CYTOGENETIC STUDIES ON ROMANIAN CATTLE BREEDS

ESTUDIOS CITOGENÉTICOS EN RAZAS BOVINAS RUMANAS

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Genetics prophylaxis.

PALABRAS CLAVE ADICIONALES

Profilaxis genética.

SUMMARY

Taking into consideration the present and future interest of the genetic diseases, this work review the results obtained in the last 25 years in genetic prophylaxis of cattle in Romania. Started since 1965 in the Laboratory of Genetics of Research Institute for Animal Breeding, these researches developed beginning 1970 in three important Romanian institutes: Research Institute for Bovine Breeding-Balotesti, Agronomic Institute-Cluj-Napoca and *Pasteur* Institute-Bucarest.

Under the conditions of the increasing of artificial insemination in cattle, the studies were directed to the bull sires and a Program of genetic prophylaxis was created in the National Program of bovine improvement. In this way, 2364 bulls of Romanian cattle breeds were investigated between 1975 and 2000. A several kind of abnormalities were identified: chimerism XX/XY (24 cases), translocation 1/29 (12 cases), translocation 3/27 (1 case), translocation 5/23 (1 case), translocation 11/21 (1 case), traslocation 14/20 (1 case), tandem translocation (2 cases), chromosomal constitution 2n=59,XX t (1;29) (1 case), dicentric chromosom (1 case), autonom XX/XY (2 cases), mosaic 60,XX/61,XXY/78,XXY (1 case).

Consequently, an eradication policy of these abnormalities was adopted with very important economic effects.

RESUMEN

Teniendo en cuenta el interés presente y futuro de las enfermedades genéticas, este trabajo revisa los resultados obtenidos en los últimos 25 años, en la profilaxis genética en Rumanía. Iniciados en 1965 en el Laboratorio de Genética del Instituto de Investigaciones sobre Cría Animal, esas investigaciones se desarrollaron al comienzo de 1970, en tres importantes institutos rumanos: Instituto de Investigación sobre Cría Bovina Balotesti, el Instituto Agronómico Cluj-Napoca y el Instituto Pasteur de Bucarest.

Bajo las condiciones del incremento de la inseminación artificial en los bovinos, los estudios se orientaron hacia los sementales y se creó un programa de profilaxis genética dentro del programa nacional de mejora bovina. De este modo, 2364 toros de razas bovinas rumanas fueron investigados entre 1975 y 2000. Una diversidad de anormalidades fue detectada: quimerismo XX/XY (24 casos), translocación 1/

29 (12 casos), translocación 3/27 (1 caso), translocación 5/23 (1 caso), translocación 11/21 (1 caso), translocación 14/20 (1 caso), translocación en tandem (2 casos), constitución cromosómica 2n=59,XX t (1;29) (1 caso), cromosoma dicéntrico (1 caso), XX/XY autónomo (2 casos) y mosaico 60,XX/61, XXY/78,XXY (1 caso).

Consecuentemente se adoptó una política de erradicación de esas enfermedades con efectos económicos muy importantes.

INTRODUCTION

In Romania the prophylaxis of chromosomal diseases is very well established but only for cattle.

The cytogenetics studies concerning this species started in 1965 in the laboratory of Genetics of Animal Breeding Research Institut in Bucarest taking as model and supported by the Human Cytogenetics Laboratory of Medical School under the scientific advisory of dr. Alexandru Caratzali, the director of this laboratory.

The first observations concerning the normal cattle karyotype belongs to P.C. Popescu and I.Granciu in 1965, followed by the first reserches of Yak chromosomes (*Bos grunniens* L.) chromosomes performed by Veturia Derlogea *et al.*, (1967), as well as the communication of the techniques of blood microculture, by Paul C. Popescu in 1968.

Since 1970 the cytogenetics research of domestic animals were developed in three laboratories: Research Institut for bovine Breeding Balotesti (B.E. Livescu and M. Samarineanu), Agricultural Institut Cluj-Napoca (D.D. Ciupercescu) and Pasteur Institut Bucharest (A. Lungeanu).

MATERIALS AND METHODS

Blood samples for chromosome analysis were taken from all the investigated bulls. Peripheral blood lymphocytes were cultured at 38,5°C for about 72 hours in MEM (minimal essential medium) with PHA-M as mitogen and 15 per cent foetal calf serum added. Metaphase chromosomes were prepared by conventional air-drying technique. Chromosomes were analysed by the conventional Giemsa stain technique. The presence of the chromosomal abnormalities were demonstrated by C-banding treatements (Sumner,72).

RESULTS AND DISCUSSION

Under the conditions of an increased utilization of the artificial insemination in cattle, the studies were focused on the bulls of the A. I. Consequently, a Program of Genetic Prophylaxis in the National Program of Cattle Amelioration was created.

