Chromosomes of Normal and Dwarf Cattle

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Chromosomes of normal domestic cattle have been recently observed by several investigators with the help of the modern technique of tissue culture. Sasaki and Makino (1962) observed the chromosomes from cultured cells of kidney or skin obtained from adult specimens. Nichols et al. (1962), Biggers and McFeely (1963), and Ulbrich and Weinhold (1963) observed the chromosomes by peripheral blood culture technique. On the other hand, Ohno et al. (1962) observed directly the chromosomes from the gonads and femoral bone marrow.

The intensive examination of human chromosomes during the past few years has led to important discoveries which helped to understand certain disorders. In domestic animals, however, similar studies are very few. The present authors have undertaken the study of chromosomes of a genetic malformation in cattle known as dwarfism. In the present paper the results are reported.

Material and Methods

Cattle, both normal and dwarf, were obtained from the "Bay-Manor-Farm" Lewis, Delaware, U. S. A., by courtesy of Mr. Dale Liable, manager of the farm. They were two months old "Hereford" strain. The underside of an animal’s body was shaved, then wiped with ethyl alcohol. For biopsy of the skin 20 mm x 20 mm squares approximately 4 mm thick, were cut out and the specimens were placed in a jar containing Eagles 2 (pH 7.2) with 20% calf serum.

5 or 6 hours after specimens were returned to the laboratory, they were washed three times with normal saline. The tissue was again transferred to another sterile petri dish and minced finely with scissors, then resuspended in 10 ml of the above medium. 5 ml of the suspension was placed in a 2 oz. prescription bottle. The caps on the bottles were left loose, then the bottles were stored in a CO₂ incubator with 5% CO₂ at 37°C and approximately 80% humidity. The cells started to grow and spread after 48 hours. At this time the medium was poured off and the bottles were refilled with fresh medium. After 48 hours the cells were scraped off the wall with

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a rubber "policeman" and the suspension was vigorously pipetted. 2.5 ml of the suspension was added to another 2 oz. prescription bottle, plus 2.5 ml of fresh medium. This time the caps were tightened and stored in the incubator again for 72 hours. Then, 0.1 ml colchicine solution (1:10,000) was added to the culture medium for 2 hours whereupon the medium was decanted, and trypsin solution was added to dislodge the cells from the bottle walls. The cell suspension thus obtained was centrifuged for five minutes at 1,000 r.p.m. Drops of the precipitant were placed on clean

Figures 1 to 6. Metaphasic chromosomes in cells of normal female and male and female dwarf cattle. Figs. 1 and 4, normal female cells. Figs. 2 and 3, normal male cells. Figs. 5 and 6, dwarf female cells. X and Y chromosomes are denoted by X and Y. All figures were drawn with camera lucida at magnification of about 2000X.
slides and squashed after pretreatment with hypotonic hyaluronidase solution (30 usp/cc alidase) according to acetic dahlia squash method. Camera lucida drawings were used for karyotype analysis.

**Results of Observations**

1. **Karyotype of normal females**

   The number of chromosomes in diploid cells of females was 60 as found previously by several investigators (Figs. 1, 4). Morphology of individual chromosomes was analysed on the basis of size and shape. Idiogram analysis of several reliable cells revealed that the chromosomes can be assorted into 30 homologous pairs. Among them 29 pairs were characterized by telocentric centromers, and one pair by submedian centromere (Figs. 9, 10). By comparison of chromesomes in males and females it was recognized that these submedian metacentric chromosomes are the X's. They seemed to rank in relative magnitude with the first and second autosomal pairs.

2. **Karyotype of normal males**

![Figures 7 and 8. Photomicrographs of normal male and dwarf female cells. Fig. 7, normal male cell. Fig. 8, dwarf female cell. The cell shown in Fig. 7 is the same as that in Fig. 3. The cell shown in Fig. 8 is the same as that in Fig. 5.](image-url)
The number of diploid chromosomes in males was 60 like in female cells (Figs. 2, 3, and 7). Morphology of autosomes in males was also the same as in the females. Sex chromosomes in the male, however, were represented by an X and a Y chromosome. Shape and length of X-chromosome were the same as in the female cells. Y chromosome had a median metacentric centromere and seemed to rank in size between the first and second smallest autosome (Fig. 13).

3. Karyotype of dwarf females

Morphology of autosomes and sex chromosomes in female dwarfs was exactly the same as in normal females (Figs. 5, 6 and 8). They have 58 autosomes characterized by a telocentric centromere, and two X elements with submedian centromere. From the observation of chromosomes, the dwarf karyotypes showed no difference from the karyotype of normal females (Figs. 11, 12).

Discussion

Studies of chromosomes of cattle with the help of the modern cytological technique have been carried out by several investigators (Sasaki and Makino 1962, Nicols et al. 1962, Ohno et al. 1962, Biggers and McFeely 1963, Ulbrich and Weinhold 1963). They observed chromosomes of normal domestic cattle and reported the male karyo-
type as consisting of 58 rod shaped autosomes and a submedian-X and a median-Y chromosome. Our observation of chromosomes in normal and dwarf female cattle showed that they have 60 chromosomes and that there is no difference in the karyotypes between normal and dwarf.

Chromosomes of human dwarfism were observed by Marshall and Crawfurd (1961), and Makino et al. (1961). They observed apparently a normal chromosome condition in these patients. A genetical study of dwarfism in cattle was carried out by Mead et al. (1942). According to them this character is produced by a single autosomal recessive gene. Dwarfism of mice was also studied by several investigators (Snell 1939, Kemp 1933, Gruneberg 1952). They too found that a single recessive gene controls this character. Dwarf female cattle have normal karyotype, this character being controlled by one autosomal gene.

Summary

Chromosomes of dwarf female cattle were observed in comparison with those of normal females and males. Normal as well as dwarf female cattle had 60 chromosomes, namely 29 telocentric autosome pairs and one submedian metacentric X pair. There was no difference in the karyotype between normal and dwarf cattle. Male normal cattle had 29 telocentric autosome pairs and in addition a submedian X and a median metacentric Y chromosome. From the above investigation, it was considered that the character of dwarfism is not reflected in the karyotype being due to a single autosomal gene mutation.

Literature Cited

Mead, S. W., Gregory, P. W. and Regan, M. W. 1942 J. Hered. 33: 411-436.