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## Seasonal changes in LH profiles of ewes selected for and against an early lambing date

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### ABSTRACT

Differences between breeds in seasonal breeding activity have been linked to seasonal shifts in both LH levels and the magnitude of oestradiol negative feedback. Studies were undertaken to determine the endocrine differences in ewes of the same breed with high or low breeding values (BVs) for date of lambing.

Forty-eight ewes (24 BV- early lambing and 24 BV+ later lambing) were selected from the flocks and 16 ewes (8 of each BV class) were ovariectomized (ovx) and implanted with silastic implants containing oestradiol-17 $\beta$ . All ewes were bled 2 x week for a 12 month period commencing in November to provide data on basal LH. The ovx ewes were subjected to rapid sampling (every 20 min for 24h) using automated sampling equipment, on 5 occasions approximately 1 month after each equinox and solstice in that year and samples were analysed for LH pulse frequency.

Basal LH levels showed a seasonal shift with the ovx ewes having a greater amplitude of change than the entire ewes. LH levels in the ovx ewes were lower in November to January, and higher in March, April, May and June; and lower again in August, September, October than in entire ewes. There was no evidence of a major difference in pattern due to BV. In the ovx ewes the values for the BV- animals tended to be lower than those for the BV+ ewes during March through July.

At the initial rapid bleed no animals showed pulse activity. In April the BV- group had more pulses per 24 h (6.0 v 2.8). In July there was no difference in proportion having pulses (8/8) nor in the pulse frequency (5.3 v 5.6/24 h). In October the BV- animals had a higher pulse frequency (2.8 v 1.0), while the proportion of animals (3/8) exhibiting pulses was the same. More BV- ewes had pulses in January (6 v 3) and of those with pulses the BV- group had higher frequency (7.7 v 4.7).

These data confirm the seasonal shifts in basal LH levels and in LH pulse frequency. Selection for earlier lambing had no effect on the pattern of basal levels. In the ovariectomized ewe the differences in LH pulse frequency due to BV detected in October and final bleed in January were not as marked as expected for the differences in the breeding activity of the two flocks but they confirm that differences in sensitivity to oestradiol 17 $\beta$  negative feedback are associated with differences in the onset of breeding season of ewes selected for earlier lambing within one breed. It would appear that selection for earlier lambing has disturbed the association between day length and LH pulse activity and the sensitivity of the pituitary to GnRH.

**Keywords:** LH; seasonal profile; selection; date of lambing; breeding value.

### INTRODUCTION

Differences between breeds in seasonal breeding activity has been linked to seasonal shifts in both LH levels and the magnitude of the oestradiol negative feedback (Legan and Karsch, 1979; Webster and Haresign, 1983; Thomas *et al.*, 1988). Thus the onset of cyclic activity in the entire ewe coincides with the drop in the negative oestradiol feedback on LH production and the increase of LH pulse activity in oestradiol implanted ovariectomised ewes of that breed. However, little data appears to be available on these relationships for lines of the same breed that have undergone divergent selection for onset of breeding season.

Flocks selected for and against an earlier date of lambing have been established and a relatively high heritability for this trait has been recorded (Smith *et al.*, 1993). These flocks have different onsets to the breeding seasons (Smith, unpublished data) but no information on their hormonal profiles is available.

This trial was conducted to determine if the seasonal pattern of LH profiles differed between ewes selected for or against an early date of lambing.

### MATERIALS AND METHODS

**Animals** Twenty-four ewes were selected from each of the two selection flocks (BV- early lambing and BV+ later lambing) on the basis of their breeding value (BV) for date of lambing (Smith *et al.*, 1992). Eight ewes from each of these subgroups were subjected to ovariectomy (ovx) and were implanted (subcutaneously) with silastic tubing containing oestradiol-17 $\beta$ .

**Bleeding schedule** All ewes (16 BV- entire, 16 BV+ entire, 8 BV- ovx and 8 BV+ ovx) were bled 2 x each week for a period of 12 months commencing in November 1992.

The 16 ovx ewes (8 BV- and 8 BV+) were also subjected to a series of rapid blood samplings. Ewes had intravenous cannular inserted into the jugular vein and were connected to computer controlled bleeding equipment. Samples were collected simultaneously from the 16 ewes every 20 minutes for a period of 24 hours (80 samples). This rapid sampling procedure was performed 6 times at approximately 1 month after each solstice and equinox.

