The National and University Institute of Agriculture

THE VOLCANI INSTITUTE OF AGRICULTURAL RESEARCH

Prelim.
Rep. No. 641
Project No..
00502/21

CHROMOSOMES OF CATTLE (Bos taurus L.) OBTAINED FROM PERIPHERAL BLOOD AND FROM TISSUE CULTURE OF THE TESTICLES AND EPIDIDYMIS

By

M. Wysoki, B. Padeh and R. Volcani

Preliminary Report

Division of Scientific Publications, Rehovot

May, 1969

CHROMOSOMES OF CATTLE (Bos taurus L.) OBTAINED FROM PERIPHERAL BLOOD AND FROM TISSUE CULTURE OF THE TESTICLES AND EPIDIDYMIS

By

M.Wysóki*,B. Padeh** and R. Volcani*

S U M M A R Y

A short-term culture for leucocytes and a different culture for the testicle and epididymis of a bull were set up in a laboratory in order to obtain the chromosomes. The methods of processing and the results are discussed and summarized. The question of the Y-chromosome and its morphology is discussed.

---

* The Volcani Institute of Agricultural Research, Rehovot.

** Cytogenetics Laboratory, Tel Hashomer Hospital, Ramat Gan.

INTRODUCTION

The newer methods of short-term cultures of leucocytes and cultures of different tissues permit a simple determination of the chromosome constitution of animals.

The diploid number of chromosomes in cattle is 2n=60, of which 29 pairs are autosomes and one pair consists of sex chromosomes. There is some difference of opinion about the Y-chromosome: Chiarelli et al. (2) describe it as acrocentric, while Crosley and Clarke (3) describe it as metacentric. We therefore decided to examine it by short-term culture of leucocytes and by tissue culture of the testicles and epididymis.

MATERIALS AND METHODS

For a short-term culture of leucocytes we used the method of Moorhead et al. (4), with our own modification. Ten ml of blood was drawn from the jugular vein with a disposable syringe which contained 0.2 ml of heparin. After centrifugation for 15 minutes at 3000 rpm, the plasma rich in leucocytes was separated from the blood. In the culture which was set up, the number of leucocytes was 1 million per cc of M-199 with the addition of phytohemagglutinin (Bacto-Phytohemagglutinin [PHA] is a muco-protein extracted from Phaseolus vulgaris). When PHA comes in contact with small mature lymphocytes, blastogenesis occurs and mitosis starts. The continuous process of transformation from the beginning until division lasts for 72 hours in the incubator (at 40°C). After 72 hours, 0.2 ml colchicin was added to the culture for 4-6 hours; after centrifugation, a hypotonic solution of 0.95% sodium citrate was added and fixation was achieved with Carnoy fixative (ethyl alcohol: glacial acetic acid, 3 : 1). Slides were prepared and dried by warm air. After staining with 1% acetorcein and dehydration with 75%, 90% and 100% alcohol, the preparations were closed with euparal.

Tissues of the testicle and epididymis were taken for sterile culture from a bull at the time of slaughtering. The tissue was cut into small pieces of approximately 0.3 ml, and grown in plastic Falcon flasks in M-199 with the addition of Fetal Calf Serum; some of the pieces were cultured with the addition of Bovine Testis Extract. The culture was grown in a CO_2 atmosphere, and after

a large number of fibroblasts appeared they were separated with trypsin versen and a sub-culture was set. When the bottom of the sub-culture was full of fibroblasts another sub-culture was set up. After two or three days colchicin at 0.2 mg/100 ml was added for 4-6 hours, or at 0.2 mg/1000 ml for 18 hours, to stop the division at metaphase. Slides were prepared in the same way as with the leucocyte cultures described above.

RESULTS

From the leucocyte culture of the cow 25 metaphases were counted, and from that of the bull 29 metaphases were counted. Among the 25 cells of the cow only one metaphase was polyploid, and in one metaphase one autosomal chromosome was missing, which is most probably an artifact. In the bull only one polyploid cell was found. From the tissue culture of a testicle 20 metaphases were counted, and from that of the epididymis also 20 were counted.

