \pm 0.04{ }^{\text {a }}$ | $2.71 \pm 0.05^{\text {b }}$ |
| 14 | $2.71 \pm 0.04$ | $2.66 \pm 0.05$ |
| 15 | $2.57 \pm 0.05$ | $2.55 \pm 0.05$ |
| 16 | $2.45 \pm 0.04$ | $2.47 \pm 0.04$ |
| 17 | $2.34 \pm 0.05$ | $2.36 \pm 0.05$ |
| 18 | $2.19 \pm 0.05$ | $2.28 \pm 0.05$ |
| 19 | $2.12 \pm 0.05$ | $2.18 \pm 0.05$ |
| 20 | $2.04 \pm 0.06$ | $2.10 \pm 0.05$ |
| 21 | $1.94 \pm 0.04$ | $2.01 \pm 0.05$ |
| 22 | $1.85 \pm 0.04$ | $1.93 \pm 0.05$ |
| 23 | $1.76 \pm 0.04$ | $1.82 \pm 0.04$ |
| 24 | $1.67 \pm 0.03$ | $1.74 \pm 0.04$ |
| 25 | $1.54 \pm 0.04$ | $1.61 \pm 0.06$ |
| 26 | $1.39 \pm 0.05$ | $1.48 \pm 0.06$ |
| Sex chromosome |  |  |
| X | $5.66 \pm 0.15$ | $5.83 \pm 0.17$ |
| Y | $1.20 \pm 0.02$ | $1.27 \pm 0.06$ |

The mean of a row having different superscript differ significantly ( $p \leq 0.05$ )
The mean relative length of first 26 pairs of autosomes and one pair of sex chromosome ( X and X) of Garole and Bonpala females were presented in Table 3 and Fig. 8. For Garole female the mean relative length of autosomes ranged from $1.43 \pm 0.06$ to $10.80 \pm 0.20$ and in Bonpala female it ranged from $1.42 \pm 0.04$ to $11.42 \pm 0.36$. The mean relative length of X chromosome of Garole female was $5.51 \pm 0.13$ and that of Bonpala female it was $5.61 \pm 0.15$. These findings were in agreement with Selvakumar (2003) and Thanikaivel (2004).

The mean relative length of autosomes and sex chromosome ( X and Y ) of Garole and Bonpala breeds were presented in Table 4 and Fig. 9. The mean relative length of autosomes of Garole breed was ranged from $1.41 \pm 0.04$ to $11.12 \pm 0.14$ and that of Bonpala breed it ranged from $1.45 \pm 0.04$ to $11.55 \pm 0.22$. The mean relative length of X-chromosome of Garole breed was estimated to be $5.59 \pm 0.10$ and Bonpala breed it was $5.72 \pm 0.11$. The relative length of Y -chromosome of Garole was found to be $1.20 \pm 0.18$ and that of Bonpala it was $1.27 \pm 0.06$.


Fig. 8: Comparative idiogram for chromosomes of Garole and Bonpala females

Table 3: Relative length (expressed in percentage) of chromosomes of Garole and Bonpala female sheep (Mean $\pm$ SEM)

