Study of LH response to GnRH in the young male as a criterion of genetic merit for female reproduction in sheep

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Summary. A high and a low response line in sheep were selected on the basis of the mean concentration of LH in 10-week-old Finn-Dorset ram lambs after an i.v. injection of 5 µg GnRH. After 8 male generations the mean LH response of the high line was more than 5-fold that of the low line and the heritability of the selected trait was estimated at 0.44 ± 0.015 . Highly significant line differences in mean LH response to GnRH were also found in males at 20 weeks of age and females at 10 and 20 weeks of age and the genetic correlations between the four LH response traits appear to be close to unity. Large line differences in the mean FSH response to GnRH were also found in both males and females at 10 and 20 weeks of age. Selection had little effect on the physical characteristics of lambs. High-response line ewes entering their first breeding season at about 7 months of age showed oestrus earlier in the season and had higher ovulation rates and numbers of lambs born per ewe lambing than did low-response line ewes. In the second breeding season, at about 19 months of age, the only line difference was a higher ovulation rate early in the breeding season in high-line ewes. It is suggested that these changes may be mediated by a more rapid response in high-line ewes to increased GnRH stimulation at puberty or at the beginning of the breeding season.

Keywords: genetic selection; LH release; GnRH; sheep

Introduction

The rate of response to genetic selection for reproduction traits is constrained by the sex and age limited nature of the traits and their generally low heritabilities. Litter size, for example, can only be measured in mature females and has a mean realized heritability of about 0.07 in sheep (Bradford, 1985). Selection pressure for litter size is necessarily limited by the need to select sufficient females to maintain the flock and a long generation interval is dictated by the need to measure the trait in mature females. Selection of males would allow higher selection intensities, but male genetic merit can currently only be measured on the basis of their female relatives' performance.

The possibility that traits directly measurable in males could provide indicators of their genetic merit for litter size has been suggested (Land, 1973). The benefits of this indirect selection, instead of, or in combination with, direct selection for litter size, have been quantified by Walkley & Smith (1980) who demonstrated that the additional response using indirect indicators of genetic merit alongside litter size could be substantial. With moderate heritabilities for the indirect indicator trait and a high genetic correlation with litter size, the predicted rate of response for litter size could be doubled. However, Walkley & Smith (1980) stressed the requirement for accurate estimates of the genetic parameters, otherwise selection pressure could be wasted on inappropriate indicators. Research has focussed on the identification of indicator traits measurable in young males (see reviews by Hanrahan, 1982; Land *et al.*, 1982; Ricordeau, 1982; Haley *et al.*, 1987).

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One area which has been investigated for potential indirect indicators of genetic merit is the dynamics of gonadotrophin release which is central to the control of reproduction in both sexes. Research on follicle-stimulating hormone (FSH) currently suggests a positive correlation between peripheral plasma concentrations of FSH in the lamb and genetic merit for litter size in the female, but the relationship was significant only in the female (Ricordeau *et al.*, 1984). Luteinizing hormone (LH) release is pulsatile, resulting in a low correlation between repeated measures from the same animal and making it a poor candidate for an indirect indicator trait. LH release after injection of gonadotrophin-releasing hormone (GnRH) is more highly repeatable, and studies of lambs of the prolific Finnish Landrace breed and its crosses in comparison with lambs from less prolific breeds suggested a correlation of this trait in lambs with prolificacy (Land & Carr, 1979). However, the later study of Hanrahan *et al.* (1981) did not support this conclusion.

The present study was initiated to evaluate the use of LH response to GnRH stimulus in young male sheep as a potential indirect indicator of genetic merit for reproductive performance. The genetic variance for the selected trait and the genetic regressions of other traits, particularly reproductive performance in mature females, on the selected trait were estimated. Two selection lines were established, one for high and another for low LH response to GnRH, and correlated responses were followed in young males and females and in the reproductive performance of mature females. This paper reports the results of this selection.

Materials and Methods

Animals and selection procedure. The animals used were Finn-Dorset sheep, a synthetic breed containing 50% Finnish Landrace and 50% Dorset Horn genes, which was created between 1965 and 1970 (Land & McClelland, 1971). The sheep were maintained at the Institute's Blythbank farm in Southern Scotland and managed on a traditional upland grassland sheep farming system. Mating took place in November and December for a 6-week period, with lambing the following April.

The experiment was started in 1978 by mating a group of females born in 1977 and 1978 to males, born in 1978, classified as high or low responders, on the basis of their LH response to GnRH. In 1979 and subsequent years, 7 7-month-old-rams in each line were selected, with no more than 2 from each half-sib group, and mated to a total of 40 7-month-old and 40 19-month-old ewes from the same line. Rams were randomly mated to approximately equal numbers of ewes, but mating between individuals with common grandparents was avoided. Males were only used for a single season and females for two seasons. Females were unselected, any surplus lambs being culled so as to make half-sib groups as equal in number as possible.

Selection criterion. At 10 weeks $\pm$ 3 days of age, male lambs were given an injection of 5 μ g GnRH (Hoechst, Frankfurt, FRG) directly into the jugular vein. Jugular blood samples were taken 30, 50 and 70 min after the injection of GnRH and assayed for LH. To normalize the 3 samples and equalize their variances and to linearize the decline in LH between samples, the values were $\log_{10}$ transformed and the selection criterion was the mean $\log_{10}$ LH concentration of the three sampling times after GnRH injection. Unless otherwise stated, all mean hormone responses quoted are mean $\log_{10}$ hormone responses in response to GnRH calculated as described above.

