Endocrine and Genetic Control of Seasonal Breeding in Sheep

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PREFACE

The contents of this thesis represent original work conducted by the author under the supervision of Associate Professor M.F. McDonald, Professor S.N. McCutcheon and Dr. H.T. Blair, Department of Animal Science, Massey University.

Publications arising from this thesis are as follows:

1. Harris, P.M., Xu, Z.Z., Blair, H.T., Dellow, D.W., McCutcheon, S.N. & Cockrem, J., 1989. The effect of exogenous melatonin, administered in summer, on wool growth and testis diameter of Romneys. *Proceedings of the New Zealand Society of Animal Production*, **49**: 35-38. [Based in part on Chapter III]

2. Xu, Z.Z., McDonald, M.F., McCutcheon, S.N. & Blair, H.T., 1991. Seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH in rams of two breeds differing in time of onset of the breeding season. *Animal Reproduction Science*, (In press). [Based on Chapter IV]

3. Xu, Z.Z., McDonald, M.F., McCutcheon, S.N. & Blair, H.T., 1991. Effects of season and testosterone treatment on gonadotrophin secretion and pituitary responsiveness to gonadotrophin releasing hormone (GnRH) in castrated Romney and Poll Dorset rams. *Journal of Reproduction and Fertility*, (In press). [Based on Chapter VII]

ABSTRACT

Xu, Z.Z., 1991. *Endocrine and Genetic Control of Seasonal Breeding in Sheep*. PhD thesis, Massey University, Palmerston North, New Zealand. 117pp.

A series of related experiments were conducted to investigate genetic variation in the pattern of seasonal changes in testis size, gonadotrophin secretion and pituitary responsiveness to an intravenous gonadotrophin releasing hormone (GnRH) challenge in rams during the transitional period from the nonbreeding to the breeding season, with the objective of identifying potential genetic markers in the ram for date of onset of the breeding season in the ewe.

Because the accurate measurement of changes in testis diameter was fundamental to the assessment of seasonality in rams used in the research programme, two preliminary trials were conducted to measure the reliability of this technique. In the first trial, testis diameters of 18 Romney rams were measured twice 1 h apart using a pair of dial calipers. Repeatability of measurements obtained from this trial was 0.90±0.05 (mean±SEM). In the second trial, testis diameters of 24 Southdown rams were measured before and after the animals were slaughtered. Pearson correlation coefficients between the live measurements and the diameter and weight of the dissected testes were 0.91 and 0.90 respectively ($P < 0.001$). An opportunity also arose to investigate the effects of continuous melatonin treatment for five weeks in early summer on testis growth in rams from the Massey University fleeceweight-selected (FW) and control (C) lines. The melatonin treatment significantly ($P < 0.001$) altered the pattern of seasonal variation in testis size. Compared with that of the untreated animals, testis growth of the melatonin-treated animals was significantly stimulated during the treatment period, but significantly depressed after termination of the treatment. The testis diameter of FW rams was significantly greater than that of C rams as the breeding season approached. There were indications that the FW rams were less responsive to melatonin treatment than the C rams. This trial also confirmed that seasonal changes in testis diameter could be detected using caliper measurements made on the live animals.

In the first major experiment, differences between rams (aged 2 years) of breeds with short (Romney) and long (Poll Dorset) breeding seasons in the pattern of seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to an exogenous GnRH challenge were compared during the transitional period from the

nonbreeding to the breeding season. Testis size of rams of both breeds varied significantly during the trial period, with a significant breed difference in the timing and magnitude of this variation. Increases in testis size occurred earlier, but the magnitude of seasonal variation was smaller, in Poll Dorset rams. Overall, mean LH concentration was higher ($P < 0.05$) in Romney rams due mainly to a difference ($P < 0.001$) in the frequency of LH pulses. There was also an effect of sampling time ($P < 0.01$) and a significant ($P < 0.05$) breed x time interaction in LH pulse frequency. Mean FSH concentrations exhibited significant ($P < 0.01$) variation with sampling time and the increase in FSH concentrations occurred earlier ($P < 0.10$) in Poll Dorset rams. There was an effect of sampling time on both the peak ($P < 0.01$) and the total ($P < 0.05$) LH responses to the GnRH challenge, but no significant effects of breed or breed x time interactions were detected.

The between-breed differences in the pattern of seasonal variation in gonadotrophin secretion were shown to be due to breed differences in sensitivity to both the steroid-dependent and steroid-independent effects of season. In castrated Romney and Poll Dorset rams, depression by testosterone treatment of LH pulse frequency, basal and mean LH concentrations, mean FSH concentration and peak and total LH responses to exogenous GnRH was greater ($P < 0.01$) during the nonbreeding season than during the breeding season. Poll Dorset rams were less sensitive to testosterone treatment than Romney rams ($P < 0.05$). In rams not receiving testosterone treatment, LH pulse frequency was lower ($P < 0.05$) during the nonbreeding season than during the breeding season in the Romneys (15.8±0.9 vs. 12.0±0.4 pulses/8h), but not in the Poll Dorsets (13.6±1.2 vs. 12.8±0.8 pulses/8h).

In another experiment, the magnitude of differences between rams (aged 4 years) of the Romney breed in the pattern of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH was studied. There were marked differences between Romney rams in the pattern of seasonal variation in testis size. Rams which had an early increase in testis size prior to the onset of the breeding season (the "early" group) had a greater magnitude of seasonal variation in testis size than those which had a late increase in testis size (the "late" group). The pattern of seasonal changes in testis diameter appeared to be repeatable from year to year. Differences between the groups in the pattern of seasonal variation in testis size were associated with group differences in endocrine function. Thus rams in the early group had a higher LH pulse frequency in March than those in the late group (4.4±0.4 vs. 1.7±0.3 pulses/8h, $P < 0.01$) and the seasonal increase in plasma FSH concentrations occurred earlier in the early group than in the late group. There were group differences in the pattern of seasonal variation in total LH response to the GnRH challenge.

The potential usefulness of testicular and endocrine parameters as predictors of genetic merit for date of onset of the breeding season was investigated in two progeny tests involving rams which had previously been studied for these parameters. The first progeny test involved rams of the Romney and Poll Dorset breeds. Results from this trial showed that, compared with straightbred Romney hoggets, a higher proportion of Poll Dorset cross hoggets reached puberty during the first breeding season (79.6 vs. 59.9%, $P < 0.05$). Poll Dorset cross hoggets also reached puberty earlier (13 May±3 vs. 22 May±3, $P < 0.10$) and at a younger age (264±3 vs. 276±3 days, $P < 0.10$) and had more oestrous cycles (2.7±0.2 vs. 2.0±0.2, $P < 0.05$) than Romney hoggets. Sire within breed had a significant ($P < 0.05$) effect on the number of pubertal oestrous cycles but not on the date of, or age at, onset of puberty. Liveweight at the beginning of the breeding season influenced both the proportion of hoggets reaching puberty during the first breeding season ($P < 0.01$) and the number of pubertal oestrous cycles ($P < 0.05$). There were significant effects of breed ($P < 0.001$) and sire within breed ($P < 0.05$) on the date of onset of the second breeding season. In the second progeny test, only rams of the Romney breed were studied. There was a significant ($P < 0.05$) sire effect on date of onset of the second breeding season but not on any of the measured pubertal oestrous parameters. While differences between Romney and Poll Dorset rams in the pattern of seasonal variation in testis size and gonadotrophin secretion were associated with breed differences in pubertal oestrous activity and date of onset of the breeding season, the within-breed correlations between the testicular and endocrine parameters in the rams and date of onset of the breeding season in their female offspring were low.

In conclusion, the present study has identified several physiological and endocrinological parameters in rams that might potentially be used as predictors of genetic merit for date of onset of the breeding season in ewes. Further, and more extensive, studies designed specifically to establish the genetic correlations between these potential parameters and date of onset of the breeding season are needed before these parameters can be incorporated into selection programmes. Finally, this study has also demonstrated that seasonality of rams is regulated by steroid-dependent and steroid-independent mechanisms similar to those operating in the ewe.

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LIST OF ABBREVIATIONS

The following abbreviations have been used in the text without prior definition:

Units:

°C	degree Celcius
h	hour
kg	kilogram
min	minute
mm	millimetre
ml	millilitre
ng	nanogram
μ	micron
μg	microgram
μl	microlitre

Hormones:

bTSH	bovine thyroid stimulating hormone
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
LH	luteinizing hormone
oFSH	ovine follicle stimulating hormone
oGH	ovine growth hormone
oLH	ovine luteinizing hormone
oPRL	ovine prolactin

Statistical:

SEM	standard error of the mean
NS	non-significant
+	$P < 0.10$
*	$P < 0.05$
**	$P < 0.01$
***	$P < 0.001$

C H A P T E R I:

INTRODUCTION

CHAPTER I:

INTRODUCTION

1. The benefits of producing lambs out of season

In sheep, the seasonal nature of breeding activity imposes a major limitation to increasing the efficiency of well-managed sheep production systems. While seasonal breeding can be regarded as an evolutionary adaptation to ensure the birth and development of the newly born lambs at the most favourable time of the year, modern husbandry techniques have made it possible to at least partially overcome seasonal problems such as inclement weather and feed shortage. Although the marked seasonality of pasture growth will dictate, to some extent, the seasonal nature of lamb production in countries like New Zealand where sheep production is based on pasture, the ability to produce lambs at any time of the year would still be a decided advantage to sheep farmers in many parts of the country (Andrewes & Taylor, 1986). As a result, there exists considerable interest in increasing the breeding frequency of sheep flocks and/or modifying the time of lambing (Ricordeau, 1982; Andrewes & Taylor, 1986).

The practice of out-of-season lambing offers many advantages to both the meat industry and the individual farmer. From an industry point of view, out-of-season lambing allows more efficient use of the expensive capital installations by spreading the lamb killing season. This will lead to increased returns to farmers because the largest internal cost to affect sheep farmers is that for killing and processing (Taylor, 1982). From a marketing perspective, expansion of the world markets that require a constant supply of chilled lamb throughout the year also points towards a more consistent year-round breeding policy (Ward, 1987). In New Zealand, out-of-season lambing may also bring advantages to farmers in areas where there is severe summer drought. Lambing at an earlier time of the season means that lambs can be sold and ewes culled before the dry season sets in, so conserving feed for other classes of stock (e.g. replacement females) during the summer (Fisk, 1984). However, most of the dual-purpose breeds of sheep used for lamb production in New Zealand breed only during a certain period of the year. This means that the production of out-of-season lambs will inevitably involve either the induction of oestrus during the ewe's anoestrous season or the genetic modification of the time of the breeding season.

2. Current techniques for oestrous induction

The concept of out-of-season breeding is not new. Techniques for inducing oestrous activity in ewes outside their normal breeding season have been known for many years (Robinson, 1967; Welch & Tervit, 1970). The earliest technique developed involves progesterone priming followed by an injection of pregnant mare's serum gonadotrophin (PMSG). Since then, several other techniques for oestrous induction, including the use of the "ram effect" (Knight, 1983), gonadotrophin releasing hormone (GnRH, McLeod et al., 1988), melatonin (Kennaway et al., 1987) and modification of length of photoperiod (Ducker & Bowman, 1972; Thimonier & Ortavant, 1985), have also been studied and shown to have some commercial potential. At present, the most reliable treatment of commercial importance used for oestrous induction is to prime the anoestrous ewes with progesterone for 10-14 days followed by an injection of PMSG on termination of the progesterone treatment. The development and commercial marketing in New Zealand of the controlled internal drug release (CIDR) dispenser for progesterone delivery has further aided the commercial utilization of this technique (Welch, 1985). When the time of induction of oestrus is close to the ewe's natural breeding season, the "ram effect" can be used in conjunction with progesterone treatment to eliminate the need for using PMSG (Knight, 1983; Welch, 1985; Taylor & Andrewes, 1987).

The most important factor limiting the commercial utilization of this technique for out-of-season lamb production is the cost of drugs and labour incurred in treating the animals (Andrewes & Taylor, 1986; Ward, 1987). This will eliminate much of the potential profit that sheep farmers could expect to return from producing out-of-season lambs. Unless the ewes are treated close to their breeding season, they will return to anoestrus after the treatment. This, together with the low fertility of ewes induced to breed outside their normal breeding season, further increases the cost of producing out-of-season lambs (McManus, 1983; Andrewes & Taylor, 1986; Taylor & Andrewes, 1987). Therefore, if a long-term commitment is to be made to producing out-of-season lambs which supply the specialist world market, it would be preferable to develop a suitable type of sheep that can breed throughout most of the year.

3. Genetic manipulation of the breeding season in the ewe

3.1. Genetic variation in timing of the breeding season

The basis of any genetic improvement programme is the exploitation of genetic variation by increasing the frequency of favourable genes. Genetic variation in the time of onset and duration of the breeding season exists both between and within breeds and thus may be exploited in breeding programmes.

3.1.1. Variation between breeds

Breed differences in the time of onset and duration of the breeding season have long been recognized and information concerning this has been reviewed by Robinson (1951), Hafez (1952), Eckstein & Zuckerman (1956), Quirke & Hanrahan (1985), and Hanrahan & Quirke (1986). Considerable variation in the length of the breeding season has been found between breeds, ranging from some primitive breeds with a very short breeding season to breeds such as the Dorset (Poll Dorset and Dorset Horn) and Merino which are capable of breeding for long periods of the year. This variation has been attributed to the different environmental conditions (particularly latitude, altitude and nutrition) under which these different sheep breeds have developed (Hafez, 1952).

The genetic basis for breed differences in timing of the breeding season has been clearly demonstrated by studies in which different breeds were compared under the same environment (Table 1.1; see also Kelley & Shaw, 1943). However, for any particular breed type, the timing of the breeding season may be modified by latitude, altitude and nutrition (Kelley & Shaw, 1943; Hafez, 1952; Knight et al., 1989). From the viewpoint of discussing the genetic variation in timing of the breeding season, it seems pertinent to consider only the breed differences found in a common environment. Breed differences in the length of the breeding season are achieved by delayed entry into anoestrus (e.g. Finnish Landrace and Romanov) and/or by early onset of cycling (e.g. Merino and Dorset Horn) (Hanrahan & Quirke, 1986). However, there seems to be some independence between the dates of onset and cessation of the breeding season, suggesting that these two events may have different genetic bases (Quirke & Hanrahan, 1985). Earliness of onset of cycling is not necessarily associated with a particularly late cessation of oestrous activity, and vice versa (Hafez, 1952; Kelly et al., 1976; Wheeler & Land, 1977).