|  | Females |  |
| :---: | :---: | :---: |
| Chromosome pair | Garole | Bonpala |
| 1 | $10.80 \pm 0.20$ | $11.42 \pm 0.36$ |
| 2 | $9.54 \pm 0.1461$ | $9.95 \pm 0.32$ |
| 3 | $8.43 \pm 0.25$ | $8.93 \pm 0.29$ |
| 4 | $4.90 \pm 0.20$ | $4.75 \pm 0.10$ |
| 5 | $4.53 \pm 0.17$ | $4.35 \pm 0.09$ |
| 6 | $4.19 \pm 0.14$ | $4.07 \pm 0.07$ |
| 7 | $3.95 \pm 0.11$ | $3.90 \pm 0.07$ |
| 8 | $3.78 \pm 0.10$ | $3.65 \pm 0.05$ |
| 9 | $3.61 \pm 0.09$ | $3.51 \pm 0.05$ |
| 10 | $3.43 \pm 0.09$ | $3.34 \pm 0.04$ |
| 11 | $3.22 \pm 0.06$ | $3.24 \pm 0.03$ |
| 12 | $3.09 \pm 0.05$ | $3.09 \pm 0.03$ |
| 13 | $2.99 \pm 0.05$ | $2.98 \pm 0.04$ |
| 14 | $2.86 \pm 0.05$ | $2.86 \pm 0.05$ |
| 15 | $2.77 \pm 0.04$ | $2.72 \pm 0.07$ |
| 16 | $2.60 \pm 0.05$ | $2.62 \pm 0.06$ |
| 17 | $2.48 \pm 0.05$ | $2.46 \pm 0.07$ |
| 18 | $2.41 \pm 0.05$ | $2.43 \pm 0.09$ |
| 19 | $2.32 \pm 0.05$ | $2.21 \pm 0.09$ |
| 20 | $2.21 \pm 0.07$ | $2.16 \pm 0.09$ |
| 21 | $2.15 \pm 0.06$ | $2.03 \pm 0.09$ |
| 22 | $2.06 \pm 0.07$ | $1.94 \pm 0.08$ |
| 23 | $1.97 \pm 0.08$ | $1.84 \pm 0.08$ |
| 24 | $1.81 \pm 0.10$ | $1.73 \pm 0.06$ |
| 25 | $1.65 \pm 0.05$ | $1.58 \pm 0.05$ |
| 26 | $1.43 \pm 0.06$ | $1.42 \pm 0.04$ |
| Sex chromosome |  |  |
| X | $5.51 \pm 0.13$ | $5.61 \pm 0.15$ |



Fig. 9: Comparative idiogram for chromosomes of Garole and Bonpala breeds

Table 4: Relative length (expressed in percentage) of chromosomes of Garole and Bonpala sheep breeds (Mean $\pm$ SEM)

| Chromosome pair | Breeds |  |
| :---: | :---: | :---: |
|  | Garole | Bonpala |
| 1 | $11.12 \pm 0.14$ | $11.55 \pm 0.22$ |
| 2 | $9.83 \pm 0.12$ | $10.02 \pm 0.20$ |
| 3 | $8.58 \pm 0.18$ | $8.75 \pm 0.20$ |
| 4 | $4.93 \pm 0.12$ | $4.89 \pm 0.08$ |
| 5 | $4.45 \pm 0.09$ | $4.34 \pm 0.06$ |
| 6 | $4.12 \pm 0.08$ | $4.01 \pm 0.05$ |
| 7 | $3.88 \pm 0.07$ | $3.81 \pm 0.05$ |
| 8 | $3.68 \pm 0.06$ | $3.55 \pm 0.05$ |
| 9 | $3.48 \pm 0.06$ | $3.40 \pm 0.04$ |
| 10 | $3.32 \pm 0.06$ | $3.25 \pm 0.04$ |
| 11 | $3.14 \pm 0.04$ | $3.13 \pm 0.04$ |
| 12 | $3.03 \pm 0.04$ | $2.98 \pm 0.04$ |
| 13 | $2.91 \pm 0.03$ | $2.84 \pm 0.04$ |
| 14 | $2.79 \pm 0.03$ | $2.76 \pm 0.04$ |
| 15 | $2.64 \pm 0.04$ | $2.63 \pm 0.05$ |
| 16 | $2.53 \pm 0.04$ | $2.55 \pm 0.04$ |
| 17 | $2.41 \pm 0.04$ | $2.41 \pm 0.04$ |
| 18 | $2.30 \pm 0.04$ | $2.36 \pm 0.05$ |
| 19 | $2.22 \pm 0.04$ | $2.20 \pm 0.05$ |
| 20 | $2.12 \pm 0.05$ | $2.13 \pm 0.05$ |
| 21 | $2.04 \pm 0.04$ | $2.02 \pm 0.05$ |
| 22 | $1.95 \pm 0.05$ | $1.94 \pm 0.05$ |
| 23 | $1.86 \pm 0.05$ | $1.83 \pm 0.04$ |
| 24 | $1.74 \pm 0.05$ | $1.73 \pm 0.04$ |
| 25 | $1.59 \pm 0.04$ | $1.60 \pm 0.04$ |
| 26 | $1.41 \pm 0.04$ | $1.45 \pm 0.04$ |
| Sex chromosome |  |  |
| X | $5.59 \pm 0.10$ | $5.72 \pm 0.11$ |
| Y | $1.20 \pm 0.18$ | $1.27 \pm 0.06$ |

