Genet Sel Evol (1991) 23, suppl 1, 126s-130s

XX/XY chromosomal chimerism in infertile sheep of the *Cambridge* breed

JJB Gill[1], DAR Davies[2]

[1] *University of Liverpool, Department of Genetics and Microbiology, Liverpool;*
[2] *University of Liverpool, Department of Animal Husbandry, Liverpool, UK*

(Proceedings of the 9th European Colloquium on Cytogenetics of Domestic Animals; Toulouse-Auzeville, 10-13 July 1990)

sheep / chimerism / XX/XY / infertility

The *Cambridge* sheep has been developed since the mid 1960s as a high-fecundity breed from crosses between the *Finnish Landrace* and various British breeds, notably the *Clun Forest.* Although the *Cambridge* is now selected for various morphological characters, the initial and continuing selection has been for the number of lambs born to a ewe at one parturition (litter size). The flock of 150 ewes owned by the University of Liverpool has an average litter size near to 2.9 and around 65% of the mature ewes have at least 3 lambs per litter. Approximately 2% of the females have 6 lambs per litter.

In recent years, the occurrence of sterile females has been monitored and, in mixed-sex litters of 3 or more lambs, a frequency of about 0.05 has been found. In similarly sized, all-female litters, sterile females are much less common at a frequency of about 0.001. No male sterility has been noted and, in any litter, only one affected female has been found. One affected animal with a possible inguinal testis has been noted but this, although it showed male-like aggression and mounting behavior, had, like all the others, entirely normal female-type external genitalia. Apart from this animal, all the ultimately infertile females showed no sign of abnormality until the usual time of puberty, after which, juvenile teat and udder development persisted. All the animals thus identified failed to conceive when run with proven fertile males for at least one season.

Initial chromosome studies on lymphocytes showed that some of the infertile females were XX/XY chimeras and these investigations have been extended to include the available sibs of affected animals. Thirteen infertile ewes have been examined and, for 9 of these, at least some co-sibs were available. The results are summarized in table I.

Most of the affected females were produced from different and only distantly related parents but litters 5 and 6 were from the same dam in consecutive years, and 7 and 8 shared a sire. No other close genetic relationships were apparent among the animals.

Table I. Cell mixtures in sterile female sheep and their available co-sibs.

Litter no	*Litter composition*		*Animals sampled (sex)*	*No of cells (mixture)*		*Morphology at puberty*
	♀	♂		*XX*	*XY*	
1	1	3	♀	58	42	small teats
2	2	1	♀	94	6	small teats
3	2	1	♀	73	27	small teats
4			♀	98	2	small teats
5	2	2	♀	84	16	small teats
			♀	100	0	normal ♀
			♂	2	98	normal ♂
6	1	2	♀	70	30	small teats
			♂	7	93	normal ♂
			♂	0	100	normal ♂
7	1	3	♀	85	15	small teats, inguinal testis
			♂	0	100	normal ♂
			♂	0	100	normal ♂
8	3	1	♀	100	0	small teats
			♀	100	0	normal ♀
9	4	1	♀	100	0	♀ small teats
			♀	100	0	normal ♀
			♂	0	100	normal ♂
10	4	0	♀	100	0	♀ small teats
			♀	100	0	normal ♀
11	1	2	♀	79	21	♀ small teats
			♂	1	99	normal ♂
12	3	1	♀	53	47	♀ small teats
			♂	54	46	normal ♂
13	2	1	♀	53	47	♀ small teats
			♀	100	0	normal ♀

All the affected females, except those in litter 10, had at least one male co-sib recorded and 10 of the 13 were XX/XY chromosomal chimeras. There were considerable differences between the cell ratios of the chimeric females but all had more XX than XY cells. The highest frequency of XX cells was 0.98 and the lowest 0.53. In litters 5, 6, 7, 9, 11 and 12, at least one male co-sib was available. In 4 of these litters (5, 6, 11 and 12), an XX/XY chimeric male was identified. In litter 12, the cell ratios of the chimeric co-twins were almost identical but, in the other pairs, a very great difference between the members of the pairs was present. In each of these cases there was an obvious preponderance of XX cells in the female and XY cells in the male.

The correlation between the persistence of juvenile teat and udder development, and ultimate sterility in *Cambridge* sheep shows that the first is a powerful indicator

of the second. The demonstration of XX/XY lymphocyte chimerism in many of the sterile animals suggests that at least some of the infertility is due to freemartinism. This suggestion is supported by Dobson and Davies (1989), who showed that some of the sterile, XX/XY females had inhibition of Müllerian duct derivatives.

Freemartinism is well known in cattle and the studies of Marcum *et al* (1972) and Darré *et al* (1972) have, respectively, demonstrated correlation coefficients for the percentages of male cells between the members of male and female pairs of 0.97 and 0.92. Marcum (1974) has calculated that the mean XX/XY cell ratio in such animals approaches 1, with a mean frequency of XY cells in the females of 0.48 and in the males of 0.52. The data for the few freemartin sheep recorded and for which phenotypic details are available (table II) show a mean frequency of XY cells of 0.47 and a range of 0.04–0.94. These are very similar to the data for cattle and, together with the high correlation of cell ratios between the members of chimeric lamb pairs recorded in the only 4 sheep pairs reported (Dain, 1974), have led to the assumption that the origins of freemartinism in sheep and cattle are alike. Considerable masculinization of the freemartin seems, however, more common in sheep than in cattle, but this may reflect only the fact that many of the sheep were initially recognized as freemartins because of this masculinization.

