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Genetic relationship between prepuberal plasma FSH levels and reproductive performance in *Lacaune* ewe lambs

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Summary

In this study, we have estimated the phenotypic and genetic relationships between prepuberal plasma FSH level (FSH and logFSH) of ewe lambs of the *Lacaune meat* breed and their reproductive performance at first mating (fertility and prolificacy). Hormonal levels were assayed in a single blood sample collected at approximately 5 weeks of age (mean ± S.D. = 33.7 ± 3.2 days) from 829 ewe lambs born in July-September 1983, in 30 flocks, from 33 AI sires. Mating took place over two periods, in early breeding season at 11 months of age and in October-November at 15 months. Fertility analysis was conducted only on ewe lambs mated in June-July (n = 737), while prolificacy was analyzed for all pregnant ewe lambs (n = 732).

For plasma FSH and log plasma FSH the effects of birth type, blood sampling date and time, as well as age at blood sampling were not significant ($P > 0.05$). The flock and sire effects were highly significant ($P \leq 0.01$) and heritability was 0.44 and 0.50 for plasma FSH and log plasma FSH respectively. The phenotypic correlation between prolificacy and FSH levels was positive but non-significant, the genetic correlation was 0.35 for plasma FSH and 0.41 for log plasma FSH. Prepuberal FSH levels of ewe lambs which remained barren after their first mating early in the breeding season were significantly higher than that of pregnant ones ($P \leq 0.05$). Nevertheless the genetic correlation between fertility and logFSH was significantly positive ($P \leq 0.05$).

Key words : ewe, prepuberal plasma FSH, fertility, prolificacy, heritability, genetic correlation.

Résumé

Relation génétique entre la concentration de FSH plasmatique au stade impubère et les performances de reproduction des agnelles de race Lacaune viande

Cette étude estime les relations phénotypiques et génétiques entre le taux plasmatique impubère de FSH (et logFSH) des agnelles *Lacaune viande* et leurs premières performances de reproduction (fertilité et prolificité). Les niveaux hormonaux sont estimés à partir d'un seul échantillon de sang prélevé à environ 5 semaines d'âge (moyenne ± écart type = 33.7 ± 3.2 jours) sur 829 jeunes agnelles nées de juillet à septembre 1983, dans 30 élevages, et issues de 33 pères utilisés en insémination. La lutte a lieu en deux périodes, en début de saison sexuelle à 11 mois, et en octobre-novembre à 15 mois. L'analyse de la fertilité concerne uniquement les agnelles mises en lutte à la première période (n = 737), alors que l'analyse de la prolificité porte sur l'ensemble des agnelles fécondées (n = 732).

La mode de naissance, la date, l'heure, ainsi que l'âge au prélèvement n'ont pas d'effet significatif ($P > 0.05$) sur la concentration de FSH ou sa transformée logarithmique. Les effets troupeaux et pères sont très hautement significatifs ($P \leq 0.01$). L'héritabilité est de 0.44 pour FSH et 0.50 pour logFSH. La corrélation phénotypique entre prolificité et niveau FSH est positive mais non significative, les corrélations génétiques sont : 0.35 pour FSH et 0.41 pour logFSH. Le niveau plasmatique de FSH des agnelles vides après la première lutte de début de saison sexuelle est significativement supérieure à celui des agnelles fécondées à la même période ($P \leq 0.05$). Cependant, la corrélation génétique entre fertilité et logFSH est significativement positive ($P \leq 0.05$).

Mots clés : brebis, FSH plasmatique impubère, fertilité, prolificité, héritabilité, corrélation génétique.

I. Introduction

The observations of FINDLAY & BINDON (1976), BINDON *et al.* (1985) as well as indirect response to divergent selection of *Lacaune* sires on their breeding value for prolificacy (RICORDEAU *et al.*, 1984), suggest that plasma concentration of follicle stimulating hormone (FSH) in ewe lambs from 3 to 7 weeks of age could be a suitable early criterion for prolificacy selection. However, these results have been obtained on limited samples and have compared ewe lambs of different genotypes.

This study was conducted on commercial farms with the *Lacaune meat* breed, within the framework of its selection scheme for prolificacy in an accelerated lambing system. Only one sampling at 5 weeks of age was choosen as it has been previously shown that (1) FSH release in prepuberal ewe lambs is not pulsatile (BLANC & POIRIER ; personal observation) and that a high correlation was observed beetwen plasma FSH concentration at 5 weeks of age and that observed at 3 and 7 weeks and, (2) at 5 weeks of age the highest values were acheived for genetic variability and phenotypic correlation with ovulation rate (RICORDEAU *et al.*, 1984). This work follows our previous paper concerning the heritability of plasma FSH concentration at 5 weeks of age (BODIN *et al.*, 1986) and aims at assessing the genetic correlation between FSH level of ewe lambs and their reproductive performance at their first mating.

