

# CHROMOSOME COMPLEMENT AND EXTERNAL PHENOTYPE OF 25 INFERTILE SHEEP

## COMPLEMENTO CROMOSÓMICO Y FENOTIPO EXTERNO DE 25 OVEJAS INFÉRTILES

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### Additional keywords

Freemartin. Intersex. Ovarian hypoplasia. XO/XX mosaic.

### Palabras clave adicionales

Freemartin. Intersexo. Hipoplasia ovárica. Mosaico XO/XX.

### SUMMARY

Twenty five infertile sheep were obtained from various sources.

Cytogenetic analyses were carried out on conventionally stained chromosomes from peripheral blood lymphocytes and peritoneal fibroblasts. On the basis of chromosome complement and *post mortem* findings, 21 were diagnosed as freemartins (54,XX/54,XY), 2 as intersexes (54,XX male pseudohermaphrodites), 1 as a female with ovarian hypoplasia (54,XX) and 1 as a presumptive 53,XO/54,XX mosaic.

The 21 freemartins were from the following breeds: 7 Suffolks, 7 British Frieslands, 2 Jacobs, 1 Bleu demaine, 1 Exmoor Horn, 1 Cambridge, 1 Mule and 1 meat type of indeterminate breed. Seven of the freemartins were known to have male litter mates. Three other freemartins, all Suffolks, were confirmed 54,XX when fibroblasts were examined.

The presumptive XO/XX mosaic animal was infertile but had produced mummified lambs.

The phenotype of the infertile animals will be discussed in relation to their chromosome complement.

### RESUMEN

Veinticinco ejemplares ovinos infértiles se obtuvieron a partir de varios orígenes.

Los análisis citogenéticos se realizaron mediante cromosomas teñidos de forma convencional, obtenidos a partir de cultivos de linfocitos de sangre periférica y de fibroblastos peritoneales.

A partir del complemento cromosómico y análisis *post mortem*, se diagnosticaron 21 *freemartins* (54,XX/54,XY), 2 intersexos (54,XX, machos pseudohermafroditas), 1 como hembra con hipoplasia ovárica (54,XX) y 1 como presunto mosaico (53,XO/54,XX).

Las 21 hembras *freemartin*, pertenecían a las siguientes razas: 7 Suffolks, 7 Frisonas británicas, 2 Jacobs, 1 Bleu demaine, 1 Exmoor Horn, 1 Cambridge, 1 Mule y un cruce para carne de raza indeterminada. Se sabe que siete de las *freemartin* tuvieron compañeros macho de camada. Otras tres *freemartin*, todas Suffolk, fueron confirmadas como 54,XX cuando se examinaron los fibroblastos.

El presunto animal mosaico XO/XX, fue infértil, aunque produjo corderos momificados.

El fenotipo de los animales infértiles será discutido en relación a su complemento cromosómico.

## INTRODUCTION

The incidence of chromosome anomalies was investigated as part of a larger investigation into infertility in sheep. In addition, the phenotype of each animal was carefully noted with a view to determining whether a veterinary surgeon or experienced farmer would be able to recognise that the sheep were abnormal without further investigation.

## MATERIALS AND METHODS

Twenty four sheep, of various breeds, that had failed to be served by the ram in the previous breeding season were obtained from local farms. One additional ewe (no. 10), that had bred but produced mummified lambs in two successive seasons, was also included (**table I**). The animals were examined and the size and shape of the vulva and

**Table I.** Chromosome complement of infertile sheep. (Complemento cromosómico de ovejas infértiles).

Nº	Sheep Breed	Lymphocytes			Fibroblasts		Diagnosis
		XX	XY	XO	XX	XY	
1	British Friesland	3	4	0	-	-	Freemartin
2	Bleu demaine	3	3	0	-	-	Freemartin
3	British Friesland	8	2	0	-	-	Freemartin
4	Jacob	1	8	0	-	-	Freemartin
5	Mule	5	23	0	-	-	Freemartin
6	Suffolk	16	2	0	-	-	Freemartin
7	British Friesland	1	3	0	-	-	Freemartin
8*	British Friesland	2	4	0	-	-	Freemartin
9*	British Friesland	30	0	0	-	-	Hypoplasia
10	Texal	6	0	7	-	-	Mosaic
11	British Friesland	1	2	0	-	-	Freemartin
12	Mule	47	0	0	-	-	Male pseudohermaphrodite
13	Jacob	12	15	0	-	-	Freemartin
14	Exmoor horn	5	1	0	-	-	Freemartin
15	British Friesland	30	0	0	-	-	Male pseudohermaphrodite
16	Suffolk	1	74	0	30	0	Freemartin
17	Suffolk	11	7	0	30	0	Freemartin
18	Suffolk	2	5	0	30	0	Freemartin
19	Meatline	7	8	0	-	-	Freemartin
20	Cambridge	4	5	0	-	-	Freemartin
21	British Friesland	3	2	0	-	-	Freemartin
22	British Friesland	5	5	0	-	-	Freemartin
23	Suffolk	3	3	0	-	-	Freemartin
24	Suffolk	8	22	0	-	-	Freemartin
25	Suffolk	9	21	0	-	-	Freemartin

\* litter mates

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clitoris, the length of the vagina, size of teats and the presence or absence of inguinal gonads were recorded.