Table 1.1. Differences in the mean dates of onset and cessation, and the mean and range of duration of the breeding season, among various sheep breeds.[1]

Breed	Date of onset	Date of cessation	Duration (days) mean±SEM	range	Source (country)
Blackface M.	25 Oct	19 Feb	139± 9.4	103-176	Hafez,1952
Border Leicester	09 Oct	10 Feb	131±11.7	83-154	(Britain)
Romney Marsh	04 Oct	02 Mar	171± 8.9	131-186	
Suffolk	03 Oct	17 Mar	189± 7.8	161-214	
Welsh Mountain	25 Oct	17 Feb	133± 6.8	103-148	
Dorset Horn	24 July	02 Mar	223±13.7	199-277	
Welsh M.xDorset H.	01 Oct	10 Mar	179±10.5	167-220	
Galway	14 Sept	17 Feb	156		Quirke et al.,
Suffolk x Galway	13 Sept	24 Feb	164		1986 (Ireland)
Fingalway	11 Sept	28 Mar	198		
Finn-Dorset	30 Aug	01 Apr	214		
Scottish Blackface	11 Nov	02 Feb	85		
Dorset	08 Aug	02 Mar	206±10.2		Dufour, 1974
Leicester	13 Sept	16 Feb	157± 9.9		(Canada)
Suffolk	16 Sept	24 Jan	132± 9.9		
DLS	28 July	11 Mar	227±10.4		
Romney	22 Mar	29 July	128±5		Kelly et al., 1976
Coopworth	20 Mar	13 Aug	146±6		(New Zealand)
Perendale	23 Mar	05 Aug	128±4		
Merino	14 Mar	12 July	-		
Dorset Horn	27 Feb	05 Aug	-		
Finnish Landrace	09 Oct	21 May	224		Wheeler & Land,
Tasmanian Merino	29 Aug	18 Feb	173		1977 (Britain)
Scottish Blackface	01 Oct	01 Mar	151		

[1] M = Mountain; H = Horn; DLS = a synthetic population of 50% Dorset, 25% Border Leicester and 25% Suffolk.

3.1.2. Variation within breeds

In addition to between-breed variation in the timing of the breeding season, there is also evidence of considerable variation between ewes within a breed when maintained under a common environment (Kelley & Shaw, 1943; Table 1.2). This variation includes differences in the timing of both the onset and cessation of the breeding season (Kelley & Shaw, 1943; Hafez, 1952; Quirke et al., 1986). As with the between-breed situation, the within-breed correlation between the dates of onset and cessation of the breeding season is very low (Quirke et al., 1986; Rodriguez Iglesias et al., 1988).

Table 1.2. Repeatability (r) and heritability (h^2) estimates for components of the breeding season and some related traits.

Trait	r	h^2	Breed	Source[a]
Onset of breeding season	0.12	-	Iceland	1
(mature ewes)	0.22	-	Suffolk, Texel	2
	0.37	-	Galway, Cheviot	2
	0.35	0.32	Welsh Mountain	3
	0.40	-	5 genotypes	4
	0.38	-	6 genotypes	5
	0.33-0.42	-	Southdown,Hampshire	6
	-	0.06	Southdown	7
End of breeding season	0.25	-	5 genotypes	4
Duration of anoestrum	0.30	-	5 genotypes	4
Date of lambing	-	0.24	Southdown	7
	0.28	-	4 genotypes	8
	0.33	0.14	DLS[b]	9
Number of hogget oestrus	-	0.27-0.32	Romney	10
	-	0.30-0.49	Romney	11

[a] (1) Dyrmundsson, 1978
(2) Hanrahan, 1982
(3) Purser, 1972
(4) Quirke et al., 1986
(5) Ricordeau et al., 1976
(6) Ruegg et al., 1967
(7) Thrift et al., 1971
(8) McGloughlin & Curran, 1969
(9) Fahmy, 1982
(10) Ch'ang & Rae, 1970
(11) Baker et al., 1974, 1979

[b] DLS = a synthetic population of 50% Dorset, 25% Border Leicester, 25% Suffolk.

There is very limited information on the genetic basis for the within-breed variation and most of the information in this area is concerned only with time of onset of the breeding season (Table 1.2). There appears to be no estimate of the heritability of breeding season length in mature ewes. From the available information summarized in Table 1.2, it can be concluded that date of onset of the breeding season is moderately repeatable and some of this variation is of genetic origin.

3.2. Methods for effecting genetic changes in the breeding season

There are two main methods that can be used to exploit genetic variation. The first is by crossing different genotypes, usually breeds, utilizing additive effects and any heterosis that may be available. The second is based on selection within a breed.

3.2.1. Crossbreeding

Due to the large breed differences in the timing of the breeding season, crossbreeding will offer a fast and effective way to alter the seasonal breeding pattern of a particular breed (McQueen & Reid, 1988). Hanrahan & Quirke (1986) summarized results from several crossbreeding studies and found that with a few exceptions (e.g. Dufour, 1974; Aboul-Naga et al., 1985), heterosis for breeding season length is small or non-existent. Thus crossbreeding between breeds for the breeding season length will mainly involve exploitation of additive genetic effects. The time of onset and/or cessation of the breeding season may have a pattern of inheritance different from that of the duration of the breeding season (Kelley & Shaw, 1943; Hafez, 1952; Hanrahan & Quirke, 1986; Table 1.1).

A major drawback of crossbreeding is that the production characters of an established breed may be greatly changed following crossbreeding. For example, the use of crossbreeding to increase the breeding season length in New Zealand would most likely involve crossing Dorset rams with ewes of Romney or Romney-based breeds. While the crossbred animals may be superior in terms of lamb production, wool production is reduced and the wool quality is changed in the crossbred animals compared to the straightbreds (Carter & Cox, 1982). The desirability of these changes in production characteristics is dictated by the relative prices of wool and lamb which fluctuate from time to time. One way to overcome this problem is to use backcrossing with the original breed while applying selection pressure to retain genes for the particular trait concerned, in this case timing of the breeding season.

3.2.2. Within-breed selection

Selection within a breed offers an opportunity to change some of the productive characters of an established population. The effectiveness of this method depends on the rate at which genetic gains can be achieved. Major factors that affect the annual rate of response to selection are the selection differential, generation interval and heritability (which, from a breeding point of view, is the proportion of selection differential that can be passed on to the next generation). Heritability determines the accuracy of selection and hence the rate of genetic gain. There have been no estimates of the heritability of the duration of the breeding season. From the information summarized in Table 1.2, it can be seen that date of onset of the breeding season is moderately repeatable and some of this is attributable to a genetic component which can be exploited by selection. Unfortunately, no selection experiment on the length of the breeding season has been reported. Selection for date of lambing in a synthetic population (50% Dorset, 25% Border Leicester and 25% Suffolk) led to advancement in the date of onset of the breeding season and lambing at a rate of 3 days per generation (Fahmy et al., 1980; Dufour et al., 1982).

In summary, attempts to increase the breeding season length of breeds such as the Romney may involve the use of either crossbreeding or within-breed selection. In a crossbreeding programme, ewes are mated to rams of breeds with an extended breeding season. Their female progeny are then backcrossed to Romney rams with selection pressure being applied to retain the genes controlling the breeding season. Use of within-breed selection relies on identifying individuals with superior performance. Neither programme is likely to bring about rapid changes to the timing of the breeding season because of the low selection pressure that can be applied on the ewe, the fact that onset and duration of the breeding season is a sex-limited trait for which selection pressure cannot be applied prior to puberty, and the likely low heritability of this trait.

Consider, as an example, selection based on date of the onset of the breeding season in a ram-breeding Romney flock of 1,000 breeding ewes.

Assuming:
a) All breeding ewes are culled after the fourth lambing and a 5% death rate in the ewe. This gives a replacement rate of 27% each year.
b) Replacement ewes will be mated at 18 months of age.
c) A lamb weaning rate of 100% and a sex ratio of 1:1.

d) No selection is made on the rams with a random sample being chosen as replacements and being used once at 18 months of age.
e) The heritability (h^2) is 0.32 (Purser, 1972).
f) The standard deviation (s) is 10.3 (Hafez, 1952).

Under these assumptions, the number of replacement ewes each year would be 270, which is 54% of the 500 ewe lambs born each year. The generation interval (L) calculated is 2.72 years and the selection intensity or the standardized selection differential (i) is 0.735. Thus the genetic gain per annum (ΔG) using ewe selection alone is only 0.45 days ($\Delta G = i \cdot h^2 \cdot s \cdot 0.5/L$).

Thus in order to advance the date of onset of the breeding season in sheep through within-breed selection, it would be advantageous to develop techniques to increase the rate of response to selection. One such technique could involve selection in the ram. Land (1973) argued that both the male and the female carry virtually the same autosomal genes and that the same gonadotrophins control reproductive processes in both sexes. This suggests that the quantitative expression of sexual activity in males and females may well be genetically correlated. The benefit of using indirect selection in the male instead of, or in combination with, direct selection in the female for reproductive traits has been demonstrated (Walkley & Smith, 1980). At a moderate to high heritability for the indicator trait and a high genetic correlation between the indicator trait and the production trait of interest, the predicted rate of response could be substantially increased.

The above example can be extended to allow selection only on the ram. In this case, selection in the ram is equivalent to an indirect selection. With an indirect selection, the genetic gain achieved for the desired trait (ΔG) is affected not only by the heritability of the desired trait (h^2_x) but also by the heritability of the indicator trait used in selection (h^2_y) and the genetic correlation (r) between the desired and indicator traits ($\Delta G = i \cdot h_x \cdot h_y \cdot r \cdot s \cdot 0.5/L$). Assume a ram to ewe ratio of 2%, that replacement ewes are randomly selected, and that all relevant assumptions made in the above example remain the same. Under these assumptions, it is possible to make predictions on the relative rate of genetic gain resulting from selection on the ram alone as compared with that resulting from selection on the ewe alone (Table 1.3). It can be concluded from Table 1.3 that a substantial increase in the rate of genetic gain could be achieved by using ram selection at a moderate heritability of the ram trait and a moderate to high genetic correlation between the ewe and the ram traits.

Table 1.3. Relative rates of genetic gains (in percentage) resulting from selection on the ram alone as compared with selection on the ewe alone (100) at different combinations of heritability (h^2) of the indicator trait in the ram and genetic correlation (r) between the desired and the indicator traits

r	h^2					
	0.1	0.2	0.3	0.4	0.5	0.6
0.3	49	70	85	98	110	121
0.4	66	93	114	131	147	161
0.5	82	116	142	164	183	201
0.6	98	139	170	197	220	241
0.7	115	162	199	230	257	281
0.8	131	186	227	262	293	321

The successful use of this selection technique would depend on finding an appropriate indicator trait in the ram that could be used as a selection criterion. Ideally, this indicator trait should neither be sex- nor age-limited, and should be measured cheaply and accurately (Blair et al., 1990). The purpose of this study was therefore to identify, and to investigate the usefulness of, some physiological and endocrinological characteristics in the ram that might be used as selection criteria in breeding programmes for early onset of the breeding season in the ewe. The search for a useful selection criterion in the ram for early onset of the breeding season in the ewe depends on understanding the mechanisms responsible for the seasonal variation in breeding activity in both sexes. In the following sections, the seasonal variation in breeding activity of the ram together with the endocrine mechanisms controlling seasonal breeding in both the ram and the ewe are briefly reviewed.

4. Seasonal variation in breeding activity of the ram

Like the seasonal changes in the oestrous and ovulatory activities in the ewe, seasonal variation in reproductive activity exists in the ram. Although rams of most breeds are capable of producing semen throughout the year, there is usually a period of subfertility, corresponding to the anoestrous season in the ewe, during which there is a decrease in semen production and sexual activity (Lees, 1965; Haynes & Schanbacher, 1983). Such seasonal variation in the breeding performance of the ram is associated with marked seasonal changes in both the structure and function of the testis.

4.1. Testis size and structure

Seasonal variation in the testis size of the ram has been documented by many studies (Islam & Land, 1977; Lincoln & Short, 1980; Bremner et al., 1984; Dufour et al., 1984). Generally, testis size of the ram is at a minimum in the spring when daylength is increasing, and increases to reach its maximum some weeks before the females of the corresponding breed come into the breeding season. However, while the sheep is considered to be a short-day breeder, studies have shown that the seasonal decrease in testis size tends to start before the winter solstice and the seasonal increase in testis size begins before the summer solstice (Lincoln & Davidson, 1977; Lincoln & Short, 1980; Pelletier et al., 1981). This suggests that, under natural lighting conditions, the photoperiodic change does not drive the seasonal testis cycle, but merely entrains an endogenous rhythm as revealed in animals reared under constant conditions (Howles et al., 1982).

Breed differences exist in both the magnitude and timing of seasonal variation in testis size (see review by Pelletier & Almeida, 1987) and this seems to be related to seasonal variation in the reproductive activity of the ram's female relatives (Islam & Land, 1977; Bremner et al., 1984). In breeds like the Merino, whose ewes have a short, shallow anoestrous season, seasonal variation in testis size is less conspicuous and the increase in testis size occurs earlier than in other breeds such as the Finnish Landrace. Furthermore, the difference between these two breeds in date of the seasonal increase

in testis size corresponds roughly to their difference in date of onset of the breeding season (Wheeler & Land, 1977). Within breeds, there are also differences among individual rams in the magnitude of seasonal variation in testis size and these differences seem to be repeatable from year to year (Colas et al., 1988; Ringwall et al., 1989).

