

KARYOTYPIC STUDY OF A CANCHIM CATTLE HERD

Antonio Junqueira Tambasco

ABSTRACT

A karyotypic study was conducted on 626 animals of a Canchim (5/8 Charolais + 3/8 Zebu) cattle herd using metaphase spreads obtained from peripheral blood lymphocyte cultures and G- and C-banding techniques. Eleven animals (1.76%), five of which were phenotypically normal, showed karyotype alterations. The results emphasize the need for karyotype analysis as a criterion for the selection of animals to be used for reproduction.

INTRODUCTION

After the development of special chromosome analysis techniques, the cytogenetic study of cattle herds has acquired great importance by contributing to the elucidation of changes that have occurred in the species through the study of karyotype evolution, and by permitting the establishment of associations between karyotype and phenotype in cases of hereditary defects, embryo death, and spontaneous abortions, among others.

After the discovery of the 1/29 Robertsonian translocation in Red and White Swedish cattle and of the relationship of this translocation with low fertility (Gustavsson, 1969), several cytogenetic studies have been conducted on cattle populations in order to determine whether chromosome aberrations may be interfering with fertility, with consequent economic losses. Thus far, more than 30 breeds have been found to have the 1/29 translocation at a frequency ranging from 0.2 to 50% worldwide (Tambasco, 1988).

Today, cytogenetic studies are performed routinely for the early detection and culling of animals carrying transmissible chromosome aberrations. The present study is a report of karyotypic analyses carried out in a Brazilian herd of Canchim cattle.

MATERIAL AND METHODS

The study was conducted on 626 Canchim (5/8 Charolais + 3/8 Zebu) animals born from August 1976 to July 1987 and belonging to the herd of the Canchim Farm, "Empresa Brasileira de Pesquisa Agropecuária", São Carlos, State of São Paulo, Brazil.

The karyotypic study was conducted using metaphase spreads obtained from peripheral blood lymphocyte cultures by the technique of Moorhead *et al.* (1960). All material was analysed with standard giemsa staining. However, some of the samples were also submitted to either G (Drets and Shaw, 1971) or C (Arrighi and Hsu, 1971) banding. Some other samples were analysed with both G and C banding. A minimum of ten metaphases from each animal were evaluated. Whenever chromosome aberrations were found, further analyses were carried out up to a limit of 100 metaphases.

RESULTS AND DISCUSSION

The results are shown in Table I. Eleven of the 626 animals (1.76%) had karyotypic alterations while the remaining animals had normal 60, XY and 60, XX karyotypes. All Canchim males had a Y chromosome of the metacentric type, normal for the species *Bos taurus*. Since some of the alterations were detected in phenotypically normal animals which could have been chosen for reproduction, they could have been transmitted to the offspring. This would not occur with chromosome aberrations of animals already barred from reproduction because of multiple physical abnormalities or because of abnormal sexual development. The transmission of chromosome aberrations by phenotypically normal animals has been found to occur in several studies of cattle populations, especially with respect to the 1/29 Robertsonian translocation (Gustavsson, 1980). Even though this aberration has been found in the Charolais cattle, one of the breeds forming Canchim cattle (Harvey, 1976; Moraes and Mattevi, 1980; Popescu, 1980), it was not detected in the present sample.

The animal with 61,XY + mar karyotype (Figure 1) was phenotypically normal and of normal fertility. The origin of this marker chromosome, which is smaller than the smallest acrocentric autosome, could not be identified by G banding. C banding might have clarified this, but, unfortunately was not used. In some cells in which the marker was not detected or even in metaphases in which it was present, no struc-

tural anomalies were detected by the standard method or by G banding. Twelve members of the family to which this animal belonged were analyzed and all of them showed normal karyotypes.

Table I - Chromosome aberrations detected in a Canchim cattle herd consisting of 626 animals.