Asian J. Anim. Vet. Adv., 10 (2): 48-61, 2015

Table 5: Mean arm ratio (expressed in percentage) of chromosomes of Garole and Bonpala males (Mean $\pm$ SEM)

| Chromosome pair | Males |  |
| :---: | :---: | :---: |
|  | Garole | Bonpala |
| 1 | $1.26 \pm 0.04$ | $1.19 \pm 0.04$ |
| 2 | $1.25 \pm 0.06$ | $1.21 \pm 0.05$ |
| 3 | $1.20 \pm 0.04$ | $1.24 \pm 0.04$ |

Table 6: Mean arm ratio (expressed in percentage) of chromosomes of Garole and Bonpala females (Mean $\pm$ SEM)

| Chromosome pair | Males |  |
| :---: | :---: | :---: |
|  | Garole | Bonpala |
| 1 | $1.29 \pm 0.04$ | $1.22 \pm 0.03$ |
| 2 | $1.27 \pm 0.04{ }^{\text {a }}$ | $1.14 \pm 0.03^{\text {b }}$ |
| 3 | $1.27 \pm 0.05$ | $1.24 \pm 0.06$ |

The mean of a row having different superscript differ significantly ( $\mathrm{p} \leq 0.05$ )

These results were almost similar to the observations of Langhe et al. (1993) reported that higher values of relative length in Bannur sheep for long chromosomes. However, lower mean relative length of autosomes were reported in Muzzafarnagari (Benjamin and Bhat, 1978), Munjal, Magra and Pattanwadi (Bhatia and Shanker, 1994, 1996, 1999), Nellore (Amareswar et al., 2005), Mecheri (Karunanithi et al., 2005), Deccani (Prakash et al., 2008) and Coimbatore sheep (Devendran et al., 2009).

Mean arm ratio: The mean arm ratio of first 3 pairs of autosomes of Garole and Bonpala male sheep were presented in Table 5. The mean arm ratio of 1st and 2nd pairs of autosomes of Garole male was higher than Bonpala male and the mean arm ratio of 3rd pair of autosomes of Bonpala male was higher than Garole male; however these mean arm ratio values were not statistically significant ( $\mathrm{p} \leq 0.05$ ).

The mean arm ratio of first 3 submetacentric chromosome pairs of autosomes of Garole and Bonpala female sheep are presented in Table 6. The mean arm ratio of first 3 pairs of autosomes of Garole female was higher than Bonpala female. The values of 1st and 2nd pairs of autosomes were not statistically significant ( $p \leq 0.05$ ) but the values of 3rd pair of autosome were significantly different.

In the present study, the mean arm ratio values of first 3 pairs of autosomes of Garole breed was $1.28 \pm 0.03,1.26 \pm 0.03$ and $1.24 \pm 0.03$, respectively. In Bonpala the corresponding values were $1.21 \pm 0.03,1.18 \pm 0.03$ and $1.24 \pm 0.03$, respectively. The values are not significantly different between Garole and Bonpala breeds. In the present study, the arm ratio decreased from first to third autosomes in Garole breed. These findings were in agreement with the reports made in other Indian sheep breeds viz. Munjal (Bhatia and Shanker, 1994), Malpura (Gupta and Gupta, 1995), Pattanwadi (Bhatia and Shanker, 1999), Nellore (Amareswar et al., 2005) and Deccani (Prakash et al., 2008) sheep. However, Langhe et al. (1993) reported higher values in Bannur sheep and Karunanithi et al. (2005) reported lower values in Mecheri sheep.