Cytogenetic analyses were carried

out on chromosomes from peripheral blood lymphocytes of all 25 animals. Heparinised whole blood was cultured for 72hrs in MEM supplemented with

**Table II.** External phenotype of infertile sheep. (Fenotipo externo de las ovejas infértiles).

Sheep nº	Vulva		Clitoris		Vaginal length cm	Teat size	Inguinal gonad		
	size	shape	within fossa	size cm			+/-	L size*	R size*
1	S	N	+	/	4.8	S	-	/	/
2	VS	VT	+	/	6.2	VS	-	/	/
3	VS	F	-	1.5	4.6	VS	+	1.8	2.2
	dil								
4	VS	F	+	/	6.5	VS	-	/	/
5	VS	VT	+	/	6.0	VS	-	/	/
6	VS	F	-	0.5	7.0	VS	-	/	/
	dil								
7	S	N	+	/	6.0	S	-	/	/
8	VS	VT	+	/	6.5	VS	-	/	/
9	S	N	+	/	12.0	S	-	/	/
10	N	N	+	/	19.0	N	-	/	/
11	VS	F	+	/	5.5	VS	-	/	/
12	L	F	-	2.0	3.4	S	+	2.8	2.8
	dil								
13	S	N	+	/	6.8	S	-	/	/
14	N	N	-	1.5	3.1	S	+	1.8	2.0
	dil								
15	S	F	+	/	4.5	VS	+	1.4	3.1
16	L	F	-	1.6	6.5	S	-	/	/
	dil								
17	N	F	+	0.5	4.0	VS	+	4.0	5.4
18	L	F	-	1.0	5.0	VS	+	2.0	1.8
19	S	N	-	0.8	5.5	S	+	2.0	1.5
20	S	F	-	1.0	3.2	S	+	1.5	5.0
21	S	N	-	/	3.5	S	-	/	/
22	S	F	-	0.5	4.2	N	+	5.0	2.5
23	S	F	-	1.0	6.0	S	+	2.5	3.5
24	S	I	+	I	I	S	-	/	/
25	S	I	+	I	I	S	-	/	/

Key:

VS = vey small; S = small; L= large; N = normal; F = fish hook shape; dil = dilated.

VT = ventral tip; \* circumference in cms, + = present, - = absent, / = not recorded.

20 p. cent foetal calf serum, 1 p. cent L-glutamine, 1 p. cent antibiotics and 3 p. cent poke weed mitogen. Forty five minutes prior to harvesting mitotic division was arrested by the addition of 0.2 µg/ml of colcemid. Harvesting and slide preparation were carried out in a routine manner and staining was with 10 p. cent giemsa at pH 6.8.

In addition, peritoneal fibroblast cultures, from material collected *post mortem*, were examined in 3 animals. A minimum of 30 cells were examined except when two cell lines were established before this number was counted.

## RESULTS

The results of the chromosome examinations are shown in **table I**. Twenty one animals were peripheral blood sex chromosome chimaeras (54,XX/54,XY) and were diagnosed as freemartins. Three of these animals were confirmed 54,XX when fibroblasts were examined.

Three animals had a chromosome complement of 54,XX. Two were diagnosed as male pseudo-hermaphrodites and one as having ovarian hypoplasia on the basis of the anatomical findings (**table II**).

One animal was a presumptive 53,XO/54,XX on the basis of 13 cells.

This animal died before re-examination could occur so that the finding may be a technical artefact since the X chromosome was not definitively identified by means of G,TG staining. This animal had been served in the past two seasons but only produced mummified lambs.

The external phenotypes of the infertile animals are shown in **table II**.

## DISCUSSION

Despite the fact that the reported incidence of freemartinism in sheep is less than 7 p. cent, even in the highly fecund breeds, (Dain, 1971; Long, 1980; Bindon *et al.*, 1985; Power *et al.*, 1985; Matejka *et al.*, 1987; Cribiu *et al.*, 1990; Hanrahan, 1990; Gill and Davies, 1991; Szatkowska, 1995) it is nevertheless an important cause of failure to breed. In this work, 21 of the 25 infertile animals (84 p. cent) were diagnosed as freemartins on the basis of sex chromosome chimaerism. All of the animals, except the presumptive XO/XX mosaic, had one or more anatomical feature that enabled the veterinary surgeon and/or the farmer to suspect that they were not normal. Therefore, whilst the use of cytogenetic screening was helpful in establishing a definite diagnosis the farmer could identify 96 p. cent of the infertile animals on physical inspection alone.

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