Chromosome aberrations	Sex	Nº of animals	Phenotype
60,XX/61,XY + mar (44.4% - + mar)	M	1	normal
60,XX - gap in the X	F	1	normal
60,XY chromosome breaks	M	2	normal
60,XY chromosome breaks	M	3	altered sperm count
60,XX/60,XY(85.0% and 80.0% - XY)	F	2	Freemartin
60,XX/61,XXY(23.8% - XXY)	F	1	Freemartin
60,XY + t (17/21-22)	M	1	normal

Cytogenetic studies on humans have revealed some cases of apparently innocuous chromosome abnormalities mainly related to marker chromosomes. Froland *et al.* (1963) first reported the presence of marker chromosomes in children with physical abnormalities, an occurrence that was later detected by other researchers (Gustavson *et al.*, 1964; Ferrari *et al.*, 1968; Ishmael and Laurence, 1968; Mukherjee *et al.*, 1968; Armendares *et al.*, 1971; Finley *et al.*, 1971). However, cytogenetic studies on neonates have revealed that marker chromosomes are often detected in normal individuals and in several family members of different generations even though no abnormalities are present (Smith *et al.*, 1965; Pfeiffer *et al.*, 1967; Abbo and Zellweger, 1970; Hoehn *et al.*, 1970; Nielsen *et al.*, 1971).

A marker chromosome may have a phenotypic effect depending on its gene content. The presence of such a chromosome in normal individuals may be an indication that this extranumerary chromosome may be formed only of constitutive heterochromatin containing few or no structural genes.

In the present study, a normal female showed a gap in an X chromosome (Figure 2). In human males, this condition is related to mental retardation. Karyotypic analysis of two sons of this female did not show any anomalies.

Three of the five bulls with chromosome breaks showed sperm count alterations (Table I).

There are human pathological conditions that are accompanied by chromosome breaks due to an enzyme defect in DNA repair, such as xeroderma pigmentosum, Fanconi anemia, Bloom syndrome, ataxia telangiectasia (German, 1964; Schroeder *et al.*, 1964; Hecht *et al.*, 1966). Halnan (1975) also detected multiple

