Effect of selection for early lambing performance on the seasonal patterns of gonadotrophin levels; response to GnRH and semen characteristics in adult rams

J.C. BREWER[1,2], R.M. BRIGGS[2], J. PARR[2], R.J. WILKINS[1], J.F. SMITH[2]

[1] Postgraduate school of Animal Biology, University of Waikato, Hamilton.

ABSTRACT

Little research has been done on within breed differences in seasonality of reproduction in rams. We took 16 Dorset x Romney rams (8 BV+ and 8 BV-, selected on the basis of their breeding value for date of lambing derived for the lambing performance of their female relatives). These rams were housed indoors under natural light conditions and fed a ration of pellets and hay. Semen samples, testis diameter and body weight measurements were taken weekly. Blood samples were taken monthly (every 20mins for 26hrs, and then every 10mins for a further 4hrs following a 1ml IV injection of 0.001 mg GnRH). Samples were assayed for oLH and oFSH. The trial ran from September 1993 to August 1994. More BV- (early lambing group) rams (70% vs 25%) produced ejaculates in the first 3 months of the trial. The BV- rams had significantly ($P<0.01$) lower ejaculate volumes than the BV+ rams with both showing significant ($P<0.001$) seasonal changes. There were significant ($P<0.01$) seasonal effects on semen concentration with a January maximum of 6300×10^6 sperm/ml and a minimum in July of 3350×10^6 sperm/ml. There was a seasonal effect on testis diameter (55mm in Dec-Feb and 38mm in July). There was a significant ($P< 0.05$) seasonal shift in live weight between Feb-Apr (70kg) and July (58kg). There was a significant ($P<0.001$) seasonal effect on basal LH levels and LH pulse frequency, but there were no detectable BV effects. The BV- rams had greater pulse amplitude and longer durations of peaks than did the BV+ animals. LH response to GnRH was significantly different only in Nov (BV- rams showed greater area under the curve 162±35 vs 122±28 units). The BV- rams showed a seasonal effect with a rise in Nov and a decline Feb ($P<0.05$) while the BV+ rams showed little seasonal change. There was a significant ($P<0.01$) seasonal shift in FSH levels from a low of 2.3 ng/ml in Sept to 40.0 ng/ml in Feb then a decline to 4.3 ng/ml in April but no effect of BV group.

These results suggest that animals selected for early lambing may be more sensitive to GnRH prior to the summer solstice.

Keywords: Out-of-season lambing; ram seasonality; reproductive hormones.

INTRODUCTION

The major stumbling block in the year round production of lambs is that sheep are intrinsically seasonal breeding animals.

One of the dominant signals controlling seasonal behaviour is that of changing photoperiod (Lincoln, 1979) and it is difficult to overcome this control mechanism in order to allow all year round breeding. The most effective way has been through various hormone treatment programs. These are however both costly and time consuming (Andrews and Taylor, 1986; Smith *et al.*, 1989), thus reducing economic viability. It would therefore be desirable to develop a variety of sheep that would breed naturally for most of, if not all, the year without the need for external manipulation.

With different breeds of sheep expressing different levels of seasonality (Wheeler and Land, 1977), it appears there is a strong genetic component involved in the degree to which sheep exhibit seasonal behaviour (Williams and Heliwell, 1993).

The aim of this study was to look at both hormonal, and physical characteristics in rams from flocks selected for and against an early lambing date to determine if there were differences in the seasonal patterns resulting from selection. Differences in control mechanisms may provide an indication to an easily measured physiological marker in rams that could allow the prediction of the time of lambing in their daughters. This marker could then be used to apply selection pressure upon the rams as well as upon the ewes, and thus accelerating the rate of genetic change towards a less pronounced seasonal pattern of breeding activity.

MATERIALS AND METHODS

Animals

Sixteen Dorset x Romney interbred rams born in either 1990 or 1991 from the Ruakura out-of-season breeding selection flocks were used in this trial. They were selected on the basis of their breeding value (BV) for date of lambing (8 BV- early lambing and 8 BV+ late lambing) as described by Smith *et al.* (1992).

Animal management

The rams were housed under cover in natural light conditions in individual pens (1m x 2m) at the Ruakura Research Centre, Hamilton (latitude 37° 46' S, longitude 175° 20' E, elevation 40 m). Daily feed comprised a standard ration of 800g meadow hay and 800g sheep nuts (NRM Ltd, Mt Maunganui). A standard diet was used to remove seasonal fluctuations in feed quality and quantity as a factor influencing seasonal changes. Water was available ad lib. The experiment was conducted between the dates of 1 September 1993 and 31 August 1994.

[2] AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton.

