Chromosome Karyotype Analysis of Cultivate Somatic Cells of Ganjia Sheep

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***Abstract*— Cytogenetics studies in domestic animal are gaining importance because of their genetics and implication in breeding programmes. The present study describes the chromosome number and karyotypic characteristics of Ganjia sheep and comparison between males and females breeds. We adopt the method of cultivating somatic cells, and analyzed the chromosome karyotype of the Ganjia sheep. The result indicates the diploid chromosome of the Gangia sheep is 2n=54, in which 26 pairs of autosomes and a pair of sex chromosome were observed. Result indicates all autosomes are tip silk. The X chromosome was acrocentric and largest except the first three pairs that were metacentric. The Y chromosome was the smallest, biarmed and probably metacentric chromosome.**

***Index Terms*— Chromosome, Ganjia sheep, Karyotype**

I. Introduction

Analysis of chromosome between species has contributed significantly to our knowledge of identifying cell lines and evolutionary relationships and helped to clarify our understanding of evolutionary mechanisms and processes among population [1].

Chromosome analysis can also distinguish between normal and transformed cells, because the chromosome number is more stable in normal cells (except in mice, where the chromosome complement of normal cells can change quite rapidly after explanation into culture). Stains and preparation techniques have evolved that have made possible for a great number of discoveries in cytogenetic [2].

Karyotype is the representation of entire metaphase chromosomes in a cell, arranged in order of size. The study of karyotypes is made possible by staining. Usually, a suitable dye is applied after cells have been arrested during cell division by a colchicine solution [3].

Recent development of techniques for *in vitro growth* of tissue cells and the preparation of chromosome spreads from such cultures have generated renewed interest in the chromosomal complement of animal cells [4]. While such interest has naturally centered on human cells, some attention has been given to those of domestic animals. Most (but not all) species have a standard karyotype[5]. The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes. Normal karyotypes for females contain two X chromosomes and are denoted as 44,XX whereas males have both an X and Y chromosome denoted as 44,XY. Any variation from the standard karyotype may lead to developmental abnormalities. Meanwhile, karyotypes prepared from peripheral leucocyte cultures have been published for the pig, horse and donkey [6][7]. Successful culture methods for bovine tissue culture have been described as the classic pathway [8].

This project was undertaken to determine the requirements for *in vitro growth* of ovary cells and spermary cells of Ganjia sheep, and the chromosomal characteristics of such cells.

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II. Materials and Method

Materials

Sterile or aseptically prepared:
1. Culture of cells in log phase
2. Colcemid
3 D-PBSA (0.9%)
4. Trypsin, 0.25% crude
5. Serum (inactivate at 56°C)

Non-sterile solution
Hypotonic solution: 0.04 M KCL,0.025

Methods

Successful ovary and spermary tissue samples for this study were removed from Ganjia sheep through primary culture. Subculture tissue cells were incubated until the glass wall were covered at least 60%. Colchicine was added 6 hours before cell-harvesting and trypsin was used to detach the cells from glass. Cells were harvested followed by hypotonic solution treatment and fixed in methyl-acetic(3:1) fixative. The air-dried slides were stained with giemsa.

III. Results and Discussion

A total of 100 metaphase figures from males and 100 from female sheeps were examined, and their chromosome number distributions were given in Figure 1 and Figure 2. Figure 1 and Figure 2 show the numerical variations of chromosomes observed in 100 spermary and 100 ovary cells of Ganjia sheep. The chromosome number varied over a rather wide range with the most frequent variation lying between 40-60. The modal number of 54 chromosomes agrees with previously published values for sheep (9, 10, 11).

Figure 1 indicates the chromosome number in male sheep varied over a wide range of between 40 to 60, in which 54 chromosome is dominant in the chromosomal morphology and number. Similarly, the trends are also observed in Figure 2 of the chromosome number in female sheep. Figure 2 shows the most frequent variation lies between 48 to 60, in which 54 chromosomes is dominant. It is well known that variant cell has as one of its general characteristics, one or more stem-line cells.

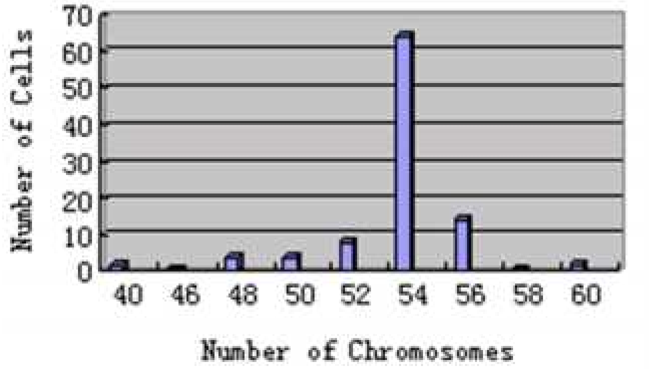

Fig 1. Chromosome number distribution observed in 100 spermary cells of male sheep