Lamb traits recorded. In males, the selection criterion was recorded every year from 1978 to 1986 and additionally the body weight (kg) and mean testis diameter (cm) were recorded at both 10 and 20 weeks of age. To measure testis size the animal was held in a semi-reclining position and each testis was held tight against the scrotum; its diameter was recorded as the smallest separation of callipers which could be passed freely over the scrotal surface from the head to the tail of the epididymis. In 1978, 1982 and 1984 the mean LH response was recorded at 20 weeks of age in males and, together with the body weight, at 10 and 20 weeks of age in females. The mean FSH response to GnRH was recorded in males and females at 10 and 20 weeks of age in 1982 and 1984 and in males alone at 10 weeks of age in 1985. All mean LH and FSH responses were calculated in the same way as male 10-week LH response.

Hormone assays. Peripheral plasma LH concentrations were measured using a modified double-antibody radioimmunoassay (Martensz *et al.*, 1976; Webb *et al.*, 1985). The limit of detection was 0.43 ± 0.08 ng oLH-S18/ml. The inter-assay and intra-assay coefficients of variation were 5.8% and 8.8% respectively. FSH was measured using an homologous radioimmunoassay supplied by NIAMDD. The limit of detection was 0.22 ± 0.05 ng oFSH-RP1/ml. The inter-assay and intra-assay coefficients of variation were 10.2% and 3.9% respectively.

Female reproductive traits recorded. Each year, vasectomized rams were run with the ewes from early August and the day of first oestrus (confirmed by a second oestrus 14–20 days later) was recorded for each female as number of days counted from 1 January. The pre-mating weight was recorded for each animal on the first day of the mating period in early November each year.

Ovulation rates were recorded using laparoscopy to count corpora lutea for females born in 1977, 1978, 1980, 1982 and 1984 in their first and second breeding season. In the first season, the ovulation rate of each female was recorded 3–13 days after the oestrous at which she was first mated. In the second season, the ovulation rate of each female was recorded twice in consecutive oestrous cycles, immediately before and after the oestrus at which she was first mated. Ovulation rate was not recorded when animals returned to oestrus and were remated. The litter size of all ewes lambing was recorded.

Analyses. The inbreeding coefficient of each animal was calculated according to the numerator relationship method of Quaas (1976), assuming that the parental foundation animals were unrelated.

The heritability of the selected trait was estimated using the recursive method of J. Juga & R. Thompson (unpublished). The method predicts the breeding values of animals using parental information, but changing the regression coefficients every generation to allow for the effects of selection. Iteration on the heritability is performed to minimize the squared differences of the observed from the predicted observations. The traditional methods of analysing selection experiments with overlapping generations, regressing the selection response on the selection differentials calculated by the method proposed by Pattie (1965), or by the method proposed by Newman *et al.* (1973) as discussed by James (1986), are essentially subsets of the recursive method (J. Juga & R. Thompson, unpublished). However, for comparison purposes, selection differentials were calculated by both the Pattie (1965) and the Newman *et al.* (1973) methods and observed values regressed on these using least squares as discussed below.

Most of the analyses were performed using the LSML76 computer package (Harvey, 1977). The data were analysed fitting fixed effects of line (high or low), year of birth of lamb (up to 9 classes, depending on the trait), the litter size at birth (4 classes) and the age of the dam at birth of the lamb (1 or 2 years). The day of birth of the lamb (day number counted from 1 January) was fitted as an independent variate, as was the inbreeding level of the lamb and that of its dam.

In the genetic analyses of response to selection the line factor was omitted and additional independent variates fitted. For the selected trait, fitting either the Pattie (1965) or the Newman *et al.* (1973) selection differentials provide estimates of the realized heritability. For the remainder of the traits, a regression on the individual breeding values for the selected trait, as predicted by the recursive method of J. Juga & R. Thompson (unpublished), was fitted as an independent variate. This then provides an estimate of the genetic regression of the chosen trait on the selected trait.

In the analyses performed, the effects of genetic drift have not been taken into account. This will not result in biased estimates of selection responses, but will lead to the underestimation of the variance of the estimates (Hill, 1972). In order to make some estimate of drift variance, estimates of the genetic variance of traits are required, but these are not provided for the correlated traits by the analyses performed here. Furthermore, analytical techniques incorporating drift variance with overlapping generations are not yet well developed.

Results

Inbreeding

Inbreeding accumulated from 1981 onwards and by 1986 had reached about 5% in both lines, accumulating at about 1% *per annum* from 1983 to 1986.

Line differences

Lamb traits. The means for each year and line combination for the lamb traits after adjustment for other fixed effects (age of dam, litter size at birth, day of birth of lamb and inbreeding level of lamb and dam) are shown in Table 1. The means of the hormone traits are shown on the observed scale, obtained by analysis of the geometric mean hormone responses to GnRH. Large and highly significant differences were observed between the line means for LH response to GnRH in 10-week-old ram lambs, the difference between the high and low lines was greater than 3-fold on the observed scale in 1984 and greater than 5-fold in both 1984 and 1986. The correlated mean LH response at 20 weeks of age in males was as large as the direct response at 10 weeks with a greater than 3-fold difference between lines in 1984. Large, significant line differences were also observed in mean LH response at 10 and 20 weeks of age in females. Highly significant line differences in the mean FSH response were found in both sexes at both ages, although of smaller magnitude than those found for mean LH response.