II. Materials and methods

A. *Animals and reproduction system*

Data in this paper were collected from 829 *Lacaune* ewe lambs born between July and September 1983, as the result of inseminations made from 33 sires in 30 flocks. These flocks are part of a *Lacaune meat* selection scheme aimed at increasing prolificacy and operating since 1975, through a sire progeny test. All sires used were young males on progeny test thus providing a suitable sample for estimation of genetic parameters. Each ram's progeny averaged 25 daughters spread among 7 different flocks, so that each flock had daughters of about 8 sires.

Ewe lambs were raised indoors and allowed to feed on their mothers' milk, except that artificial feeding was provided for some twins and triplets.

The breeding schedule was slightly different on the different farms but mating was always natural on unsynchronized cycles.

For 25 flocks, the ewe lambs (n = 737) were first mated in June-July 1984 when they averaged 11 months of age. Those non-pregnant (n = 242) were mated again in October-November of the same year at 15 months of age. At the end of this time, 68 ewes remained barren.

In 5 other flocks, the 92 ewe lambs available were first mated in October-November 1984 at 15 months of age, and 29 remained barren.

Altogether in the 30 flocks, 732 ewes were pregnant in June-July or October-November.

B. *Measurements and assays*

A single blood sample was collected from a jugular vein of each ewe lamb at about 5 weeks of age (mean value ± S.D. = 33.7 ± 3.2 days). In each flock sampling was made on the same day 5 weeks after birth of the first lamb. Sampling hour was classified in four groups : 8-10 ; 10-12 ; 14-16 and 16-18 hours. Blood samples (5 ml) were collected in heparinized vacutainer tubes (Becton Dickinson) and stored in ice for no longer than one hour until centrifugation. The plasma was frozen and stored at − 12 °C until FSH assay.

FSH concentration in each plasma sample was assayed according to Blanc & Poirier (1979) and expressed as ng FSH HG 225 per ml of plasma. This reference standard is immunologically equivalent to 14 times NIH-FSH-S3. Samples were measured in duplicate and in the same assay in order to decrease variability. Sample volume was 100 µl, which gave a B/Bo value of 50 % for 7.7 ng/ml, a figure close to the mean (B = cpm bound to antibody for a given sample ; Bo = cpm bound to antibody in absence of unlabelled hormone). For FSH concentrations of 13-17, 7-8 and 3.5-4.5 ng/ml the coefficient of variation (C.V.) were 10.1, 8.9 and 12.6 % respectively, estimated from 50 unknown samples in duplicate within each range. The lower limit of detection for the assay as calculated from B/Bo = 95 % was 0.5 ng/ml.

The variables analyzed were the plasma FSH concentration and its logarithmic transformation to compensate for the asymmetric distribution. For reproduction, we considered prolificacy, as defined by litter size (lambs born/ewes lambing) and fertility (ewes lambing/ewes mated) at the first or subsequent matings.

C. *Statistical analysis*

Initially, we estimated the extent of variation caused by several factors in hormonal variables (FSH and its logarithm transformation) and in fertility and litter size.

For FSH, the variance analysis was conducted for the effects of sample hour, flock within hour, age of lamb at sampling and date of sampling as fixed effects. This model included also sire effect in order to minimize the bias when estimating the other fixed effects.

For litter size, we have considered all the pregnant females in June-July and October-November, and analyzed variation attributable to flock, birth type and date, age at lambing and date of lambing as fixed effects, sire effects being also included in the model.

The same kind of analysis was made on fertility, but only the 737 ewe lambs mated in June-July were included : the others mated in October-November were considered to be a biased sample, since it was the second mating period for most of them.

Estimates of variance and covariance components have been obtained by the Henderson III method, considering only the significant effects as possible factors of variation. Variance of estimators (h^2 and r_G) has been estimated by the methods described by ROBERTSON (1959 *a, b*).

Plasma FSH levels of barren ewes or of those ewes giving birth to 1, 2, or 3 lambs have been assessed by the method of least squares, adjusting for the flock effect.

III. Results

A. *Fertility and litter size*

The fertility of females mated for the first time at 11 months of age was 67.2 % (495/737) in June-July and 71.9 % (174/242) in October-November at the second mating, so that 90.8 % of ewe lambs were pregnant at 11 or 15 months of age. Prolificacy at these two periods was respectively 1.50 and 1.57.

For ewes mated for the first time at 15 months, the fertility was 68.5 % and prolificacy 1.43.

Mean prolificacy for all the ewes was 1.51. Although litter size is a categorical trait (54 %, 41 %, and 5 % of single, double, treble or more lambings respectively), it was analyzed as a continuous trait. Birth type and date of birth did not have significant effects, so that the best model included only the flock, age at lambing and sire effects (table 1).