DISCUSSION

As already mentioned, the diploid number of chromosomes in the cells of domestic cattle is 2n=60. All autosomal chromosomes are acrocentric and differ from each other in size. In the photokaryotype (Plate 1) they are placed in declining order according to size. The X-chromosome is a metacentric or a sub-metacentric one and is very distinct microscopically due to its special structure, which differs from the autosomal chromosomes. There is overall agreement among cytogeneticists about the autosomes and X-chromosomes. As to the morphology of the Y-chromosome, there is some controversy. Chiarelli et al. (2) describe it as a medium-sized acrocentric chromosome; Crosley and Clarke (3) and Basrur and Moon (1) describe it as sub-metacentric, and claim that it is the smallest chromosome in the cell.

Our evidence agrees with that of Crosley and Basrur. In Plate 2 is a small, somewhat metacentric chromosome which is the smallest one in the karyotype and is easily distinguishable from all other chromosomes as the X-chromosome.

The reason for the different opinions expressed in the literature is

Plate 1: Female karyotype (Bos taurus L.). Chromosomes arranged in decreasing order of size. Sex chromosomes XX metacentric, as opposed to acrocentric autosomes.

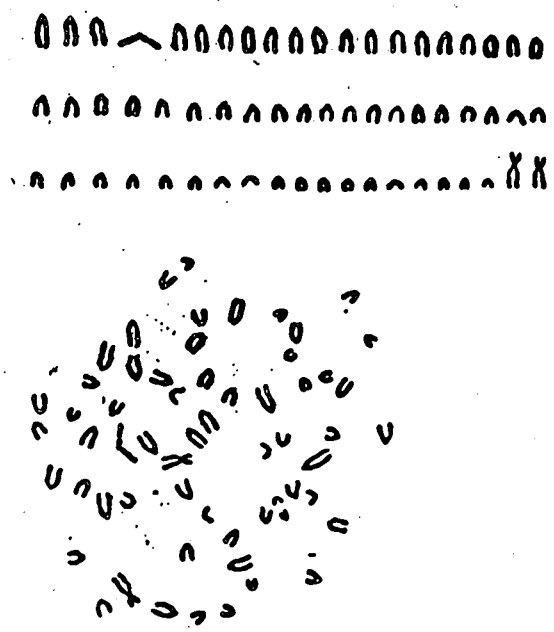
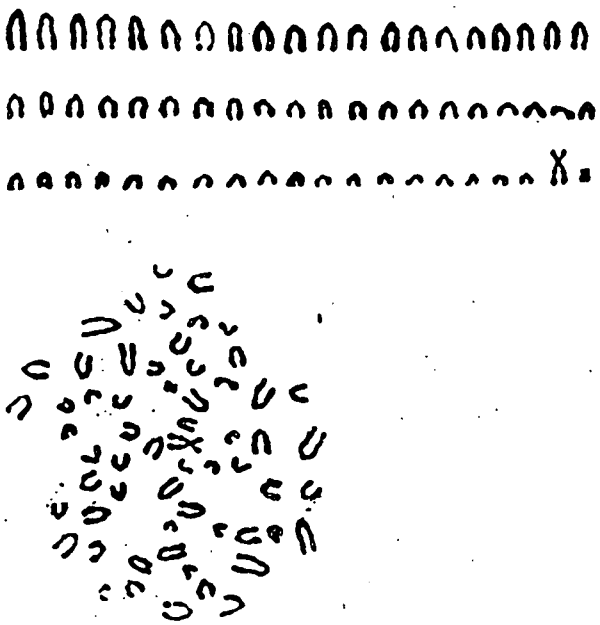


Plate 2: Male karyotype (Bos taurus L.). Sex chromosomes XY; Y small metacentric.



probably due to the various species which are related to cattle, such as Bos bison or hybrids of B. taurus and B. bison (cattalo). The cattalo differ from the domestic cattle in the morphology of the Y. In the latter the Y-chromosome is acrocentric, although the chromosomal number is also 2n=60. The autosomes and the Y-chromosome are acrocentric, whereas the X-chromosome is sub-metacentric. Crosses between the different genera of Bos are successful and fertility is high.

