Genetic and non-genetic effects on plasma LH concentrations in lambs at 4 and 8 weeks of age

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Summary. Plasma LH was measured at 4 and 8 weeks of age in purebred lambs born to Finnish Landrace, Galway, Fingalway and High Fertility ewes. The average litter sizes for these breeds vary from 1.4 to 2.5. Lambs born after the transfer of fertilized eggs (all Fingalway) to Finnish Landrace, Galway and Fingalway ewes were also studied. There was evidence for a significant positive effect of selection for litter size within the Galway breed on plasma LH at 8 weeks of age. Finnish Landrace lambs had significantly higher levels of plasma LH at 8 weeks of age than did Galway Control lambs. These two groups did not differ with respect to plasma LH at 4 weeks of age. Non-additive genetic effects were significant at 4 weeks of age. The ranking of genetic groups on plasma LH was not consistent with their ranking for litter size. There was no evidence for a maternal effect associated with breed of dam on plasma LH and the effects of litter size were generally insignificant. Female lambs had significantly higher levels of LH at 4 weeks of age but there was no sex difference at 8 weeks because LH levels in males had increased. The correlation between the level of plasma LH at 4 and 8 weeks of age was -0.04 . It is concluded that plasma LH is unlikely to prove a practical aid to selection for increased fecundity in sheep.

Introduction

A measurable characteristic in males which is a good indicator of breeding value for prolificacy would increase the efficiency of breeding programmes for increased litter size. Such an index should be adaptable for use under field conditions. The possibility that circulating levels of gonadotrophin might be used as an index of a male's genotype for prolificacy (Land, 1973) received support from reports of Bindon (1973), Thimonier & Pelletier (1973) and Bindon & Turner (1974). The results of Thimonier & Pelletier (1973), however, suggest that the relationship between breed differences in plasma LH and prolificacy is rather complex. The findings of Bindon & Turner (1974) must be confirmed and possible sources of non-genetic variation also need to be examined. The present experiment was undertaken to examine the impact of the genetic factors of breed and selection for increased litter size on plasma LH and also the effects of litter size, maternal breed and sex.

Materials and Methods

Animals

The lambs studied were all born in March/April 1974 and consisted of two distinct groups (Table 1). Group I contained Fingalway lambs born to either Finnish Landrace, Galway or Fingalway ewes after the transfer of fertilized eggs. All these Fingalway lambs were F_1 generation animals of the Finn $\times$ Galway. Group II lambs were produced by intrabreed matings of the Finnish Landrace, Galway Elite, Galway Control, Fingalway and High Fertility breeds. The Fingalway lambs in this group were all at least F_2 generation. The High Fertility sheep are a flock selected for high litter size from a foundation of extremely prolific ewes of various breed types purchased originally from com-

mercial farms (Timon, 1964). The Galway Elite ewes represented the best 5% of the recorded pedigree population as far as litter size was concerned and they were mated with Galway rams from ewes of similar merit. The effects of this selection in Galway ewes has been estimated (Hanrahan, 1976) to increase litter size by approximately 0.1.

Table 1. Source of lambs and number samples at 4 and 8 weeks of age

Breed of dam	Egg-transfer of Fingalway lambs (Group I)	Purebred lambs (Group II)	Breed litter size*
Finn	19	23	2.5
Fingalway	16	19	2.0
High Fertility	—	12	2.0
Galway Elite	—	16	1.5†
Galway Control	28	14	1.4

* Breeding flock averages, 1973–1975, for 2-, 3- and 4-year-old ewes.

† Predicted genetic mean (Hanrahan, 1976),

Assay

Blood samples were taken from a jugular vein into evacuated tubes containing EDTA. All samples were placed immediately in an ice-bath and allowed to cool before centrifugation. The plasma was then poured off and stored at -20°C until assayed. The samples were obtained from all lambs at 4 and 8 weeks of age and on each occasion four samples were collected from each lamb at 30-min intervals beginning at 10.00 h.

