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# KARYOLOGY OF KARI SHEEP

S. AHMAD AND M. S. KHAN

Department of Animal Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

## ABSTRACT

Kari sheep is an important genetic resource of Pakistan endemic to Chitral in North Western Frontier Province of Pakistan. The objective of the present study was to establish karyotype of this breed. Blood samples from five representative specimens of Kari sheep, including one ram and four ewes, were examined for chromosomal spread from metaphase of the lymphocyte culture. Animals for this purpose were brought to Peshawar, as traveling to nearest laboratory with 12 hours was difficult. The homologous chromosomes were arranged in pairs in their descending order and size. Diploid number of chromosomes in Kari sheep were 54 (27 pairs), including 26 autosome pairs and one sex-chromosome pair. The chromosome array in ewes was similar to ram, except for the sex chromosome. Both the homologous sex chromosomes (XX) in ewes were large but similar. The results confirm that Kari falls within the category of domestic sheep (*Ovis aries*).

**Key words**: Karyotype, sheep, Kari.

## INTRODUCTION

Sheep domestication during the last 10,000 years has resulted in an increase in body size, a decrease in horn size and a change from a hairy molting fleece to a white wooly fleece (Ryder, 1983). Hundreds of local breeds and strains have been developed for different production systems throughout the world, carrying different genetic material organized mainly in the chromosomes within a cell. The chromosomes are classified on the basis of their size, shape and the position of the centromere. There is enormous diversity in chromosome number and structure (karyotype) between mammalian species. Sheep is one of such species, having variable chromosome morphology and count.

Nadler *et al.* (1973a) recognized different groups of wild sheep; these were: Mouflon (*O. mousimon/orientalis*, 2n=54), Urial (*O. vignei*, 2n=58), Argali (*O. ammon*, 2n=56), amphiberingian dall (*O. dalli*, 2n=54), big horn (*O. canadensis*, 2n=54) and snow sheep (*O. nivicola*, 2n=52). However, the Siberian snow were later shown to have a karyotype of 2n=52 (Geist, 1991). Despite the differences in chromosomes number, different species of the genus *Ovis* can hybridize in captivity (Nadler *et al.*, 1973b) and in some cases in natural habitats where ranges overlap (Nadler *et al.*, 1971; Valdez *et al.*, 1978) and can produce fertile offsprings. Accordingly, Mouflon/Urial hybrids display intermediate chromosomes number of 55 to 57, observed in Northern (Nadler *et al.*, 1971) and Southern Iran (Valdez *et al.*, 1978). It was suggested that the domestic sheep are descended from the Asiatic Mouflon (*Ovis orinetalis*) of Western and Southwest Iran. The mitochondrial DNA sequence variations have further confirmed these early findings (Hiendleder *et al.*, 1998).

Shorter gestation period and shorter lambing intervals in Kari sheep (Ahmad *et al.*, 2002) raised many questions including its speciation and karyology. Gestation period of 90 days duration was repeatedly narrated by the Kari farmers during the referred survey. While studies to confirm these claims are being carried out, the current study was carried out to determine the number of chromosomes for Kari sheep.

## MATERIALS AND METHODS

Blood samples from five representative specimens of Kari sheep located in the Lotkho Tehsil of Chitral district of North Western Frontier Province of Pakistan, including one ram and four ewes, were collected in heparin coated vacutainers and were brought to laboratory within 12 hours at $37^0$C. As it was difficult to travel from Chitral to nearest laboratory (at Peshawar) in 12 hours, animals were brought to Peshawar. Blood lymphocytes were cultured in a media containing fetal bovine serum (20 ml), penicillin and streptomycin (1 ml), L-glutamine (1 ml) and sodium heparin (1 ml).

Approximately 0.8 ml blood was inoculated in a sterile media (5 ml) having 20 µl phytohaemagglutinin. After 72 hours of incubation at $37^0$C, 200 µl colchicin was added and incubated again at the same temperature for 30 minutes. Supernatant was drained and the pellet thus obtained was treated with hypotonic solution of

0.075M potassium cloride (8 ml), added drop by drop on tube walls to the pellet, keeping it at 15-25$^0$C for 20 minutes. Approximately 5 ml of fixative (60 ml methanol and 20 ml glacial acetic acid) was added drop by drop. After centrifugation for 7 minutes (without heating or cooling the sample), the pellet was obtained and was repeatedly treated with fixative 3 times. Finally, 2-3 ml of fixative was added to the pellet for slide preparation (Babar, 1987).