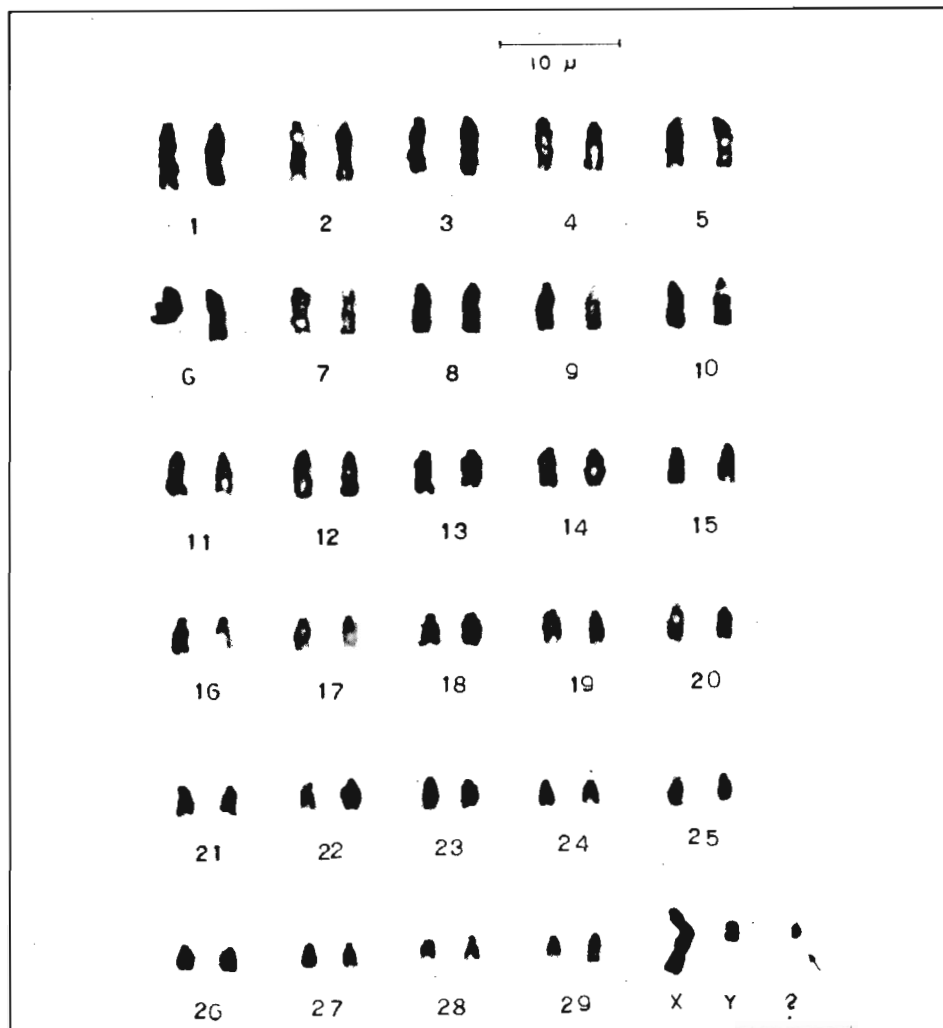


Figure 1 - Karyotype of a cell 61,XY + mar (44.4%) from a bull, 60,XY/61,XY + mar.

chromosome breaks among animals with reproductive problems. However, the causes of these aberrations have not yet been fully elucidated. It is known that the action of certain viruses in higher organisms produces nuclear division anomalies that give rise to nuclei with altered structures and chromosome numbers, as well as gene alterations. In cattle, this is the case for bovine leukosis (Basrur *et al.*, 1964; Gustavsson and Rockborn, 1964; Lungeanu *et al.*, 1978; Stanik and Izarikova, 1979; Stanik *et al.*, 1981).

Of the three sterile females diagnosed as Freemartins in the present study, two had XX/XY chromosomal chimerism and one had either chimerism or mosaicism

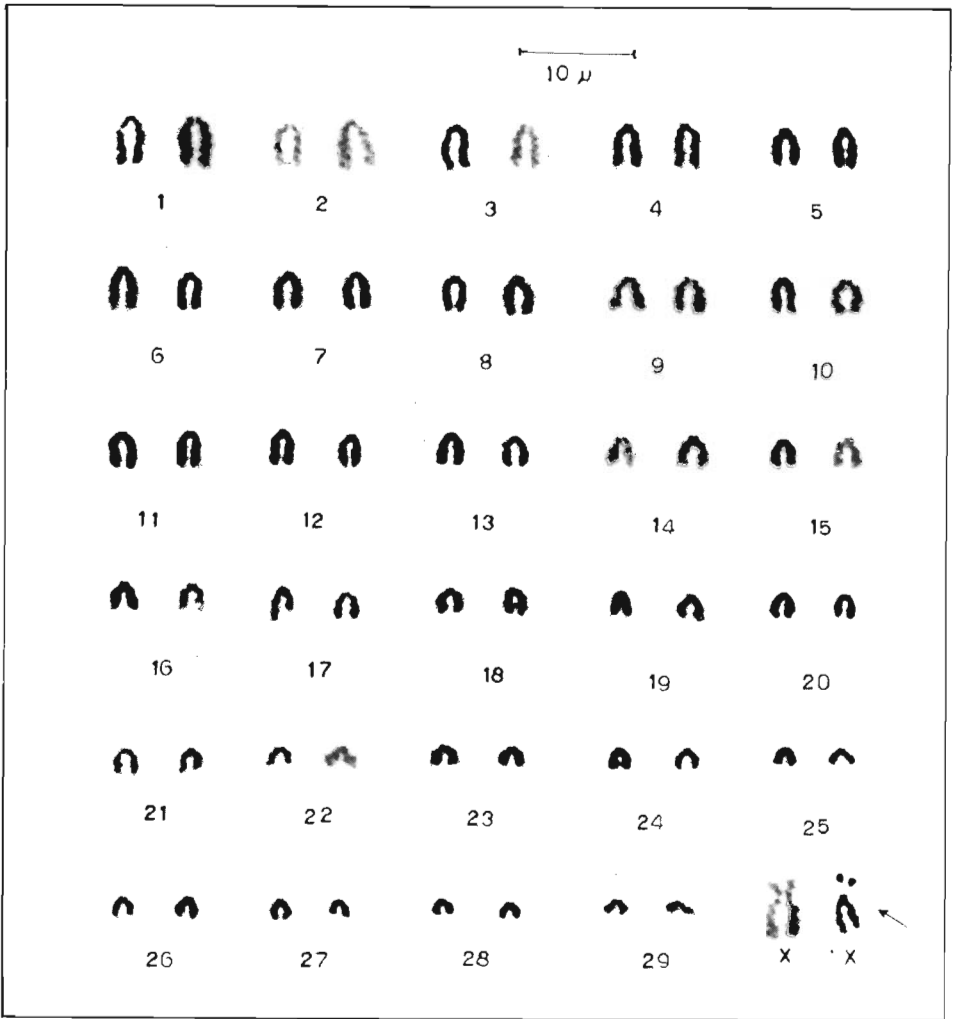


Figure 2 - Karyotype of a cow 60,XX with a gap in an X chromosome.