Data collection

Body weight (using a walk on weigh crate and electronic scales) and scrotal or testis diameter (using a set of electronic callipers) were measured on a weekly basis.

A semen sample was collected on a weekly basis using an artificial vagina. The density of the raw semen was obtained using a spectrophotometer and a standardised semen concentration curve was used to estimate the number of spermatozoa per ml. Ejaculate volume was also noted.

Intensive blood sampling was undertaken on a monthly basis. On day 1, indwelling intravenous catheters were inserted into the jugular vein under mild general anaesthetic (Rompun 0.1ml/10kg body weight). Following surgery the rams were connected to a computer controlled blood sampling system. A 2 minute pulse infusion of 0.5% Heparin in Saline solution (6mls) was given every half hour for the next 16 hours (overnight). Intensive sampling started between the hours of 8:00 and 9:00am on day 2 and continued for 26hrs. A 5ml blood sample was collected every 20 minutes and immediately cooled to 4°C. These samples were then centrifuged at 2000rpm for 20 minutes at 4°C. Plasma was harvested and frozen for subsequent assay. At the completion of this 26hr sampling period the rams were given a 1ml intravenous injection of 1μgml^{-1} GnRH (Peninsula Laboratories Ltd, code 7201). Sampling was then re-initiated (5mls every 10 minutes) for a further 4 hours with the plasma being processed and frozen as above.

Assays

Both oLH and oFSH were measured using established RIA techniques (Scaramuzzi *et al.*, 1970; McNatty *et al.*, 1987). All samples from the initial 26hr sampling period (80 samples per ram per month) were assayed for oLH. Fourteen samples per ram per month (one sample for every 2hr of the sampling period) were assayed for oFSH. All the samples collected after the GnRH challenge (25 samples per ram per month) were also assayed for oLH.

The within and between assay coefficient of variation for the oLH pulse samples were (8.6% and 11.2%) for the (1.0 ng/ml) control samples. For the oLH response to GnRH using 4.0 ng/ml control samples the values were 8.2% (within assay) and 14.6% (between assay). The respective values for the oFSH assay using a 4.0 ng/ml control were 8.9% and 11.7%.

Statistical analysis

The oLH pulse data was analysed using the "Pulsar" programme and the following parameters were derived for each ram in each month: pulse frequency, pulse amplitude, pulse duration, interpulse interval and basal level. These variates and the data on semen characteristics, testis diameter, oFSH and the total oLH response to GnRH were subjected to restricted maximum likelihood (REML) procedures to fit sine and cosine curves to determine annual seasonal patterns and differences between the BV groups in that pattern (Genstat v 5.3 statistical package - Rothamsted Experimental Station). Year of birth of the rams was included as a factor in the analyses but as there were no interactions with BV or season effects it is not presented as a factor in the results.

