The phenomenon of cell chimerism in goats

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ABSTRACT: Cell chimerism was diagnosed in goats with test reagents that identify erythrocyte antigens and with bovine probes that paint sex chromosomes. Same-sex and opposite-sex twins and their parents, representing the Fawn Improved breed, were used in the study. Ovine test reagents (anti-Aa, -Be, -Bi, -Bd, -Bb, -Ca, -R) were used to analyse the blood groups of twins. Cytogenetic analysis was based on FISH technique. Identical antigens and incomplete results of the reaction of blood cells with some immune sera showed that these animals had two populations of erythrocytes differing in antigens A_1, B_2, B_3, B_{15} and R. The analysis of 100 metaphase plates for each animal, which were subjected to FISH technique using bovine sex chromosome painting probes, showed the presence of two cell lines: 60,XX and 60,XY.

Keywords: goats; cell chimerism; blood groups; molecular probes; FISH

The phenomenon of cell chimerism in *Bovidae* has been examined for many years (Dunn et al., 1979; Rejduch, 2001). It occurs in dizygotic twins of the same or opposite sex and results from the exchange of haematopoietic tissue cells which takes place through anastomosed vessels of foetal membranes which are formed in the early postimplantation stage of intrauterine life (Dain, 1971; Zhang et al., 1994). Depending on the diagnostic method, cell chimerism can be found in the populations of erythrocytes and lymphocytes (Rejduch et al., 1998b, 2004). The analysis of cell chimerism in the family Bovidae concerning erythrocyte antigens (Rychlik et al., 2003) allowed to choose, out of the set of test reagents used for the blood typing of sheep, those reagents that could be used for diagnosing cell chimerism in goats. In turn, cytogenetic studies performed by an FISH technique made it possible to use probes painting sex chromosomes to distinguish cell lines present in animals with the leukocytic chimerism (Rejduch, 2001; Kozubska-Sobocinska et al., 2003; Rejduch et al., 2004).

This study was aimed to diagnose cell chimerism in goats with ovine test reagents that identify erythrocyte antigens and with bovine sex chromosome specific probes.

MATERIAL AND METHODS

Fifteen same-sex and opposite-sex twins and their parents, goats of the Fawn Improved breed originating from the Experimental Station belonging to National Research Institute of Animal Production, were investigated.

Antigens of red blood cells were determined by a haemolytic test on microplates using 7 selected sheep reagents (anti-Aa, -Be, -Bi, -Bd, -Bb, -Ca, -R) prepared in the National Research Institute of Animal Production in Balice (Poland). The reagents identify erythrocyte antigens of goats A_1 (A blood group system), B_2, B_3, B_8, B_{15} (B blood system), C_{12} (C blood system) and R (R blood system) (Nguyen, 1990; Kaczor et al., 1999). The microplates were incubated at 30°C (± 2) and the reaction (positive or negative) was read out twice after 2 h incubation.

For the cytogenetic diagnosis of leukocytic chimerism in goats, fluorescence *in situ* hybridization (FISH) was applied following the procedure of Pinkel et al. (1986) and Solinas-Toldo et al. (1993), using bovine X and Y specific probes. These probes were obtained through microdissection of 20 heterosomes from metaphases plates. The X probe was amplified and labelled with DOP-PCR using

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the bio-16-dUTP nucleotide (Goldammer et al., 1996). This probe was obtained from ETH Zürich Breeding Biology Group. The Y fragments were amplified by PCR as described by Guan et al. (1992) and PCR labelled with digoxigenin-11-dUTP (Boehringer Mannheim, Germany) according to the manufacturer's instruction. The chromosome probe was obtained from the Swedish University of Agricultural Sciences (Uppsala). Intense fluorescent signals were observed under an OPTON-Axiophot fluorescent microscope using triple attenuation filters – DAPI/FITC/Texas Red. Selected cells were recorded and evaluated using the image analysis system LUCIA-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

RESULTS AND DISCUSSION

A total of 21 antigens belonging to 6 blood group systems A, B, C, E, F and R have so far been identified in goats, but the knowledge of blood group systems in this species remains insufficient. Previous investigations involved a number of experiments detecting erythrocyte antigens by alloimmunization and heteroimmunization of goats (Nguyen, 1990). It was stated, among others, that 5 blood group systems in sheep are homologous with blood group systems found in goats (Stormont and Suzuki, 1961). As shown subsequently by Nguyen (1990), some erythrocyte antigens in goats can be identified by test sera used for the typing of blood groups in sheep.

In the present study, selected ovine test reagents were used to determine the antigenic composition of kids derived from twins. In the haemolytic test, two pairs of twins showed incomplete reactions with several test reagents. Identical antigens and incomplete results of the reaction of blood cells with some immune sera indicate that these animals had two populations of erythrocytes that differed in antigens A_1, B_2, B_3, B_{15} and R (Table 1).

The pair of opposite-sex twins with the confirmed erythrocyte chimerism was subjected to cytogenetic analysis (FISH technique) to diagnose the leukocytic chimerism which is manifested by the presence of two cell lines having a different set of sex chromosomes.

The synthenic and conservative nature of cattle and goat heterosomes shown by FISH (Kozubska-Sobocinska et al., 2003) makes bovine sex chromosome specific probes useful for the diagnosis of chimerism in the leukocyte population in goats.