## CONCLUSION

The present study of cytogenetic evaluation showed that the diploid chromosome number of Garole and Bonpala breeds of sheep was $2 \mathrm{n}=54$, comprising of 26 pairs of autosomes and a pair of sex chromosome. The metaphase chromosome spread and karyotypic pattern was more or less

Asian J. Anim. Vet. Adv., 10 (2): 48-61, 2015
similar to those reported in descript breeds of sheep in India. The chromosome number, morphology and morphometric measurements of the autosomes and sex chromosomes showed that both the Garole and Bonpala breeds of sheep are genetically very close. They might have originated from the same source. Morphologically, both the breeds are very alike except some difference in behaviour and fecundity. Cytogenetic characterization of Garole and Bonpala breeds of sheep performed in the present study forms the basis for molecular genetic characterization and physical gene mapping like FISH (Fluorescent in-situ Hybridization). Routine cytogenetic screening enables early detection and culling of animals with chromosomal abnormalities and reduces economic loss. Further, the chromosome organization information revealed by karyotyping may aid in thorough understanding of evolution gene expression and characterization which in turn may result in the conservation of these sheep breeds.

## ACKNOWLEDGMENT

All the authors of this manuscript thanks and acknowledge their respective University/Institute for providing necessary facilities for carrying out this research work.

## REFERENCES

Acharya, R.M., 2000. Management and conservation of livestock genetic resources-impact analysis. Proceedings of the National Workshop on Conservation and Management of Genetic Resources of Livestock, (NWCMGRL'00), New Delhi, India, pp: 9-23.
Ali, I., S. Ali, R H. Mirza and M. Afzal, 1999. Identification of individual chromosome pairs by Giemsa banding in Lohi sheep. J. Anim. Plant Sci., 9: 85-88.
Amareswar, P., B.R. Gupta, G.N. Rao and G.V.N. Reddy, 2005. Cytogenetic characterization of Nellore sheep. Ind. J. Anim. Sci., 75: 433-437.
Arora, R., S. Bhatia and B.P. Mishra, 2010. Genetic analysis of Deccani sheep. Indian Vet. J., 87: 1109-1112.
Arslan, A. and J. Zima, 2011. Banded karyotype of the Konya wild sheep (Ovis orientalis anatolica Valenciennes, 1856) from Turkey. Comp. Cytogenet., 5: 81-89.
Banerjee, R., P.K. Mandal, U.K. Pal and K. Ray, 2010. Productivity and genetic potential of garole sheep of India: A review. Asian J. Anim. Sci., 4: 170-189.
Banerjee, S., S.M. Galloway and G.H. Davis, 2011. Distribution of prolific Garole sheep in West Bengal, India. Anim. Genet. Resour., 48: 29-35.
Benjamin, B.R. and P.P. Bhat, 1978. A note on the study of sheep chromosomes by cell culture technique. Indian J. Anim. Sci., 48: 234-237.
Bhatia, S. and V. Shankar, 1989. Chromosomes of nali sheep. Indian J. Anim. Sc., 59: 297-299.
Bhatia, S. and V. Shanker, 1994. Cytogenetic characteristics of Munjal sheep. Indian J. Anim. Sci., 64: 975-977.
Bhatia, S. and V. Shanker, 1996. Chromosomes of Magra sheep. Indian J. Anim. Sci., 66: 511-515.
Bhatia, S. and V. Shanker, 1999. Cytogenetic studies in Patanwadi sheep. Cheiron, 28: 34-39.
DAHDF., 2012. Annual report-2012-13. Department of Animal Husbandry Dairying and Fisheries, Ministry of Agriculture, Government of India, New Delhi.
Devendran, P., N. Murali, S. Panneerselvam and N. Kandasamy, 2009. Cytogenetic characterization of Coimbatore sheep. Indian J. Small Rumin., 15: 55-61.
Di Meo, G.P., A. Perucatti, S. Floriot, D. Incarnato and R. Rullo et al., 2005. Chromosome evolution and improved cytogenetic maps of the $Y$ chromosome in cattle, zebu, river buffalo, sheep and goat. Chromosome Res., 13: 349-355.

