

EVALUATION OF THREE CRITERIA USED TO IDENTIFY F-GENE CARRIERS IN A ROMNEY x MERINO BOORoola SHEEP FLOCK IN SOUTH BRAZIL

José Carlos Ferrugem Moraes, Nelson Manzoni de Oliveira and
Arturo Selaive-Villarroel

ABSTRACT

The continuous search for higher fertility in sheep has led to the identification of major genes related to high ovulation rates. Among them, the so called F-gene has been found in a strain of Australian Merino sheep. This gene has been employed in crossing programmes to quickly increase fertility rates. Presently, the identification of F-gene carriers has been made by measuring ovulation rates and/or litter size in ewes, and by progeny tests in rams. In a 3/4 Romney-1/4 Merino Booroola population the precision of criteria employed to screen gene carriers was evaluated. The results showed that although the criteria examined had a satisfactory precision, they disturbed the segregational analysis, highlighting the need for development of improved methods of gene carrier identification.

INTRODUCTION

High fertility has probably been the most sought after trait in animal production. In sheep, at least three different genes with major effects on prolificacy have been discovered in the past ten years. Among them, the Booroola F-gene, found in an Australian Merino strain is the most widespread one (Piper *et al.*, 1988). The fast increment in fertility due to higher ovulation rates, introduces a new approach for fertility selection, which involves simple crossbreeding or introgression using major genes. However there have been great problems associated with the use of fertility genes in the

lamb industry (Smith, 1985). Currently, F-gene carrier identification is performed by measuring ovulation rates and/or litter sizes in ewes, and by progeny tests in males. Consequently segregational analysis could be biased by errors in genotype identification.

We examined the efficiency of three criteria employed to screen carriers of F-gene in a 3/4 Romney-1/4 Merino Booroola population, derived from low prolificacy foundation Romney ewes raised under southern Brazil environmental conditions.

MATERIAL AND METHODS

During 1981-82 three 1/2 Romney-1/2 Merino Booroola rams, imported from New Zealand, were mated to Romney ewes, from a flock which originally presented a twinning rate of about 0.5%. From 1983 to 1987 the reproductive performance of 97 3/4 Romney-1/4 Merino Booroola (Rom x Boo) ewes mated with four different Rom x Boo rams, was evaluated. From the available information a litter size of ≥ 2 during the first three breeding years was used as CRITERION 1 to select those ewes carrying the F-gene (F+). From 1988 onwards, all ++ ewes (non-carrier females) classified by CRITERION 1, as well as all the 18 month old Rom x Boo hoggets had their ovulation rates scored at mid-cycle by laparoscopy just before being joined. This procedure, assuming ovulation rates ≥ 3 and ≥ 5 to identify F+ and FF ewes, respectively (Davis *et al.*, 1982), was considered as CRITERION 2 in the present study. A further criterion (CRITERION 3), grouping as F+ ewes those having at least one litter size ≥ 3 during their reproductive lifetime, as employed by Davis *et al.* (1982), was examined on the same 52 ewes to evaluate the efficiency of CRITERION 1 suggested in this work.

All ewe groups were single sire mated and their progenies identified at birth. Segregational analyses were performed in the three overlapping generations. Data were analysed either by simple chi-square, goodness of fit chi-square for segregational studies or by analysis of variance to investigate the effects of age of ewe and sampling year on the number of lambs born/ewe joined (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

During the experimental period the rate of lambs born/ewe joined for the Rom x Boo genotypes ranged between 1.11 and 1.81 (Table I). There was a significant effect of year ($P < 0.05$), but it appears to be somewhat confounded with the proportion of F+ ewes within each year. This trend was previously expected. The non-significant effect of age of ewes on lambs born/ewe joined supports our conclusion that there was no additional source of bias in these data analyses.

Table I - Mean values of lambs born/ewe joined in a 3/4 Romney-1/4 Merino Booroola flock during seven years.