The analysis of 100 metaphase plates in each animal, subjected to FISH with the use bovine probes identifying sex chromosomes showed the presence of two cell lines: 60,XX and 60,XY. The effect of hybridization was two yellow, intensely fluorescing signals on 60,XX plates (Figure 1), and two signals differing in both colour and size in line 60, XY (Figure 2). The applied probes, by giving very clear signals in a large number of plates, made it possible not only to identify chimerism but also to determine the ratio of individual cell lines (XX : XY), which was 66 : 34% in females and 63 : 37% in males.

The cell chimerism is a frequent syndrome in dizygotic twins. It is known that in the family Bovidae the female partners are infertile and referred to as freemartins (Miyake et al., 1990).

Table 1. Blood typing results for same-sex and opposite-sex twins demonstrating erythrocyte chimerism

Animal	Blood groups			
Pair I				
♂	$A_1^{\pm}$	$B_2^{\pm}B_3^{\pm}B_8B_{15}$	C_{12}	R
♀	$A_1^{\pm}$	$B_2^{\pm}B_3^{\pm}B_8B_{15}$	C_{12}	R
M	–/–	B_{15}/–	C_{12}	R
O	A_1/–	B_2B_3/B_8	C_{12}	R
Pair II				
♀	$A_1^{\pm}$	$B_2B_3^{\pm}B_8B_{15}^{\pm}$		$R^{\pm}$
♀	$A_1^{\pm}$	$B_2B_3^{\pm}B_8B_{15}^{\pm}$		$R^{\pm}$
M	–/–	B_8/B_{15}	C_{12}/–	R
O	A_1/–	B_2/B_3	C_{12}/–	R

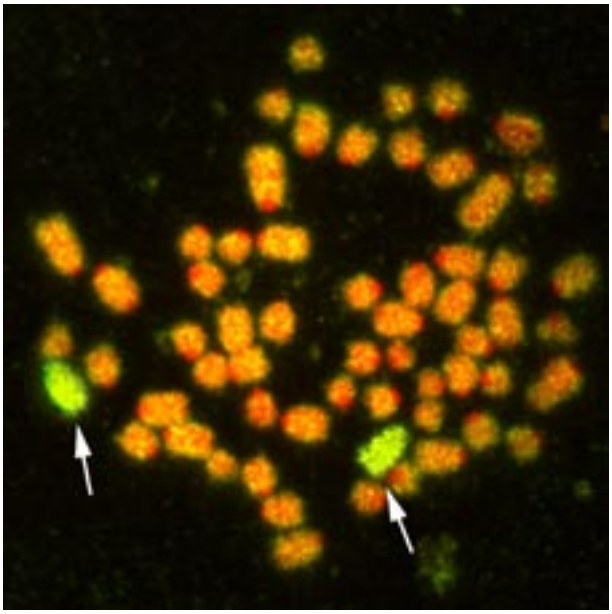

Figure 1. FISH technique. Arrows indicate heterosomes in the cell line 60,XX in goat No. 442

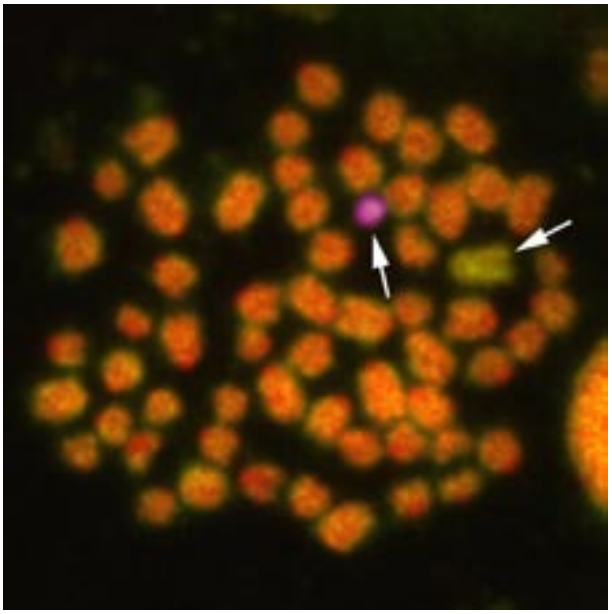

Figure 2. FISH technique. Arrows indicate heterosomes in the cell line 60,XY in goat No. 442

The frequency of freemartins in cattle ranges from 82 to 95% of heterosexual twins (Zhang et al., 1994), in sheep from 1.2% in Finish Mountain to 11.2% in Booroola breed (Dain, 1971; Keszka et al., 2001) and only about 1% in goats (Yadav et al., 1993). The impact of karyotype abnormality such as cell chimerism carrying males is arguable. However, Rynkiewicz-Szatkowska (1992) in rams and Rejduch et al. (1998a) in bulls – carriers of XX/XY chimerism showed lower semen parameters (volume, motile spermatozoa and sperm concentration) in comparison with males characterized by normal karyotype.

Cytogenetic and immmunogenetic methods can also be employed to detect cell chimerism in the blood of single-born animals (Miyake et al., 1990). The presence of an additional cell line in such animals may result from simultaneous fertilization of the egg cell and the polar body by two spermatozoa or from an exchange of haematopoietic tissue between twin foetuses, one of which became dead during its early development (Miyake et al., 1990).

The application of quick evaluation methods will improve the diagnosis of chimerism, enabling undesirable carriers to be eliminated at an early stage. This, in turn, will reduce economic losses resulting from the need to keep animals carrying this karyotype abnormality.

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