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Frequency of lymphocytic XX/XY chimerism in Leine sheep coming from heterosexual twin and multiple births

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Abstract. The performed cytogenetic analysis included 454 young Leine ewes, aged 3-8 months, coming from heterosexual twins and multiplets. Out of them, the studied 431 animals had a normal diploid chromosome number – 54,XX. Leukocytic 54,XX/54,XY chimerism was identified in 23 young ewes, which makes 5.06% of the studied group of animals.

Key words: chromosomal chimerism XX/XY, hererosexual twins, infertile ewes, multiplets.

Freemartinism is a malformation of reproductive system in females coming from heterosexul twins and multiplets (FORBES 1946, GOODFELLOW et al. 1965). A direct cause of that anomaly is the appearance of anastomoses, that is vascular junctions between placentae of twin embryos in early embryonic stage, which leads in consequence to restraining effect of substances secreted by male gonads of a twin brother upon the development of female reproductive system (ALEXANDER, WILLIAMS 1964, MELLOR 1969). Clinical changes of reproductive tracts are accompanied among others, by leukocytic chimerism, which is a result of primary haematopoietic cells' passage from one embryo to another throughout the anastomoses. That last feature was repeatedly implemented into diagnostics of the discussed phenomenon using identification of cell lines with XX and XY chromosomes (WILKES, MUNRO 1978, CHAFFAUX et al. 1987).

Attempts to determine an average frequency of freemartinism in the sheep by cytogenetic studies were made many times, establishing at a 1.1% level (LONG 1980) for sheep without a documentary evidence of origin and 5-21.4%

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for animals coming from heterosexual multiplets (STORMONT et al. 1953, MATEJKA et al. 1987, JASZCZAK et al. 1993). The incidence of ewes with cellular XX/XY chimerism, which had been culled from the herd due to their sterility, was also determined. It was found, that 73-78% of such ewes demonstrated XX/XY chimerism (CRIBIU et al. 1990, GILL, DAVIES 1991). However, these studies were carried out on a small number of animals, thus the determination of negative results of freemartinism in sheep breeding is still a matter for debate.

The aim of this work was to determine the frequency of cellular XX/XY chimerism occurrence in a group of 454 young Leine ewes coming from heterosexual twin and multiple births.

Material and methods

Cytogenetic analysis covered 454 young, 3-8-month-old Leine ewes coming from heterosexual twins and multiplets. The studies were carried out within a breeding herd (POHZ Cerekwica) for four consecutive years (1990-1993).

Lymphocyte cultures from entire blood were grown using the method described by ARAKAKI and SPARKES (1963). The culture medium consisted of Eagle's medium suplemented with FCS, mitogen (LF-7) and antibiotics (penicillin and streptomycin).

For routine microscopic analysis the Giemsa staining technique was employed. For each animal 30-40 metaphase plates were analysed. In the case of diagnosed chimerism the number of analysed metaphasal plates increased to 80-100. According to sheep karyotype standard (ISCNDA, 1990), the Y chromosome was identified as the smallest metacentric chromosome in the karyotype, while the X chromosome – as the largest acocentric one with a very short p arm. However, the identification of the X chromosome in conventionally stained preparations was very difficult. Therefore, in the case of animals identified initially as the XX/XY chimerism carriers a complementary staining by CBG method was applied (SUMMER et al. 1972). Using this method, the X chromosome can be easily identified, since it is the only chromosome in sheep karyotype, which has no positive C band.

Results

Out of 454 cytogenetically studied young ewes (coming) from heterosexual twins or multiplets, 431 ones had a normal diploid chromosome number – 54,XX (Fig. 1).