The plasma LH concentrations were assayed in triplicate (50 μl aliquots) by a modification of the liquid-phase double-antibody method of Niswender, Reichert, Midgley & Nalbandov (1969) using ovine LH (LER 1374A) as tracer, ovine LH (NIH-LH-S18) as standard and rabbit anti-ovine LH antibody absorbed with TSH (D.B. 5/5, kindly supplied by Dr Graham Jenkin). Dextran T-40 was added with the second antibody (raised in rams against rabbit γ -globulins) to hasten the reaction between first and second antibody molecules, enabling centrifugation to be performed within 40 min (Martin & Landon, 1975). Data transformation (logit-log), standard curve fitting (unweighted linear least squares) and dose interpolation were carried out with a Hewlett Packard 9830 calculator. The sensitivity of the assay system was 0.34 ± 0.16 ng/ml ($n = 21$). Samples were assigned to assay runs so that all genetic groups were represented in each run. This prevented confounding of inter-assay variation with the effects of breed. Samples taken at 4 and 8 weeks of age were assayed in separate runs.

Statistical analysis

Before the final analyses the variation among replicate determinations and among samples from the same animal at a single age was examined. It was decided that the average value for all samples on each occasion would be the most useful measure for analysis. The distribution of these values was extremely skewed (to the right) and logarithmic transformation was used to normalize the distribution and reduce heterogeneity of variance among the various subgroups. Because some animals had zero values for LH the transformation used was $\log(\text{LH} + 1)$. Least squares procedures were used in the final analyses with models which included litter size and sex as main effects in addition to genetic groups. The coefficient of variation was 85% and 78% at 4 and 8 weeks of age, respectively, and within-assay measurement error was 33%.

The experiment was undertaken to examine aspects of genetic effects and the following *a priori* contrasts were evaluated by using a *t*-test: (i) the effect of selection for increased litter size within the Galway breed, (ii) the difference between Finnish Landrace and Galway Controls (compares low

and high prolificacy types), (iii) difference between the Fingalway and High Fertility breeds (compares equal prolificacy types) and (iv) non-additive genetic effects.

Results

Breed of dam effects

The analysis of data from the Group I lambs (least squares means in Table 2) revealed no effect of breed of recipient dam on LH levels at 4 or 8 weeks of age.

Table 2. Least squares means ($\pm$ S.E.M.) showing the effect of dam breed on plasma LH in Group I lambs (egg transfer)

Breed of dam	Log plasma LH (ng/ml)	
	4 weeks	8 weeks
Finn	0.40 $\pm$ 0.11	0.50 $\pm$ 0.12
Galway	0.49 $\pm$ 0.08	0.52 $\pm$ 0.09
Fingalway	0.45 $\pm$ 0.11	0.62 $\pm$ 0.12

Litter size effects

The effects of litter size were examined in separate analyses for the two lamb groups (see Table 3). In Group I litter size effects would be purely environmental while in Group II the genetic and environmental components of any litter size effects are confounded. There was no evidence for any effect of litter size except for the LH levels at 8 weeks in Group I lambs in which the effect of litter size was quadratic ($P < 0.05$), with the lowest level being observed for twin-born lambs.

Table 3. Litter size and plasma LH in lambs at 4 and 8 weeks of age (mean $\pm$ S.E.M.)

Group	Litter size	Log plasma LH (ng/ml)	
		4 weeks	8 weeks
I (egg transfer)	1	0.47 $\pm$ 0.14	0.56 $\pm$ 0.15
	2	0.42 $\pm$ 0.09	0.36 $\pm$ 0.06
	3	0.44 $\pm$ 0.10	0.72 $\pm$ 0.11
II (natural mating)	1	0.46 $\pm$ 0.10	0.50 $\pm$ 0.12
	2	0.45 $\pm$ 0.05	0.50 $\pm$ 0.06
	>3*	0.26 $\pm$ 0.08	0.49 $\pm$ 0.09

* Includes 3 quadruplet and 4 quintuplet lambs.

Lamb genotype and sex effects

Data from both groups were combined in a final analysis with breed of lamb and sex as the only main effects (Tables 4 and 5). The two types of Fingalway lambs, F₁ in Group I and F₂ in Group II, were distinguished in the analysis. The only effect which had a significant influence was sex at 4 weeks of age: female lambs had higher LH levels than males. There was no evidence for any genetic group $\times$ sex interaction.