B. *FSH and prolificacy*

Plasma concentrations of FSH at 5 weeks of age in the 732 ewe lambs which subsequently lambed were highly variable (mean value 7.6 ng/ml, S.D. = 4.5 ng/ml) and the distribution of the data was asymmetric (varying from 0.9 to 43.2 ng/ml, mode = 3.8 ng/ml). Of the fixed effects examined only the flock effect was significant ($P \leq 0.01$).

Transformation to logarithms induced a better skewness and curtosis of residuals (Pearson b1 and b2 * were 10.6 and 18.0 for FSH and 0.30 and 4.6 for logFSH respectively). However, this transformation did not normalize the residual distribution.

* For a normal distribution b1 = 0 and b2 = 3.

Heritability was significantly positive for FSH (0.44, $P \leq 0.05$), for logFSH (0.50, $P \leq 0.01$) and for litter size (0.37, $P \leq 0.05$). The genetic correlation between FSH or logFSH and litter size was estimated to be 0.35 (s.e. = 0.24) and 0.41 (s.e. = 0.23) respectively (table 2).

The mean level of FSH and logFSH for females which had a single lamb were lower than that of females having two or more lambs, but these differences were not significant (table 3).

TABLE 1

Mean squares, variance components and heritabilities for the concentration at 5 weeks of age of plasma FSH, its logarithm and litter size

Variance components and heritabilities	d.f.	FSH	logFSH	Litter size
μ		7.58	1.91	1.51
F Flock	28	2.37 **	2.29 **	1.82 **
F Sire	32	3.32 **	3.70 *	2.94 **
F Lambing age	3	1.32 NS	1.64 NS	3.04 *
Total Variance	731	19.94	0.21	0.371
Residual Variance	668	17.22	0.18	0.32
R^2		13 %	15 %	14 %
Sire component σ_s^2		2.115	0.026	0.033
h^2		0.44	0.50	0.37
$\sigma(h^2)$		0.15	0.16	0.13

TABLE 2

Phenotypic and genetic correlations (with standard errors) between litter size and prepuberal hormonal criteria

r_G \ r_p	FSH	logFSH	Litter size
FSH		0.912	0.053
logFSH	0.999 (0.01)		0.074
Litter size	0.353 (0.24)	0.412 (0.23)	

TABLE 3

Least squares means (and standard errors) of plasma FSH and log plasma FSH level adjusted for flock and age at lambing, for ewes having one or more than one lamb

Litter size	FSH	logFSH
1	7.45 (0.16)	1.89 (0.016)
≥ 2	7.74 (0.19)	1.93 (0.019)

C. *FSH and fertility*

Of the 829 ewe lambs mated in June-July or October-November, 97 (i.e. 11.7 %) did not conceive and were classed as subfertile. The mean level of FSH and logFSH (mean = 8.89 ng/ml and 2.04 log ng/ml respectively) for these females was significantly higher ($P \leq 0.01$) than those becoming pregnant during one of the two mating periods (mean = 7.58 ng/ml and 1.91 log ng/ml).

For the data obtained from ewes mated for the first time in June-July, analysis of variance showed that only the flock effect was significant ($P \leq 0.01$) (table 4). The birth type had no effect on the fertility at first mating ; likewise there was no influence of the sire, considered as a fixed effect. Heritability of fertility was only 0.04 in this group.

TABLE 4

Mean squares, variance components and heritabilities for the concentration at 5 weeks of age of plasma FSH, its logarithm and fertility at first mating

Variance components and heritabilities	d.f.	FSH	logFSH	Fertility
μ		7.71	1.92	0.67
F Flock	24	2.54 **	2.80 **	3.16 **
F Sire	32	3.11 **	3.55 **	1.22 NS
Total Variance	736	22.25	0.22	0.22
Residual Variance	680	19.46	0.19	0.20
R^2		13 %	14 %	11 %
Sire component σ_s^2		2.13	0.025	0.022
h^2		0.40	0.47	0.044
$\sigma\,(h^2)$		0.14	0.15	0.06

TABLE 5

Phenotypic and genetic correlations (with standard errors) between fertility at first mating and prepuberal hormonal criteria

r_G \ r_P	FSH	logFSH	Fertility
FSH		0.917	− 0.083
logFSH	1.001 (0.01)		− 0.087
Fertility	0.146 (0.25)	0.429 (0.24)	

The phenotypic correlation between the FSH or logFSH level at 5 weeks of age and fertility at first mating was negative but small (− 0.08 and 0.10, table 5). The genetic correlation between these same variables was positive. It should be noted however that the relationship between FSH and fertility (r_G = 0.15, s.e. = 0.25) was lower than that between logFSH and fertility (r_G = 0.43, s.e. = 0.24) although this difference was not significant.