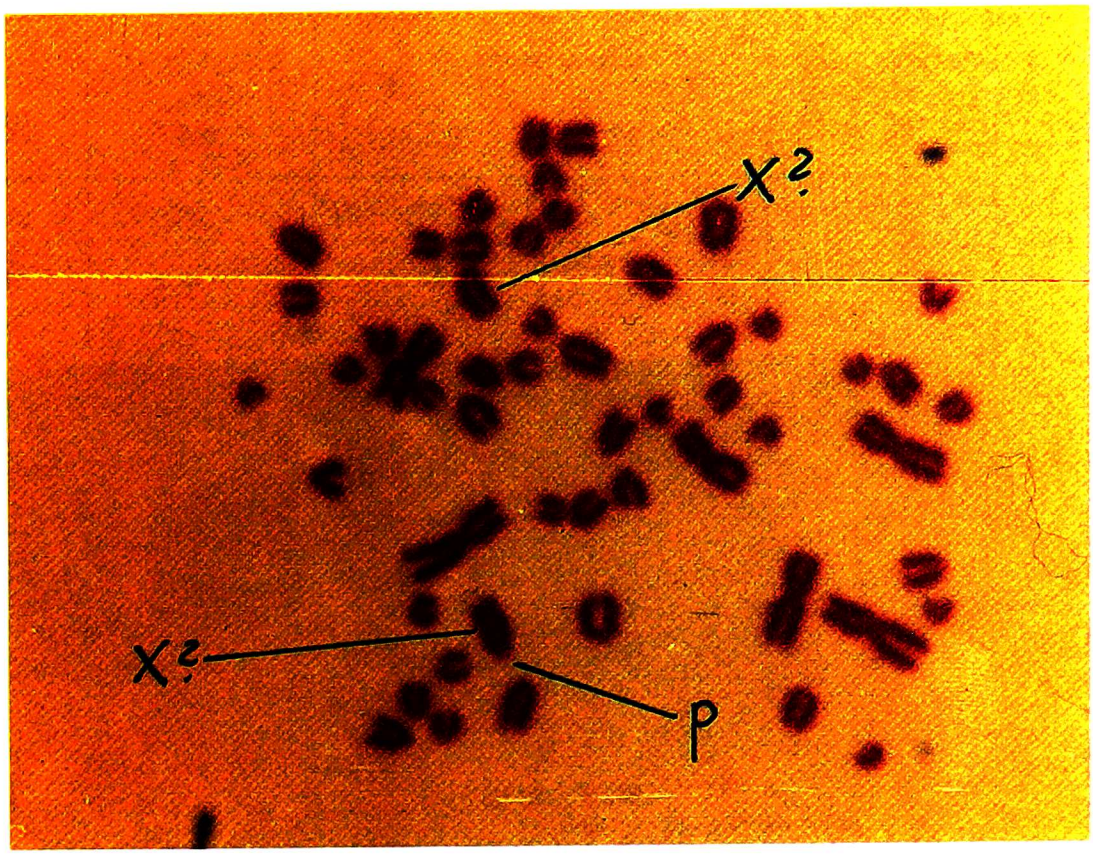

Fig. 1. Karyotype of female sheep, 2n=54,XX (Giemsa staining)

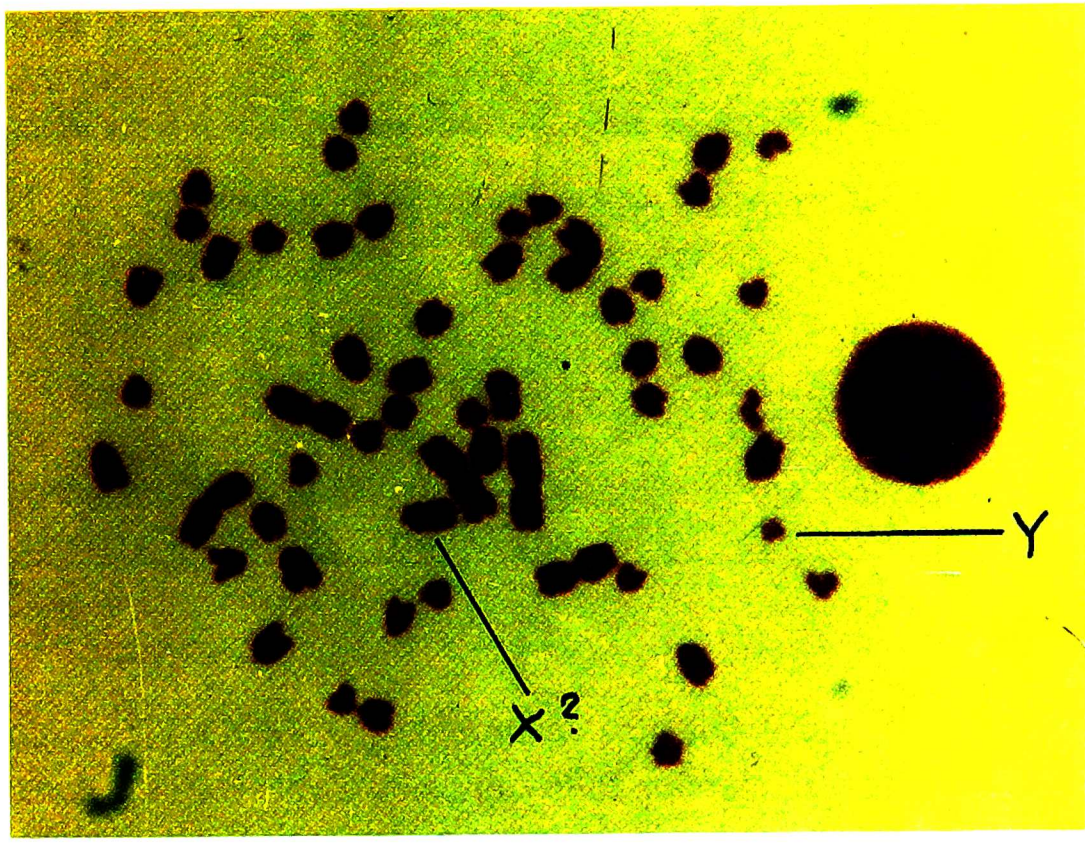

Fig. 2. Karyotype of male sheep, 2n=54,XY (Giemsa staining)