A priori contrasts

The evaluation of the *a priori* contrasts is presented in Table 6. There is evidence that the Galway Elite lambs have significantly more circulating LH than the Galway Controls at 8 weeks of age

Table 4. Least squares means ($\pm$ S.E.M.) showing the effects of lamb genotype and sex on plasma LH

Effect	Log plasma LH (ng/ml)	
	4 weeks	8 weeks
(1) Genetic group		
Finnish Landrace	0.30 $\pm$ 0.07	0.64 $\pm$ 0.08
Galway Control	0.30 $\pm$ 0.09	0.34 $\pm$ 0.11
Galway Elite	0.43 $\pm$ 0.09	0.64 $\pm$ 0.10
Fingalway—F ₁	0.45 $\pm$ 0.04	0.57 $\pm$ 0.05
Fingalway—F ₂	0.54 $\pm$ 0.09	0.50 $\pm$ 0.10
High Fertility	0.42 $\pm$ 0.10	0.37 $\pm$ 0.12
(2) Sex		
Male	0.31 $\pm$ 0.05	0.52 $\pm$ 0.06
Female	0.50 $\pm$ 0.04	0.50 $\pm$ 0.05

Table 5. Analysis of variance of log plasma LH in lambs

Source	d.f.	Mean Squares	
		4 weeks	8 weeks
Genetic groups (G)	5	0.176	0.260
Sex (S)	1	0.957**	0.011
G $\times$ S interaction	5	0.093	0.060
Residual	135	0.120	0.159

** $P < 0.01$.** Table 6.** Estimates of *a priori* contrasts

Definition of contrast	4 weeks	8 weeks
Selection (GE—G)	0.14 $\pm$ 0.13	0.29 $\pm$ 0.15*
Prolificacy (F—G)	0.00 $\pm$ 0.12	0.29 $\pm$ 0.14†
Heterosis (FG(F ₁) + FG(F ₂)—F—G)	0.40 $\pm$ 0.15‡	0.09 $\pm$ 0.17
Breed (FG—HF)	0.13 $\pm$ 0.13	0.12 $\pm$ 0.15

F = Finn; G = Galway Control; GE = Galway Elite; FG = Fingalway; HF = High Fertility.

* $P = 0.05$; † $P < 0.05$; ‡ $P < 0.01$.

($P = 0.05$). While there was no evidence for any difference in plasma LH between Galway Control and Finn lambs at 4 weeks the difference observed at 8 weeks was significant ($P < 0.05$). Differences between High Fertility and Fingalway lambs were not significant. The importance of non-additive genetic effects in the cross of the Finn and Galway breeds was evaluated by computing the difference between the average of the LH levels in F₁ and F₂ Fingalway lambs and the corresponding average for Finn and Galway Control lambs. This contrast was significant ($P < 0.01$) at 4 weeks but not at 8 weeks of age.

Relationship between LH level at two ages

The correlation between LH level at 4 and 8 weeks of age was computed within genetic group $\times$ sex subclasses. The resulting value was -0.04 which is not significantly different from zero.

Discussion

The present experiment did not reveal differences among the genetic groups in plasma LH consistent with differences among these groups with respect to fecundity. The comparison of the present results with previous studies is complicated by differences in the age of experimental animals. Blanc *et al.* (cited by Thimonier, 1975) have shown that breed rankings vary with age. Analysis of the importance of breed $\times$ age interaction in the present data, however, showed that the effect was not significant. This result is not necessarily in conflict with that of Blanc *et al.* (1975) because in the present data the interaction tested was associated with 5 degrees of freedom. The possibility therefore exists that specific breed $\times$ age interactions may be masked in the overall test for interaction. Another limitation of the present experiment on this point is that it only extends to two ages. The results of the present experiment can be most closely compared with those of Bindon & Turner (1974), who studied 4-week-old lambs, and Echternkamp & Laster (1976) who initiated breed comparisons when the lambs were 9 weeks old.