The data available on the *Cambridge* sheep, however, do not support this assumption of similarity with cattle freemartins. Even if the 3 infertile ewes not showing XX/XY chimerism are removed from the sample as having infertility not proven to be associated with placental or embryonic fusion, the frequency of XY cells in the 10 chimeric females is 0.25. These data are, however, compatible with those of Power *et al* (1985), where between 26 and 48% male cells were recorded in 8 infertile ewes from multiple birth cohorts. According to Hanrahan (personal communication), all these ewes were from high-fecundity breeds. It therefore appears that, in some high-fertility sheep, the cell mixtures established in the female chimeras are not random with a cell ratio of 1 but that there is considerable preponderance of XX types. That in some such sheep the constitutions of the established cell populations are not random but tend to match the zygotic cell type is also suggested by the very clear differences in the cell ratios between the females and males from lines 5, 6 and 11, where there are very obvious dominances of XX cells in the females and XY cells in the males.

These deviations from random cell mixtures could have a number of possible origins: 1) the exchange of hemopoietic cells between developing fetuses may be restricted to only a portion of the cells; 2) fusions between fetuses may involve more than pairs, so that in some fusion complexes there will be an imbalance of cell types; 3) there may be selection against 'foreign' cells, either at the time of fusion or in subsequent development.

If the first is true, it might be expected that any exchange would be reciprocal so that the frequency of 'foreign' cells in twin recipients would be similar. Inspection of the ratios in the chimeric pairs in table I suggests that this is not so. The data are not, however, sufficient to totally rule out this possibility.

If multiple placental fusions, with random mixture of hemopoietic cells were taking place, it might be expected that the predominant cell type in the chimeras would be that of the most common sex in the litter. No such relationship is apparent in the data and the presence of markedly different cell ratios in co-sibs suggests that

Table II. Records of freemartin sheep.

Author	*Breed*	*Sibship*	*Cell mixture (%)*		*Genitalia phenotype*
			XX	*XY*	
Gerneke (1965)	*Afrikaner* × *Persian*	twin to ♂	58.5	41.5	♂intersex
Bruère and Mcnab (1968)	*Welsh Mountain*	sib to 2♂ + 1♀	45.3	54.7	♂intersex
	Clun	twin to ♂	12.4	87.6	♂intersex
	Scots BF[a] × *Welsh*	twin to ♂	26.7	73.8	♂intersex
	Scots BF[a] × *Welsh*	sib to 1♂ + 1♀	6.5	93.5	♂intersex
	Scots BF[a] × *Cheviot*	twin to ♂	93.4	6.6	normal ♀
	Cheviot	single born	42.8	57.2	normal ♀
Hulot and Popescu (1969)	*Southdown*	sib to 1♂ + 1♀	96	4	♂intersex
	Southdown × *Rova*	single born	35	65	♂intersex
Jonsson and Gustavsson (1969)	*Leicester* × *Swedish Landrace*	sib to 2♂	35.4	64.6	
Dain (1974)	*Clun*	sib to 2♂ + 1♀	63	38	enlarged clitoris ♀ posterior vagina
	Clun	sib to 2♂	95	5	〃 〃 ♀
	Finnish Landrace	sib to 1♂ rig	45	55	〃 〃 ♀
	Merino X	sib to 1♂	86	24	〃 〃 ♀
	Suffolk	sib to 1♂	32	68	〃 〃 ♀
	Clun	sib to 1♂	35	65	enlarged clitoris ♀
	Finnish Landrace	not known	80	20	enlarged clitoris ♀
Wilkes *et al* (1978)	*Dorset Horn*	sib to 1♂ + 1♂ dead	34.1	65.9	♂intersex
Matejka *et al* (1987)	*Romanov*		55	45	normal ♀
	Romanov		44	56	normal ♀
	Romanov		83.4	16.6	large clitoris ♀
	Romanov		25.3	74.7	normal ♀
	Romanov		90.8	9.2	normal ♀

[a] *Scots Blackface.*

this does not happen. Indeed, the presence of only a single affected female in any litter suggests that multiple placental anastomosis either does not take place or is extremely rare.

Dain's results (1974) did record changes in leukocyte frequencies with age in sheep chimeras and these were alike in differently sexed individuals in the chimeric pairs. It is therefore clear that some competition between cell types can occur. Such competition could lead to dominance of zygotic cell types.

It is clear that, in any successful multipartuate species, some system to prevent freemartinism is likely to be present. In sheep this is probably initially achieved by the normal separation of twins to different uterine horns. According to Mellor (1969), there is also the formation of a suture line between fused sheep placentas and major blood vessels rarely cross this line. The production of such high-fecundity breeds as the *Cambridge* must, however, produce a much greater frequency of placental interactions than has been common in sheep and is therefore likely to increase the selection pressure against freemartin production above previous levels. This could well include the development of new genetic systems against the condition.

The possibility of the induction of any such systems is at present being investigated.

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