In both lines, the mean LH response decreased slightly in males between 10 and 20 weeks of age but increased in females. The mean FSH response approximately halved in males between 10 and 20 weeks of age, but tended to increase slightly over this period in females.

Table 1. Geometric mean LH and FSH responses (ng/ml) to a 5 µg intravenous injection of GnRH, body weight (kg), and mean testis diameter (cm) at 10 and 20 weeks and number of lambs recorded for LH response at 10 weeks for each combination of year of birth and line

Year of birth	Line	No. of animals	LH (ng/ml)†		FSH (ng/ml)†		Body wt (kg)		Testis diam. (cm)	
			At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks
Male lambs										
1978	Base	101	29.67	21.61			22.56	32.78	3.03	5.73
1979	High	39	48.16				19.61		2.91	
	Low	36	24.58 **				20.68		2.85	
1980	High	43	33.74				21.69	30.35	2.96	5.59
	Low	45	19.03 ***				20.71	29.98	2.73 *	5.40
1981	High	26	30.62				18.86	27.54	2.64	4.99
	Low	50	13.97 ***				18.61	27.78	2.60	5.24
1982	High	58	43.67	41.63	4.18	2.00	19.32	29.75	2.77	5.24
	Low	57	14.72 ***	10.82 ***	2.21 ***	1.11 ***	19.76	29.76	2.65 *	5.39
1983	High	43	35.13				17.38	26.32	2.47	4.74
	Low	35	22.82 ***				16.97	24.43	2.39	4.53
1984	High	54	44.07	35.55	4.62	2.26	20.09	28.36	2.81	4.95
	Low	42	13.94 ***	11.15 ***	3.09 ***	1.19 ***	20.06	28.28	2.91	5.15
1985	High	60	41.87		6.24		20.54	31.26	2.90	5.55
	Low	45	7.75 ***		3.25 ***		19.06	29.93	2.66 **	5.36
1986	High	45	57.03							
	Low	38	10.25 ***							
Female lambs										
1978	Base	112	21.71	28.18			19.12	28.97		
1982	High	66	32.94	50.18	7.48	8.15	18.47	30.11		
	Low	48	14.29 ***	15.98 ***	4.86 ***	5.00 ***	19.34	31.16		
1984	High	62	29.28	42.46	5.45	5.77	18.78	26.96		
	Low	60	14.07 ***	16.82 ***	4.06 ***	3.84 ***	18.66	26.65		

The level of significance of the difference between line means within years obtained from least squares analysis after adjustment for fixed effects is shown under each pair of means: **P* = 0.05–0.01; ***P* = 0.01–0.001; ****P* < 0.001, otherwise *P* > 0.05.

†Geometric mean concentrations 30, 50 and 70 min after i.v. injection of 5 µg GnRH.

No significant line differences in body weight were found in either sex at either age. There was a suggestion of a line difference in testis size at 10 weeks, with values for the high line being significantly greater in 1980, 1982 and 1985, but there was no evidence for a line difference at 20 weeks of age.

Female reproduction traits. Table 2 gives the mean for each year and line combination for the adult female reproduction traits in the first and second breeding season. Two measures of litter size are given in Table 2, LS1 is the number of lambs born per ewe eligible for mating and alive at lambing and LS2 is the number of lambs born per ewe lambing. The mean pre-mating body weight

Table 2. Means for each combination of year and line of traits recorded on adult females

Year	Line	No.	DFO1	PM1	OV1	LS1.1	LS2.1	DFO2	PM2	OV2.1	OV2.2	LS1.2	LS2.2
1977	Base	144	296	38.4	1.99	1.36	1.54	250	50.4	2.30	2.02	1.56	1.78
1978													
1979	High	39	325	33.8		1.44	1.88	271	54.2			1.43	1.94
	Low	37	321	34.0		1.48	1.69	257	54.7			1.91	2.12
1980	High	48	320	33.6	2.41	1.04	1.84	255	53.9	2.56	2.41	2.11	2.15
	Low	47	323	35.7	2.32	1.05	1.66	255	55.8	2.57	2.55	2.18	2.30
1981	High	38	310	34.6		1.68	1.75	251	50.9			2.26	2.29
	Low	32	335	34.1		1.16	1.57	250	51.0			1.89	2.32
			***			*							
1982	High	42	318	31.5	2.45	1.02	1.62	249	47.9	2.49	2.36	2.12	2.42
	Low	44	327	32.8	2.12	1.07	1.59	253	48.2	2.17	2.30	2.08	1.97
			*		*					*			*
1983	High	36	313	32.1		1.69	1.86	254	49.5			2.27	2.43
	Low	24	314	30.8		1.35	1.47	244	48.0			2.21	2.29
							*						
1984	High	40	316	33.3	2.42	1.70	1.69	243	48.8	2.49	2.51	2.01	2.21
	Low	40	324	32.9	2.04	1.61	1.70	247	48.4	2.15	2.65	1.96	2.27
			*		*					*			

Abbreviations: No., number of records; DFO1 and DFO2, day of first oestrus in the first and second breeding seasons respectively; PM1 and PM2, premating body weight (kg) in the first and second breeding seasons respectively; OV1, OV2.1 and OV2.2, ovulation rate in the first season and the first and second records in the second season respectively; LS1.1 and LS1.2, lambs born per ewe mated and alive at lambing in the first and second breeding seasons respectively; LS2.1 and LS2.2, lambs born per ewe lambing in the first and second breeding seasons respectively. The level of significance of the difference between line means within years obtained from least squares analysis after adjustment for fixed effects is shown under each pair of means: * $P = 0.05-0.01$; *** $P < 0.001$.

increased from 33.3 to 51.0 kg from the first to the second breeding season. There was no difference between lines in the premating body weight, either in the first season or in the second season.