Seasonal variation in testis size is caused by changes in the histological structure of the testis. Annual changes in testis size are accompanied by parallel changes in the length and diameter of the seminiferous tubules (Hochereau-de Reviers et al., 1976; Schanbacher & Ford, 1979; Lincoln & Short, 1980). This is associated with changes in the size of Sertoli cells and the number and size of germ cells (Hochereau-de Reviers et al., 1976; Schanbacher & Ford, 1979; Lincoln & Short, 1980; Lincoln, 1981). The intertubular tissue, including the Leydig cells, of the testis also undergoes changes related to season (Hochereau-de Reviers et al., 1976; Lincoln, 1981).

4.2. Testicular functions

4.2.1. Spermatogenic function

Marked seasonal variation in sperm production has been reported in studies which involved counting the number of sperm present in the testis (Hochereau-de Reviers et al., 1976; Schanbacher & Ford, 1979), rete testis fluid (Dacheux et al., 1981), and ejaculate (Cupps et al., 1960; Smyth & Gordon, 1967; Islam & Land, 1977; Dufour et al., 1984; Folch, 1984; Boland et al., 1985). Sperm production is significantly decreased during the non-breeding season, with large breed differences being evident in the magnitude of this seasonal change (Islam & Land, 1977; Dacheux et al., 1981; Folch, 1984). It is generally agreed that the magnitude of the seasonal variation in semen production is less prominent in breeds with longer breeding seasons, suggesting breed differences in their sensitivity to the seasonal effect (Dacheux et al., 1981; Folch, 1984). Quantitative studies on the number of germ cells in the seminiferous tubules at various stages of mitosis and meiosis have revealed that the reduced sperm production in spring compared to autumn is due to reductions in: the total number of type A spermatogonia; efficiency of the mitotic divisions of the spermatogonia; efficiency of the meiotic divisions of the primary spermatocytes; and the proportion of germ cells completing the final processes of spermiogenesis (Ortavant et al., 1969; Hochereau-de Reviers et al., 1976; Schanbacher & Ford, 1979; Lincoln, 1981).

Season affects not only the quantity but also the quality of sperm produced, albeit to a lesser degree. During the nonbreeding season, when testes are regressing or regressed, there are often increases in the number of immature, morphologically abnormal and dead sperm, and/or a reduction in sperm motility (Cupps et al., 1960; Fowler, 1965; Smyth & Gordon, 1967; Colas, 1981, 1983; Dufour et al., 1984; Boland et al., 1985; Amir et al., 1986a). The fertilizing capacity of the semen also varies with season, being higher in autumn than in spring (Colas, 1981, 1983; Amir et al., 1986a). Seasonal variation in the metabolic activity of the spermatozoa has also been reported (Amir & Volcani, 1965).

4.2.2. Steroidogenic function

Studies with a variety of breeds have demonstrated a marked seasonal variation in testosterone secretion in rams (Katongole et al., 1974; Schanbacher & Lunstra, 1976; Davies et al., 1977; Lincoln & Davidson, 1977; Sanford et al., 1977, 1984a; Wilson & Lapwood, 1978; Pelletier et al., 1982; D'Occhio & Brooks, 1983; Dufour et al., 1984; Amir et al., 1986b). The mean serum concentrations of testosterone are low during the spring months when the testes are regressed, but increase gradually with increasing stages of testicular development until the maximum is attained in rams with fully developed testes. Breeds differ in the extent and time-course of the seasonal variation in testicular steroidogenic function (Pelletier et al., 1982; D'Occhio & Brooks, 1983; Bremner et al., 1984; Dufour et al., 1984; Pelletier & Almeida, 1987). The seasonal variation in plasma testosterone concentrations is less conspicuous, and the seasonal increase takes place earlier, in the Poll Dorset and Dorset Horn as compared to other more seasonal breeds (D'Occhio & Brooks, 1983; Boland et al., 1985). In another study, it was found that plasma testosterone concentrations started to increase earlier in Préalpes du Sud than in Ile-de-France rams (Pelletier et al., 1982). Within breeds, rams differing in magnitude of the seasonal variation in testis size also differ in seasonal variation in plasma testosterone concentrations (Ringwall et al., 1990). In most sheep breeds studied, with the exception of the Finnish Landrace (Sanford et al., 1977), the seasonal increase in plasma testosterone concentrations starts before the summer solstice (see Pelletier & Almeida, 1987).

Testosterone secretion from the testis is stimulated mainly by luteinizing hormone (LH) (Amann & Schanbacher, 1983). There are seasonal changes in the testicular response to LH stimulation throughout the year (Lincoln, 1978; Sanford et al., 1984a). During the sexually inactive season, testosterone release in response to either endogenous LH pulses or exogenous LH injection is small. It increases to reach a maximum when the testes are fully active. At this time, a small increase in LH is followed by a rapid and sustained increase in plasma testosterone concentrations. This seasonal variation in testicular androgen production is undoubtedly related to the seasonal growth and regression of the testicular steroid-producing cells. Furthermore, studies have shown similar seasonal changes in the androgenic capacity of testicular steroidogenic cells (Lincoln, 1981).

4.3. Sexual activity

Many studies have shown that sexual activity in the ram is affected by season and is at its highest during the autumn mating season (Schanbacher & Lunstra, 1976; Mattner, 1979; Howles et al., 1980; D'Occhio & Brooks, 1983; Dufour et al., 1984). There are differences between breeds, and between rams within a breed, in their sensitivity to this seasonal effect, suggesting a genetic basis for variation in this trait (Schanbacher & Lunstra, 1976; D'Occhio & Brooks, 1983). Despite the well-established concept that testicular hormones are involved in the control of sexual behaviour (Haynes & Schanbacher, 1983), the relationship between testosterone concentrations and sexual behaviour has not been established. The correlation between an individual's mating performance and its serum concentrations of testosterone is very low (D'Occhio & Brooks, 1980, 1983). On the other hand, significant correlations between serum concentrations of testosterone and mating performance have been reported for groups of rams throughout the annual sexual cycle and for individual animals at the beginning of the mating season (Schanbacher & Lunstra, 1976; Sanford et al., 1977; D'Occhio & Brooks, 1983; Dufour et al., 1984). These results, together with the finding that libido of sexually active rams is not improved with testosterone treatment (Knight, 1973; Mattner & Braden, 1975), suggest that there is a threshold concentration of testosterone required for maximal sexual activity.

5. The endocrinology of seasonal breeding in sheep

The seasonal variation in breeding activities of both the ram and the ewe is undoubtedly caused by more fundamental changes in the mechanisms controlling reproduction. In the following section, a brief account of the endocrine mechanisms controlling reproduction in sheep is presented first, followed by a review of the endocrine causes of seasonal breeding in sheep. The ram and the ewe are considered together in order to highlight the similarities in the mechanisms controlling seasonal breeding in both sexes. Within the context of genetic selection in the ram for aseasonality of breeding in the ewe, such information is very important to the choice of appropriate selection criteria.

5.1. Endocrine control of reproduction in sheep

The endocrine mechanisms responsible for the control of reproductive activity in both the ram and the ewe are governed principally by the hypothalamo-pituitary axis, which is in turn modified by feedback mechanisms involving gonadal steroids and inhibin (Haresign et al., 1983; Haynes & Schanbacher, 1983; Fink, 1988). Activity of the hypothalamus is further modulated by neural pathways impinging on it from extra-hypothalamic centres in the brain. In addition, the function of the hypothalamo-pituitary axis is under the influence of factors in the external environment, such as photoperiod and temperature, by way of the central nervous system. This ensures that the activity of the hypothalamo-pituitary axis, and hence the reproductive processes controlled by it, is appropriate to the external environment the animal is subjected to.

In both the male and the female, gonadal function is controlled mainly by gonadotrophins, namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH), secreted from the anterior pituitary gland. The secretion of FSH and LH is stimulated by the hypothalamic gonadotrophin-releasing hormone (GnRH) which is synthesized and released by neurons in the preoptic area and travels to the anterior pituitary gland via the portal blood system. It has been shown that GnRH controls the secretion of both FSH and LH (Lincoln & Short, 1980; Fraser et al., 1984; Schanbacher, 1984; Clarke et al., 1986). However, there is probably a sex difference in

the hypothalamic areas involved in the control of gonadotrophin secretion in sheep. While tentative evidence indicates two separate hypothalamic areas regulating the preovulatory LH surge and the pulsatile mode of secretion, respectively, in the ewe (Haresign et al., 1983; Clarke, 1984), it seems probable that in the ram only the area regulating pulsatile LH release is functional.

Apart from the preovulatory LH surge at oestrus in the ewe, the patterns of LH secretion in both the ram and the ewe are very similar. In both sexes, LH secretion is pulsatile, basal levels of LH being interrupted by small, short-lived pulses, the frequency of which varies with the physiological status of the animal (Baird et al., 1976; Hauger et al., 1977; Lincoln & Short, 1980; Martin, 1984). Studies in both the ram and the ewe have shown that each LH pulse is caused by a pulse release of GnRH from the hypothalamus (Lincoln & Short, 1980; Clarke & Cummins, 1982; Levine et al., 1982; McLeod et al., 1982a,b; Clarke et al., 1986). The LH pulse amplitude is affected by the quantity of GnRH released from the hypothalamus and the pituitary sensitivity to GnRH, both of which are controlled by gonadal feedback mechanisms (Haresign et al., 1983). In addition, GnRH pulse frequency also affects LH pulse amplitude (Clarke & Cummins, 1985). When GnRH pulse frequency increases, the pituitary LH response to the same amount of GnRH decreases.

In contrast to LH, the pattern of FSH secretion is less variable (Haresign et al., 1983; Schanbacher, 1984). Small pulses of LH may not be accompanied by concurrent increases in FSH concentrations. Studies involving administration of exogenous GnRH to hypothalamo-pituitary disconnected, ovariectomized ewes have shown that FSH secretion responds differently from that of LH and a pulsatile pattern of GnRH secretion may not be necessary to maintain FSH secretion (Lincoln & Short, 1980; Clarke et al., 1986).

The activity of the hypothalamo-pituitary axis is under the control of negative feedback mechanisms from the gonads, as demonstrated by the post-castration rise in plasma gonadotrophin concentrations. The principal factors controlling gonadotrophin secretion are the gonadal steroids, namely oestradiol and progesterone in the ewe and testosterone in the ram (Karsch et al., 1978; Haresign et al., 1983; Schanbacher, 1984). In addition, a peptide factor known as inhibin is also involved in the control of FSH secretion (de Jong & Robertson, 1985).

5.2. Endocrine causes of seasonal breeding in sheep

Since gonadal functions in both the ram and the ewe are controlled by pituitary gonadotrophins, a great deal of research effort has been devoted to identifying seasonal changes in gonadotrophin secretion that might account for the seasonal variation in breeding activity.

5.2.1. LH

Marked seasonal fluctuations in LH secretion have been found in both the ram and the ewe. Early studies in the ram reported higher mean LH concentrations during the breeding season or in animals exposed to short photoperiods than during the nonbreeding season or in animals exposed to long photoperiods (Katongole et al., 1974; Gomes & Joyce, 1975; Pelletier & Ortavant, 1975a; Barrell & Lapwood, 1978/1979). Recent studies involving intensive blood sampling revealed that the seasonal change in LH secretion occurs in pulse frequency as well as in mean plasma concentrations. LH pulse frequency is lower during the nonbreeding season or in rams maintained under long photoperiods than during the breeding season or in rams under short photoperiods (Lincoln, 1976; Schanbacher & Ford, 1976; Wilson & Lapwood, 1978; Pelletier et al., 1982; Sanford et al., 1977, 1978, 1984a).

There are breed differences in the timing and magnitude of seasonal variation in plasma LH concentrations. Barrell & Lapwood (1978/1979) showed that the seasonal variation in mean LH concentrations was less conspicuous in Merino than in Romney and Poll Dorset rams. Differences between Merino and Romney rams in the seasonal variation in both mean LH concentration and LH pulse frequency have also been reported (Bremner et al., 1984). The seasonal increase in LH pulse frequency took place earlier and the magnitude of variation was greater in Préalpes du Sud rams than in Ile-de-France rams (Pelletier et al., 1982). Within a breed, rams differing in the magnitude of seasonal variation in testis size also have different patterns of seasonal variation in LH secretion (Ringwall et al., 1990).

In the ewe the most prominent change associated with seasonal anoestrus is the reduction in LH pulse frequency (Scaramuzzi & Baird, 1977; Yuthasastrakosol et al., 1977; Jackson & Davis, 1979; Walton et al., 1980; McNatty et al., 1984). During seasonal anoestrus, the frequency of LH pulses is lower than that found during the

luteal phase of the oestrous cycle, although the basal concentration of LH is not significantly reduced. Since the sustained increase in LH frequency during the follicular phase of the oestrous cycle is responsible for driving follicles through their final stages of development, and for stimulating the increased oestradiol production required to induce the preovulatory LH surge and oestrus (Baird & McNeilly, 1981; Haresign et al., 1983), it appears that seasonal anoestrus is caused by a reduction in the frequency of pulsatile LH secretion to a level below that required to stimulate the final stages of follicle growth and maturation. Indeed, artificial elevation of LH pulse frequency in anoestrous ewes, to a level similar to that observed during the follicular phase of the oestrous cycle, by injection of exogenous LH or low doses of GnRH has been shown to result in normal follicular development, ovulation and normal luteal function (McNatty et al., 1981; McLeod et al., 1982a,b; McNeilly et al., 1982, 1985).

Seasonal variation in the LH pulse amplitude has also been reported. In the ewe, the LH pulse amplitude is greater during anoestrus than during the luteal phase of the oestrous cycle (Yuthasastrakosol et al., 1977; Jackson & Davies, 1979; Scaramuzzi & Baird, 1979). In the ram, LH pulse amplitude is greatest at the time when testis size is increasing rapidly. It decreases to reach its annual minimum when the testes are maximally active (Lincoln, 1976, 1978; Sanford et al., 1984a).

