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The frequency of chromosomal XX/XY chimerism occurrence in Merino Booroola sheep

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Abstract. Cytogenetic analysis was carried out in a total of 59 females and 45 males from 48 litters of Merino Booroola sheep. Karyotypes were investigated only in sheep coming from multiple heterosexual pregnancies. The study was performed over three consecutive years. Lymphocyte 54,XX/54,XY chimerism was identified in 16 ewes and 16 rams, i.e. 30.8% of the karyotyped group of animals and 10.32% of all animals born alive in the flock.

Key words: Booroola Merino sheep, chromosomal chimerism XX/XY, freemartinism, multiple pregnancies.

Introduction

Polish Booroola sheep breeding began with imported Booroola embryos from New Zealand in 1988 (CZŁONKOWSKA et al. 1991, KLEWIEC et al. 1991). Animals from the Booroola line of Australian Merino sheep are carriers of the Fec B gene, which controls ovulation rate and litter size (BINDON, PIPER, 1986, DODDS et al. 1991, MONTGOMERY et al. 1993, LANNELUC et al. 1994).

The Booroola Merino strain as well as other highly prolific sheep is often used for cross-breeding to increase fecundity in other breeds (BINDON, PIPER, 1986, CZŁONKOWSKA et al. 1991). It is known that leukocyte and erythrocyte chimerism is observed in some multiple heterosexual litters (STORMONT et al. 1953, DARRE et al. 1972, GILL, DAVIES 1991, JASZCZAK et al. 1993, KUBIEŃ et al. 1993, SZATKOWSKA 1995). Chimeric animals have two genetically

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different blood cell lines, with a chromosome set 54, XX and 54, XY, respectively. Females with 54,XX/54,XY chimerism, the so-called freemartins, are sterile. This sterility arises from pathological changes of the reproductive tract. Moreover, they are usually masculinized and can also show male-like aggression and mounting behaviour (BRUERE, MACNAB 1968, DAIN 1971, WILKES et al. 1978, MATEJKA et al. 1985, 1987, GILL, DAVIES 1991).

The chimeric rams do not show any phenotypic abnormalities. SMOKOVITIS et al. (1992) however, suggested that the abnormality in sperm plasminogen activator activity of a ram with the XY/XX chromosomal chimerism could affect fertility, although sperm morphology, viability and motility showed no divergence from rams with normal karyotype 54,XY.

The masculinization of female fetuses in heterosexual multiple pregnancies is well known in cattle as freemartinism. Freemartinism is caused by placental anastamosis and exchange of the primordial blood cells in uterine life (LONG 1979). In sheep also, investigations have shown the existence of blood vessel connections between fetuses and the exchange of blood cells, but some authors indicated that this occurred rarely (ALEXANDER, WILLIAMS 1964, MELLOR 1969, MATEJKA et al. 1987). The occasional existence of cellular XX/XY chimerism was found in such highly prolific breeds as Friesian, Romanov, Olkuska, Clun Forest, Finnish Landrance and Cambridge (STORMONT et al. 1953, DAIN 1974, MATEJKA et al. 1985, 1987, GILL, DAVIES 1991, KUBIEŃ et al. 1993).

The aim of this study was to determine the frequency of leukocyte 54,XX/54,XY chimerism in the Booroola strain.

Material and methods

A cytogenetic analysis was done on 59 females and 48 males from 48 litters of a Merino Booroola flock. The karyotypes were investigated only in sheep born from multiple heterosexual pregnancies. The study was conducted in the years 1992-1994. Chromosome preparations were obtained from in vitro cultured leukocytes. The lymphocyte culture was performed by a modification of the method of MOORHEAD et al. (1960). The preparations were air-dried and stained with Giemsa.

Two cultures were grown from one blood sample of each animal. At least 40 metaphase spreads were counted from each culture. The chimerism was assessed examining a total of 80-100 spreads per individual from two cultures.

Results and discussion

The problem of the leukocyte 54,XX/54,XY chimerism and associated freemartinism have been considered in many studies taking into account three main aspects – cytogenetic, anatomic and histological. However, only a few authors have given data on the frequency of XX/XY chromosomal chimerism in sheep. The given data are often not comparable to each other because different methods were used for their determination.