In this way, 2364 bulls from different centers of A.I. have been investigated between 1975 and 2000 **(table I)**. The most important investigated bulls belongs to the Romanian Spotted (BR) Romanian Black Spotted (BNR) and Brown (B) which are the most common breeds in the country.

In the bulls population systematically controlled in Romania, the most important weight is hold by the XX/XY chimerism as chromosome abnormality **(table II)**. Because the opinions about the reproductive performances of the carriers are different, the limitation of reproductive activity,

even theirs elimination was recommended.

Five kind of different centric fusion (1/29, 3/27, 5/23, 11/21, 14:20) have also described (Samarineanu *et al.*,1976; Livescu *et al.*, 1979; Ciupercescu *et al.*, 1980) involving chromosomes of different pairs (1/29, 3/27, 5/23, 11/21 14/20), but the most common was the translocation 1:29 **(figures 1a and b)** which have had a large distribution in Romanian Spotted Breed. This situation could be explained, on one side, by the important number of studied animals, and on the other side by the massive import of animals and frozen

Table I. *The number of bulls investigated between 1975 and 2000.* (Número de toros investigados entre 1975 y 2000).

Year	Laboratory			Total	Breed				
	A	B	C		B.R.	B.N.R.	B.	Charolais	Others
1975	21	-	-	21	-	11	8	2	-
1976	34	-	-	34	-	33	1	-	-
1977	25	5	-	30	5	21	4	-	-
1978	50	70	-	120	44	56	20	-	-
1979	50	50	-	100	50	48	2	-	-
1980	50	30	-	80	27	40	13	-	-
1981	53	75	50	178	65	63	50	-	-
1982	50	80	67	197	70	67	60	-	-
1983	60	80	60	200	120	53	27	-	-
1984	60	-	-	60	60	-	-	-	-
1985	80	-	-	80	75	5	-	-	-
1986	102	-	-	102	98	4	-	-	-
1987	122	-	-	122	114	6	-	-	2
1988	131	-	-	131	70	61	-	-	-
1989	250	-	-	250	127	123	-	-	-
1990	229	-	-	229	118	111	-	-	-
1991	50	-	-	50	-	50	-	-	-
1992	101	-	-	101	21	61	19	-	-
1993	85	-	-	85	-	52	33	-	-
1994	65	-	-	65	-	59	6	-	-
1995	47	-	-	47	22	25	-	-	-
1996	24	-	-	24	18	6	-	-	-
1997	12	-	-	12	-	6	6	-	-
1998	15	-	-	15	-	4	5	-	6
1999	11	-	-	11	4	5	2	-	-
2000	20	-	-	20	9	-	11	-	-
TOTAL	1797	390	177	2364	1117	970	267	2	8

Table II. *The breeding distribution of the chromosome abnormalities identified in the examinated bulls since 1975 to 2000.* (Distribución por razas de las anomalías cromosómicas identificadas en los toros examinados entre 1975 y 2000).

Abnormalitie	Nr. of bulls	Breed			
		B.R.	B.N.R.	B.	Charolais
Chimerism XX/XY	24	13	11	-	-
Translocation 1/29	12	9	-	2	1
Translocation 3/27	1	-	1	-	-
Translocation 5/23	1	-	-	1	-
Translocation11/21	1	-	-	1	-
Translocation14/20	1	1	-	-	-
Tandem translocation	2	-	2	-	-
2n=59,XX t(1 ;29)	1	1	-	-	-
Dicentric	1	-	1	-	-
Autonom XX/XY	2	-	1	1	-
Mosaic 60,XX/61,XXY/78,XXY	1	-	1	-	-
Polyploidy	39	10	28	1	-
Chromatid breakage	3	-	2	1	-

seminal material of Simmenthal breed. The carriers of robertsonian translocation which negative effect on reproduction was demonstrated, have been eliminated from the herd. As a mater of fact, our results are in accord with those reported by many other authors (Havrankova *et al.*, 1987;

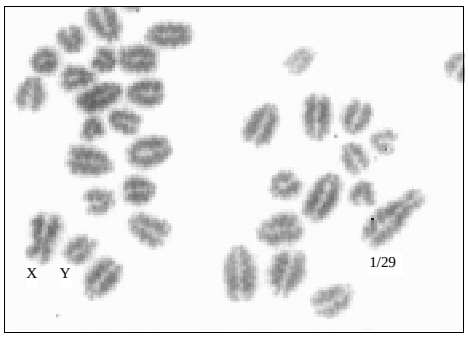

Figure 1a. *A partial metaphase plate of a male heterozygous for the 1/29 Robertsonian translocation.* (Metafase parcial de un macho heterocigoto para la translocación robertsoniana 1/29).