REFERENCES

1. Basrur, P.K. and Moon, Y.S. (1967) Chromosomes of cattle, bison and their hybrid, the cattalo. Am. J. Vet. Res. 28 (126) : 13-19.

2. Chiarelli, B., De Carli, L. and Nuzzo, F. (1960) Analisi morfometrica dei cromosomi di Bos taurus L. Caryologia 13 (3) : 766-772.

3. Crosley, R. and Clarke, G. (1962) The application of tissue culture techniques to the chromosomal analysis of Bos taurus. Genet.Res. 3 : 167-168.

4. Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.M. and Hungerford, D.A. (1960) Chromosome preparations of leucocytes cultured from human peripheral blood. Exp. Cell Res.20 : 613-616.

כרומוסומים של פרות (Bos taurus L.) שהופקו מדם פריפרי ומתרבית רקמה של אשכים ויתרת-האשך

מאת

מ' ויסוקי*, ב' פדה**, ר' וולקני*

תרבית קצרת מועד מלויקוציטים ותרבית מרקמת האשך ויתרת-האשך של פר גודלו במעבדה לצורך עיבוד כרומוסומאלי.

כרומוסומים מתרבית לויקוציטים נבדקו בשיטתם של Moorhead וחובריו (4), שהכנסנו בה שינויים משלנו.

הלויקוציטים הופרדו מדם שהכיל 0.2 ml /10cc הפרין וגודלו במדיום M-199, בריכוז של 10^6 מ"ל/לויקוציטים בתוספת של PHA.

לאחר 72 שעות של אינקובאציה הוספו 0.2 ml כולכיצין. לאחר טיפול היפוטוני ופיקסאציה, הוכנו פרפאראטים שנצבעו באצטואורצאין, עברו סידרת אלכוהולים ונסגרו ב- Euparal.

הרקמות של האשכים ויתרת-האשך גודלו בבקבוק פלסטיק פלקון, במדיום M-199. בתוספת של Fetal Calf Serum, או של Bovine Testis Extract ובאווירה של CO_2. פיברובלאסטים הופרדו בעזרת טריפסין ורסן והועמדה תת-תרבית. כשהתחחית של הבקבוק היתה מלאה תאים נעשתה העברה שנייה, וכעבור 2-3 ימים הוסף כולכיצין 0.2mg/100ml אחת ל-4-6 שעות, או 0.2mg/1000ml אחת ל-18 שעות. פרפאראטים נעשו באותה השיטה שהוכנו מתרבית לויקוציטים.

המספר הדיפלואידי של כרומוסומים של בקר הוא 2n=60 והאוטוזומים הם כולם אקרוצנטריים ונבדלים זה מזה בגודלם. הכרומוסום X הוא סובמטאצנטרי, והכרומוסום Y הוא אקרוצנטרי בינוני, לפי Chiarelli וחובריו (2), וסובמטאצנטרי לפי Crosley and Clarke -(1). Basrur and Moon.

המימצאים שלנו תואמים את אלה של קרוסלי ובסרור.

---

* האגף לבעלי חיים, מכון וולקני לחקר החקלאות, רחובות.

** מעבדה ציטוגנטית, ביה"ח תל-השומר, רמת-גן.

המכון הלאומי והאוניברסיטאי לחקלאות
מכון וולקני לחקר החקלאות

כרומוסומים של פרות (Bos taurus L.) שהופקו מדם צריפרי ומתרבית רקמה של אשכים ויתרת-האשך

מאת

מ׳ ויסוקי, ב׳ פדה, ר׳ וולקני

המחלקה לפיזיולוגיה מדעית
בית-דגן, מאי 1969