In the first breeding season the first oestrus mainly occurred in early to mid-November. By the second breeding season the day of first oestrus had advanced to about mid-September. In the first season there were significant differences between the lines in the day of first oestrus, with the high line showing oestrus between 1 and 25 days earlier than the low line over the last 4 seasons' records (Table 2). There is little evidence for this same effect in the second breeding season of a ewe.

Ovulation rate increased from 2.29 to 2.43 between the first and second breeding seasons, the means of the first and second records in the second breeding season being similar. There was evidence for a difference between lines in ovulation rate in the first season, with the high line having a significantly greater ovulation rate in animals born in 1982 and 1984, the differences being 0.33 and 0.38 ova in the 2 years respectively (Table 2). In the ewes' second breeding season, ovulation rate behaved differently in the two consecutive records made in the season. At the first record, the high-line ovulation rate was significantly greater than the low-line ovulation rate in animals born in both 1982 and 1984 (the differences being 0.32 and 0.34 ova in the 2 years respectively), whereas at the second record there was no evidence of any line difference.

Litter size as measured by lambs born per ewe eligible for mating and alive at lambing (LS1) increased from 1.36 to 2.04 between the first and second breeding seasons and the number of lambs born per ewe lambing (LS2) increased from 1.70 to 2.21 between breeding seasons. There were few significant between-line differences for either litter size criterion in either breeding season and there was no consistent pattern in the between-line differences (Table 2). However, those line differences which were significant (litter size per ewe mated in the first season of animals born in 1981 and litter size per ewe lambing in the first season of ewes born in 1983 and in the second season of ewes born in 1982) were in favour of the high line.

Table 3. Residual variances of lamb traits (bold type, on diagonal) and correlations between lamb traits (above diagonal) obtained from least squares analysis after adjustment for all fixed effects

	LH (ng/ml)†		FSH (ng/ml)†		Body wt (kg)		Testis diam. (cm)	
	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks
Male lambs								
No. of records	716	200	299	200	626	527	626	527
LH (ng/ml)† at 10 weeks	0.039	0.56***	0.40***	0.25**	0.11*	0.12**	0.14**	0.09*
LH (ng/ml)† at 20 weeks		0.046	0.35***	0.34***	0.07	0.12**	0.13**	0.01
FSH (ng/ml)† at 10 weeks			0.036	0.38***	0.07	0.08	0.19**	0.08
FSH (ng/ml)† at 20 weeks				0.054	0.06	0.07	0.18**	-0.02
Body wt (kg) at 10 weeks					13.09	0.79***	0.78***	0.58***
Body wt (kg) at 20 weeks						24.24	0.65***	0.67***
Testis diam. (cm) at 10 weeks							0.186	0.70***
Testis diam. (cm) at 20 weeks								0.538
Female lambs								
No. of records	236	236	236	236	236	236		
LH (ng/ml)† at 10 weeks	0.041	0.56***	0.49***	0.27***	-0.18**	-0.12*		
LH (ng/ml)† at 20 weeks		0.045	0.27***	0.41***	0.05	0.07		
FSH (ng/ml)† at 10 weeks			0.028	0.40***	-0.21**	-0.22**		
FSH (ng/ml)† at 20 weeks				0.039	-0.11	-0.24**		
Body wt (kg) at 10 weeks					12.28	0.81***		
Body wt (kg) at 20 weeks						17.60		

Level of significance of difference of residual correlations from zero: * $P = 0.05-0.01$; ** $P = 0.01-0.001$; *** $P < 0.001$.
†Geometric mean concentrations 30, 50 and 70 min after i.v. injection of 5 µg GnRH.

Table 4. Residual variances of adult female traits (bold type, on diagonal) and correlations between traits (above diagonal) after least squares adjustment for fixed effects

	DFO1	PM1	OV1	LS1.1	LS2.1	DFO2	PM2	OV2.1	OV2.2	LS1.2	LS2.2
No. of records	467	467	228	467	326	467	467	228	228	467	326
DFO1	532.0	-0.28**	-0.04	-0.24**	-0.06	0.10	-0.13*	-0.16	-0.00	-0.12*	0.04
PM1		20.77	0.21*	0.22**	0.19**	0.02	0.63***	0.18**	0.09	0.14**	0.19***
OV1			0.476	0.21**	0.30**	0.09	0.14**	0.36**	0.28**	0.23**	0.21**
LS1.1				0.674	—	0.01	-0.09	0.14**	0.06	0.09	—
LS2.1					0.349	-0.05	0.12*	0.07	0.13	—	0.20**
DFO2						404.6	-0.10	-0.06	0.08	-0.06	0.02
PM2							33.52	0.23**	0.23**	0.17**	0.22**
OV2.1								0.393	0.46***	0.31***	0.33***
OV2.2									0.445	0.41***	0.51***
LS1.2										0.717	—
LS2.2											0.514

Abbreviations: No., number of records; DFO1 and DFO2, day of first oestrus in the first and second breeding seasons respectively; PM1 and PM2, premating body weight (kg) in the first and second breeding seasons respectively; OV1, OV2.1 and OV2.2, ovulation rate in the first season and the first and second records in the second season respectively; LS1.1 and LS1.2, lambs born per ewe mated and alive at lambing in the first and second breeding seasons respectively; LS2.1 and LS2.2, lambs born per ewe lambing in the first and second breeding seasons respectively.
Levels of significance of difference of residual correlations from zero: * $P = 0.05-0.01$; ** $P = 0.01-0.001$; *** $P < 0.001$.