5.2.2. FSH

Conspicuous seasonal variation in FSH secretion occurs in the ram. FSH concentrations are low during the nonbreeding season when the testes are regressed. It increases coincident with, or preceding, the increase in testis size with maximum concentrations being reached some weeks before the testes are fully grown (i.e. at a time when the testes are growing rapidly). Thereafter, plasma FSH concentrations decrease while the testis size continues to increase (Lincoln & Davidson, 1977; Lincoln et al., 1977; Sanford et al., 1977, 1984a). The decrease in plasma FSH concentration when testis size is increasing rapidly is probably due to increased feedback inhibition from the testis which secretes increasing amounts of testosterone, and possibly inhibin, with increasing testis size (Schanbacher, 1984). A breed difference in the magnitude of seasonal variation in FSH concentrations has been found between Merino and Romney rams (Bremner et al., 1984).

Conflicting results have been reported on the seasonal changes in FSH secretion in the ewe. While some studies have reported reductions in FSH concentrations during seasonal anoestrus (Findlay & Cumming, 1976), others have found similar (Walton et

al., 1977, 1980) or even higher (McNatty et al., 1984) FSH concentrations during seasonal anoestrus than during the breeding season. These discrepancies may reflect breed differences in the extent of seasonal variation in gonadotrophin secretion. In sheep breeds with very deep anoestrus, such as the Scottish Blackface and Ile-de-France, injection of purified LH is ineffective in inducing ovulation during mid-anoestrus, whereas a sequence of FSH and LH injections is effective (McNeilly et al., 1985). These results suggest that, in these breeds, FSH secretion in the middle of the anoestrous season may be insufficient to stimulate early follicular growth to a stage where follicles can respond fully to LH stimulation.

5.2.3. Prolactin

Apart from FSH and LH, prolactin secretion is also affected by seasonal changes in daylength. The circulating concentrations of prolactin are higher during the non-breeding season than during the breeding season in both the ram (Ravault, 1976; Barrell & Lapwood, 1978/1979) and the ewe (Walton et al., 1977, 1980; Thimonier et al., 1978; Jackson & Davies, 1979). This inverse relationship between circulating prolactin concentrations and gonadal activity suggests that prolactin may play a role in mediating the seasonal effects on breeding activity in sheep. Studies in hamsters have shown that prolactin is involved in regulating the photoperiod-induced variation in gonadal function (McNeilly, 1986, 1987). However, the hamster is a long-day breeder which starts breeding in the spring when daylength is increasing and circulating concentrations of prolactin are high. While it is possible that prolactin may have opposite effects in different species, there is considerable evidence arguing against prolactin being an intermediary factor mediating the photoperiod effects on gonadal function in sheep. First, suppression of prolactin secretion during anoestrus does not result in a return to oestrus (Baird & McNeilly, 1981), nor does it have any effect on tonic LH secretion or the ability of oestrogen to induce an LH surge (McNeilly & Land, 1979; Land et al., 1980). Second, ovulation and normal luteal function can be induced during anoestrus, when prolactin concentrations are elevated, by timed injections of LH. Third, breeds of ewes coming into the breeding season at different times of the year have been shown to have similar temporal changes in prolactin concentrations (Webster & Haresign, 1983). Therefore, the increased prolactin secretion during the nonbreeding season is most likely to be a coincident effect of the prevailing photoperiod which has little, if any, effect on the seasonal changes in gonadal function in sheep (Worthy & Haresign, 1983).

To summarize, gonadotrophin secretion in both the ram and the ewe varies with season and this appears to be the immediate cause of the seasonal changes in gonadal activities. The relative importance of the seasonal variation in FSH and LH secretion in the control of the annual cycle in testis function in the ram is not clear. Unlike the situation in the ewe, where the role of FSH in the control of seasonal anoestrus is permissive (at least in the less seasonal breeds), marked seasonal variation in FSH concentrations exists in the ram and this has been found to be closely correlated with the annual cycle in testis activity (Lincoln, 1981). Injection of multiple, small doses of GnRH is able to induce early growth and function of the testes (Lincoln, 1979), but both FSH and LH concentrations were elevated by this treatment. It seems most likely that both FSH and LH are responsible for the seasonal variation in testicular function in the ram. Seasonal variation in prolactin secretion does not seem to have a measurable effect on gonadal function in sheep.

5.3. Effect of season on the mechanisms regulating gonadotrophin secretion

Gonadotrophin secretion from the pituitary gland is controlled by GnRH secreted from the hypothalamus. A change in gonadotrophin secretion would suggest changes in GnRH secretion from the hypothalamus and/or the pituitary responsiveness to GnRH, both of which are controlled by the negative feedback mechanisms from the gonads.

5.3.1. GnRH secretion

Since each LH pulse is caused by a pulse release of GnRH (Clarke & Cummins, 1982; Levine et al., 1982), the marked seasonal variation in the frequency of LH pulses implies a major effect of photoperiod on the hypothalamic GnRH pulse generator. Two effects of photoperiod on the LH pulse generator have been identified. The first is the "direct" effect of photoperiod, as observed by monitoring LH pulse frequency in long-term gonadectomized animals in different seasons of the year or when they are exposed to different artificial photoperiods. LH pulse frequency in castrated ewes (Goodman et al., 1982; Robinson et al., 1985; Montgomery et al., 1985) or rams (Parrott & Davies, 1979; Lincoln & Short, 1980; Sanford et al., 1984b) is lower under long photoperiods than under short photoperiods. However, there seem to be breed differences in this direct effect of photoperiod, which may reflect breed differences in their depth of anoestrus. No direct effect of photoperiod on LH pulse frequency was

observed in ovariectomized Merino ewes (Martin et al., 1983; Thomas et al., 1988), while the same study observed a seasonal difference in LH pulse frequency in the more seasonal Suffolk ewes (Thomas et al., 1988). No comparative study has been conducted in the ram.

The second effect of photoperiod, which is much more dramatic, manifests itself as a marked seasonal shift in the sensitivity of the hypothalamus to the negative feedback effects of gonadal steroids, namely oestradiol in the ewe and testosterone in the ram. In the ewe, this has been clearly demonstrated in ovariectomized animals implanted with Silastic capsules to maintain a constant concentration of oestradiol in the circulation (Legan et al., 1977; Karsch et al., 1978; Goodman et al., 1982; Martin et al., 1983; Webster & Haresign, 1983; see also review by Karsch et al., 1984). During anoestrus, oestradiol alone is a potent inhibitor of pulsatile LH secretion but, during the breeding season, the inhibitory effect of oestradiol on LH pulse frequency is lost and the potentiating effect of progesterone is required for full negative feedback (Karsch et al., 1984). Of particular interest is the finding that the seasonal shift in the sensitivity of the hypothalamus to the negative feedback effect of oestradiol in ovariectomized ewes is coincident with the change in the reproductive status of entire ewes of the same breed (Legan & Karsch, 1979; Webster & Haresign, 1983). This relationship between changes in the sensitivity to the negative feedback action of oestradiol in ovariectomized ewes and changes in cyclic activity of entire ewes exists even for breeds with very different breeding season lengths (such as Dorset Horn and Welsh Mountain; Webster & Haresign, 1983). A difference between Merino and Suffolk ewes in the hypothalamic sensitivity to the negative feedback effect of oestradiol has also been found (Thomas et al., 1988).

In the ram, available evidence suggests that the seasonal variation in GnRH secretion may similarly be caused by a seasonal change in the responsiveness of the hypothalamus to the negative feedback effects of testosterone. Pelletier & Ortavant (1975b) found that intramuscular injection of testosterone propionate produced a greater inhibition of LH secretion for a longer period in rams maintained under long photoperiods than in rams maintained under short photoperiods. More recently, seasonal variation in the sensitivity of the hypothalamo-pituitary axis to the negative feedback effect of testosterone has been demonstrated in castrated Soay rams exposed to artificial photoperiod and bearing testosterone implants to maintain a relatively constant plasma testosterone concentration (Lincoln & Ebling, 1985). However, in another study on castrated rams bearing constant-release implants of either testosterone or oestradiol, Schanbacher (1980a) did not find any significant seasonal effect on the ability of testosterone or oestradiol to inhibit LH pulse frequency. Results from this

study should be interpreted with caution because LH pulse frequency of castrated or steroid-treated rams did not differ significantly from that of intact animals. Clearly, further studies involving castrated rams bearing constant-release implants of testosterone and maintained under natural lighting conditions are needed to clarify the effects of photoperiod on the sensitivity of the hypothalamus to the negative feedback effects of testosterone. No comparative study of breed differences in hypothalamic sensitivity to the negative feedback of testosterone has been conducted in the ram.

5.3.2. Pituitary sensitivity to GnRH

Seasonal changes in pituitary responsiveness to GnRH have also been observed. In the ram, LH pulse amplitude in response to endogenous GnRH pulses is greatest at the time when testis size is increasing rapidly. It decreases to reach its annual minimum when the testes are maximally active (Lincoln, 1976, 1978; Sanford et al., 1984a). In the ewe, the LH pulse amplitude is higher during anoestrus than during the breeding season (Scaramuzzi & Baird, 1979). This seasonal variation in LH pulse amplitude could be due to variation in GnRH pulse frequency, the amount of GnRH released in each pulse and/or in the sensitivity of the pituitary gland to GnRH, all of which are controlled by the negative feedback mechanisms from the gonads. Unfortunately, the effects of these factors are confounded and their relative importance is not clear. The pituitary responses to exogenous GnRH treatment in the ram also undergo changes related to season (Lincoln, 1976, 1978; D'Occhio et al., 1988). LH response to exogenous GnRH is greatest at the time when testis size is increasing. In addition, the pattern of LH response following GnRH challenge varies with the stage of the annual sexual cycle. During the period of gonadal regression, LH secretion in response to exogenous GnRH injection is characterized by a high amplitude and short duration of LH release, which changes to a low-amplitude and more sustained release during the period of maximum testicular activity (Lincoln, 1976, 1977; Lincoln & Short, 1980; D'Occhio et al., 1988). Since the clearance rate of LH does not change during the sexual cycle of the ram (Lincoln, 1978), it follows that the change in LH response to GnRH reflects a difference in the pattern of secretion, being rapid during the regressed phase and sustained during the active phase. This results in a less clear seasonal variation in the amount of LH released in response to a standard dose of GnRH (Sanford et al., 1984a). Since the pituitary content of LH is higher during the breeding season than during the nonbreeding season (Ortavant et al., 1964), these results suggest that there is a seasonal change in the form of LH stored in the pituitary gland (Lincoln, 1976, 1977; Lincoln & Short, 1980). Gonadal steroids can affect the responsiveness of the pituitary gland to GnRH (Schanbacher, 1984), so the seasonal variation in pituitary

responsiveness to GnRH in intact animals may be confounded with the seasonal variation in testosterone concentrations. Schanbacher (1980a) showed that the LH responses to GnRH challenges were greater during exposure to short photoperiods than during exposure to long photoperiods in castrated rams treated with constant-release implants of testosterone or oestradiol.

To summarize, photoperiod affects gonadotrophin secretion through its effects on both GnRH secretion from the hypothalamus and the pituitary sensitivity to GnRH. The effects of photoperiod on GnRH secretion appear to be mediated via both steroid-independent and steroid-dependent mechanisms and breed differences in sensitivity to these two effects of photoperiod have been found in the ewe. Experimentally, it appears that there are two different ways in which photoperiod is capable of altering hypothalamic GnRH secretion. However, while the steroid-dependent and steroid-independent processes are operationally distinct from each other, it is not known whether the two processes are functionally coupled. For example, during exposure to an inhibitory photoperiod, there may be a steroid-independent inhibition of the hypothalamo-pituitary axis which automatically leads to increased sensitivity to steroid feedback (Turek & Ellis, 1981). The mechanism by which photoperiod affects pituitary responsiveness to GnRH is less clearly defined. Since GnRH controls the biosynthesis and release of gonadotrophins as well as its own receptors in the pituitary (Clayton, 1989), the seasonal variation in pituitary responsiveness to GnRH could be the result of the seasonal effects on GnRH secretion. It is not known to what extent photoperiod affects pituitary responsiveness to GnRH by mechanisms other than its effects on the hypothalamic GnRH secretion.

5.4. Mechanisms of photoperiodic action

Studies in sheep and other photoperiodic species have identified major portions of the pathway by which photoperiodic information is perceived and transmitted to the neuroendocrine systems controlling reproduction and other seasonal processes (Karsch et al., 1984). Photic information picked up by the retina is transmitted via neural routes to the pineal gland where the coding for photoperiodic information is translated from neural to humoral signals through the secretion of melatonin (Arendt, 1986). Pinealectomy, or interruption of the sympathetic innervation of the pineal gland, renders animals incapable of responding to changes in photoperiod. Melatonin secretion is restricted to the dark phase of the day and the duration of elevated plasma

melatonin concentrations parallels that of the dark period (Bittman, 1985; Arendt, 1986). It has been shown that in sheep the duration of the night-time melatonin secretion codes daylength (Bittman, 1985; Wayne et al., 1988). Despite this convincing demonstration that melatonin is involved in photoperiodic control of seasonal breeding in sheep, its target sites and mode of action are not clear. It is believed that melatonin acts mainly on central nervous sites that regulate reproductive function (Glass, 1984). Recently melatonin receptors have been found in the pituitary gland (Stankov & Reiter, 1990; Stankov et al., 1991). Nevertheless, the acute response of the pituitary gland to GnRH is not influenced by melatonin in the ewe (Symons & Arendt, 1982; Robinson et al., 1986).

6. Purpose and scope of the study

Evidence reviewed in this chapter highlights some potential physiological and endocrinological characteristics in the ram that might be used in selection programmes for early onset of the breeding season. Although a change in the responsiveness of the hypothalamo-pituitary axis to the negative feedback effects is the main factor determining the between- and within-breed variation in seasonal breeding activities, it cannot be utilized as a selection criterion because its measurement requires the removal of the testes. The consequences of this seasonal variation in the negative feedback mechanisms controlling gonadotrophin secretion are manifested in seasonal variation in gonadotrophin secretion and in the pituitary responsiveness to GnRH. These in turn drive the seasonal variation in testis size and function. In view of the similarities in the mechanisms controlling seasonal breeding in the ram and the ewe, the pattern (timing and/or magnitude) of seasonal variation in testis size and some endocrinological parameters might be useful characteristics in the ram that can be used in selection programmes for early onset of the breeding season. The greater selection pressure that can be applied to the ram as compared to the ewe means that potentially greater genetic progress might be achieved through ram selection. However, there has been no detailed study of the genetic basis for the seasonal variation in gonadotrophin secretion, testis size and testis function. Furthermore, no selection programme based on the pattern of seasonal variation in these ram characteristics has been reported.