Experimental year	No. of EJ	LB/EJ (s.e.)	Proportion of F+ ewes*
1983	18	1.25 (0.22)	20.00
1984	44	1.28 (0.15)	27.54
1985	41	1.13 (0.16)	36.36
1986	22	1.81 (0.19)	100.00
1987	22	1.70 (0.18)	90.91
1988	49	1.34 (0.13)	51.02
1989	47	1.11 (0.12)	53.19

EJ, ewes joined; LB, lambs born; *, in accordance to criteria 1 and/or 2.

All information about the number of ewes, genotype identification and reproductive performance is presented in Table II. Despite any difference in accuracy among criteria, ewes classified as F-gene carriers presented, as expected, the highest values of both ovulation rates and litter sizes. Assuming that foundation rams were F+, half the progeny from ++ Romney Marsh ewes should be carriers, in order to agree with the major gene hypothesis. In the first generation of Rom x Boo ewes it was observed that only CRITERION 1 selects F+ ewes as expected theoretically, at a frequency of 57% (25 in 44 ewes), differing from CRITERION 3 which selects only 20% (9 in 44 ewes). The chi-square for heterogeneity showed that these criteria were different ($\chi^2 = 4.10$; 1 D.F.; $P < 0.05$) and that, overall, CRITERION 3 disturbs the expected segregation of the single gene.

CRITERION 1 was based on litter size ≥ 2 assuming embryo mortality ranging from 20 to 30% (Edey, 1969). One may therefore suppose that when triplet births were used for F-gene classification, mother ewes should have had ovulation rates of at least 4. This figure may be higher if one assumes those values of embryo mortality reported by Hanrahan (1980) and Bindon *et al.* (1982). In addition, there was a low frequency of triplets in this study within the Rom x Boo population (5.7% in 243 births).

Another approach for comparison among the criteria employed is how they classified ewes into possible genotypes with respect to reproductive performance. Considering the results presented in Table III, some details can be highlighted:

- surprisingly, among the ewes separated by CRITERION 2 the highest and most significant percentage of dry ewes occurred. This could be due to laparoscopic examination procedures;

Table II - Number of ewes, ovulation rate and litter size in Rom x Boo ewes screened by the three criteria for F- gene classification.

Criteria	Genotype	No. of ewes	Ovulation rate (s.e.)	No. of joinings	Litter size (s.e.)
1	F+	30	-	129	1.72 (0.06)
	++	22	-	77	1.05 (0.03)
2	FF	2	5.50 (0.71)	1	2.00 -
	F+	12	3.14 (0.86)	20	1.36 (0.06)
3	++	49	1.30 (0.47)	83	1.16 (0.05)
	F+	9	-	45	2.10 (0.12)
	++	43	-	161	1.35 (0.04)

- there were no differences in single lambing rates among the criteria;

- the significant difference among criteria for the proportion of ++ ewes having twin births, suggests that CRITERION 1 concentrated almost all twin products in F+ ewes, and, CRITERION 3, as expected, grouped a significant proportion of assumed ++ ewes lambing twins;

- no triplets or quadruplets were found in F+ ewes grouped by CRITERION 2, which justifies a further investigation on levels of misclassification between criteria 1 and 2, concerning the adequacy of three or more ovulations to class an F+ ewe in any breed group in which this gene was introduced.

Under our environmental conditions, the Rom x Boo population had an overall frequency of twinning of 0.35. By employing the usual formulae (Piper *et al.*, 1985), the proportion of F+ ewes classified is $1-(1-y)^n$ where, y is the twinning rate and n is the number of parturitions controlled. Thus, for CRITERION 1 there was a theoretical probability of 0.72 to class correctly an F+ ewe. However, as the frequency of ovulations ≥ 3 (CRITERION 2) was 0.22, the efficiency in identifying a carrier ewe in one measurement is only 0.22, it being necessary to observe five ovulations in order to obtain an efficiency similar to that found in CRITERION 1. It seems therefore reasonable to infer that CRITERION 1, though time consuming, identifies a proportion of F+ ewes similar to that expected theoretically. We presume that the low number of ewes detected as F+ by ovulation rates in Rom x Boo ewes (Table II), may firstly have been due to the grouping in CRITERION 2 of both the ewes classified as ++ by CRITERION 1 and the

females 18 months old at the beginning of the reproductive season. Secondly, there may have been modifier genes in the highly complex metabolic cycle of the foundation Romney genotype, within which the F-gene product acts.

