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SEASONAL VARIATION IN THE OESTROGEN INDUCED LH DISCHARGE OF OVARIECTOMIZED *FINNISH LANDRACE* AND *SCOTTISH BLACKFACE* EWES

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SUMMARY

The discharge of LH following the injection of 50 μg oestradiol benzoate was studied in 12 ovariectomized *Blackface* and 12 ovariectomized *Finn* ewes on 6 occasions (November, January, March, May, July and November).

Both breeds were more sensitive to the oestradiol during the breeding season than during anoestrus. The *Finn* ewes were less sensitive than the *Blackfaces* as judged by the proportion of ewes discharging LH, the maximum plasma concentration of LH attained, the interval to the discharge of LH, and the absolute reduction in LH concentration immediately following injection.

The failure of oestrogen to trigger the preovulatory discharge of LH may be one of the components of seasonal anoestrus and it is suggested that some of the variation in the ovulation rate of sheep may arise in part from differences in sensitivity to feed-back from developing follicles.

INTRODUCTION

The study of sources of variation in the reproductive performance of the sheep is complicated by the difficulty of separating the causes from the effects. This is particularly so in the case of the role of gonadotrophin secretion in the mediation of seasonal variation in the presence or absence of cyclicity, and in variation in ovulation rate within cycles. Differences in gonadotrophin secretion between cyclic and acyclic ewes could be the consequence of the presence or absence of cyclicity rather than the cause. Further, although THIMONIER and PELLETIER (1971) and LAND, PELLETIER, THIMONIER and MAULÉON (1973) observed phenotypic and genetic relationships respectively between the number of corpora lutea formed and the characteristics of the preovulatory discharge of luteinising hormone (LH), these

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relationships could have arisen from the development and ovulation of different numbers of follicles rather than be the expression of a basic difference in the characteristics of LH release which may be associated with the differences in ovulation rate.

In order to overcome these difficulties of interpretation, LH release was studied at different times of the year in ovariectomized females of the *Scottish Blackface* (*Blackface*), and the *Finnish Landrace* (*Finn*) breeds, where females naturally shed 1.3 and 2.9 eggs per cycle respectively (WHEELER and LAND, 1973). In addition to the difference in the number of follicles which normally develop and ovulate, the breeding season of the *Blackfaces* ends earlier than that of the *Finns*, so that although they both start at around the same time (October), they differ in duration (146 and 199 days) (WHEELER and LAND, 1973). In the absence of ovaries, variation in LH release would be independent of cyclicity and of the number of follicles developing or corpora lutea present, and therefore a direct effect of environment or genotype.

MATERIAL AND METHODS

Twelve ewes of each breed, which had been ovariectomized more than 12 months beforehand, were allocated to this experiment in summer 1972, and maintained throughout at the Animal Breeding Research Organisation, Dryden Field Laboratory, Roslin, Midlothian.

LH was measured before and after the intramuscular injection of 50 µg of oestradiol benzoate in 2 ml of oil at 11.00 hours GMT (PELLETIER and SIGNORET, 1970). Blood samples were taken by puncture of the jugular vein using heparinised evacuated tubes at 10.00 and 21.30 hours on the day of injection, and subsequently at 1½ hour intervals until 17.00 hours on the day after injection. Oestrus was determined by the introduction of vasectomized rams immediately after the second and subsequent blood collections, and ewes standing for mating with either of two rams were recorded to be in oestrus. Ewes which had not shown oestrus by the time of the last bleeding were tested morning and afternoon for a further two days. The ewes were housed from two days before to two days after injection.

Blood samples were immediately placed in iced water, centrifuged and frozen until assay. Plasma LH concentration was measured by double antibody radioimmunoassay (CARR and LAND, 1975), and expressed in terms of NIH-LH-S17.

All ewes were injected at the end of September, 1972, and subsequently at the end of every second month until November, 1973. They were all bled following injection in November, 1972, January, March, May, July and November, 1973, so that any progression from the breeding season to anoestrus could be followed, the final bleeding in November, 1973 enabling any circannual seasonal changes to be separated from any possible age or linear time changes.