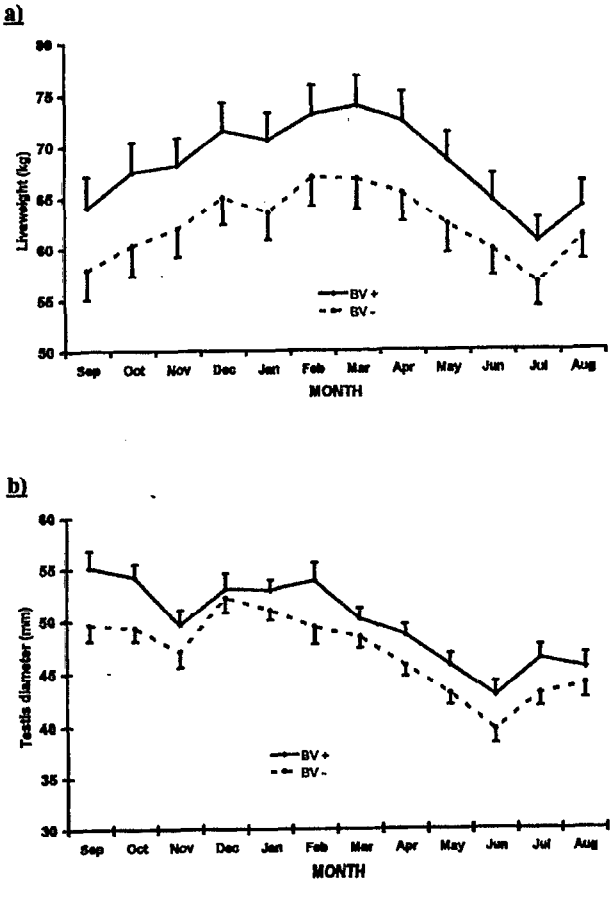
RESULTS

There was a significant ($P<0.01$) seasonal effect on live weight with the rams of both BV groups showing a rise to a maximum of 71kgs in February and then a decline to 58 kgs in July (Figure 1a). Testis diameter also showed a significant ($P<0.01$) seasonal pattern with both BV groups reaching a maximum (53mm) in December, and declining through to June (Figure 1b). There was a significant ($P<0.01$) seasonal effect on semen concentration with a maximum in January ($6300x10^6$ sperm/ml) and a low in July ($3350x10^6$ sperm/ml), however there was no BV group interactions (Figure 2a). Semen volume exhibited significant BV group ($P<0.01$) and seasonal effects ($P<0.001$) with the BV+ rams being consistently higher than the BV- rams (Figure 2b). Both BV group rams reached a high in April (1.00±0.21 and 0.97±0.74 respectively) from the preceeding low in December (0.51±0.28 and 0.55±0.17).

Table 1 presents the seasonal and BV group means for the LH pulse parameters, LH response to GnRH and oFSH levels.

There was a significant ($P< 0.01$) seasonal effect on average basal LH levels with a rise to maximum in November and drop off subsequent to that, but no detectable BV group effects. No significant difference in LH pulse frequency was observed between the two BV groups but again there was a strong ($P<0.001$) seasonal effect. On average the BV- rams had significantly ($P<0.001$) longer LH peak duration than the BV+ rams. Significant BV group ($P<0.05$) and season

FIGURE 1: Effect of season on the ram values for (a) ram liveweight and (b) testis diameter for the two BV groups. Values are means (± sem for 32 individual observations per BV groups per month.

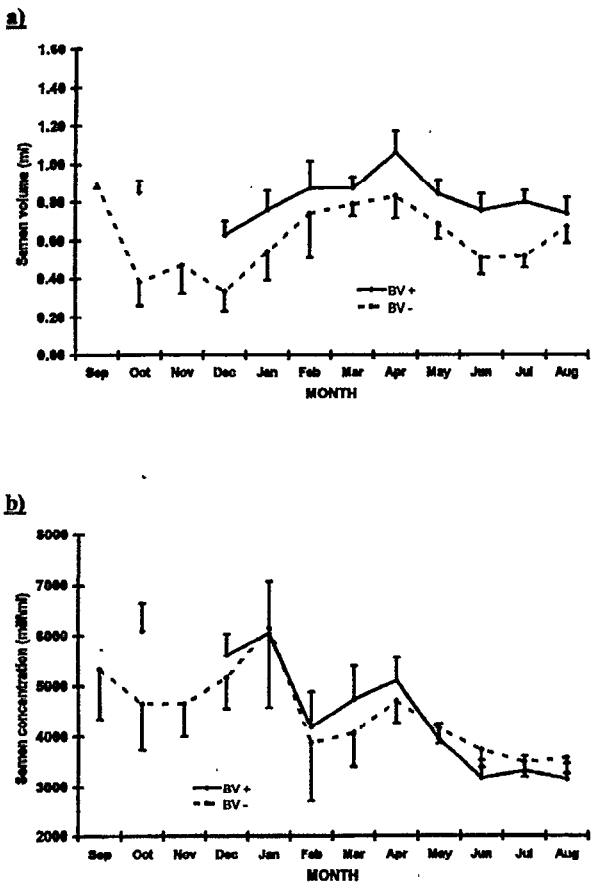


(P<0.001) effects on LH peak amplitude was seen with the BV+ rams showing a much more uniform rise to a maximum (4.00ng/ml) in November and a subsequent decline through to April. The BV- rams showed a more erratic pattern of rise and fall and finished at a higher amplitude (1.07ng/ml vs 0.40ng/ml) in April.

A significant (P<0.01) seasonal pattern was observed in the LH response to a GnRH challenge as well as an interaction between season and BV group (P<0.05). Both groups had approximately the same value at the beginning of the trial (114 units). The BV- rams rose to a maximal response (162 units) in November whereas the BV+ rams remained static. In December the BV- rams fell slightly and in January both groups were similar and this trend continued until April.

Plasma levels of FSH showed a significant (P<0.05) seasonal cycle with a low (2.40 and 2.24ng/ml for BV- and BV+ rams) in September and a peak (36.3ng/ml and 42.6ng/ml for the BV- and BV+ rams respectively) in February. Although the FSH levels for the BV+ rams were consistently higher throughout the trial there was no significant difference between the two BV groups.

FIGURE 2: Effect of season and BV group on (a) the value of ejaculate and (b) semen concentration. Values are means (± sem for 32 individual observations per BV groups per month.