Table III - Comparison of the proportion of lambs born/ewe joined according to the criteria employed to identify F-gene carrier ewes.

No. lambs born/ewe joined	Criteria							
	1		2		3		χ^2 (P)	
	F+	++	F+	++	F+	++	F+	++
0	6.98	25.97	23.81	26.51	13.33	15.23	6.15 (< 0.05)	6.32 (< 0.05)
1	36.43	70.13	42.86	62.65	22.22	54.30	3.67 (> 0.10)	5.12 (> 0.05)
2	46.51	3.90	33.33	12.16	35.56	30.47	2.39 (> 0.30)	27.70 (< 0.01)
3/4	10.08	-	0.00	0.00	28.89	-	8.31 (< 0.01)	-

All chi-square have 2 degrees of freedom.

Although both criteria 1 and 2 were found to be of low accuracy for identifying carrier ewes, they showed a good level of agreement (83%) among eighteen ewes examined simultaneously and, because they are the methods presently in use, the results support their practical employment.

Table IV presents the probability of seven rams being F+ from information about their female progeny. In fact, the results came from a genealogical study, which included an expected genotype of sires by examining the observed genotypes of both the daughters and their mothers. Ram n^o 905 was considered as F+ only when the criteria employed were assumed to be 100% efficient. The low probabilities of being heterozygous when the amount of misclassifying error of both criteria are taken into account can also be seen in Table IV.

Despite the evidence for F-gene presence in these Romney x Booroola crosses, acting in the same manner as that in the pure Merino breed or in their worldwide crosses, our results revealed that the disturbance of segregational analysis can be greater than suggested by Piper *et al.* (1985). The employment of this major gene under farm conditions to achieve better reproductive performance in breeding flocks could be desirable economically. However, considering genetic aspects, the commercial use of the

Booroola F-gene depends on the development of advanced methods for identification of gene carriers as well as genetic markers (protein or DNA; as already suggested by Montgomery *et al.*, 1989), or even some other chromosomal markers.

Table IV - Heterozigosity of Rom x Boo rams, considering the genotypes of daughters and ewes.

Ram	Daughter genotypes		Presumptive genotypes (P)	
	% FF, F+	Total	a	b
905	42.11	19	F+ (0.094)	++ (0.022)
41035	20.00	10	++ (0.016)	++ (0.003)
51055	11.76	17	++ (0.000)	++ (0.000)
1699	75.00	16	F+ (0.989)	F+ (0.175)
1644	57.14	7	F+ (0.773)	F+ (0.273)
65035	57.14	7	F+ (0.206)	F+ (0.080)
41007	27.27	22	++ (0.004)	++ (0.000)

a, Considering that CRITERION 1 or 2 had P = 1.00 to classify correctly an F+ ewe;

b, Considering that CRITERION 1 had P = 0.72 and CRITERION 2 had P = 0.22 to classify correctly an F+ ewe.

RESUMO

A constante busca de maior fertilidade nos ovinos levou a descoberta de genes principais responsáveis por altas taxas de ovulação, entre estes, o gene F na linhagem Booroola da raça Merino Australiano. Este gene tem sido introduzido em raças menos prolíficas objetivando rápidos acréscimos na fertilidade. A identificação dos portadores é pela taxa de ovulação e/ou número de cordeiros nascidos por parto, nas fêmeas; e via teste de progênie, nos machos. Numa população 3/4 Romney Marsh-1/4 Merino Booroola foi estudada a precisão com que foram identificados os portadores do gene F. Os resultados permitiram concluir que, embora os critérios utilizados sejam aceitáveis para o estado atual do conhecimento, os erros de classificação genotípica afetam as análises segregacionais do gene F. Este fato, dificulta o emprego deste gene em rebanhos comerciais e salienta a necessidade de métodos avançados para a identificação de portadores.

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