Seasonal and breed variation was assessed in terms of the induction of a « preovulatory » LH discharge and the display of oestrus. The characteristics of the LH discharge were estimated by least squares analysis, when constants were fitted for the month when treatment took place, breed and individuals within breeds for each of the characters studied : *a*) initial plasma concentration, *b*) the reduction in concentration between that preceeding injection and the next collection, and *c*) the maximum concentration reached after injection.

RESULTS

1. — *The presence of a « preovulatory discharge »*

The number of ewes in which the plasma concentration of LH exceeded 20 ng/ml is given in table 1 for each of the two breeds. Taking this threshold of 20 ng/ml of plasma as indicative of a positive response, all ewes responded to the injection of

oestrogen in November of both years. During May, however, the concentration of LH exceeded 20 ng/ml in only 4 of the Finn ewes, and 8 of the *Blackfaces*, indicating that the response was reduced, and that the response of the *Blackfaces* was greater than that of the *Finns*. The effects of the season are also shown by a reduction to 10 in the number of *Finn* ewes where the concentration exceeded 20 ng/ml in January, March and July.

TABLE I

The effect of season on the positive feedback of an injection of 50 μg ODB on plasma LH levels in 12 Blackface (B) *and 12* Finn (F) *ovariectomized ewes*

	November		January		March		May		July		November	
	B	F	B	F	B	F	B	F	B	F	B	F
N° of ewes exceeding a plasma LH concentration of 20 ng/ml	12	12	12	10	12	10	8	4	12	10	12	12
N° showing oestrus	12	12	12	12	12	12	0	5	0	2	12	12
Plasma LH ng/ml												
a) before injection	8.2	6.2	9.6	7.2	11.0	8.5	10.3	9.3	14.6	10.7	11.2	9.2
b) mean maximum concentration	101	50	94	53	94	44	96	34	77	46	108	51

2. — *The characteristics of LH release*

a) *The concentration before injection* (*i.e.* at 10.00 hrs) is tabulated in table 1 for both breeds, and the fitted deviations from the overall mean in table 2. The *Blackfaces* exceeded the *Finns* ($P < 0.001$), and the differences between the months were statistically significant. The nature of any seasonal variation is not clear; but the initial concentration increased from the middle of the first breeding season through to anoestrus.

b) *The reduction in LH concentration* from an overall mean of 9.65 to an overall mean of 5.24 ng/ml, was greater in the *Blackfaces* than the *Finns* when measured on a linear scale ($P < 0.01$) but not when measured on a log scale. Although the absolute reduction was greater, the relative reduction was not, so that the concentration of plasma LH was only 0.9 ng/ml greater in the *Blackfaces* than the *Finns* after injection, whereas the mean difference was 2.34 ng/ml beforehand. As in the case of the initial concentration, the reduction differed from month to month ($P < 0.001$) and again, there is an indication that the reduction increased from the first breeding season through to anoestrus, indicating that the negative feed-back sensitivity to oestrogen was greater during anoestrus. The mean fitted reduction for the three sampling occasions during the breeding season (November, January and November) was 2.06 ng/ml less than the mean reduction for the three months of anoestrus (March, May and July). Compared to the overall mean reduction of 4.41 ng/ml, this represents a seasonal change of approximately 50 p. 100 of the mean.

c) *The maximum concentration recorded* was affected by both season and breed ($P < 0.001$), without any interaction between the two sources of variation. The *Blackfaces* responded more to the injection of oestrogen indicating their greater sensitivity to oestrogen than the Finns (table 2). Both breeds were less sensitive during anoestrus.