DISCUSSION

The results described confirm previous reports on the general seasonal patterns of gonadotrophin secretion, testis diameter and semen characteristics of rams (Xu *et al.* 1991). It would seem that the rams of the two selection (BV) lines are reacting the same way to changing photoperiod. However, it is interesting to note that selection for date of lambing (BV group) has not shown any of the differences that were seen in studies of the two individual breeds (Xu *et al.* 1991) that contribute to the genotype under selection.

One effect that has been brought to light is the possible consequences of selection on pituitary responsiveness to GnRH where the BV- rams appear more sensitive in November prior to the summer solistice. This narrow window of increased sensitivity is in agreement with the findings of Lincoln and Short (1980) and Sanford *et al.* (1984). As there

TABLE 1: Effect of month of year and BV group on the attributes of the oLH pulses, the oLH response to GnRH and levels of oFSH. Values are means (±sem)

Month		Amplitude (ng/ml)[2]	Duration (minutes)[2]	Basal level (ng/ml)[2]	Peak numbers[2]	GnRH response[1,3]	oFSH ng/ml[4]
Sep	BV+	1.02 (.54)	43.27 (9.28)	0.46 (.084)	3.05 (1.03)	115.9(13.7)	2.24 (1.11)
	BV-	3.90 (.51)	77.37 (9.12)	0.66 (.079)	3.00 (.97)	113.7(12.7)	2.46 (1.07)
Oct	BV+	2.23 (.51)	86.93 (9.12)	0.66 (.079)	5.00 (.97)	123.2(18.3)	3.09 (1.15)
	BV-	2.25 (.51)	85.88 (9.12)	0.67 (.079)	4.75 (.97)	135.1(16.5)	2.95 (1.10)
Nov	BV+	4.00 (.51)	91.10 (9.12)	0.92 (.079)	5.38 (.97)	111.7(27.9)	3.80 (1.29)
	BV-	2.70 (.51)	90.34 (9.12)	0.93 (.079)	6.38 (.97)	162.0 (35.9)	4.07 (1.38)
Dec	BV+	3.70 (.51)	90.46 (9.12)	0.69 (.079)	3.88 (.97)	118.2 (23.4)	6.46 (1.29)
	BV-	3.23 (.51)	107.08 (9.12)	0.80 (.079)	4.75 (.97)	143.1 (39.3)	6.17 (1.48)
Jan	BV+	1.83 (.51)	80.08 (9.12)	0.78 (.079)	4.25 (.97)	114.8 (31.1)	15.49 (1.41)
	BV-	2.41 (.51)	99.04 (9.12)	0.77 (.097)	3.63 (.97)	101.8 (14.2)	12.59 (1.70)
Feb	BV+	1.05 (.51)	74.43 (9.12)	0.64 (.079)	8.13 (.97)	105.2 (15.0)	42.66 (1.55)
	BV-	1.20 (.51)	69.74 (9.12)	0.65 (.079)	9.87 (.97)	105.7 (11.6)	36.31 (2.04)
Mar	BV+	0.64 (.51)	53.93 (9.12)	0.59 (.079)	9.37 (.97)	63.5 (9.1)	10.23 (1.29)
	BV-	1.13 (.51)	79.17 (9.12)	0.60 (.079)	6.00 (.97)	66.6 (6.8)	6.61 (1.15)
April	BV+	0.40 (.54)	38.14 (9.73)	0.61 (.084)	4.99 (1.03)	69.1 (12.4)	5.25 (1.23)
	BV-	1.07 (.54)	57.74 (9.73)	0.63 (.084)	3.80 (1.03)	83.2 (9.4)	3.31 (1.20)

[1] Calculated as the area under the oLH response curve to injection of GnRH.
[2] Values are means from 632 individual LH measurements per BV group per month.
[3] Values are means of 200 individual samples per BV group per month.
[4] Values are means of 104 individual samples per BV group per month.

were no detectable differences between the BV groups in the frequency of LH pulses this indicates that GnRH pulse frequency is not different between the two BV groups. When looking at the LH response to a GnRH challenge it can be seen that, at a time when daylength is expected to inhibit gonadotrophin secretion (in November) the BV- rams are at their maximum. This would thus suggest that the pituitary responsiveness to GnRH has been enhanced. This data is supported by similar findings with oestradiol implanted ovariectomised ewes from the same selection lines (Smith *et al.*, 1995). Their results showed that the BV- ewes had a higher LH pulse frequency in January and that this was associated with greater pulse amplitude and longer pulse duration. This indicates both increased frequency of GnRH release and a greater LH response to that GnRH release.

In conclusion these results show that there is potential for an identifiable marker in the LH response to GnRH. The mechanism and repeatability of these differences however need further investigation before any useful application can be made.

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