The purpose of the present study was to identify and investigate the usefulness of some physiological and endocrinological characteristics in the ram that might be used as selection criteria in breeding programmes for early onset of the breeding season. The main emphasis of this study centred on the seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH challenge.

Although some other characteristics, such as seasonal variation in semen production or libido, could also be useful, they were not considered in the present study either because they are difficult to measure or because the repeatability of their measurements is likely to be low. It is the objective of any selection programme to estimate genetic potential as early as possible. However, seasonal changes in testis size and gonadotrophin secretion in ram lambs are confounded with growth and puberty. This will present problems in practice unless a reliable method to remove these confounding effects can be found. Therefore, the present study was conducted on mature (2 year and over) rams only.

The approaches to find useful predictors of genetic merit have been discussed by Blair et al. (1990). As a first approach in the present study, the differences between Romney and Poll Dorset rams in seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH challenge were compared. Because of the large difference between the two breeds in date of onset of the breeding season, it was expected that any differences in these characteristics were likely to be greater than those that could be found in a within-breed situation (Blair et al., 1990). However, results obtained from breed comparison studies do not guarantee a cause-effect relationship and have to be verified in a within-population situation. Therefore, in a second experiment, differences among Romney rams in seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH challenge were investigated. Results from this trial were expected to give some indications as to the magnitude of variation in these traits among rams of the same breed. Because the objective was to advance the onset of the breeding season, these studies were restricted to the transitional period from the nonbreeding to the breeding season.

The likely usefulness of the differences found in the above studies was investigated in two progeny tests. One of the progeny tests involved a comparison between Romney and Poll Dorset rams used in the first experiment and the other a comparison among the Romney rams used in the second experiment. Results from these progeny tests were related to differences in seasonal variation in testis size and other endocrine parameters found between breeds, or between rams within a breed, to evaluate the likely usefulness of these indicator traits in predicting a ram's genetic merit for early onset of the breeding season. The ultimate test of their usefulness would be to carry out selection experiments based on these indicator traits, but this was not possible within the time frame of this study. In addition, another experiment was conducted to investigate the breed differences in the mechanisms by which season affects gonadotrophin secretion in the ram.

The studies which comprised this programme were therefore designed to address the following questions:

1) To what extent are there differences between Poll Dorset and Romney rams, and among Romney rams, in the pattern of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH challenge during the transitional period from the nonbreeding to the breeding season?

2) What is the likely usefulness of the pattern of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH challenge during the transitional period from the nonbreeding to the breeding season in predicting a ram's genetic merit for early onset of the breeding season?

3) What differences exist between Romney and Poll Dorset rams in the mechanisms by which season affects gonadotrophin secretion?

C H A P T E R II:

GENERAL MATERIALS AND METHODS

CHAPTER II:

GENERAL MATERIALS AND METHODS

This chapter describes materials and methods which were used in more than one experiment. Materials and methods which were unique to a particular experiment are described in the chapter relating to that experiment.

1. Animal management and housing

Animals used in trials described in this thesis were grazed on mixed ryegrass and clover pastures under normal farm management except during the indoor trial periods. At each indoor trial period, the rams involved were brought indoors and housed in metabolism crates for 10 days before the planned blood sampling date in order to allow them to adjust to the experimental conditions and handling. The room in which the animals were housed had large windows so that rams were exposed only to natural light (except on the night of blood sampling when artificial light was used until completion of the sampling). While indoors, they received a maintenance diet of chaffed lucerne hay supplemented with minerals (59% sodium chloride, 37% sodium sulphate and 4% sodium molybdate) at a rate of 1 g/day to counteract possible copper toxicity (Rattray, 1986). Fresh water was available *ad libitum* at all times. The rams were returned to pasture 3 days after completion of each blood sampling period. The latitude of the location where all trials were conducted is 40.1°S. At this latitude the annual variation in photoperiod is from 9.3 h (22 June) to 15.1 h (22 December).

2. Jugular cannulation, blood sampling and GnRH challenge

Jugular vein cannulae were inserted as described by Carter et al. (1989) one day before the date of blood sampling. All blood samples were collected via the cannulae, using 6 ml syringes, into centrifuge tubes containing 100 iu sodium heparin (batch 2091098, New Zealand Pharmaceuticals, Palmerston North) and immediately placed on ice. Within 25 min of collection they were centrifuged at 3,000 g and 4°C for 20 min. The plasma was harvested and stored in duplicate vials at -20°C until hormone analyses were conducted.

The pituitary responsiveness to GnRH was measured by the LH response to an intravenous injection of synthetic GnRH (Batch #2, NIDDK, NIH) administered at a dose of 50 ng/kg liveweight (Wilson & Lapwood, 1978). The GnRH was dissolved at a concentration of 1 μg/ml in saline 1 h prior to injection and administered via the

cannulae followed by 4 ml of heparinized saline to flush the cannulae. Further blood samples were collected at 10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min after GnRH injection.

3. Technique for testis diameter measurements

Measurements on testis diameters were carried out using a pair of dial calipers (Mitutoyo, Japan). The maximum diameter of each testis was measured in the anterior-posterior plane with the ram being held in a sitting position and its testes being lightly pulled down to the bottom of the scrotum. Three measurements were taken alternately on both testes and the average of the six measurements was taken as the testis diameter. Wool on the scrotum was clipped at every second measurement period. In order to test the reliability of the technique for testis diameter measurement, two preliminary studies were conducted. In the first study, testis diameters of 18 Romney rams were measured twice 1 h apart. Repeatability of measurements obtained from this study was 0.90±0.05 (mean±SEM). In the second study, testis diameters of 24 Southdown rams were measured before and after the animals were slaughtered. Pearson correlation coefficients between the live measurements and the diameter and weight of the dissected testes were 0.91 and 0.90 respectively ($P < 0.001$). These relationships are shown graphically in Figure 2.1.

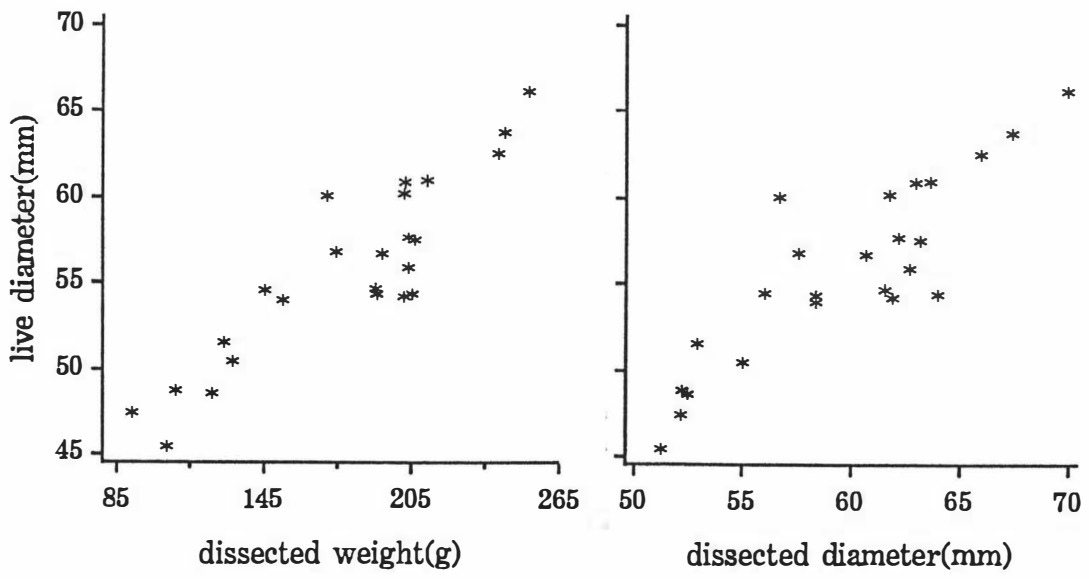

Figure 2.1. Diagrammatical illustration of the relationship between testis diameter measured before slaughter and the weight (left panel) and diameter (right panel) of the dissected testes after slaughter.

4. Set-up and validation of the hormone assays

The following section describes in detail the procedures followed in setting up and validating the radioimmunoassays for LH and FSH

4.1. Luteinizing Hormone

The LH assay was a double-antibody radioimmunoassay based on a kit and protocol supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, and the National Hormone and Pituitary Program, University of Maryland School of Medicine, U.S.A.

4.1.1. Components of the assay

4.1.1.1. Standard

Ovine LH (NIADDK-oLH-25, AFP-5551B) with a biological potency of 2.3 times that of NIH-LH-S1 was used as the reference standard. A stock solution of this material at 10 μg/ml in 1% (w/v) bovine serum albumin (BSA, Fraction V, Lot No. 11686421-35, Boehringer Mannheim GmbH, Germany) in 0.01 M phosphate buffered saline (PBS) was created. This 10 μg/ml LH solution was then used to create the standard curve concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 ng/ml by serial dilution in 0.3% BSA-PBS.

4.1.1.2. First antibody

The antiserum used in this assay was rabbit anti-ovine LH (NIADDK-anti-oLH-1, AFP-192279). For use in the assay, it was diluted with 0.5% normal rabbit serum in 0.01 M PBS to give a final tube dilution of 1:2,000,000.

4.1.1.3. ^{125}I-labelled LH

Ovine LH (NIDDK-oLH-I-3, AFP-9598B) was used for iodination by the chloramine-T method (Greenwood et al., 1963). Prior to each iodination, a small aliquot of this hormone was weighed out and solubilized in 0.01 M PBS at 500 μg/ml. This solubilized hormone was used for iodination. At the time of iodination, 1 mCi Na^{125}I (10 μl, Amersham Laboratories, England) was first added to the iodination vial (a siliconized, V-bottomed glass vial). Next, 10 μl of the 500 μg/ml LH solution was added to the vial. Immediately thereafter, the iodination process was started by the addition of 16 μg of chloramine-T in 10 μl volume (1.6 mg/ml in 0.5 M phosphate

buffer, pH 7.5). The reaction was allowed to proceed for 30 seconds before being terminated by the addition of 100 µg of sodium metabisulphate in 25 µl volume (4 mg/ml in 0.5 M phosphate buffer, pH 7.5). 100 µl of 2% BSA-PBS was then added to the vial to act as a protection buffer. The contents of the vial were immediately transferred to a 0.8 cm x 20 cm column of Sephadex G-50 (medium, particle size 50-150 µ, Pharmacia, Uppsala, Sweden). The column was then eluted with 0.05 M phosphate buffer (pH 7.5) to separate the labelled hormone from the free iodine. Seven drops of the eluate were collected into each of 35 LP3 tubes (Luckham Ltd, Sussex, England) containing 50 µl of 2% BSA-PBS. A 5 µl aliquot from each tube was then counted and the elution profile established. A typical elution profile is shown in Figure 2.2. The LH fraction with the highest count was stored frozen and used in the assay within 3 weeks.

For use in each assay, the radio-labelled hormone was diluted with 0.5% normal rabbit serum in 0.01 M PBS to give about 10,000 counts per minute (CPM) in a 100 µl aliquot, which was the volume of trace added to each tube.

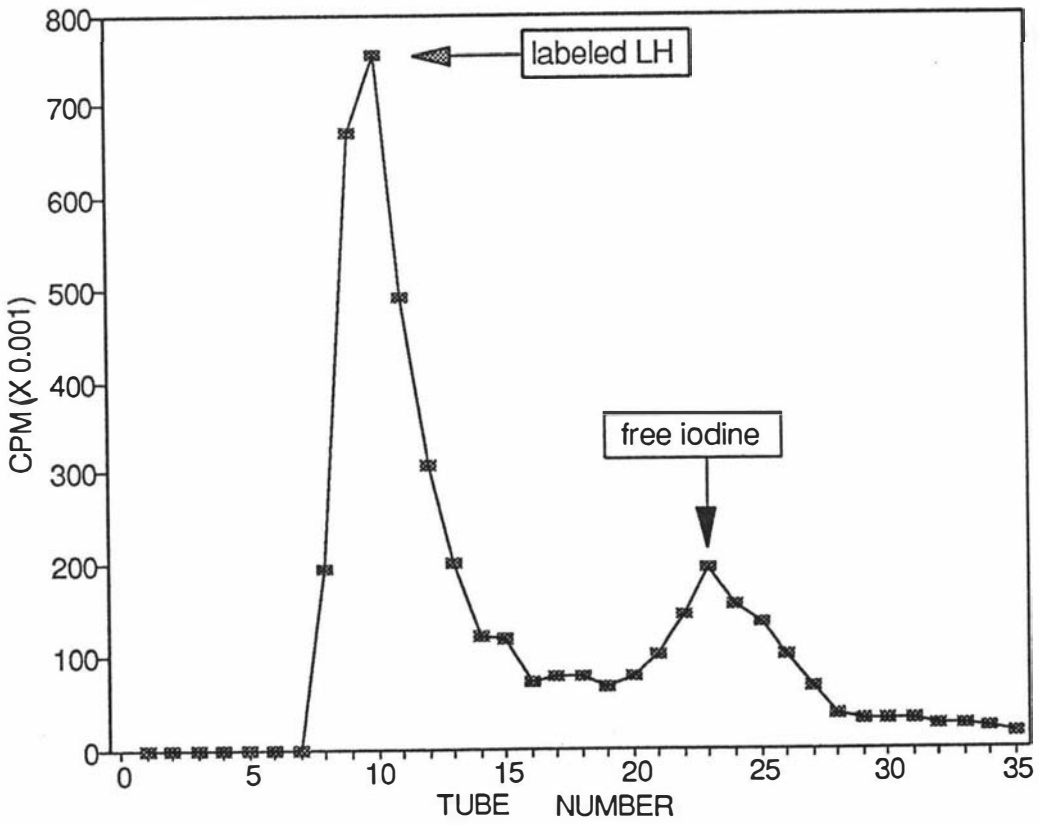

Figure 2.2. A typical elution profile for the LH iodination procedure. Pictured in this diagram is the CPM in a 5 µl aliquot from each of 35 tubes collected in sequence.