TABLE 2

Plasma LH concentrations (ng/ml) *of the* Finn *and* Blackface *ewes expressed as fitted linear deviations from the overall mean, together with the seasonal deviations for (a) initial concentration before injection, (b) the reduction in levels following injection, and (c) the maximum concentration observed.*

	(*a*) Initial	(*b*) Reduction	(*c*) Maximum
Overall mean	9.65	4.41	70.70
Blackface	1.17	0.72	24.3
Finn	— 1.17	— 0.72	— 24.3
November	— 2.48	— 2.28	4.75
January	— 1.28	— 1.30	3.02
March	0.12	— 0.27	— 1.60
May	0.11	0.41	— 5.84
July	2.95	2.95	— 9.33
November	0.58	0.49	9.00

d) *The interval between the injection of oestrogen and the release of LH.* All animals of both breeds released LH during the breeding season (November and January) as defined by plasma concentrations greater than 10 ng/ml. The crossing of this threshold was therefore used for the calculation of the time interval between the injection of oestrogen and the release of LH, for it enabled all ewes to be included on all occasions. This was 14.1 hours in the *Blackface* ewes, compared to 15.2 for the *Finns*. The statistical comparison of these means indicated that the difference was significant, indicating a shorter time lag and again greater sensitivity of *Blackface* compared to *Finn* ewes. There was no significant effect of month within the breeding season (F for 2 + 66 d.f. = 0.5), or of an interaction between breed and month (F for 2 + 66 d.f. = 1.4).

3. — *The display of oestrus*

The number of ewes of both breeds showing oestrus in response to the single injection of 50 μg oestradiol benzoate is given in table 1 for each occasion when the discharge of LH was studied. The most striking effect of the season is the reduction in the summer of animals showing oestrus in May and July, followed by a return to the full response in November. This reduction was more marked in the *Blackfaces*, where none of the ewes showed oestrus in either month, compared to the *Finns*, where 5 and 2 were observed in oestrus in May and July respectively.

The time from the injection of oestrogen to the onset of oestrus is given in table 3 for those months when all the ewes showed heat during the period of intensive blood sampling *i.e.* the months of November, 1972, and January and November, 1973. On these three occasions, the *Finn* ewes showed oestrus approximately 3 hours before the *Blackfaces*. Furthermore in March, when some ewes did not show oestrus until after the period of bleeding, eleven *Finn* ewes showed oestrus during the sampling period compared to only seven *Blackfaces*. In terms of oestrus therefore, the *Finns* are more sensitive to oestrogen than are the *Blackfaces*. When rams of both breeds were used for all tests after November, 1972, there was a tendency for the ewes to stand to rams of their own breed before they would stand to those of the other.

TABLE 3

The time (hrs) *from the injection of oestrogen to the mean time of onset of oestrus for ewes of both breeds when all showed oestrus during the period of intensive bleeding*

Breed of ewe (1)	*Blackface* (*BF*)		*Finn* (*F*)	
Breed of ram	*BF*	*F*	*BF*	*F*
November, 1972	—	20.93	—	16.75
January	18.00	18.13	15.12	15.37
November, 1973	20.87	21.00	19.37	17.25

(1) *Finn* < *BF* ; P < 0.05.

DISCUSSION

Two main conclusions can be drawn. Firstly, that ovariectomized ewes are less sensitive to the positive feed-back effects of oestrogen on the release of LH during the months of anoestrus than during the months of the breeding season. Secondly, ewes of the *Blackface* breed were more sensitive to oestrogen than those of the *Finnish Landrace* breed.

The observation of a progressive change in positive feed-back from season to season contrasted with the constant response observed by BECK and REEVES (1973) using intact ewes, but is compatible with the observation that the response of anoestrous intact ewes to the infusion of hypothalamic extracts is dependent upon the stage of anoestrus (DOMANSKI and KOCHMAN, 1968). The differences between these results may arise from differences in the breeds of sheep used, but the resumption of cyclic ovarian activity in the sheep used by BECK and REEVES (1973) may, as they point out, have masked an increase in sensitivity at the end of anoestrus.

The role of this change in sensitivity to positive feed back in the determination of the normal breeding season is not certain, for the natural breeding season is depen-

dent upon the integration of hypothalamic, pituitary and ovarian activity, and the design of the experiment deliberately excluded variation in the latter. It was only by excluding endogenous oestrogen production that it was possible to study and detect the present variation. Clearly the change in sensitivity to oestrogen is not the sole determinant of the duration of the breeding season, for it was the same in both breeds, whereas the end of the normal breeding season differs. It is possible therefore that endogenous oestrogen production is lower in the *Blackfaces*, and declines more rapidly as the breeding season progresses. Indeed the greater production of oestrogen by *Finn* ewes was inferred from the experiments of LAND, THOMSON and BAIRD (1972).