A negative phenotypic correlation between FSH and fertility was also apparent from the significant difference between the hormone levels of females which were pregnant or non-pregnant after the first mating at 11 months of age ($P \leq 0.05$, table 6).

TABLE 6

Least squares means (and standard errors) of plasma FSH and log plasma FSH level adjusted for flock, for pregnant and non-pregnant ewe lambs

Fertility	FSH	logFSH
Pregnant ewes	7.48 (0.13)	1.89 (0.012)
Non-pregnant ewes	8.18 (0.26)	1.97 (0.026)

IV. Discussion

Our estimate of heritability for prolificacy (h^2 = 0.37) is higher than earlier estimates for the *Lacaune milk* breed (h^2 = 0.03 ; BODIN, 1979) and higher than values in the literature reviewed by RICORDEAU *et al.* (1979). This is probably due to the system of selection for the *Lacaune meat* breed, which has favoured the selection of very good males without eliminating the poorest, causing higher sire variance.

The high value of the estimate for the heritability of plasma FSH at 5 weeks of age is partly explained by the independence of the concentration of FSH during the prepuberal period, with variation in such factors as age of the dam, birth type, body weight or growth rate of the individual. This independence has already been reported by several authors (RICORDEAU *et al.*, 1984 ; BINDON *et al.*, 1985 ; FERNANDEZ ABELLA, 1985). In a previous paper which concerned a slightly different sample of the ewe lambs analyzed here, we also reported an absence of effects associated with birth type and growth rate on prepuberal FSH plasma levels (BODIN *et al.*, 1986). The small influence of the environmental factors on plasma FSH levels has also been observed in experiments on food restriction in prepuberal male rats (SISK & BRONSON, 1986). In mature ewes the enhanced FSH plasma concentrations 3 to 5 days before oestrus claimed to be due to increased body weight in lupin supplemented ewes (BRIEN *et al.*, 1976), is apparently associated with lupin feeding *per se* rather than an indirect influence through body weight change (KNIGHT *et al.*, 1981).

The present paper reports for the first time the existence of a high genetic correlation between an early hormonal parameter and prolificacy. Some other results published on this topic are conflicting. Thus, lambs of a highly prolific genetic type had plasma FSH levels during the prepuberal period which were greater than those of the less prolific controls (FINDLAY & BINDON, 1976 ; RICORDEAU *et al.*, 1984 ; BINDON *et al.*,

1985). On the other hand, in two flocks of *New Zealand Romneys,* selected for prolificacy, the relationship between FSH during the prepuberal period and ovulation rate induced at 3.5 months of age was positive for one flock and negative for the other (BODIN, CLARKE, PETERSON, SMITH, personal observation). Finally, in strains of rats selected for their ovarian response to PMSG, a negative relationship was found between the number of follicles and the level of FSH during the prepuberal period (DE REVIERS & MAULÉON, 1979). However, these discrepancies observed between breeds cannot question the relevance of the FSH levels as an early criterion for selection within *Lacaune* breed. These contradictions no doubt reflect the fact that selection for prolificacy could operate by several physiological mechanisms. Thus, in the *Lacaune* breed, selection could perhaps be achieved through genes which control the function (activity or feed-back sensitivity) of gonadotroph cells in the pituitary rather than through those affecting the activity or sensitivity of the gonad.

The FSH concentrations observed in the present paper are lower than those found by FINDLAY & BINDON (1976) (group mean : 27-159 ng/ml). This discrepancy is probably accounted for by the references standard used, or by different slopes of the respective standard curves.

The use of the transformed variable can be justified by its distribution which suits better statistical analysis and because it gave higher heritability and genetic correlation with prolificacy than untransformed data. However, the distribution shape may have a particular biological meaning which is not yet understood ; this needs further investigation.

Subfertile ewes, which remained non-pregnant after their first mating period, had significantly higher FSH levels than those of pregnant ewes. Fertility at the beginning of the breeding season and at an age of 11 months implies not only a precocious sexual maturity but also an ability to breed outside the normal breeding season. These two characteristics most likely depend on the removal of inhibition to pituitary activity either by oestrogens (FOSTER & RYAN, 1979), or inhibin (RIVIER & VALE, 1987). Thus, the higher values of FSH observed in non-pregnant ewes might reflect a delay in the development of negative feed-back sensitivity by the pituitary. This relationship between prepuberal FSH plasma levels and fertility also requires further study.

Prepuberal FSH plasma levels could be used for selection of ewes before puberty. Nevertheless, a higher increase of genetic progress will come from the fact that sires could be classified by a progeny test at an early age (before the decision concerning ewe replacement must be made). For this early estimation of sire breeding value the total number of offsprings and therefore the number of sires on test will not be limited by the ewe replacement rate. Thus, the use of this early criterion should be optimized and incorporated together with other specific parameters in selection schemes for reproductive rate.

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