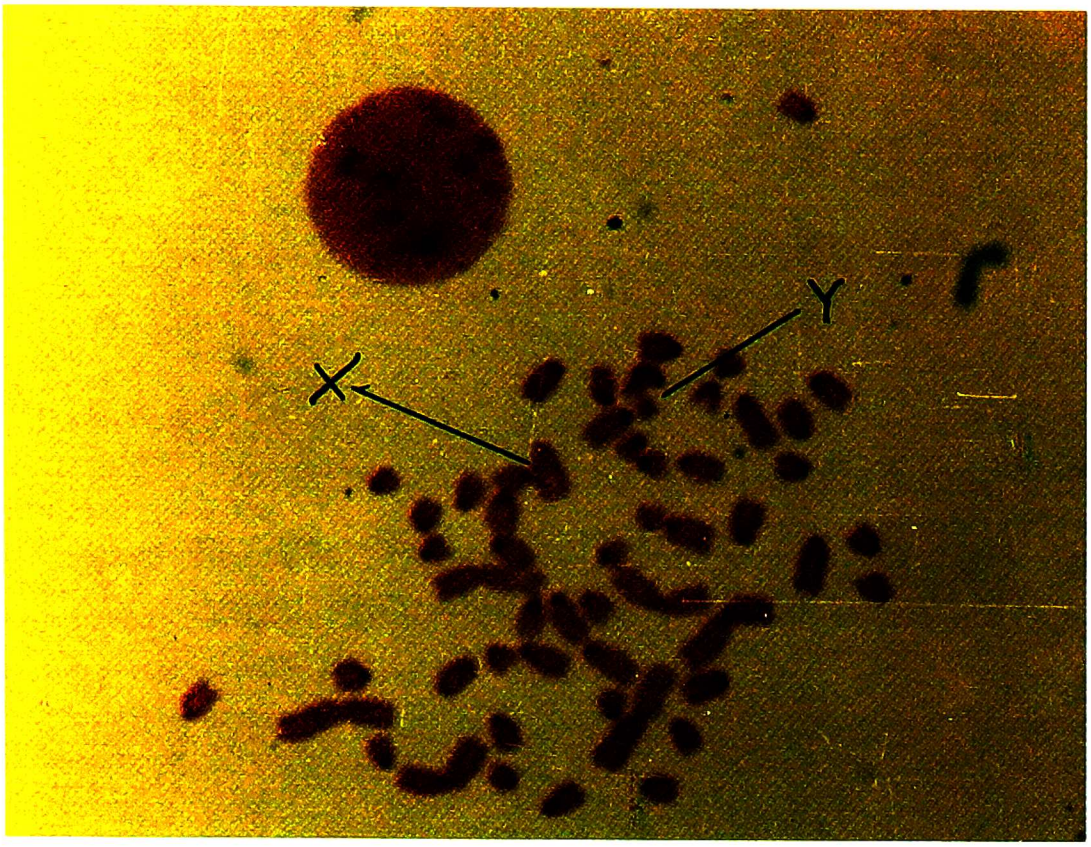

Fig. 3. Karyotype of male sheep, 2n=54,XY (C-banding)

Table 1. Proportions of XX and XY cell lines in the studied sheep

Designation of sheep	Investigated cell No.	Number of cells		Percentage of cells	
		54,XX	54,XY	54,XX	54,XY
2912	96	71	25	73.9	26.1
3082	100	33	67	33.0	67.0
3092	101	29	72	28.7	71.3
3125	95	58	37	61.0	39.0
3243	88	47	41	53.4	46.6
3309	87	62	25	71.3	28.7
3621	82	21	61	25.6	74.4
3791	77	47	30	61.0	39.0
3335	79	31	48	39.2	60.2
3795	79	65	14	82.3	17.7
3843	89	67	21	76.4	23.6
3860	80	44	36	55.0	45.0
3868	94	52	42	55.3	44.7
3892	94	28	66	29.8	70.2
3893	82	24	61	25.7	74.3
3875	89	64	25	71.9	28.1
3940	108	96	12	88.8	11.2
4405	91	68	23	74.7	25.3
4460	92	60	32	65.2	34.8
4464	83	26	57	31.3	68.7
4526	81	34	47	41.6	58.4
4769	90	16	74	17.8	82.2
4852	86	15	71	17.5	82.5

