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Native cattle breeds of Southern Italy: karyological profile

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ABSTRACT - Italian typical products of animal origin are strictly linked to native breeds. Their protection requires control of their reproductive and productive abilities. Hence the need for karyological studies to identify subjects with chromosome abnormalities linked to hypofertility or sterility. We report the results of karyological analyses carried out from January 2008 to December 2008 on 145 cattle of native breeds (Agerolese, Cinisara, Modicana and Podolica) reared in Southern Italy so as to evaluate and characterize the presence of chromosome abnormalities in subjects with normal phenotypes. Besides the 128 karyologically normal subjects (2n=60, XY and 2n=60, XX), 17 were carriers of rob (1;29) and one male was a carrier of cellular chimerism 2n=60, XX/XY. According to our data there is a high frequency of rob (1;29) in Cinisara and Podolica breeds while in Agerolese there was only one case of rob (1;29) and none in Modicana.

Key words: Cattle, Karyological profile, Chromosome abnormality, Robertsonian translocation.

Introduction - The Italian livestock system is variegated and most opportunities are represented by typical high-quality products, linked to places of origin and to particular production and processing techniques. These products have an important role for the ecologically sustainable economy of local production systems.

Threats to animal biodiversity come mainly from human activities: farmland expansion, increase in plant and animal production, deforestation, unregulated urbanisation, air, water and soil pollution. As regards livestock species, selection for production traits of economic types is the reason for the decreasing interest and sometimes for the lack of rearing low-production native breeds.

All native breeds, thanks to natural selection, are adapted to their specific environments: native breeds are more disease-resistant, able to survive, reproduce and produce in harsh environmental conditions in which other more productive breeds fail to capitalise on their genetic potential. This is why native breeds are an important tool for conservation.

Clearly, the importance of the protection and recovery of native breeds and the institution of birth registers (RA) and national herd books (LG) is the first step for increasing the number of animals concerned. Moreover, in a few cases, the absence of proper livestock management is the reason why some chromosome abnormalities, whether numerical or structural, not linked to particular phenotypes but to fertility alterations, have spread, thus reducing reproductive and productive abilities of native breeds (Molteni *et al.*, 2005). An in-depth study of the karyological profile for each of these breeds so as

to exclude sires and dams that are cytogenetically unsuitable is therefore indispensable.

The aim of this work is to assess the karyological profile of some native cattle breeds from southern Italy: Agerolese, Cinisara, Modicana, and Podolica. For Podolica an LG has been established, currently listing 15,363 females and 163 males. As for Agerolese, Cinisara, and Modicana cattle breeds, RAs have been established, listing 293 females and 48 males (Agerolese), 2,434 females and 209 males (Cinisara), and 2,567 females and 128 males (Modicana) (official data from AIA and ANABIC, 2008).

Material and methods - Karyological analyses were performed on cattle (115 females and 30 males) from Agerolese (40 females and 10 males), Cinisara (17 females and 13 males), Modicana (27 females and 3 males), and Podolica (31 females and 4 males) from January to December 2008. The animals were aged from 3 to 15 years (Table 1) and came from farms in different southern Italian regions: Agerolese from Campania (Naples), Cinisara and Modicana from Sicily (Palermo and Ragusa), and Podolica from Campania (Salerno and Avellino), Basilicata (Potenza), Puglia (Foggia), and Calabria (Cosenza and Catanzaro).

Peripheral blood (1 ml) was cultured in RPMI medium, enriched with foetal calf serum (10%), L-glutamine (1%), and Concanavalin A (1.5%) (as mitogen) for 72 h at 37.8°C. Two types of cell cultures, without (conventional karyotype) and with addition of 5-bromodeoxyuridine (BrdU) for R-banding technique were performed. In the latter, Thymidine (300 µg/ml) was added after about 48 h, for 17 h to synchronize cells in S-phase. Cell block was removed by washing twice with RPMI and recovering cells in fresh medium containing both 5-BrdU (15 µg/ml) and Hoechst 33258 (30 µg/ml) to obtain enhanced R-banding patterns. Cells from both types of cell cultures were harvested after Colcemid (0.3 µg/ml) treatment for 1 h and given hypotonic treatment (KCl 0.5%) and three fixations in methanol-acetic acid (3:1), the third overnight. Three drops of cell suspension were air-dried on cleaned and wet slides. Those obtained from normal cultures were stained a day later with acridine orange (0.01% in a phosphate buffer, pH 7.0) for 10 min, while those from BrdU treated cells were stained with Hoechst 33258 solution (25 µg/ml) for 20 min, washed in tap and distilled water and mounted in the same phosphate buffer. Slides were observed 24 h after staining or later (1 week).

At least 100 cells per animal were examined from slides of normal cultures to detect aneuploidy and structural aberrations, i.e. translocation. Karyotype construction, chromosome identification and banding followed the latest international chromosome nomenclature (ISCNDB2000, 2001).

Results and conclusions - On 145 animals examined 128 were karyologically normal ($2n=60$), 17 were heterozygote carriers of rob (1;29) and one male was a carrier of cellular chimerism ($2n=60$, XX/XY) (Table 2). Data show a high frequency of rob (1;29) in Cinisara and Podolica breeds, while in Agerolese there was only one case of rob (1;29) and none in Modicana.

Rob (1;29) is a chromosome abnormality characterized by the fusion, at centromeric level, of autosomes 1 and 29, giving rise to the reduction in chromosome number from $2n=60$ to $2n=59$ (in het-

Table 1. Breed and gender of examined animals and number of metaphases and chromosomes analysed.

Native cattle breeds	Animals	Females	Males	Metaphases	Chromosomes
	n	n	n	n	n
Agerolese	50	40	10	5,000	299,900
Cinisara	30	17	13	3,000	179,300
Modicana	30	27	3	3,000	180,000
Podolica	35	31	4	3,500	209,200
Total	145	115	30	14,500	868,400

erozygotic carriers) and $2n=58$ (in homozygotic carriers). The animals with this abnormality, whether males or females, are phenotypically normal but have reproductive problems: hypofertility is shown by irregular heat and increased periods between deliveries (Gustavsson, 1969). Although rob (1;29) has been found worldwide and in 70 different breeds (Popescu *et al.*, 1991; Ciotola *et al.*, 2003; Iannuzzi *et al.*, 2008), including some Italian breeds (Piemontese, Romagnola, Podolica, Marchigiana, Chianina, Pisana, Maremmana, Modicana, Grigio Alpina, Agerolese), this is the first time that it has been diagnosed in the Cinisara cattle breed.

The large number of animals with rob (1;29) found in Cinisara and Podolica suggests the advisability of karyological analyses in routine tests used for selecting sires and dams to avoid further spread of these or other abnormalities that reduce fertility in these endangered breeds.

Table 2. Karyological results.

Native cattle breeds	Chromosome abnormalities	Animals with chromosome abnormalities	Females	Males
			n	n
Agerolese	rob (1;29)	1 (2)	0	1
	$2n=60$, XX/XY	1 (2)	0	1
Cinisara	rob (1;29)	7 (23.3)	3	4
Modicana	0	0	0	0
Podolica	rob (1;29)	8 (22.8)	7	1
Total		17 (11.7)	10	7

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