Variances of, and correlations between, traits

Lamb traits. The phenotypic variances of lamb traits and correlations between them were estimated as the residual variances and correlations after adjustment for the fixed effects and are given in Table 3. These values were calculated using the $\log_{10}$ scale for the hormonal traits. Also given in Table 3 are the numbers of individuals contributing to the variance estimates. Individuals not having both traits recorded were omitted for the purpose of calculating correlations. The correlations between mean hormonal responses were all positive and highly significant within both sexes. The lowest correlations were found between the mean responses of the two hormones when sampled on the two different occasions. In general, the correlations between mean hormonal responses were of similar magnitude in the two sexes. In males there was a tendency for small positive correlations between the hormonal traits and body weight, with several achieving significance, whereas in females there was evidence of small negative correlations between the hormonal traits and body weight. All the correlations between hormonal traits and testis size at 10 weeks were significantly positive, although of small size, but there was little evidence for similar correlations with testis size at 20 weeks of age. In both sexes the correlation between body weight at 10 and 20 weeks was large and highly significant, as were the correlations between testis size at 10 and 20 weeks and between testis size and body weight.

Table 5. Residual correlations between female lamb and adult traits after least squares adjustment for fixed effects

	No. of records	LH (ng/ml)		FSH (ng/ml)		Body wt (kg)	
		At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks	At 10 weeks	At 20 weeks
DFO1	150	0.14	0.03	-0.04	0.06	-0.31**	-0.29**
PM1	155	-0.13	-0.02	-0.16*	-0.22**	0.74***	0.85***
OV1	139	-0.05	-0.10	-0.04	0.02	0.25**	0.26**
LS1.1	155	-0.09	0.08	-0.07	0.01	0.02	0.02
LS2.1	116	-0.04	-0.06	-0.11	-0.06	-0.01	0.03
DFO2	155	0.02	-0.20**	0.05	-0.04	-0.14	-0.12
PM2	155	-0.09	-0.05	-0.06	-0.17*	0.54***	0.66***
OV2.1	139	-0.02	0.01	-0.01	-0.15	0.27**	0.20*
OC2.2	139	0.02	0.02	0.03	0.05	0.08	0.17*
LS1.2	155	-0.07	-0.04	-0.11	-0.05	0.11	0.04
LS2.2	116	-0.14	-0.04	-0.21*	-0.02	0.15	0.13

Abbreviations: No., number of records; DFO1 and DFO2, day of first oestrus in the first and second breeding seasons respectively; PM1 and PM2, pre-mating body weight (kg) in the first and second breeding seasons respectively; OV1, OV2.1 and OV2.2, ovulation rate in the first season and the first and second records in the second season respectively; LS1.1 and LS1.2, lambs born per ewe mated and alive at lambing in the first and second breeding seasons respectively; LS2.1 and LS2.2, lambs born per ewe lambing in the first and second breeding seasons respectively.

Level of significance of difference of residual correlations from zero: * $P = 0.05$ – 0.01 ; ** $P = 0.01$ – 0.001 ; *** $P < 0.001$.

Female reproduction traits. Table 4 gives the residual variances and correlations between the adult female traits after adjustment for fixed effects. The day of first oestrus in the first season was negatively correlated with pre-mating weight and with LS1 in both seasons. Animals which cycled earlier have higher body weights and LS1 values.

Premating body weights in the first and second season were positively correlated with each other and with ovulation rates and measures of litter size in both seasons.

Ovulation rates were positively correlated between seasons and within the second season and were positively correlated with LS1 and LS2. In the second season, the correlations of LS1 and LS2

Fig. 1. Peripheral plasma LH concentrations (a, log₁₀ scale; b, nominal scale) in 10-week-old ram lambs in the high and low selection lines 30, 50 and 70 min after intravenous injection of 5 µg GnRH. Least squares estimates after adjustment for fixed effects.

with the second ovulation rate record, taken after mating, were higher than those with the first. The correlation between LS1 in the first and second season was positive but not significant and that between LS2 in the first and second seasons positive and significant but small.

Correlations between lamb and adult traits in females. Residual correlations after adjustment for fixed effects between traits recorded in female lambs and those recorded in adults are shown in Table 5. Lamb body weights at 10 and at 20 weeks of age behaved similarly in their correlations with adult traits. Their correlations were highly positive with premating weights, moderately positive with ovulation rates and moderately negative with day of first oestrus. The correlations between lamb body weights and litter size were generally positive but not significant.

There was little evidence for phenotypic correlations between mean LH response at 10 and 20 weeks and any recorded traits in adult females. A significant negative correlation between mean LH response at 20 weeks of age and day of first oestrus in the second breeding season was observed. All of the correlations between mean FSH response at 10 and 20 weeks of age and premating weight in both the first or second season were low and negative, but 3 were significant. Correlations between mean FSH responses and the 2 criteria of litter size generally gave low negative values, but only that between mean FSH response at 10 weeks and LS1 in the second season is significant. There is no evidence of correlations of mean FSH responses with day of first oestrus or with ovulation rates.