Leukocytic 54,XX/54,XY chimerism was identified in 23 young ewes, which makes 5.06% of the studied group of animals. It was manifested by the occurrence of two cell lines differing in sex chromosomes in the same animal. The Y chromosome as the smallest metacentric one was easy to find in well dispersed metaphasal plates (Fig. 2), nevertheless in the case of animals with diagnosed chimerism, a complete identification of the X and Y sex chromo-

somes was possible due to CBG technique, revealing aggregates of constitutive heterochromatin (Fig. 3).

The degree of cellular chimerism expressed by percentage of twin brother cells to its own lymphocytes, and the number of metaphasal plates analysed for each animal are given in Table 1. The percentage of foreign lymphocytes in the blood circulation system of the studied sheep ranged very vastly – from 11.2% to 82%.

Discussion

Freemartinism aroused a justified interest in the past mostly in case of cattle breeding, since in over 80% of females of this species, born together with a male twin brother, the development of their reproductive system was found to be inhibited (ŚWITOŃSKI 1992). However, this phenomenon is not of great importance in case of cattle, for merely 2% of cows give twins. Besides, this vastly described developmental anomaly enables breeders to cull heifers before they reach their sexual maturity.

Multiple births are observed more often in sheep than in cattle, but at the same time the percentage of freemartins between heterosexual twins is clearly lower, in spite of large anatomical similarity in the structure of placentae of both species. The placenta in ruminants is multiple and cotyledonic. About 80-100 placentomas are found in cattle, whereas 90-120 ones in sheep. However, fusion between main branches of allantochorione veins and arteries in sheep embryos occurs more rarely, and its occurrence is still debatable.

The frequency of cellular chimerism in Leine sheep determined on the basis of the performed analysis of leukocytic chromosomes was 5.06%. Thus, this value does not vary from those in the literature (Table 2), although the data given in the table cannot be always compared in view of different methods used for their determination. Eventually, it is difficult to interpret unequivocally results of one's studies in the context of already obtained values. When focusing on a five percent range of freemartinism one should wonder wheather this value is worthy of general interest for sheep breeders. If we consider this level along with the fertility, which determines the range of freemartinism occurring solely between heterosexual twins, then undoubtedly with a low percentage of multiple pregnancies this value will not limit significantly the reproductive performance of the whole herd. If we, however, include in the above considerations some highly fertile breeds or the herds, in which the repair is performed

Table 2. Freemartinism frequency in different sheep breeds given by other authors and in the present study

Studied sheep No.	Chimeric sheep No.	Freemartinism %	Breed	References
52 sheep from twins (no data on sex)	1 pair of twins with erythrocyte chime-rism	5 ? (factually 3.8)	no data	STORMONT C. et al. 1953
169 sheep from heterosexual twins	2	1.2	Clun Forest and Finish Landrace sheep	DAIN A.R. 1971
261 (no data on birth)	3	1.1	no data	LONG S.E. 1980
125 ewes from heterosexual twins	6	4.8	Romanov sheep	MATEJKA M.P. et al. 1987
no data	no data	5	Cambridge sheep	GILL J.J.B. and DAVIS D.A.R. 1990
29 female-lambs from heterosexual twins	2	6.9	Booroola Merino sheep	CRIBIU E.P. et al. 1990
28 ewes from heterosexual twins	6	21.4	Booroola Merino sheep	JASZCZAK K. et. al. 1993
454 lamb-females from heterosexual twins	23	5.06	Leine sheep	results of the present study

on the basis of twin material, then it can be proved, that a five percent ratio will diminish significantly the fertility, expressed as percentage of effectively covered ewes. STORMONT et al. (1953), just as JONNSON and GUSTAVSSON (1969), have already described single cases of sheep freemartins, and have also noticed this possibility. Similar conclusions were drawn by British and French scientists on the basis of cellular chimerism estimated in ewes culled from breeding due to their sterility, who proposed to undertake a discussion on using that information about sheep freemartinism in programmes aimed at the improvement of insemination efficiency. Similar suggestions were made on the basis of an experiment carried out previously in the aforenamed Leine sheep herd (SZATKOWSKA 1991), in which the repair is based on animals coming from twin births. A lower fertility index, observed among ewes born as heterosexual twins, can be considered as a confirmation of freemartinism in Leine sheep at a level showing in a statistically justified way a lower reproductive value of this group of sheep (difference at a 4% level).

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