XX/XXY (Figure 3). Cases similar to the first two have been extensively reported and discussed in the literature since 1962 (Ohno *et al.*, 1962) on the basis of the discovery by Owen (1945) that heterosexual twin gestation in cattle led to intrauterine blood exchange through coriovascular anastomoses. The third Freemartin case with either chimerism or mosaicism 60,XX/61,XXY was an animal born in a twin birth with a male co-twin which died at an early age and whose phenotypic characteristics were not recorded. If we consider this animal to be a 60,XX/61,XXY mosaic, the loss of the Y chromosome may have occurred in one of the cells during the first division of the egg cell, with the development of two populations. If this abnormality had

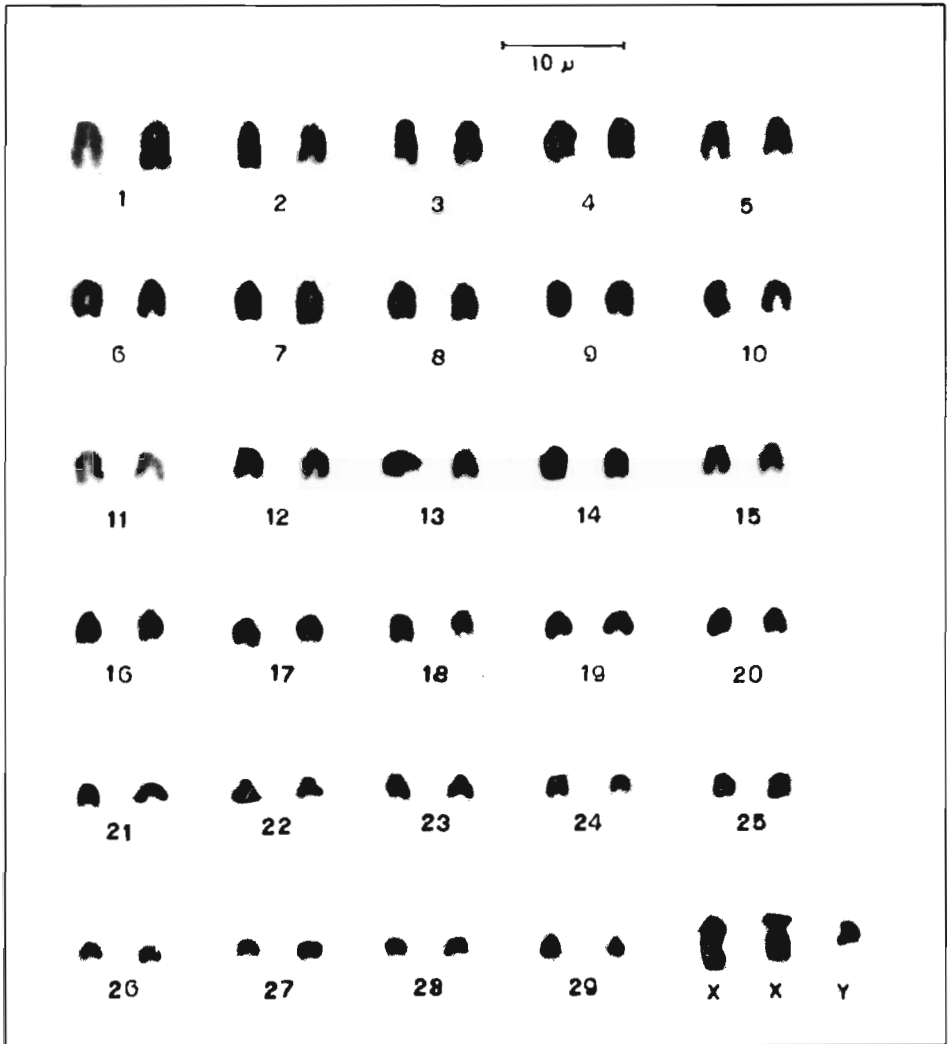


Figure 3 - Karyotype of a cell 60,XXY (23.8%) from a sterile female 60,XX/61,XXY.

reached structures responsible for gonadal development, intersexuality could have been exteriorized. If this is a case of chimerism, the co-twin would certainly have had a karyotype similar to that observed in the human condition known as Klinefelter syndrome.