The present study and that of Bindon & Turner (1974), who studied different lines of the Australian Merino, agree in finding a positive effect of selection for increased litter size on plasma LH. Galway Elite lambs had higher LH levels at 4 weeks compared with Controls and this difference increased up to 8 weeks when it was then just significant ($P = 0.05$). The fact that 4-week-old Finn lambs had LH levels equal to those of Galway Controls emphasizes the need to exercise extreme caution in extrapolating from between-breed differences to within-breed genetic relationships. At 8 weeks of age the Finn lambs showed plasma LH concentrations which were significantly higher than those of the Galway Control lambs. While this result is consistent with the findings of Carr & Land (1975), who studied male lambs from the Finn, Border Leicester and Cheviot breeds, the breed rankings for LH level at 8 weeks of age are not consistent with their rankings with respect to litter size. This latter observation is supported by the data of Echternkamp & Laster (1976). Thimonier & Pelletier (1973) found a positive association between plasma LH concentration in lambs and breed fecundity level but this was complicated by an apparent breed $\times$ sex interaction. No evidence was found for such interactions in the present data. However, significant breed $\times$ sex interactions have been found when the effect of Gn-RH on plasma LH was studied in the same genetic groups as were used in the present experiment (unpublished data). Obviously, there is no single or simple expectation tenable for the effects of genetic group on plasma LH.

The data from the egg-transfer lambs (Group I) shows no evidence for any maternal effect, associated with breed, on plasma LH, thus supporting the genetic interpretation of the difference, observed among litter size classes by Bindon (1973) and Bindon & Turner (1974). The significant effect of litter size on plasma LH at 8 weeks of age in the Group I lambs can probably be attributed to random sampling since it suggested that there were lower levels of LH for twin-born animals than for triplet or singleton lambs. Any effect of litter size in this group of egg-transfer lambs would be environmental because the genotype of the lambs was not related to that of the ewe. For the Group II lambs litter size did not have a significant effect at either age (Table 3), a result which does not appear to agree with the findings of Bindon (1973) or Bindon & Turner (1974). However, Bindon & Turner (1974) were unable to detect a significant difference between single and twin-born lambs in one flock and they also reported no effect of litter size on plasma LH in another flock in which most of the multiple births were of twins.

The effect of sex on plasma LH observed in the present study at 4 weeks of age is in agreement with the observations of Bindon (1973). The findings of Thimonier & Pelletier (1973), who reported that LH levels in males increased between 4 and 11 weeks of age whereas changes in the females depended on the genetic group, are also in agreement with the present results to the extent that we observed a significant rise in plasma LH of male but not female lambs between 4 and 8 weeks of age. This pattern was reflected in a significant sex $\times$ age interaction ($P < 0.01$).

The effects of crossbreeding on plasma LH were estimated from the two types of Fingalway crossbreds by comparing them with the mean values obtained from the parental breeds. This comparison yields an estimate of $0.75 \times$ heterosis (approximately) which was significant at 4 but not at 8 weeks of age. The presence of heterosis in plasma LH is consistent with the report by Land (1973) of

heterosis in testicular growth. However, no evidence for heterosis in testicular growth has been found in the Fingalway (Hanrahan, 1974). The absence of a correlation between plasma LH at 4 and 8 weeks of age agrees with results reported by Ricordeau, Courrot, Pelletier & Thimonier (1976) who found a corresponding correlation of -0.04 in Romanov lambs. Some earlier reports on the effects of genetic groups on plasma LH used arbitrary threshold values for plasma LH to assign animals to different classes. Analyses were then performed on the distribution of animals among the classes. A similar approach in the present case with one threshold at 3 ng/ml led to the same overall conclusions about genetic group effects.

Large breed differences in body weight could also complicate the interpretation of breed differences in the plasma concentration of hormones. The fact that Fingalway lambs born to Galway ewes in Group I were approximately 40% heavier at 4 and 8 weeks of age (and therefore had 40% greater plasma volume) compared with lambs in the same group born to Finn ewes suggests that body size differences are unlikely to complicate the interpretation of breed, litter size and sex differences. The evidence that genetic differences may be a function of age (see Thimonier, 1975, p. 37; and the present work) and the absence of a correlation between LH levels at different ages indicates that LH is unlikely to prove a practical aid to selection for increased prolificacy. Testicular growth is dependent on LH and FSH (Courrot, 1970). This observation together with the reports of Land & Lee (1976) and Islam, Hill & Land (1976) that testis size is an effective selection criterion for increased ovulation rate in sheep and mice, respectively, would indicate that testicular growth rather than plasma LH would be a more useful aid to selection for increased prolificacy. However, the generality of this conclusion is questioned by the failure of successful selection for increased litter size in Galway sheep to be associated with any change in testis diameter up to approximately 17 weeks of age (Hanrahan & Quirke, 1977).

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