The results obtained in this study are presented in Tables 1 and 2. In the first year of the study, XX/XY chimerism was found in 5 ewes and 5 rams, which constituted 6.09% of all the animals born alive in the flock; in the second year the incidence was 22.97%, and in the third year it was 6.94%. The overall incidence of XX/XY chimeric individuals during three consecutive years was

Table 1. The frequency of chromosomal chimerism XX/XY in Booroola ewes and rams from multiple heterosexual litters

Year	Number of karyotyped		Ewes with karyotype no. (%)		Rams with karyotype no. (%)		Total chimeric sheep no. (%)
	litters	individuals	54,XX	54,XX/XY	54,XY	54,XY/XX	
1992	23	52	26 (50.0)	5 (9.6)	16 (30.8)	5 (9.6)	10 (19.2)
1993	16	33	7 (21.2)	9 (27.3)	9 (27.3)	8 (24.2)	17 (51.5)
1994	9	19	10 (52.6)	2 (10.5)	4 (21.0)	3 (15.8)	5 (26.3)
Total	48	104	43 (41.3)	16 (15.4)	29 (27.9)	16 (15.4)	32 (30.8)

10.32% of all animals born alive. Such a high percentage has not previously been found. The incidence of freemartinism was estimated at 5% (STORMONT et al. 1953) and at 1.2% (DAIN 1971) in sheep born from twin pregnancies. SZATKOWSKA (1995) performed cytogenetic analysis including 454 young Leine ewes from heterosexual twins and multiplets, and identified 5.06% to be 54,XX/54,XY chimeras. LONG (1980) found three freemartins among 261 ewes (1.1%) bought at a market.

Such a high number of sheep with sex chromosomes chimerism in the blood cells indicates the influence of several factors. The above cited authors reported data concerning animals from twin pregnancies or individuals selected at random from a flock, while the data obtained in our study refers to multiple pregnancies. (We define multiple pregnancies as twin pregnancies or more). Moreover, our cytogenetic analysis concerned only heterosexual pregnancies.

PETSKOI (cited by ALEXANDER, WILLIAMS 1964) found placental anastamosis to be more common in pure-bred sheep than in cross-breds. ALEXANDER, WILLIAMS (1964) emphasize the fact that closed genetically flock may favour placental vascular anastamosis. SZATKOWSKA and ŚWITOŃSKI (1996) revealed familial occurrence of the XX/XY leukocyte chimerism in the case of 15 heterosexual litters out of 22 litters studied. Therefore, it is neccessary to take into account these factors when analysing the data in this study, particulary since the frequency of anastomosis is probably higher in the high fecundity sheep (GILL, DAVIES 1991).

Table 2. The frequency of individuals with chromosomal chimerism in Booroola Merino flock over three years

Year	Number of individuals born alive	Ewes with karyotype 54,XX/XY no. (%)	Rams with karyotype 54,XY/XX no. (%)	Total chimeric sheep no. (%)
1992	164	5 (3.04)	5 (3.04)	10 (6.09)
1993	74	9 (12.16)	8 (10.81)	17 (22.97)
1994	72	2 (2.77)	3 (4.16)	5 (6.94)
Total	310	16 (5.16)	16 (5.16)	32 (10.32)

In the great majority of multiple pregnancies no chimeras were found. Some system to prevent freemartinism is likely to be present. In sheep this is probably achieved initially by the normal separation of twins into different uterine horns (GILL, DAVIES 1991). According to MELLOR (1969), there is a suture line between fused sheep placentas arising from the fusion of adjacent chorious. Major blood vessels rarely cross this line which prevents intermixing of the blood adjacent foetuses.

In cattle, the frequency of placental anastomosis and freemartins is known to be about 90-95% (DARRE et al. 1972). However, it is not a great economic problem since the incidence of twin pregnancies has been calculated to be about 2% in dairy cattle and about 0.5% in meat cattle. In some herds, e.g. one Fresian and two Milking Shorthorn, higher twinning rates occurred (3.1 to 3.3%, MORRIS, DAY 1990), but even these rates are still very low.

The mean litter size in the Booroola Merino strain of sheep is 2.5 ranging from 1 to 7 (BINDON, PIPER 1986). It is very important with such a number of lambs in a litter to be able to predict how many of them will be XX/XY chimeras. If their percentage is too high, it may be a large economic problem,

especially in small flocks. Males with the leukocyte 54,XY/54,XX chimerism do not usually show any phenotypical abnormalities and differences in fertilization ability from normal rams (SMOKOVITIS et al. 1992). Only chimeric ewes are sterile. In the karyotipically analysed group the percentage of female chimeras was 15.4%. The frequency of chimeric ewes among all the lambs born alive in the flock is 5.16%. Such an incidence of freemartins will diminish significantly the fertility of highly prolific breeds or herds, expressed as a percentage of successfully mounted ewes, especially when rotation is based on twins and multiplets (SZATKOWSKA 1995).

The evaluation of reproductive traits of rams with XY/XX chromosomal chimerism and the investigation of a possible hereditary predisposition towards leukocyte chimerism are both currently underway.

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