4.1.1.4. Second antibody

The second antibody used in the assay was either goat anti-rabbit gamma globulin (Cat. No. ALGP-050, Immuno-Chemical Products Ltd, NZ) or donkey anti-rabbit gamma globulin (part no. A-PPT1, IDS, England). It was diluted to 1:400 with 6% polyethylene glycol in 0.01 M PBS. One ml of the 1:400 solution was added to each of the assay tubes.

4.1.2. Assay procedures

All samples were assayed in triplicate. Reagents were added to the assay tubes at a single sitting in the following sequence: standards or plasma samples (200 µl); first antibody (100 µl); and trace (100 µl). The reagents were then incubated at room temperature for 24 hours prior to the addition of the second antibody (1 ml). After addition of the second antibody, the tubes were vortexed and left to stand on the bench for 1 hour before being centrifuged at 3,000 g and 4°C for 30 min. After centrifugation, the supernatant was aspirated and the pellet remaining in the tube counted on a LKB-Wallac 1261 Multigamma counter (Wallac Oy, Finland). Results were calculated using the computer programme "RiaCalc" supplied with the counter.

4.2. Follicle Stimulating Hormone

The FSH assay was a double-antibody radioimmunoassay using a kit and protocol supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health, and the National Hormone and Pituitary Program, University of Maryland School of Medicine, U.S.A.

4.2.1. Components of the assay

4.2.1.1. Standard

Ovine FSH (NIAMDD-oFSH-RP-1, AFP-5679C) was used as the reference standard. Prior to the creation of the first set of standards, one vial (5 µg/vial) of the NIAMDD-oFSH-RP-1 supplied was reconstituted with 1 ml distilled water to provide a stock solution of 5 µg/ml. Aliquots of this 5 µg/ml solution were then prepared and stored

frozen for subsequent use for a period of no longer than 6 months. FSH standard curve concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 ng/ml were created from the stock solution by serial dilution in 0.3% BSA-PBS.

4.2.1.2. First antibody

The antiserum used in this assay was rabbit anti-ovine FSH (NIDDK-anti-oFSH-1, AFP-C5288113). For use in the assay, it was diluted with 0.5% normal rabbit serum in 0.01 M PBS to give a final tube dilution of 1:80,000.

4.2.1.3. ^{125}I-labelled FSH

Ovine FSH (NIDDK-oFSH-I-1, AFP-5679C) was used for iodination by the chloramine-T method (Greenwood et al., 1963). Prior to each iodination, a small aliquot of NIDDK-oFHS-I-1 was weighed out and solubilized in 0.01 M PBS at 100 µg/ml. This solubilized hormone was used for iodination. At the time of iodination, 0.5 mCi $Na^{125}I$ (5 µl, Amersham Laboratories, England) was first added to the iodination vial (a siliconized, V-bottomed glass vial). Next, 20 µl of the 100 µg/ml FSH solution was added to the vial. Immediately thereafter, the iodination process was started by the addition of 16 µg of chloramine-T in 10 µl volume (1.6 mg/ml in 0.5 M phosphate buffer, pH 7.5). The reaction was allowed to proceed for 30 seconds before being terminated by the addition of 100 µg of sodium metabisulphate in 25 µl volume (4 mg/ml in 0.5 M phosphate buffer, pH 7.5). 200 µg of KI in 50 µl volume was then added followed by 100 µl of 2% BSA-PBS. The contents of the vial were immediately transferred to a 0.8 cm x 20 cm column of Sephadex G-50 (medium). The column was then eluted with 0.05 M phosphate buffer (pH 7.5) to separate the labelled hormone from the free iodine. Eight drops of the eluate were collected into each of 30 LP3 tubes containing 50 µl 2% BSA-PBS. A 5 µl aliquot from each tube was then counted and the elution profile established. A typical elution profile is shown in Figure 2.3. The FSH fraction with the highest count was stored frozen and used in the assay within 3 weeks.

For use in each assay, the radio-labelled hormone was diluted with 0.5% normal rabbit serum in 0.01 M PBS to give about 10,000 counts per minute (CPM) in a 50 µl aliquot, which was the volume of trace added to each tube.

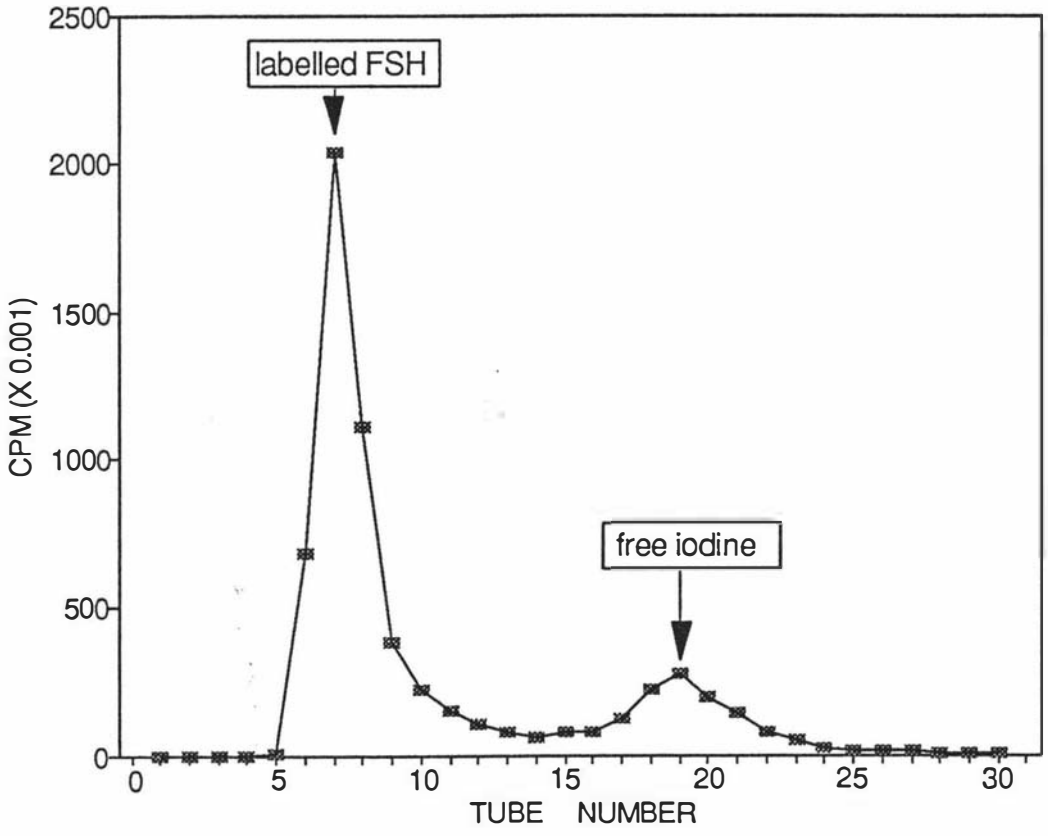

Figure 2.3. A typical elution profile for the FSH iodination procedure. Pictured in this diagram is the CPM in a 5 µl aliquot from each of 30 tubes collected in sequence.

4.2.1.4. Second antibody

The second antibody used was either goat anti-rabbit gamma globulin (Cat. No. ALGP-050, Immuno-Chemical Products Ltd, NZ) or donkey anti-rabbit gamma globulin (part no. A-PPT1, IDS, England). It was diluted to 1:400 with 6% polyethylene glycol in 0.01 M PBS. One ml of the 1:400 solution was added to each of the assay tubes.

4.2.2. Assay procedures

All samples were assayed in triplicate. Reagents were added to the assay tubes at a single sitting in the following sequence: standards or plasma samples (100 µl); first antibody (50 µl); and trace (50 µl). The reagents were then incubated at room

temperature for 24 hours prior to the addition of the second antibody (1 ml). After addition of the second antibody, the tubes were vortexed and left to stand on the bench for 1 hour before being centrifuged at 3,000 g and 4°C for 30 min. After centrifugation, the supernatant was aspirated and the pellet remaining in the tube counted on a LKB-Wallac 1261 Multigamma counter (Wallace Oy, Finland). Results were calculated using the computer programme "RiaCalc" supplied with the counter.

4.3. Validation of the LH and FSH assays

4.3.1. Specificity

Specificity of the antisera to LH and FSH was not checked in this laboratory. Information supplied by NIDDK showed that the specificity of the LH antiserum, in terms of its reactivity with highly purified pituitary hormones (relative to NIDDK-oLH-23), is: oFSH, 5.3%; bTSH, <0.2%; oGH, <1%; oPRL, <0.1%. The specificity of the FSH antiserum, in terms of its reactivity with highly purified oPRL, oLH and oGH (relative to NIAMDD-oFSH-RP-1), is <0.2% for all the hormones tested.

4.3.2. Parallelism

Reconstitution of the LH and FSH standards in 0.3% BSA-PBS buffer yielded standard curves parallel to those in which the standards were reconstituted in hypophysectomized sheep serum (Figure 2.4A & B). The parallelism between the LH and FSH standards and blood samples was demonstrated by incubating increasing volumes (50-800 µl) of sheep plasma. Sheep plasma displaced tracer in parallel with the standards (Figure 2.4A & B).

4.3.3. Sensitivity

The sensitivity of each assay was calculated from the 95% confidence limits of the buffer control tubes.

4.3.4. Precision

Assay precision was measured by including, in every assay, a sample from each of three large plasma pools containing different concentrations of LH or FSH. Results from these samples were subjected to analysis of variance to obtain the within- and between-assay coefficients of variation at each hormone concentration.

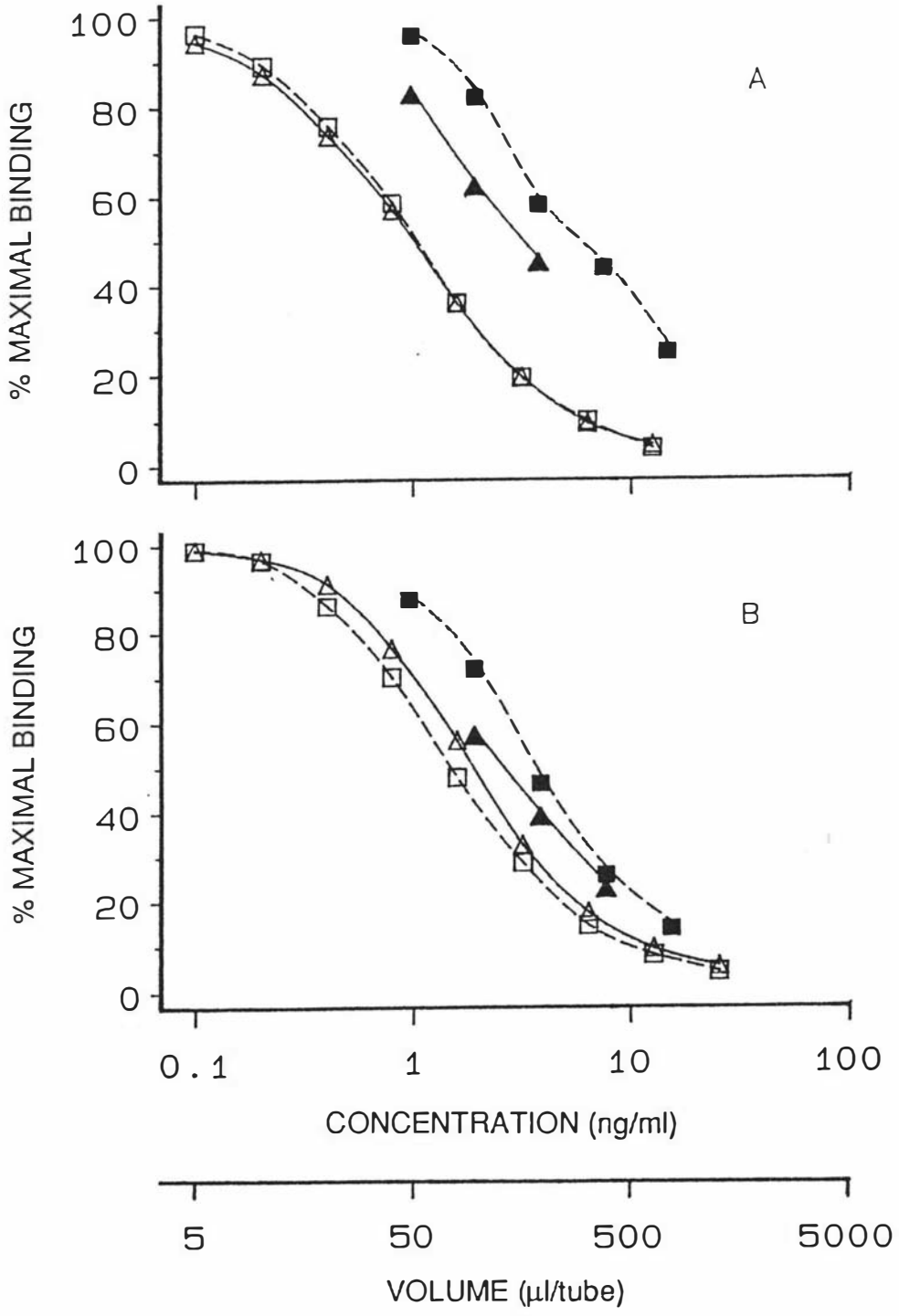

Figure 2.4. Parallelism of the LH (Panel A) and FSH (Panel B) radioimmunoassays. Shown in the Figures are two standard curves for which the standards were made up in either hypophysectomized ewe serum (△——△) or 0.3% bovine serum albumin in 0.01 M phosphate buffered saline (□---□). Parallelism between the standards and plasma samples was demonstrated by inhibition of binding during incubation of increasing volumes (50-800 µl) of ewe (▲——▲) and ram (■---■) plasma.