**Hormone assay** Levels of oLH were measured by radioimmunoassay techniques modified from that described by Scaramuzzi *et al.* (1970). The material used for iodination

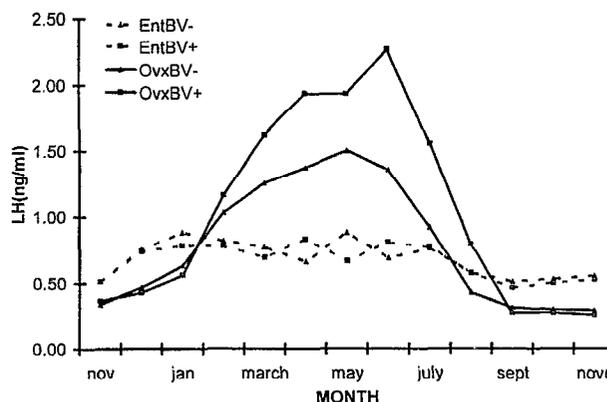
and reference standards was oLH-NIDDK-oLH-I-2, AFP7071B. The antisera was that described by McNatty *et al.* (1987) and a sheep anti rabbit (A727) was the second antibody.

**Statistical analyses** Twice weekly basal oLH data was subjected to separate analysis for each day of sampling to determine effects of BV class (- and +) and ewe type (entire vs ovx). In addition, response curves were obtained using the REML procedure in the Genstat 5.3 statistical package to fit sinusoidal regression components allowing for between ewe variation.

The oLH data from the rapid bleeding periods was subjected to pulsar analysis to define the pulse attributes for individual animals. These parameters were then analysed within and between the sampling periods to determine the effects of BV class.

All oLH data were subjected to log transformation prior to analysis.

**FIGURE 1:** Effect of ewe status (entire vs ovariectomised), BV group (- vs +) and season of the year on basal oLH levels. Values expressed are retransformed means (after log<sub>10</sub> transformation) for all samples (2 x week) collected over a month (4 weeks).



**TABLE 1:** Effect of ewe status (entire vs ovariectomised), season and BV class (- vs +) on basal levels of oLH. Values presented are means for all samples (2 x week) collected within a 4 week period (± sem) after log 10 transformation.