After cleaning each slide and washing with fixative, a drop of sample was dropped on the slide from a height of 1-1.5 ft. The slide was treated with hot steam, dried through hot plate and stained with Giemsa. After 3 minutes in the open air, the slide was dried with hot air. The slides were examined at low power (100 X) to scan the mitotic spread. The selected slides having chromosomes arrested in metaphase were coated with oil immersion and snapped. The homologous chromosomes were arranged in pairs in their descending order and size according to Book *et al.* (1960) and re-snapped.

## RESULTS AND DISCUSSION

Chromosome spread from metaphase of the lymphocyte culture from Kari sheep is presented in Fig. 1. It is evident from the slides that the diploid number of chromosomes in Kari was 54 (27 pairs), including 26 autosome pairs and one sex-chromosome pair. The autosomes spread at the metaphase stage were arranged in four groups (A-D), according to the size and form as shown in Fig. 2. Groups A, B, C and D (presented in rows in descending order) comprised of 1-6, 7-13, 14-21 and 22-27 chromosomes, respectively. Chromosomes in groups A were comparatively larger, followed by B, C, and D in the descending order. One of the homologue sex-chromosome pair in ram was large in size (X-chromosome) and the other was shorter (Y-chromosome). The X-chromosome in ram, although large in size but was grouped separately.

Chromosome spread from metaphase spread of the lymphocyte culture from Kari ewe is presented in Fig. 3. The chromosomal array in ewes was similar to ram, except for the sex chromosome. Both the homologous sex chromosomes (XX) in ewes were large but similar.

Results obtained in the present study have close agreement with many previous findings on sheep (Babar, 1987; Broad *et al.*, 1997), where 26 pairs of autosomes and two sex chromosomes have been reported. The X was the largest chromosome and the Y was a very small chromosome, which usually look like a small, square dot. The X chromosome could be recognized in unbanded preparations because of the presence of small short arm (Broad *et al.*, 1997). The results confirmed that Kari is a member of the domestic sheep (*Ovis aries*) and could interbreed and their offsprings could be fertile.

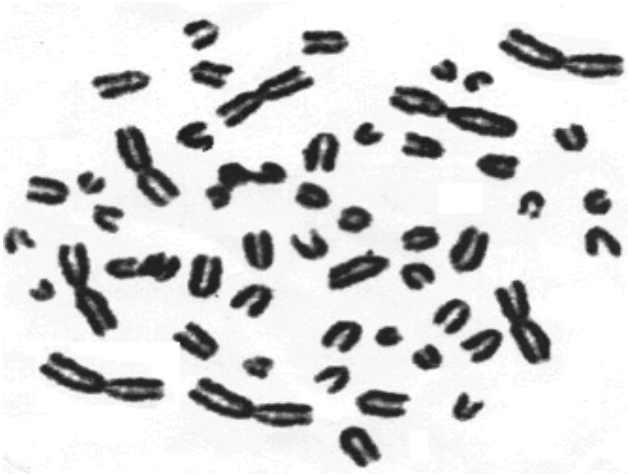

**Fig. 1: Chromosomal micrograph of a Kari sheep.**

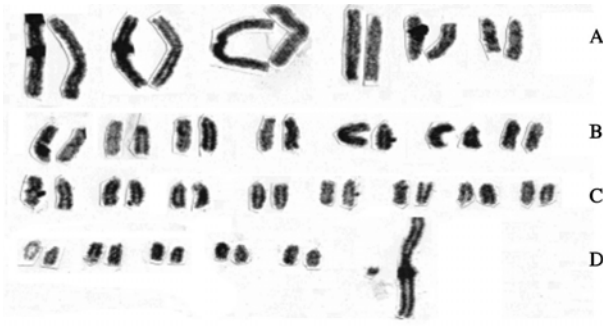


**Fig. 2: Chromosomal array of a Kari ram.**

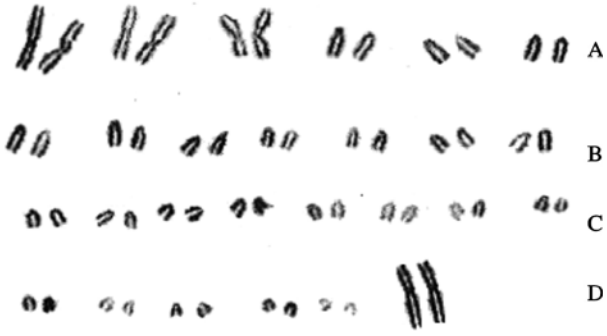


**Fig. 3: Chromosomal array of a Kari ewe.**

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