Genetic responses

Direct responses. The heritability which minimized the deviation of the observed from the predicted breeding values for male mean log₁₀ LH response to GnRH at 10 weeks of age, using the recursive method (J. Juga & R. Thompson, unpublished), was 0.44. To confirm this estimate, breeding values predicted by the recursive model were fitted as an independent covariate in the analysis of mean LH response in males at 10 weeks of age, fitting other fixed effects as discussed earlier. The regression coefficient obtained was 1.02 ± 0.035 , the trivial deviation from the expected value of unity being due to the small influence of fixed effects other than year. Thus the realized heritability can be taken as 0.44 ± 0.015 . For comparison, the selection differentials calculated by the methods of Pattie (1965) or Newman *et al.* (1973) were calculated. These selection differentials were fitted as independent covariates in the analysis of male 10-week mean LH response and gave almost identical estimates of the realized heritability of 0.43 ± 0.014 in each case. There is therefore little difference between the estimated heritabilities derived by the 3 methods for these data.

Table 6. Genetic regressions of lamb traits on estimated breeding value for male 10-week mean log₁₀ LH response to GnRH and estimated genetic correlations

Trait	Males				Females			
	Genetic regression	s.e.	Assumed heritability	Genetic correlation	Genetic regression	s.e.	Assumed heritability	Genetic correlation
LH (ng/ml) at 10 weeks	1.020	0.035***	—	—	1.031	0.076***	0.44	1.02
LH (ng/ml) at 20 weeks	1.220	0.080***	0.44	1.12	1.146	0.079***	0.44	1.07
FSH (ng/ml) at 10 weeks	0.540	0.049***	0.44	0.56	0.395	0.061***	0.44	0.47
FSH (ng/ml) at 20 weeks	0.632	0.085***	0.44	0.54	0.461	0.069***	0.44	0.46
Body wt (kg) at 10 weeks	1.083	0.768	0.3	0.07	0.278	1.229	0.3	0.02
Body wt (kg) at 20 weeks	1.485	1.054	0.3	0.07	0.730	1.522	0.3	0.04
Testis diam. (cm) at 10 weeks	0.210	0.090*	0.3	0.12				
Testis diam. (cm) at 20 weeks	0.013	0.153	0.3	0.00				

Level of significance of difference of genetic regressions from zero: * $P = 0.05$ –0.01; *** $P < 0.001$.

The observed large divergence between the selection lines in male 10-week mean LH response could have arisen in a number of ways. The release of LH in response to GnRH could be greater in the high line, the timing of the release could have changed such that the peak was closer to the sampling times or the half-life of the released LH could be greater. To examine these three possibilities, plasma LH concentrations at the three sample periods (30, 50 and 70 min after GnRH injection) in the high and low lines in males born in the 3 years 1984, 1985 and 1986 were analysed. The analysis was again performed using the LSML76 computer package (Harvey, 1977) and the factors examined were year of birth, line, litter size at birth, the sample period and interactions between line and year and sample period and line, with other fixed effects being found to be unimportant. The analyses were performed on both the $\log_{10}$ scale used for selection and on the nominal scale and the means for each line and sample period are shown plotted on the $\log_{10}$ scale in Fig. 1(a) and on the nominal scale in Fig. 1(b). The analyses of the data on the nominal scale gave a highly significant interaction between line and sample period, with the level of LH dropping much more rapidly in the high line (49.5 ng/ml between the 30- and 70-min sample periods compared with 15.0 ng/ml in the low line). However, on the $\log_{10}$ scale no interaction between line and sample period was found, indicating that the line difference was approximately constant over all 3 sample periods, and the decline between the 30- and 70-min sample periods as 0.42 $\log_{10}$ ng/ml and 0.44 $\log_{10}$ ng/ml in the high and low lines respectively. Furthermore, fitting linear and quadratic regressions of $\log_{10}$ LH concentration on time after GnRH injection, found no evidence of non-linearity in either line, with regressions of $-0.010 \log_{10}$ ng/ml per min in both lines. The linearity of these regressions and the fact that they are parallel indicate that the decline in LH concentration is proportional to the initial concentration and is the same in both lines, the measured concentration of plasma LH after GnRH stimulation halving every 30 min. The response to selection therefore seems to have been achieved by changing the initial level of LH attained after GnRH injection and not by alteration of the half-life of LH or the timing of its release.

Table 7. Genetic regressions of adult female traits on estimated breeding value for male 10 week mean $\log_{10}$ LH response to GnRH and estimated genetic correlations

Trait	Genetic regression	s.e.	Assumed heritability	Genetic correlation
DFO1	-18.679	6.880**	0.3	-0.20
PM1	-0.320	1.290	0.3	-0.02
OV1	0.831	0.244***	0.2	0.35
LS1.1	0.235	0.228	0.1	0.12
LS2.1	0.367	0.187*	0.1	0.26
DFO2	3.632	5.901	0.3	0.04
PM2	0.228	1.713	0.3	0.01
OV2.1	0.706	0.224**	0.2	0.33
OV2.2	-0.036	0.231	0.2	-0.02
LS1.2	0.054	0.235	0.1	0.03
LS2.2	0.153	0.218	0.1	0.09

Abbreviations: DFO1 and DFO2, day of first oestrus in the first and second breeding seasons respectively; PM1 and PM2, premating body weight (kg) in the first and second breeding seasons respectively; OV1, OV2.1 and OV2.2, ovulation rate in the first season and the first and second records in the second season respectively; LS1.1 and LS1.2, lambs born per ewe mated and alive at lambing in the first and second breeding seasons respectively; LS2.1 and LS2.2, lambs born per ewe lambing in the first and second breeding seasons respectively.