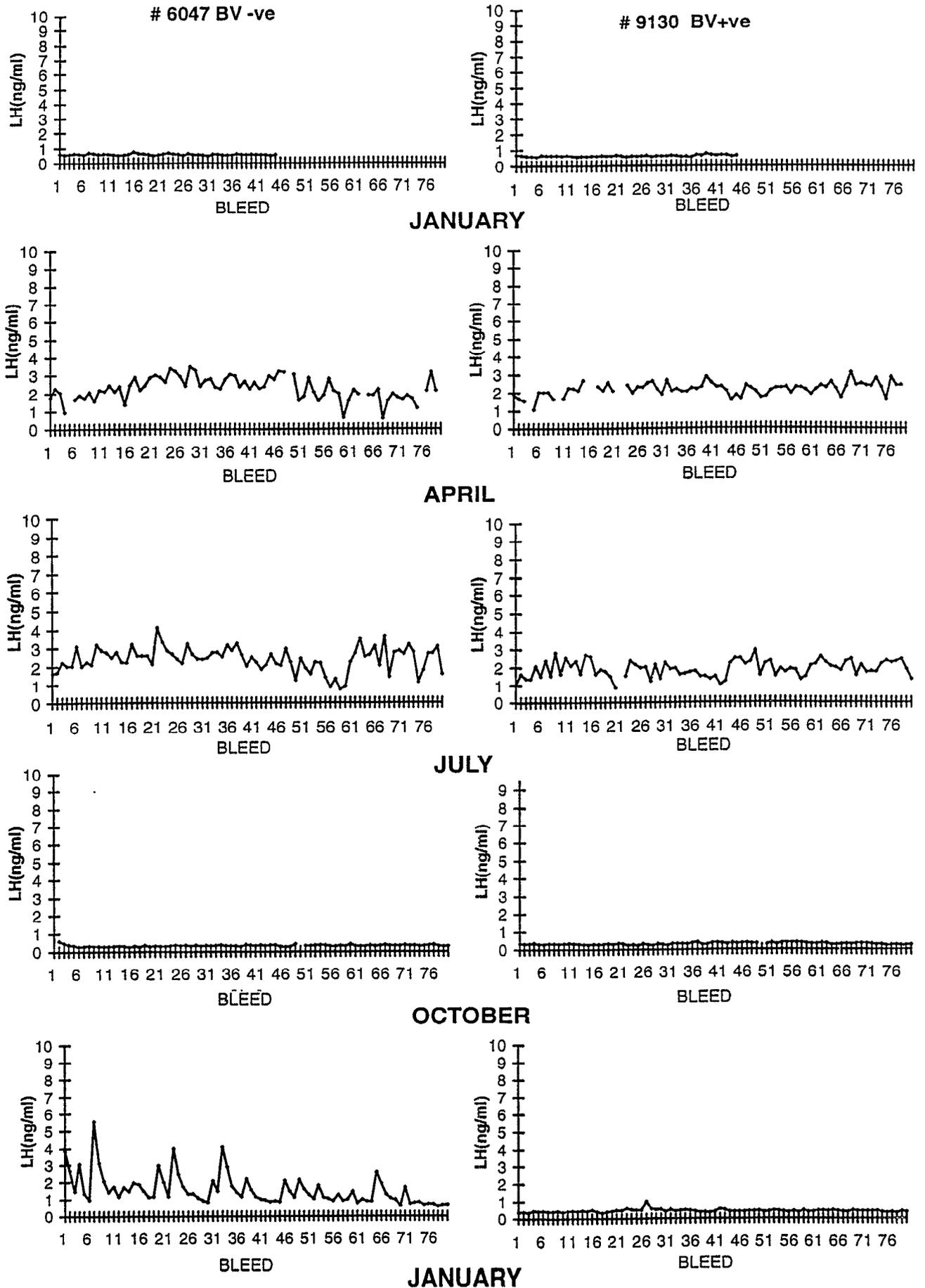
Ewe status	Entire			Ovariectomised		
Flock	BV-	BV+	Total	BV-	BV+	Total
month						
Nov.	-0.29 (0.04)	-0.29 (0.04)	-0.29 (0.04)	-0.47 (0.05)	-0.44 (0.06)	-0.46 (0.05)
Dec.	-0.12 (0.07)	-0.13 (0.05)	-0.13 (0.06)	-0.33 (0.07)	-0.37 (0.06)	-0.35 (0.07)
Jan.	-0.06 (0.07)	-0.11 (0.05)	-0.08 (0.06)	-0.20 (0.08)	-0.25 (0.06)	-0.23 (0.07)
Feb.	-0.09 (0.06)	-0.11 (0.05)	-0.10 (0.06)	0.01 (0.09)	0.07 (0.08)	0.05 (0.07)
March	-0.11 (0.09)	-0.16 (0.05)	-0.14 (0.07)	0.10 (0.08)	0.21 (0.07)	0.15 (0.07)
April	-0.18 (0.07)	-0.08 (0.08)	-0.13 (0.07)	0.14 (0.08)	0.29 (0.08)	0.21 (0.08)
May	-0.06 (0.12)	-0.18 (0.07)	-0.12 (0.09)	0.18 (0.06)	0.29 (0.08)	0.23 (0.07)
June	-0.16 (0.06)	-0.09 (0.08)	-0.18 (0.07)	0.13 (0.09)	0.35 (0.08)	0.24 (0.08)
July	-0.12 (0.09)	-0.11 (0.07)	-0.12 (0.08)	-0.04 (0.08)	0.19 (0.10)	0.08 (0.08)
August	-0.24 (0.06)	-0.24 (0.06)	-0.24 (0.06)	-0.37 (0.13)	-0.10 (0.11)	-0.24 (0.09)
Sept.	-0.29 (0.05)	-0.34 (0.04)	-0.31 (0.05)	-0.50 (0.12)	-0.57 (0.03)	-0.54 (0.08)
Oct.	-0.28 (0.05)	-0.30 (0.04)	-0.29 (0.04)	-0.52 (0.14)	-0.57 (0.04)	-0.55 (0.10)
Nov.	-0.26 (0.05)	-0.28 (0.03)	-0.27 (0.04)	-0.53 (0.11)	-0.60 (0.03)	-0.56 (0.07)

**TABLE 2:** Effect of breeding value (BV) and time of year on the LH pulse parameters of oestradiol. 17β implanted ovariectomised ewes. Values are means (± sem) determined from pulsar analysis.