Dunn *et al.* (1970) reported a case of 60,XX/60,XY karyotype with a true hermaphrodite phenotype, and Rieck *et al.* (1969) and Dain and Bridge (1978) detected XX/XY/XXY chimerisms in intersex and hypoplastic animals, respectively. Cases of an XXY sex complement in 100% of the cells analyzed were described

by Rieck (1970) and by Logue *et al.* (1979) for animals with hypoplastic testes, and a similar case was detected by the author in a phenotypically normal young animal.

The 17/21-22 translocation was detected in a bull in a mosaic form (Figure 4). The parents of this animal had normal karyotypes. Further studies will be conducted to analyze the segregation of this translocation, and to identify the translocated chromosome.

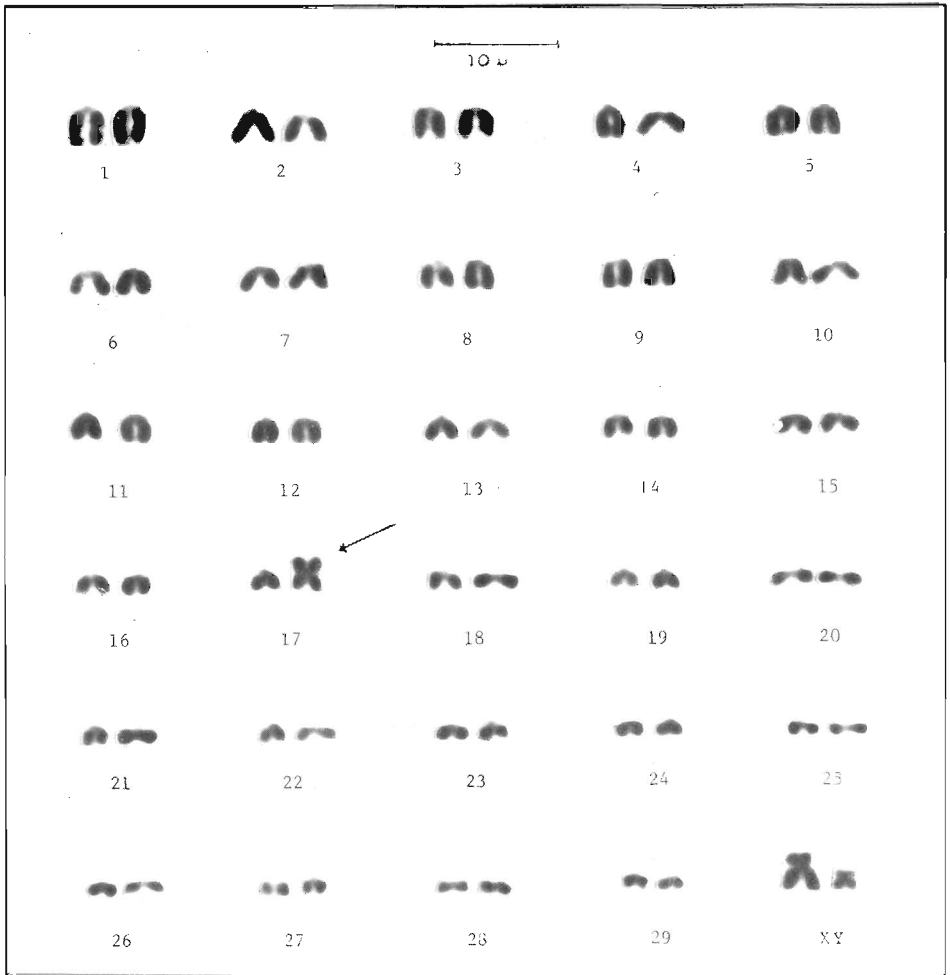


Figure 4 - Karyotype of a bull $60,XY + t(17/21-22)$.