The lower sensitivity of the *Finn* ewes was marked by a longer interval between the injection of oestrogen and the release of LH, a lower maximum concentration observed in the plasma, and possibly also by a smaller reduction in the concentration of plasma LH before the discharge. This may enable them to tolerate higher concentrations of plasma oestrogen before the release of gonadotrophin is reduced. Breed differences in follicular development and ovulation rate would therefore be compatible with similar LH concentrations during the oestrous cycle, LAND *et al.* (1973). Such an inverse relationship between female prolificacy and the sensitivity of ovariectomized females to the feed-back control of LH release corresponds to the observation in males that testicular hypertrophy following hemicastration was found to be inversely related to the ovulation rates of their female relatives (LAND and CARR, 1975), when it was proposed that variation in sensitivity to feed-back from the gonads may be one of the sources of variation in prolificacy, or even the key to such variation.

By contrast to the lower sensitivity of LH release, the *Finn* ewes were more sensitive to oestrogen as measured by the display of oestrus, this and the occurrence of preferential mating within these two breeds confirms the earlier results of LAND *et al.* (1972). The seasonal variation in the occurrence of oestrus, was compatible with earlier observations, e.g. RAESIDE and MCDONALD, 1959. The males were observed to attempt to mount and in many cases to mount ewes which would not stand, so that even though male libido may have been reduced it was unlikely to be the cause of the observed seasonal variation. As in the case of the release of LH therefore, greater ovarian activity would be required to induce oestrus during anoestrus than during the breeding season. The comparison of seasonal changes in the display of oestrus with that in the release of LH indicates that the minimum LH response is in May, whereas the minimum display of oestrus was in July. This could contribute to the different order of events at the start and end of the breeding season.

The observation that the interval between oestrogen stimulation and the display of oestrus is shorter in the *Finn* than the *Blackface* ewes, but that the interval to the discharge of LH is greater in the *Finn* ewes suggests that both components may have contributed to the longer interval between onset of oestrus and the release of LH in the highly prolific *Romanov* and *Romanov* cross ewes (LAND *et al.*, 1973).

We would suggest that the present quantitative physiological study of genetic differences between breeds of sheep with differing natural ovulation rates indicates that one of the variables which determine the ovulation rate of sheep is the sensitivity of the hypothalamo-hypophyseal axis to ovarian steroids ; and further, that

one of the physiological components of acyclicity during seasonal anoestrus is the failure of the positive feed-back loop between follicular oestrogen and the ovulatory discharge of LH.

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RÉSUMÉ

VARIATIONS SAISONNIÈRES DE LA DÉCHARGE DE LH INDUITE PAR LES ŒSTROGÈNES CHEZ DES BREBIS CASTRÉES DE RACES *FINNOISE* ET *BLACKFACE*

La décharge de LH induite par l'injection de 50 µg de benzoate d'œstradiol a été étudiée 6 fois dans l'année (novembre, janvier, mars, mai, juillet et novembre) chez 12 brebis *Blackface* et 12 brebis *Finnoises* castrées.

Les brebis des deux races sont plus sensibles à l'œstradiol pendant la saison sexuelle que pendant l'anœstrus. Mais les brebis *Finnoises* sont moins sensibles que les *Blackface*, quel que soit le critère considéré : proportion de brebis présentant une décharge de LH, concentration maximale de LH observée, intervalle entre l'injection d'œstradiol et la décharge de LH et diminution absolue de la concentration de LH dans le plasma immédiatement après l'injection d'œstradiol.

Une des composantes de l'anœstrus saisonnier doit être l'incapacité des œtrogènes à induire une décharge de LH. La variation dans le taux d'ovulation chez la Brebis pourrait provenir de différences de sensibilité du système hypothalamo-hypophysaire à la rétroaction exercée par l'œstradiol sécrété par les follicules en croissance.

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