5. Characterization of hormone secretory profiles

Plasma LH and FSH concentrations in serial samples from individual rams at each sampling period were processed by the computer programme "DETECT" to identify the presence of secretion pulses (Guardabasso et al., 1988). Parameters for the variance model used in "DETECT" were obtained using the spreadsheet programme "PREDETEC" supplied with "DETECT". The following criteria were used in deciding from the programme output if a pulse had occurred: a) a pulse must reach its peak within two sampling periods from its preceding nadir (Goodman & Karsch, 1980); and b) a pulse that occurs before the start of blood sampling or has not returned to baseline concentration at the end of a sampling period is counted as 0.5 of a pulse. Since no FSH pulses were detected, only mean FSH values were computed. The LH secretory profile was characterized by the following four parameters: a) pulse frequency, which was calculated as the total number of pulses occurring during a sampling period; b) pulse amplitude, which was determined by the highest LH concentration associated with an individual pulse minus the basal value; c) mean LH concentration, which was calculated by averaging values for all the samples taken from each ram during a sampling period; and d) basal LH concentration, which was obtained by averaging values for samples not associated with any LH pulse. The LH response to GnRH challenge was characterized by the following two parameters: a) peak response, which was the highest LH concentration post-GnRH injection minus the basal concentration; and b) total response, which was measured by the area under the response curve above basal concentration (as calculated by triangulation).

CHAPTER III:

A PRELIMINARY STUDY OF THE EFFECTS OF EXOGENOUS MELATONIN, ADMINISTERED IN EARLY SUMMER, ON TESTIS DIAMETER OF FLEECEWEIGHT-SELECTED AND CONTROL RAMS

CHAPTER III:

A PRELIMINARY STUDY OF THE EFFECTS OF EXOGENOUS MELATONIN, ADMINISTERED IN EARLY SUMMER, ON TESTIS DIAMETER OF FLEECEWEIGHT-SELECTED AND CONTROL RAMS[1]

1. Abstract

The effects of continuous melatonin treatment in early summer on testis growth prior to the onset of the breeding season were studied in rams from the Massey University fleeceweight-selected (FW) and control (C) lines. Animals from both lines were randomly divided into two groups. One group of rams from each line was injected with micro-encapsulated melatonin in adjuvant which maintained plasma melatonin concentrations above 100 pg/ml during daytime for about 5 weeks. Rams in the remaining 2 groups were injected with adjuvant only. The melatonin treatment significantly ($P < 0.001$) altered the pattern of seasonal variation in testis size. Compared with the adjuvant-only treatment, the melatonin treatment significantly stimulated testis growth during the period when plasma melatonin concentrations were high, but caused a significant depression in testis diameter after the plasma melatonin concentrations had returned to normal. The testis diameter of FW rams was significantly greater than that of C rams as the breeding season approached. There were indications that the FW rams were less responsive to melatonin treatment than the C rams.

2. Introduction

Recent studies with sheep and several other mammals have clearly shown that the pineal gland, through its secretion of melatonin, is involved in mediating the effects of changing photoperiod on reproduction and other seasonal processes (see review by Arendt, 1986). Pinealectomy, or interruption of the sympathetic innervation of the pineal gland, renders animals incapable of responding to changes in photoperiod. In sheep, melatonin secretion is restricted to the dark phase of the day and the duration of elevated plasma melatonin concentrations parallels that of the dark period (Bittman, 1985; Arendt, 1986). It has been demonstrated that the duration of the night-time melatonin secretion codes daylength in sheep (Bittman, 1985; Wayne et al., 1988).

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1 This is a secondary study conducted concurrently with a study designed to investigate the effect of exogenous melatonin, administered in summer, on wool growth (Harris et al., 1989). Therefore, the flexibility in the design of this experiment was limited.

In the ewe, attempts to advance the time of onset of the breeding season by administering exogenous melatonin through feeding, timed injection and implantation have been proved successful (Kennaway et al., 1987; Poulton et al., 1987; Waller et al., 1988). In contrast, information concerning the effect of exogenous melatonin on reproductive activity in the ram is limited. Lincoln and Ebling (1985) found that melatonin implantation in rams exposed to long days induced all the reproductive changes normally associated with an exposure to short days and rendered the animals nonresponsive to changes in photoperiod. It has also been shown that alternating 16-week periods with exogenous melatonin administered through implants to superior-cervical-ganglionectomized rams were able to induce clearly defined reproductive cycles similar to those induced by alternating exposure to short- and long-photoperiod in intact animals (Lincoln, 1988). Tekpetey & Amann (1988) found that, in the northern hemisphere, injection of melatonin late in the afternoon for 45 days starting from mid-May resulted in a non-significant increase in testis weight in early July, but a significant decrease in testis weight in September, as compared to untreated animals.

Romney hoggets from a Massey University flock selected for greasy fleece weight (the FW line; Blair, 1986) have a less marked seasonal wool growth pattern than control (C) hoggets (from a randomly bred flock) when grazed on pastures (McClelland et al., 1987). Ewe hoggets from the FW line also maintain a longer breeding season (i.e. enter anoestrus later) than C hoggets (McClelland, 1990). These results may indicate a change in the responsiveness to photoperiod of animals in the FW line. Therefore, the aims of the present trial were to study the effects of exogenous melatonin, administered in early summer prior to the onset of the breeding season, on seasonal changes in testis diameter and to investigate differences between rams of the FW and C lines in their response to exogenous melatonin with respect to testis diameter.

3. Materials and methods

3.1. Animals and treatments

The experiment was a balanced 2 x 2 (line x melatonin treatment) factorial design involving 24 FW and 24 C ram hoggets (aged 15 months). All animals were weighed and assigned randomly within line and sire to the two treatment groups on 17 November, 1987. On the following day, half of the animals in each line were injected subcutaneously in the back of the neck with micro-encapsulated melatonin in adjuvant (a melatonin preparation still under development by DSIR, Palmerston North, NZ) at a rate of 1.9 mg/kg liveweight. The remaining animals were injected with adjuvant only.

Throughout this experiment, all animals were managed as a single group and grazed on mixed ryegrass and clover pastures.

3.2. Testis measurement

At approximately 3-4 week intervals until the end of the trial in April 1988, the animals were weighed and their testis diameters measured.

3.3. Blood sampling

A single blood sample was collected from each ram by jugular venipuncture one day before treatment and at 2, 6, 10, 17, 24, 36, 59, 90 and 120 days after treatment to monitor changes in plasma melatonin concentrations. Blood sampling was carried out between 0900h and 1100h. All blood samples were collected into EDTA vacutainers, centrifuged at 2500 g and 4°C for 20 minutes and plasma recovered. Aliquots of 1 ml of plasma collected on the same day from animals in each of the four experimental groups were pooled and stored at -20°C until being assayed for melatonin using the method described by Fraser et al. (1983).

3.4. Statistical analyses

Data were subjected to analysis of variance for repeated measurements to test the effects of line, treatment, time and their interactions on testis diameter using the GLM procedure of the SAS statistical package (SAS Institute Inc., 1988). Values presented for testis diameters are least squares means (LSM) calculated from a model that included line, treatment, their interactions and the liveweight prior to start of the treatment as a covariate.

4. Results

4.1. Liveweights and Melatonin profiles

There were no significant differences between the four treatment by line groups in liveweight at any time during the trial period. Figure 3.1 shows the changes in circulating melatonin concentrations for the two groups of rams treated with

melatonin. There was a biphasic pattern of release of melatonin from the microcapsules, which maintained plasma melatonin concentrations above 100 pg/ml for about five weeks. For the remaining groups treated with adjuvant only, the circulating concentrations of melatonin rarely exceeded the assay sensitivity (6 pg/ml, data not shown).

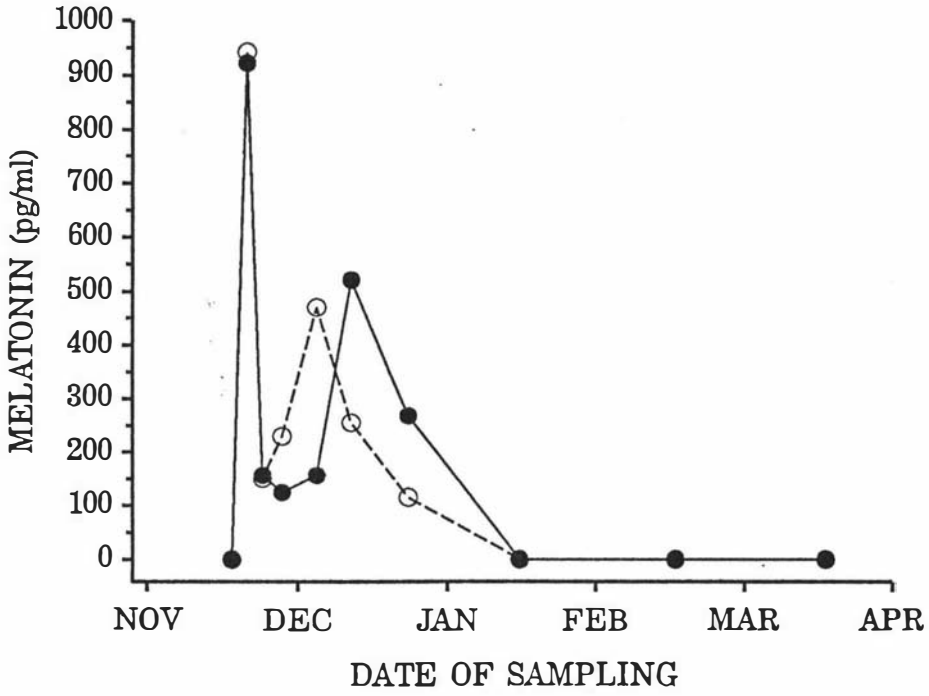


Figure 3.1. Changes in daytime plasma melatonin concentrations in rams of the FW (●——●) and C (o- - -o) lines treated with melatonin. Samples were pooled within groups at each sampling time and the means therefore have no variance.

4.2. Testis diameter

The effects of treatment and line on the seasonal changes in testis diameter over the period from November to April are shown in Figure 3.2A & B respectively. Melatonin treatment significantly affected the seasonal changes in testis diameter, as indicated by a significant ($P < 0.001$) time x treatment interaction. Testis diameter was significantly greater in melatonin-treated animals over the late-December to mid-January period as compared to untreated animals. However, the situation was reversed in February and March (i.e. after the microcapsules had expired and melatonin

concentrations returned to normal). During this time there was a significant depression in the testis diameter of melatonin-treated rams while that of untreated animals continued to increase. By late April, testis size of treated animals had recovered to a value not significantly different from that of untreated animals.

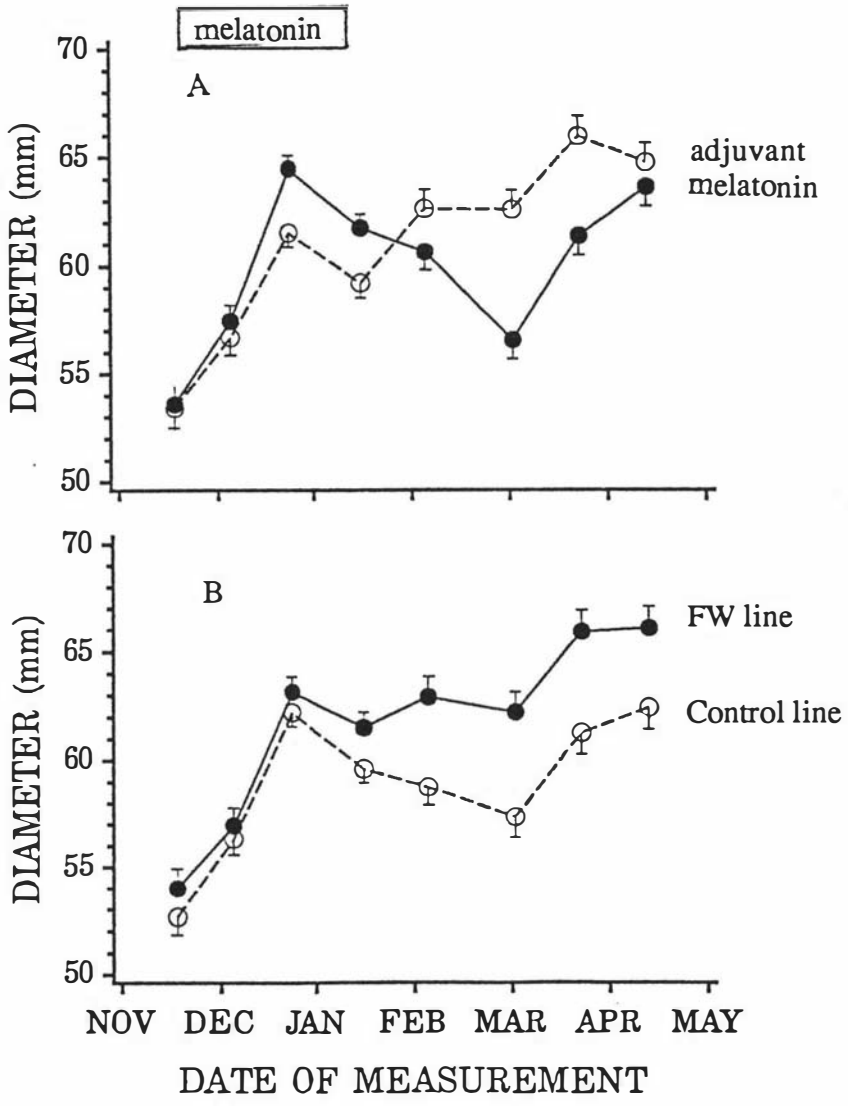


Figure 3.2. Changes in testis diameters of rams: (panel A) treated with melatonin (●——●) or adjuvant (o- - -o); (panel B) from FW (●——●) or C (o- - -o) selection lines; Approximate duration of the melatonin treatment is shown above panel A. Vertical bars represent standard errors of the least square means.

The testis diameter of FW rams was significantly ($P < 0.01$) greater than that of C rams over the January to April period. However, line differences in the pattern of seasonal changes in testis size were nonsignificant ($P > 0.10$ for line x time interaction). There was a significant ($P < 0.05$) line x treatment x time interaction, suggesting that the effects of melatonin treatment on seasonal changes in testis diameter differed between the two lines (Figure 3.3). Further analyses of the data within each time point revealed that the interactions between line and treatment were significant ($P < 0.05$) in January. At this time, the testis diameter of C rams treated with melatonin was significantly ($P < 0.01$) greater than that of C rams treated with adjuvant, but there was no significant difference between FW rams treated with melatonin and those treated with adjuvant.