The remaining animals showed normal karyotypes, i.e. 58 acrocentric autosomes and the XX or XY sex pair, with the X and the Y being metacentrics. The Canchim breed, owing to the crossing scheme followed during its formation (5/8

Charolais + 3/8 Zebu) with the participation of a Charolais bull in the last cross, does not show dimorphism for the Y chromosome.

The 1.76% frequency of karyotype anomalies detected in the present herd, although low in relation to other herds of other breeds, emphasizes the need for karyotype analysis of all animals used for reproduction since phenotypically normal animals may be carriers, balanced or not, of such anomalies.

ACKNOWLEDGMENTS

Publication supported by FAPESP.

RESUMO

Foi realizado um estudo cariotípico em 626 animais de um rebanho de bovinos da raça Canchim (5/8 Charolês + 3/8 Zebu). As análises dos cariótipos foram feitas com metáfases obtidas através de cultura de linfócitos de sangue periférico. Foram utilizadas técnicas de formação de bandas cromossômicas G e C. Onze animais (1,76%) apresentaram alterações no cariótipo, sendo que destes cinco eram fenotipicamente normais. Fica evidenciado a necessidade da realização da análise cariotípica como critério para escolha de animais para serem utilizados na reprodução.

REFERENCES

- Abbo, G. and Zellweger, H. (1970). The syndrome of the metacentric microchromosome. *Helv. Paediatr. Acta.* 25: 83-94.
- Armendares, S., Buentello, L. and Salamanca, F. (1971). An extra small metacentric autosome in a mentally boy with multiple malformations. *J. Med. Genet.* 8: 378-380.
- Arrighi, F.E. and Hsu, T.C. (1971). Localization of heterochromatin in human chromosomes. *Cytogenet. Cell Genet.* 10: 81-86.
- Basrur, P.K., Gilman, J.P.W. and McSherry, B.J. (1964). Cytological observations on a bovine lymphosarcoma. *Nature* 201: 268-371.
- Dain, A.R. and Bridge, P.S. (1978). A chimaeric calf with XX/XXY mosaicism and intersexuality. *J. Reprod. Fert.* 54: 197-201.
- Drets, M.Æ. and Shaw, M.W. (1971). Specific banding pattern of human chromosomes. *Proc. Natl. Acad. Sci.* 68: 2073-2077.
- Dunn, H.O., McEntee, K. and Hansel, W. (1970). Diploid-triploid chimerism in a bovine true hermaphrodite. *Cytogenetics* 9: 245-259.
- Ferrari, I., Braga, T. and Moura Ribeiro, V. (1968). Anormalidades físicas múltiplas associadas à presença de um cromossômo extra pequeno. *Cienc. Cult.* 20: 177.
- Finley, W.H., Finley, S.C. and Monsky, D. (1971). An extra small metacentric chromosome in association with multiple congenital abnormalities. *J. Med. Genet.* 8: 381-383.