Dorji, T., G. Tshering, T. Wangchuk, J.E.O. Rege and O. Hannote, 2003. Indigenous sheep genetic resources and management in Bhutan. Anim. Genet. Resour. Inform., 33: 81-91.
Eldridge, F.E., 1985. Cytogenetics of Livestock. AVI Publishing Co., Inc., Westport, New Zealand, ISBN: 9780870554834, Pages: 298.
Gupta, N. and S.C. Gupta, 1995. The karyotype of Malpura sheep. Indian J. Anim. Sci., 65: 101-103.
Jeremiah, Z.A., 2005. Cytogenetics and its relevance to the practice of modern medicine. J. Med. Lab. Sci., 14: 1-12.
Karunanithi, K., M. John Edwin, A.K. Thiruvenkadan and M.R. Purushothaman, 2005. Cytogenetic studies in Mecheri sheep of Tamilnadu. Indian Vet. J., 82: 953-956.
Kumar, I.S., B.P. Kumari, M.G. Prakash and J. Suresh, 2014. Cytogenetic characterization of Vizianagaram sheep. Indian J. Anim. Res., 48: 532-536.
Kumar, P., V. Choudhary, K.G. Kumar, T.K. Bhattacharya, B. Bhushan, A. Sharma and A. Mishra, 2006. Nucleotide sequencing and DNA polymorphism studies on IGFBP-3 gene in sheep and its comparison with cattle and buffalo. Small Rumin. Res., 64: 285-292.
Kumar, P., K.G. Kumar, T.K. Bhattacharya, B. Bhushan, A. Sharma and S.P.S. Ahlawat, 2009. Molecular and cytogenetic evaluation of four Indian breeds of sheep. J. Applied Anim. Res., 36: 261-266.
Langhe, R.S., S.G. Narayankhedkar and M.D. Chauhan, 1993. Cytogenetic studies in domestic sheep (Ovis aries). Cheiron, 22: 23-25.
Nimbkar, C., P.M. Ghalsasi, R.R. Ghatge and G.D. Gray, 1998. Establishment of prolific Garole sheep from West Bengal in the semi-arid Deccan Plateau of Maharashtra. Proceedings of the 6th World Congress on Genetics Applied to the Livestock Production, Volume 25, January 1116, 1998, Armidale, pp: 257-260.
Prakash, M.G., G.N. Rao, B.R. Gupta, A. Venkatramaiah and G.V.N. Reddy, 2008. Chromosomal profile of Deccani sheep. Indian J. Anim. Sci., 81: 397-400.
Rcheulishvili, M.D. and T.A. Dzhokhadze, 1985. A karyological study of Imeritian sheep. Soobshch Akad Nauk Gruz SSR., 117: 585-588.
Sahana, G., S.C. Gupta and A.E. Nivsarkar, 2001. Garole: The prolific sheep of India. Anim. Genet. Resour. Inf., 31: 55-63.
Selvakumar, C.T., 2003. Cytogenetic studies on the variabilities of metaphase chromosome in abnormal Garole sheep. M.Sc. Thesis, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata.
Shackleton, D.M., 1997. Wild Sheep and Goats and their Relatives: Status Survey and Conservation Action Plan for Caprinae. IUCN, Cambridge, England, ISBN-13: 9782831703534, Pages: 390 .
Singh, R.N., 2000. Conservation and management of sheep genetic resources. Proceedings of the National Workshop on Conservation and Management of Genetic Resources of Livestock, (NWCMGRL'00), New Delhi, India, pp: 170-180.
Snedecor, G.W. and W.G. Cochran, 1989. Statistical Methods. 8th Edn., Iowa State University Press, Amesw, Iowa, USA., ISBN-13: 978-0813815619, Pages: 503.
Tantia, M.S. and P.K. Vij, 2000. Population estimates of sheep and goat breeds of India. Indian J. Anim. Res., 34: 60-63.
Thanikaivel, V., 2004. Studies on G-banded karyotypes in Garole sheep. M.V.Sc. Thesis, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata.
Umrikar, U.D. and S.G. Narayankhedkar, 1997. Chromosome analysis of Mandya sheep. PKV Res. J., 21: 97-98.