Levels of significance of difference of genetic regressions from zero: * $P = 0.05$ -0.01; ** $P = 0.01$ -0.001; *** $P < 0.001$.

Correlated genetic responses for lamb traits. The genetic regressions of male and female lamb traits on the predicted breeding value of the selected trait given in Table 6 put the correlated responses to selection observed in the between-line differences (Table 1) on a quantitative genetic basis. In male lambs the genetic regressions for the hormonal traits are all highly significant and positive. The genetic regression for testis size at 10 weeks was just significant, but there was no evidence for a genetic regression of testis size at 20 weeks, or of weight at either age, on the predicted breeding values for mean LH response at 10 weeks. In females, the genetic regressions of all 4 mean hormonal traits on predicted breeding values for mean LH response in 10-week males were highly significant, and were similar to the genetic regressions found for the same traits in males.

Selection for mean LH response in 10-week-old ram lambs has produced a substantial direct response, with an estimated realized heritability of 0.44. In addition, there have been large correlated responses in male 20-week mean LH response and female 10- and 20-week mean LH response, with these traits having genetic regressions on male 10-week mean LH response approximately equal to unity. These results indicate that there must be a large degree of common genetic control between these four traits, with little evidence of the traits being sex or age limited over this restricted range. These results do not allow us to calculate the genetic correlations between the selected trait and the correlated traits as no estimates of the genetic variances of the correlated traits are available. However, it seems reasonable to assume that the heritabilities of the correlated mean LH responses will be of the same order of magnitude as that estimated for male 10-week mean LH response, i.e. 0.44. If this assumption is made, and using the variance estimates given in Table 3 as estimates of the phenotypic variances of the traits, the genetic correlation between male 10-week mean LH response and the other traits can be calculated as:

$$r_{(A1,2)} = (b_{(2,1)}h_1\sigma_{(P1)})/(h_2\sigma_{(P2)})$$

where $b_{(2,1)}$ is the genetic regression, h_1^2 and $\sigma_{(P1)}$ are the heritability and phenotypic standard deviation of male 10-week mean LH response and h_2^2 and $\sigma_{(P2)}$ are the heritability and the phenotypic standard deviation of the correlated trait. The estimates for genetic correlations of mean LH responses with male 10-week mean LH response are all just above one (see Table 6). Even if the assumed heritabilities of the correlated traits are slightly incorrect and estimates of other parameters are inaccurate due to sampling variance, genetic drift or other biases, it seems likely that essentially all the genes controlling mean LH response to GnRH are common in both ages and to both sexes. However, although the assumed heritabilities for the correlated traits may be considered quite high at 0.44, decreasing these assumed heritabilities would increase the estimated genetic correlations, the change being in proportion to the inverse of the square root of the heritability. Conversely, if the assumed values of the heritabilities of the correlated mean LH responses were increased, the estimates of the genetic correlations would decrease.

The genetic regressions of mean FSH response of the selected trait were also highly significant at both ages in both sexes, with values around 0.5. Again it seems reasonable to assume that the heritabilities of mean FSH response may be similar to that for male 10-week mean LH response. On this basis, genetic correlations were all estimated at around 0.5 (Table 6), again indicating a high degree of common genetic control of the traits.

The genetic correlations of lamb physical traits with male mean LH response at 10 weeks were calculated assuming heritabilities for these traits of 0.3 (Table 6) and all of these estimates are very low. The heritability of testis size in the Finn-Dorset at 10 weeks of age using a slightly different criterion of measurement from that used here has previously been estimated at 0.4 (Lee & Land, 1985); if this estimate were substituted the estimates of genetic correlations would decline still further.

Analyses similar to those performed on LH release were carried out on male 10-week FSH concentrations 30, 50 and 70 min after GnRH injection for the data collected from lambs born in 1984 and 1985. Again, on the $\log_{10}$ scale there was no evidence for line $\times$ sample period interactions and the regressions of FSH concentration on time after injection were linear and parallel for

the two lines ($-0.0014 \log_{10} \text{ ng/ml/min}$ and $-0.0012 \log_{10} \text{ ng/ml/min}$ in the high and low lines respectively), the concentration thus being halved about every 230 min. On the nominal scale, the overall decline between the 30- and 70-min sample periods was 0.60 ng/ml in the high line and 0.25 ng/ml in the low line, but this did not result in a significant line $\times$ sample period interaction. These results suggest that the correlated divergence in FSH response was also obtained by changing the initial level of FSH after GnRH injection, rather than altering the half-life of FSH in plasma or the timing of its release.

Correlated genetic responses for adult traits. The genetic regressions of the female adult traits on the predicted breeding value for the selected trait are shown in Table 7. The genetic regression for day of first oestrus was significant and negative in the first breeding season, but slightly positive, although not significant, in the second season. Genetic regressions involving body weight did not differ from zero. The genetic regressions for ovulation rate in the first season and for the first record in the second season were positive and significant, but that for the second record in the second season was virtually zero. The genetic regressions for litter size criteria were positive for the first season, with that for litter size per ewe lambing (LS2) being significant, but those for the second season were very small and not significant.