- Froland, A., Holst, G. and Terslev, F. (1963). Multiple anomalies associated with an extra small autosome. *Cytogenetics* 2: 99-106.
- German, J. (1964). Cytological evidence for crossing over *in vitro* in human lymphoid cells. *Science* 144: 298.
- Gustavson, K.H., Atkeirs, L. and Patricks, I. (1964). Diverse chromosomal anomalies in two siblings. *Acta Paediatrica* (Stockholm) 53: 371-387.
- Gustavsson, I. (1969). Cytogenetics, distribution and phenotypic effects of a translocation in Swedish cattle. *Hereditas* 63: 68-169.
- Gustavsson, I. (1980). Chromosome aberrations and their influence on the reproductive performance of domestic animals - a review. *Z. Tierzüchtg. Züchtgsbiol.* 97: 176-195.
- Gustavsson, I. and Rockborn, G. (1964). Chromosome abnormality in three cases of lymphatic leukaemia in cattle. *Nature* 203: 990.
- Halnan, C.R.E. (1975). Chromosomes of cattle: Present clinical status and promise. *Vet. Rec.* 96: 148-151.
- Harvey, M.J.A. (1976). Veterinary cytogenetics. *Vet. Rec.* 98: 479-481.
- Hecht, F., Koler, R.D., Rigas, D.A., Dahnke, G.S., Case, M.P., Tisdale, V. and Miller, R.W. (1966). Leukaemia and lymphocytes in ataxia-telangiectasia. *Lancet* 2: 1193.
- Hoehn, H., Reinwen, H. and Enfel, W. (1970). Genetic studies on a minute centric fragment transmitted through three generations. *Cytogenetics* 9: 186-198.
- Ishmael, J. and Laurence, K.M. (1968). An extra small metacentric chromosome in a mentally retarded boy. *J. Med. Genet.* 5: 335-340.
- Logue, D.N., Harvey, M.J.A., Munco, C.D. and Lennox, B. (1979). Hormonal and histological studies in a 61 XXY bull. *Vet. Rec.* 104: 500-503.
- Lungeanu, A., Paunescu, G.H., Begnescu, R., Moldovan, G., Avram, N. and Gradinaru, D. (1978). Biological significance of the cytogenetic aberrations in bovine leukosis. *Archiva Veterinaria* 13: 71-88.
- Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.M. and Hungerford, D.A. (1960). Chromosome preparation of leukocytes cultured in human peripheral blood. *Exp. Cell Res.* 20: 613-616.
- Moraes, J.C.F. and Mattevi, M.S. (1980). Estudos cromossômicos em diferentes raças do rebanho bovino do Rio Grande do Sul. *Anu. Téc. do IPZFO. Porto Alegre*, 7: 117-203.
- Mukherjee, A.B., Partington, M.W., Simpson, N.E. and Walmsley, K.A. (1968). Multiple anomalies associated with a small extra metacentric autosome. *J. Med. Genet.* 5: 329-334.
- Nielsen, J., Friedrich, U. and Tsuboi, T. (1971). Father and son with karyotype 41,XY ? Y q-. *Hum. Genet.* 11: 247-252.
- Ohno, S., Trujillo, J.M., Stenius, C., Christian, L.C. and Teplitz, R.L. (1962). Possible germ cell chimeras among newborn dizygotic twin calves (*Bos taurus*). *Cytogenetics* 1: 258-265.
- Owen, R.D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 102: 400-401.
- Pfeiffer, R.A., Dieckmann, L. and Bauchner, H. (1967). Familial occurrence of a small metacentric extra chromosome with satellites on both ends. *Ann. Génét.* 10: 124-130.
- Popescu, C.P. (1980). Cytogenetics of Domestic Animals: Present Statement and Perspectives. Anais do 1º Simpósio sobre Tópicos Avançados de Reprodução Animal. Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, UNESP.

- Rieck, G.W. (1970). Das XXY - Syndrome beim Rind (Boviner Hypogonadismus). I. Europäisches Kolloquium über Zytogenetik (chromosomenpathologie) in Veterinärmedizin und Säugetierkunde. *Gießen*: 138-145.
- Rieck, G.W., Hohn, H. and Herzog, A. (1969). Hypogonadismus intermittierender Kryptorchismus und segmentare Aplasie der Ductus Wolffii bei einem männlichen Rind mit XXY-Gonosomen-Konstellation bzw. XXY/XX/XY. Gonosomen - Mosaik. *Dtsch. Tierarztl. Wschr.* 76: 133-138.
- Schroeder, T.M., Anschutz, F. and Knopp, A. (1964). Spontane Chromosomenaberrationen bei familiärer Panmyelopathie. *Humangenetik* 1: 194.
- Smith, K.D., Steinberger, E., Steinberger, A., and Perloff, W.H. (1965). A familial centric chromosome fragment. *Cytogenetics* 4: 219-226.
- Stanik, J. and Izariková, A. (1979). Cytogenetic analysis of lymphoid blood cells in the course of bovine leucosis. *Vet. Med. Praha* 24: 641-647.
- Stanik, J., Tetrešová, J., Izariková, A. and Čierna, V. (1981). Cytogenetic study on bovine leucosis. *Vet. med. Praha* 26: 517-523.
- Tambasco, A.J. (1988). Cytogenetics of domestic animals. *Congreso Panamericano de Ciencias Veterinarias*. Lima, Peru.

(Received February 21, 1989)