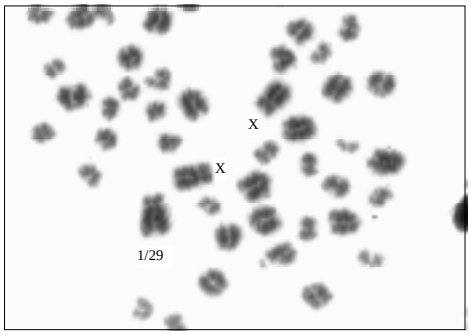

Figure 1b. *A partial metaphase plate of a female heterozygous for the 1/29 Robertsonian translocation.* (Metafase parcial de una hembra heterocigoto para la translocación robertsoniana 1/29).

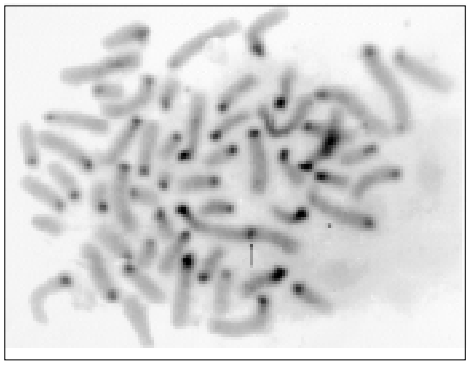

Figure 2. *A C-banded metaphase plate indicating a chromosome with a "telomere-centromere" tandem translocation. The fusion point is indicated by an arrow.* (Metafase C-bandeada indicando un cromosoma con una translocación en tandem "telómero-centrómero". El punto de fusión es indicado por una flecha).

Popescu, 1989; Wilson, 1990; Iannuzzi *et al.*, 1999).

Tandem translocation **(figure 2)** is known, in Red Holstein breed only (Hansen, 1969) and it was identified in a female (84-130) and in a male (Mapple 13) of BNR breed (Nicolae *et al.*, 1995). The genealogical research showed that the two carriers of Tan T were maternal stepbrother. Regarding the reproductive activity, the female had only two offsprings, a normal one and one having a tandem translocation. This female has also showed a lower non-return rate. The male evolution is very interesting. The two first cytogenetical examinations revealed the predominant mitosis TanT, the four next examinations (Livescu *et al.*, 1996) found the weight of these mitosis dicreased and finally having only the normal cell line. Six descendants of this bull were also examined and all of them had a normal karyotype. Consequently, we concluded that this abnormality is transmitted on maternal line.

Chromosomal constitution 2n= 59, XXt (1;29) was identified to a bull of BR breed. The presence of the female genotype could be due to a gestation partner of female sex which was either resorbed or early aborted, although it is not known if this animal comes from an heterosexual twin borne. Even if the reproductive behavior was seemingly normal, in any case, the bull was eliminated from the reproduction because of the translocation 1:29 which effects are known.

Dicentric chromosome **(figure 3)** was observed for the first time in a bull of BNR breed. This abnormality is very rare, it was identified only in human, as a rule, as a consequence of the cosmic radiation, its frequency

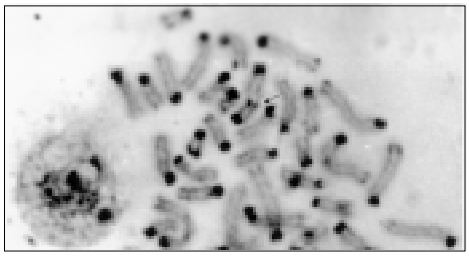

Figure 3. *A partial C-banded metaphase plate showing a dicentric chromosome. The "telomere-telomere" fusion point is indicated by an arrow.* (Metafase parcial C-bandeada mostrando un cromosoma dicéntrico. El punto de fusión "telómero-telómero" es indicado por una flecha).

being of 0,082 per cent in general population (Lloyd *et al.*, 1992).

The chromosomal constitution Pure XX/XY and cellular mosaic 60,XY/61,XXY/78, point out to the karyotypical instability of the carriers which could have effects on the descendants, but without fenotypic effects. Any fenotypic effect was observed to one of the studied bulls even if the poliploidy pointed out, especially as tetraploid cells, reached 20 per cent.

The mono and bichromatidic breakage were observed at 10 per cent of the examined cells on two bulls of BNR breed and one bull of Brown breed. Taking into consideration that in different mitosis it is not affected the same chromosome, we considered this explanation due to the culture conditions and these are not of genetic nature.

The study of an important number of bulls of A.I. had first of all economic advantages. The elimination of the abnormalities carriers from reproduction the first year of the test stopped on one side, the dissemination of abnormalities in the offsprings populations and on the other side the expenses for the complete test cycle were avoided.

Therefore, in order to eliminate unfavourable factors for reproductive performance in romanian cattle breeds an erradication policy was established and it is very important to continue this activity in the future. We intend to develop our researches by using both conventional cytogenetics and the modern molecular techniques to obtain a more precise evaluation of chromosome aberrations, a necessity also observed by Schmutz *et al.*, 1995; Kawarsky *et al.*, 1996; Joerg *et al.*, 1999; Chaves *et al.*, 2000.

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