The genetic correlations of the correlated traits with the selected trait were calculated as previously. Assumed heritabilities for the litter size traits were 0.1, for ovulation rates were 0.2 and for day of first oestrus and body weights were 0.3 (Table 7). The genetic correlations calculated where the genetic regressions were not significantly different from zero were all very small and the remaining estimated correlations were low to moderate in size. The estimated genetic correlations between male 10-week mean LH response and ovulation rate in the first breeding season and the first measure in the second season were almost the same at around 0.34, but for the second measure in the second season the correlation was almost zero.

Fig. 2. Mean ovulation rates of ewes in the high and low selection lines at oestrous cycles 2–7 after the start of the second breeding season in 1980, 1982 and 1984. Least squares estimates after adjustment for fixed effects.

The marked difference in the correlated responses of the first and second ovulation rate records in the second breeding season was investigated further. In this analysis of data from ewes in their second season which were born in 1980, 1982 and 1984, the two observations from the same animal were included as separate records and individual effects absorbed. The cycle number at which an ovulation rate was recorded was calculated as days from day of first oestrus to recording divided by 17 (the approximate mean cycle length) and the cycle number was fitted together with its interaction with line. The mean ovulation rates at each cycle in each line are shown in Fig. 2. In the second, third and fourth cycles after the start of the breeding season, sheep in the high line had a

mean ovulation rate about 0.3 ova greater than those in the low line, but in later cycles, when most of the matings took place, there was no apparent difference between lines. Thus, the genes which have a common influence on variation in male 10-week mean LH response and the first ovulation rate record in the season have no influence on variation in the second ovulation rate record. This leads to the conclusion that the genetic correlation between the two measures of ovulation rate in the second breeding season must be less than one.

Discussion

The results of this experiment provide further evidence that substantial genetic variation exists for at least some components of the physiological variation which underlie the phenotypic expression of variation in reproductive performance. Furthermore, the underlying variation for LH release is controlled by the same genes in both sexes and these genes also appear to be involved in the release of FSH. The very large changes in the hormonal traits were wrought with surprisingly little change in the physical characteristics of the lambs, and no apparent deleterious effects. An exciting challenge for the future exists to understand the genetic, biochemical and physiological mechanisms which control the line differences. The evidence at present suggests that the half life of the gonadotrophins has not been changed. The work of Kanchev *et al.* (1984, 1987) suggests that the immediate LH response (over the first 10 min) to GnRH in ewes is release of hormone from store, causing a short-duration LH peak. The early peak is followed by a larger, more sustained, peak due perhaps to de-novo synthesis of LH or conversion of LH from some other form. The initial evidence is that the timing and pattern of release is similar in male 10-week-old lambs (N. P. Evans & J. R. McNeilly, personal communication) to that observed in adult ewes by Kanchev *et al.* (1984, 1987). In the experiment reported here, selection appears to have acted by modulating LH concentrations in the period of decline just after the major peak, and this modulation has occurred by alteration of the size of the peak. This could have arisen in several ways. Firstly, the number of gonadotrophs could have been altered, by changing either the size of the anterior pituitary or the proportion of the cells that are gonadotrophs. Secondly, the activity of the gonadotrophs could have been changed. The number of GnRH receptors on the gonadotrophs could have altered, perhaps even mediated by a change in GnRH levels as GnRH stimulates production of its own receptor. Alternatively, the amount of LH stored in the pituitary or the rate of LH synthesis may have changed, resulting in the same level of intracellular signal leading to an altered level of LH release. Quantitative changes in the rate of LH synthesis could have been produced by increasing transcription or translation rates. These areas are currently being researched.

Selection for traits in the young male has produced correlated genetic changes in the reproductive performance of adult females, supporting the hypothesis of Land (1973). Some of the line differences produced are quite large, particularly in the first breeding season, and the genetic correlations which have produced them may be moderate in size. However, the observation that the only correlated response found in the second season is in the early season ovulation rate suggests that the changes produced may centre around an alteration in the age of sexual maturity. This conclusion is supported by the correlated genetic response to selection of testis diameter. The testis in Finn-Dorset lambs is growing rapidly at 10 weeks of age but has stopped by 20 weeks of age (McNeilly *et al.*, 1986). The significant correlated response at 10 weeks of age but not at 20 weeks of age indicates a more rapid growth rate of the testis in the high line not accompanied by a greater mature size, which may be a further indicator of early maturity. Earlier maturity could perhaps be mediated by a more rapid response to increasing GnRH stimulation in animals approaching maturity or entering the breeding season. Such a change is produced without any concomitant changes in body weight, either in lambs or in mature animals. The physiological changes which accompany the phenotypic changes in adult females are still under investigation.

In conclusion, the experiment has been successful in producing beneficial changes in female reproductive performance by selection on a trait in young males, but the reduced changes in older

females demonstrate the difficulties in choosing ideal selection criteria with limited initial evidence. The results support the case for an alternative strategy for identifying indirect selection criteria. This strategy would be to produce lines selected for traits of economic importance in one or a few breeds, perhaps involving several research centres worldwide, which could then be used to identify suitable indirect indicators of genetic merit for the traits of interest for application in all breeds (Haley *et al.*, 1987). As Hill (1985) points out, such lines produce all the information required to quantify the benefits of indirect selection criteria and to evaluate alternative selection regimens.

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