Parameter	Flock	Date				
		Jan	April	July	Oct	Jan
Baseline						
mean (ng/ml)	BV-	0.58 (0.03)	1.90 (0.19)	1.15 (0.23)	0.55 (0.20)	0.87 (0.19)
	BV+	0.56 (0.02)	2.42 (0.48)	1.64 (0.30)	0.39 (0.28)	0.54 (0.07)
Overall mean						
(ng/ml)	BV-	0.59 (0.03)	2.00 (0.18)	1.22 (0.25)	0.50 (0.17)	1.02 (0.25)
	BV+	0.56 (0.02)	2.47 (0.50)	1.77 (0.36)	0.39 (0.03)	0.60 (0.12)
<sup>1</sup> Peak						
Amplitude	BV-	-	0.94 (0.09)	0.75 (0.12)	0.59 (0.22)	1.17 (0.23)
(ng/ml)	BV+	-	1.11 (0.27)	0.89 (0.22)	0.20 (0.01)	0.84 (0.38)
Peak Length	BV-	-	44.3 (8.2)	36.6 (3.6)	34.5 (11.4)	52.9 (11.2)
(min)	BV+	-	53.3 (13.3)	33.1 (4.3)	25.0 (5.0)	41.8 (10.9)
Number of	BV-	0	6.0 (2.3)	5.3 (0.7)	2.8 (0.9)	7.7 (2.5)
peaks	BV+	0	2.8 (0.9)	5.6 (1.6)	1.0 (0.0)	4.7 (2.7)
No. ewes	BV-	0	8	8	3	6
with pulses	BV+	0	6	8	3	3

<sup>1</sup> Peak amplitude is expressed as mean increment in peak height above the baseline mean.

**FIGURE 2:** Profiles of LH during a 24 hr intensive sampling period of two ewes (one BV- # 6047 and one BV+ # 9130) at each of the five sampling times chosen as being typical of their BV group. Values are in ng/ml.



## RESULTS

**Basal levels LH** levels from the twice weekly sampling showed a seasonal shift in values in both the entire and the ovx ewes (Table 1). Coefficients for the annual cycle were statistically significant for both fixed and random (ewe x component) effects, and differed on average between ovx and entire ewes (all tests  $p < 0.001$ ). The same pattern of results applied to the 6-monthly cycle at the  $p < 0.05$  level. In addition, coefficients for the annual cycle differed between BV+ and BV- groups for the ovx ewes but not for the entires ( $p < 0.01$ ). The seasonal pattern was more pronounced in the ovx ewes than in the entires (Fig. 1). Levels for the ovx were lower in the November to January period, then higher from February to July, and then lower again from August to November when compared to the entire ewes. There was no difference between the entire ewe BV groups in the pattern or levels of basal LH. In the ovx ewes the BV+ animals had higher basal levels in the March to July period than the BV- ewes.

**Pulse activity** The mean pulse values for each BV class are presented in Table 2. In the initial bleeding period all animals failed to exhibit pulsatile activity. Both the overall mean value and the base line mean (omitting peak values) showed a seasonal pattern with higher values ( $P < 0.05$ ) in the April and July sampling periods (breeding season) than at other times. During the breeding season the BV+ animals tended to be higher than the BV- while this was reversed in the non-breeding season ( $P < 0.10$ ). In the final January period the BV- ewes had significantly higher overall and baseline mean values ( $P < 0.05$ ). Peak amplitude and peak length tended to follow similar patterns although both amplitude and length were high in the final January period especially for the BV- ewes. The BV- ewes tended ( $P < 0.10$ ) to have more peaks than the BV+ ewes at all times except in July. There were more BV- ewes with pulses in the April and the final January period.

These data indicate an increased level of oLH secretion by the BV- ewes at an earlier stage in the breeding season. The seasonal shifts in LH pulse activity, one of each BV class for two individual ewes is illustrated in Figure 2.

## DISCUSSION

The data obtained in this trial confirms the seasonal (day length controlled) shifts in both basal LH levels and in LH pulse frequency reported previously (Webster and Haresign 1983, Thomas *et al.*, 1988). Selection for an earlier lambing date had no effect on the pattern of basal levels in the entire ewes despite an earlier onset of oestrus cycles in the BV -ve flock (Smith, unpublished data). A similar pattern appeared to exist for the ovx ewes although their seasonal differences were more marked than that of the entire ewes. This supports the claims of seasonal changes in sensitivity to oestradiol negative feedback on the secretion and release of LH and the dampening effects of progesterone during the breeding season.

Examination of the pulse data from the frequent sampling periods suggests that selection for an earlier lambing

date (BV-) has resulted in animals that have an earlier diminution of the inhibition (negative feedback) of oestradiol on LH release. In addition the findings that the increased pulsatile activity in the BV- ewes was accompanied by peaks with greater amplitude and pulse duration suggests that either there is greater release of GnRH or that the animals are more sensitive to GnRH. Similar findings of increased LH responses to GnRH have been reported for rams from this selection line (Brewer *et al.*, 1995).

Thus selection within a genotype (breed) for an earlier lambing date has resulted in an earlier onset to the breeding season and animals with a reduced sensitivity to day length controlled negative oestradiol feedback similar to that displayed by different breeds with different seasonal breeding patterns. The actual mechanisms involved still remain to be elucidated.

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