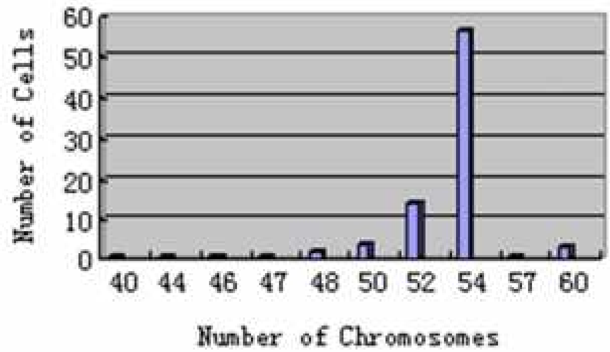

Fig 2. Chromosome number distribution observed in 100 ovary cells of female sheep

Figure 3 and 4 are karyotypic representative of male and female cells. From Figure 3, three pairs of autosomal chromosomes can be readily distinguished from the 23 autosomal chromosome by their length and structure. The first three pairs (chromosome no 1 to 3) are clearly the longest and metacentric (the centromere is located at the centre of the chromosome) while the remaining 23 autosomes (chromosome no 4 to 26) are acrocentric, in which the centromere is located quite near to one end of the chromosome. Similar pattern was also observed for female karyotype in Figure 4. Satellite chromosomes and arms with deletions were not encountered from both karyotype.

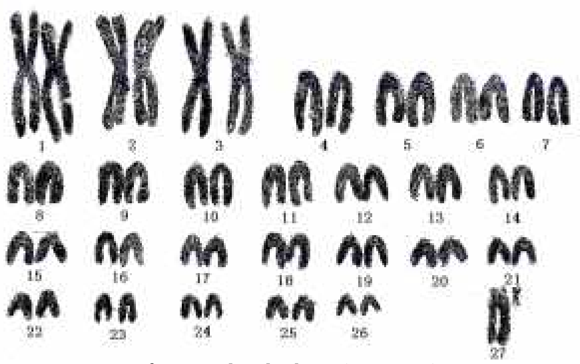

Fig 3. Karyotype of a normal male sheep (52 A+XY)

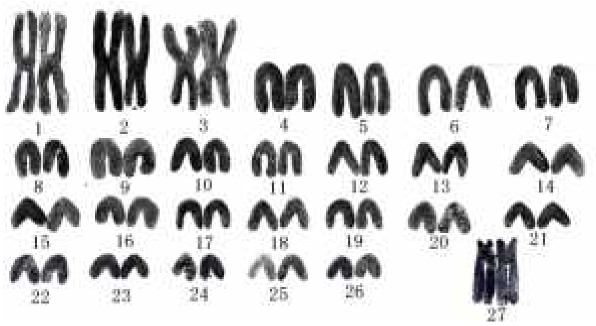

Fig 4. Karyotype of a normal female sheep (52 A+XX)

While Ravichandran *et al* (2015) illustrated both the sex chromosomes of the sheep as being quite small, our preparations indicate that the X chromosomes are the largest of the acrocentric members, having an average length approximately 1.15 times that of the next longest pair. In the female karyotype in Figure 4, sex chromosomes might easily be mistaken for an autosomal pair. However, in the male karyotype a very small and probably submetacentric Y chromosome is easily indentified.

When the karyotype was prepared, the remaining unpaired member (i.e the male X chromosome) is longer than any of the other acrocentrics and appears identical to the X chromosomes of the female. We therefore conclude that, instead of being very small, the X chromosomes of the sheep are quite prominent in terms of size.

The chromosomal complement of the sheep is far from ideal, when identification of a majority of the individual pairs is necessary. This species may, however, be preferred for further studies where quick identification of a small portion of the chromosomes is desired and where the relatively easy technique of leucocyte culture can be employed.

IV. Conclusions

This paper focus on the analysis of chromosomes of Gangia sheep by using the cultivation somatic cells technique. The distribution of chromosome number in Ganjia Sheep tissue cells were quite different from that normal cells and modal chromosome number lied in both hypodiploid (40) and diploid (60) ran. The frequency of diplochromosomes was 0.06 per 100 cells (6%), and this rate is considerably lower in comparison with that of normal cells. The frequency of chromosomes aberrations was very lower through statistic theory, T detections expressed that both two tissues were $|t| < t_{0.05(\text{both side})}$, it means the aberrations could be acceptable. The modal number of chromosomes was found to be 54. Three pairs of autosomes are readily identifiable as metacentric, while the remaining 23 pairs are acrocentric and not easily distinguishable from each other. The X chromosomes are the largest among the acrocentric members, while the Y appears as a very small and probably metacentric chromosome.

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