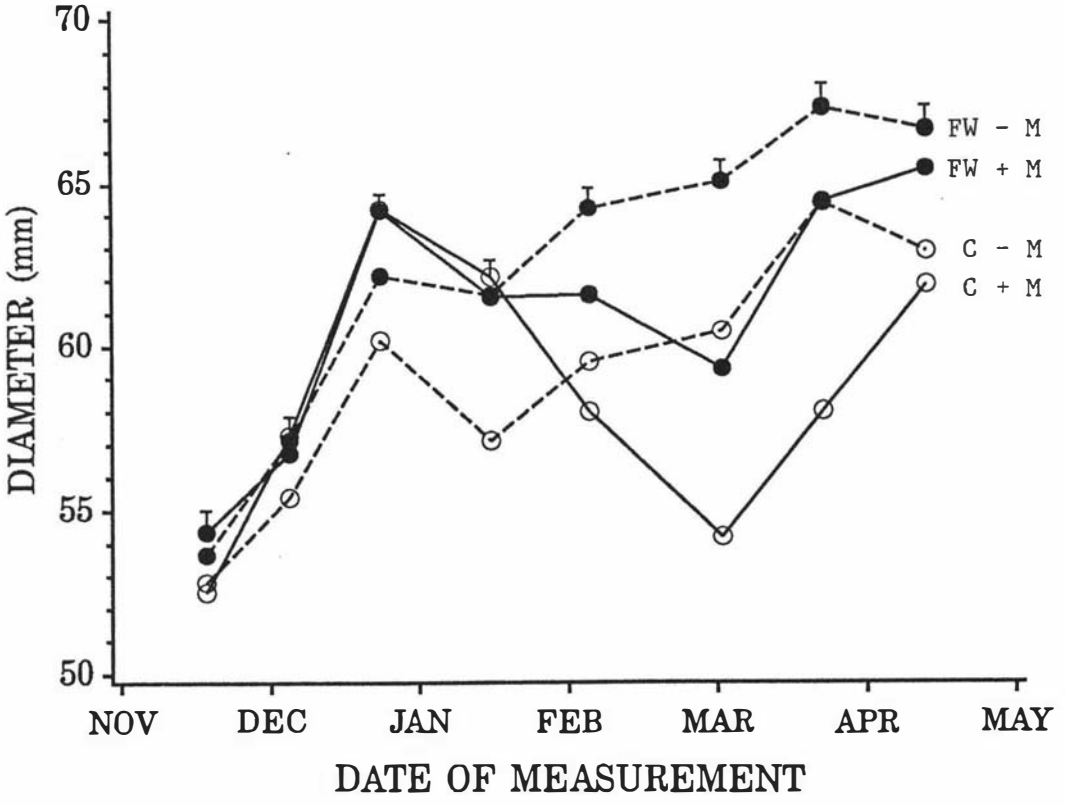


Figure 3.3. Changes in testis diameters of rams from the FW (●) and C (O) lines treated with melatonin (——) or adjuvant (------). Vertical bars represent pooled standard errors of the least square means.

5. Discussion

Results from this study clearly demonstrated that continuous administration of pharmacological doses of melatonin for five weeks prior to the onset of the breeding season was able to alter the pattern of seasonal changes in testis diameter. The initial effect of this treatment was to accelerate the rate of testis growth. This is consistent with results obtained when melatonin was implanted into rams exposed to a long photoperiod (Lincoln & Ebling, 1985). However, the stimulatory effect of the exogenous melatonin on testis growth was only temporary in the present study. Shortly after circulating concentrations of melatonin (measured during daytime) had returned to values similar to those found in untreated animals, testis growth was significantly depressed in rams previously treated with melatonin. This is in agreement with results from a recent study (Tekpetey & Amann, 1988) conducted in the northern hemisphere in which injection of melatonin late in the afternoon for 45 days starting from mid-May resulted in a non-significant increase in testis weight in early July, 48 days after the start of melatonin injection. However, compared with untreated animals, there was a significant decrease in testis weight in September, 80 days after the initiation of melatonin injection. Similarly, a daily oral administration of melatonin in the ewe for 30 days during mid-seasonal anoestrus delayed onset of the breeding season (Wigzell et al., 1988). It is unlikely that the decrease in testis size observed here was due to refractoriness to continued melatonin treatment as demonstrated by studies in which melatonin was administered for a longer period (Lincoln & Ebling, 1985). It has been shown that the continued presence of high concentrations of melatonin is interpreted by animals as a short day (Lincoln & Ebling, 1985; Wayne et al., 1988). Thus, like the situation in which animals are transferred from a long photoperiod to a short photoperiod, the melatonin treatment in this study would act to accelerate the rate of testis growth, a process which might have already commenced as a result of refractoriness to continued exposure to long days prior to melatonin treatment (Pelletier, 1986; Pelletier & Almeida, 1987). This explains the initial stimulatory effect of the melatonin treatment. It is possible that the 5 week exposure to exogenous melatonin served to break the photorefractoriness to long days. As a result, when the melatonin treatment stopped in late December, the animals interpreted the prevailing photoperiod as long days (Summer Solstice is 22 December). This might be the reason for the depression in testis size observed in February and March. However, as the daylength became shorter in March, the animals eventually responded with an increase in testis diameter, so that the difference between the two treatment groups in testis diameter was no longer significant by mid-April.

Although the patterns of seasonal changes in testis diameter were not significantly different between the two lines, rams from the FW line had significantly larger testes than those from the C line as they approached the breeding season. There were also differences between lines in their responses to melatonin treatment. Rams of the FW line appeared less sensitive to melatonin treatment, in terms of both the stimulatory and inhibitory responses, than those of the C line (see Figure 3.3). However, the exact implications of these findings to the observed differences between lines in their seasonal patterns of wool growth and oestrous activity are difficult to conceive due to the limitations of this trial. It seems likely that long-term selection for greasy fleeceweight might have reduced the animals' sensitivity to photoperiodic changes. Further studies are needed to elucidate the exact changes in the photoperiodic system.

In conclusion, results from the present trial showed that continuous administration of pharmacological doses of melatonin for five weeks in early summer was able to alter the pattern of seasonal changes in testis size. There were differences between the Massey University fleeceweight-selected and control lines in testis size during the January to April period and possibly a line difference in responses to exogenous melatonin and photoperiodic changes.

CHAPTER IV:

SEASONAL VARIATION IN TESTIS SIZE, GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN RAMS OF TWO BREEDS DIFFERING IN TIME OF ONSET OF THE BREEDING SEASON

CHAPTER IV:

SEASONAL VARIATION IN TESTIS SIZE, GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN RAMS OF TWO BREEDS DIFFERING IN TIME OF ONSET OF THE BREEDING SEASON

1. Abstract

A study was conducted to compare the variation in testis size, gonadotrophin secretion and pituitary responsiveness to an exogenous GnRH challenge during the transitional period from the non-breeding to the breeding season in rams of the Romney and Poll Dorset breeds. Testis size of rams of both breeds varied significantly ($P < 0.001$) during the trial period, with a significant ($P < 0.01$) breed difference in the timing and magnitude of this variation. Increases in testis size occurred earlier, but the magnitude of seasonal variation was smaller, in Poll Dorset rams. Overall, mean LH concentration was significantly ($P < 0.05$) higher in Romney rams due mainly to a difference ($P < 0.001$) in the frequency of LH pulses. There was also a significant ($P < 0.01$) effect of sampling time and a significant ($P < 0.05$) breed x time interaction in LH pulse frequency. Mean FSH concentrations exhibited significant ($P < 0.01$) variation with sampling time and the increase in FSH concentrations occurred earlier ($P < 0.10$) in Poll Dorset rams. There was a significant effect of sampling time on both the peak ($P < 0.01$) and the total ($P < 0.05$) LH responses to the GnRH challenge, but no significant effects of breed or breed x time interaction were detected. These results show that breed differences in seasonality are associated with differences in the pattern of variation in testis size and gonadotrophin secretion during the transitional period from the non-breeding to the breeding season. Some of these differences could potentially be used to select rams within a breed for date of onset of the breeding season in their daughters

2. Introduction

The seasonal nature of breeding in the ewe imposes a major limitation to improving the productive efficiency of well-managed sheep production systems. As a result, there is a need for the development of sheep breeds that can conceive at most times of the year. Selection for date of onset of the breeding season in the ewe will likely result in

only limited genetic progress due both to the low selection pressure that can be applied on the ewe and the fact that oestrous activity is a sex-limited trait on which selection pressure cannot be applied prior to puberty. The benefits of selecting in the male for female reproductive traits have been emphasized by Walkley and Smith (1980). Therefore, it would be desirable to identify genetic markers in the ram which could predict date of onset of the breeding season in their daughters and to use these markers in selection programmes.

Like ewes, rams exhibit marked seasonal variation in breeding performance (Lees, 1965; Haynes and Schanbacher, 1983). This seasonal variation in breeding performance is associated with variation in testis size (Islam and Land, 1977; Lincoln and Short, 1980; Dufour et al., 1984) and circulating gonadotrophin concentrations (Lincoln and Short, 1980; Sanford et al., 1984a; Pelletier and Almeida, 1987). Despite the abundance of information concerning the seasonal variation in testis size and gonadotrophin secretion, there is a lack of detailed study of the genetic differences in seasonal variation in these parameters, and their genetic relationship with timing of the breeding season in the ewe. Islam and Land (1977) observed a breed difference between Finnish Landrace and Merino rams in the time of minimum testis diameter. Within breeds, there are also differences among individual rams in the magnitude of seasonal variation in testis size and these differences seem to be repeatable from year to year (Ringwall et al., 1989). Breed differences in the seasonality of gonadotrophin secretion have been reported for Ile-de-France and Préalpes du Sud rams (Pelletier et al., 1982). However, none of these studies directly addressed the issue of identifying genetic markers in the ram for date of onset of the breeding season in the ewe. Lack of information in this area will limit the use of these potential selection criteria. If it is true that seasonal variation in testis size and/or gonadotrophin secretion are reliable genetic markers for date of onset of the breeding season, breeds differing in timing of the breeding season could also exhibit different seasonal changes in testis size and/or gonadotrophin secretion. The aims of the present study were therefore to compare the seasonal changes in testis size and gonadotrophin secretion during the transitional period from the non-breeding to the breeding season in rams of the Romney and Poll Dorset breeds, these being breeds which differ greatly in time of onset of the breeding season in the ewe (Hafez, 1952; Kelly et al., 1976; Knight et al., 1989). The normal dates of onset of the breeding season under the local conditions (40.1°S) in which this study was conducted are mid-January for Poll Dorset ewes and beginning of March for Romney ewes (Knight et al., 1989).

3. Materials and methods

Six Romney and six Poll Dorset rams (aged 2 years) were used in this trial. Beginning in September 1988 (during the nonbreeding season), testis diameters and liveweights of these rams were measured at 2-3 week intervals until the end of the trial in March 1989. On five occasions during this period (22 September, 1988; 9 November, 1988; 21 December, 1988; 2 February, 1989; and 15 March, 1989), the rams were brought indoors for intensive blood sampling and GnRH challenges. At each sampling period, 6 ml of blood was collected via indwelling cannulae at 15 min intervals for 8 hours commencing at 0900 h. After the last sample was taken (1700h), each animal received an intravenous injection of synthetic GnRH (NIDDK, NIH) at a dose of 50 ng/kg liveweight. Further blood samples were collected at 10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min after GnRH injection. All samples were analysed for FSH and LH (Chapter II) except for samples collected after the GnRH challenge which were analysed for LH only. The sensitivity of the LH assay was 0.09±0.01 ng/ml. The intra-assay coefficients of variation (c.v.) were 10.9, 5.2 and 3.6%, and the inter-assay c.v. 13.4, 11.6 and 9.6%, at mean LH concentrations of 0.5, 1.4 and 2.9 ng/ml, respectively. For the FSH assay, the sensitivity was 0.15±0.02 ng/ml. The intra-assay c.v. were 13.3, 8.5 and 6.5%, and inter-assay c.v. 16.4, 8.3 and 12.7%, at mean FSH concentrations of 1.0, 2.2 and 4.8 ng/ml respectively.

Analysis of variance for repeated measurements was used to test the overall effects of breed, sampling time and breed x time interaction on testis diameter and the endocrine parameters. In order to apply the repeated measures analysis to the testis diameter data, measurements taken on individual animals during each month were averaged to obtain a monthly mean for testis diameter. This parameter was then used in the analysis. To eliminate the heterogeneity of variance, data for LH pulse amplitude and the peak and total LH responses to GnRH were subjected to the logarithm transformation before analysis. Comparison between means at different sampling times was carried out using orthogonal contrasts. Analyses within a sampling time were conducted using univariate analysis of variance. All analyses were performed using the SAS statistical package (SAS Institute Inc., 1988).

4. Results

4.1. Testis diameter and liveweight

Mean testis diameters of Romney and Poll Dorset rams from September to March are shown in Figure 4.1. There were significant effects of time ($P < 0.001$) and breed x time interaction ($P < 0.01$) on testis diameter, indicating that testis size varied differently between the two breeds during the trial period. Testis diameter increased 28% ($P < 0.001$) in Romney rams from the smallest monthly value of 52.3 mm in November to the largest value of 64.2 mm in February. The corresponding increase in

Poll Dorset rams was 8% ($P < 0.01$) from 61.9 mm in October to 67.0 mm in January. The average liveweight of Poll Dorset rams (76.7±0.9 kg) was significantly ($P < 0.001$) greater than that of Romney rams (62.3±0.4 kg). Liveweight of the rams also varied during the experimental period (Figure 4.1). However, there was no significant association between seasonal variation in testis diameter and seasonal variation in liveweight. Correlation coefficients between testis diameter and liveweight across time were 0.38 and 0.30 ($P > 0.10$) for Romney and Poll Dorset rams respectively.

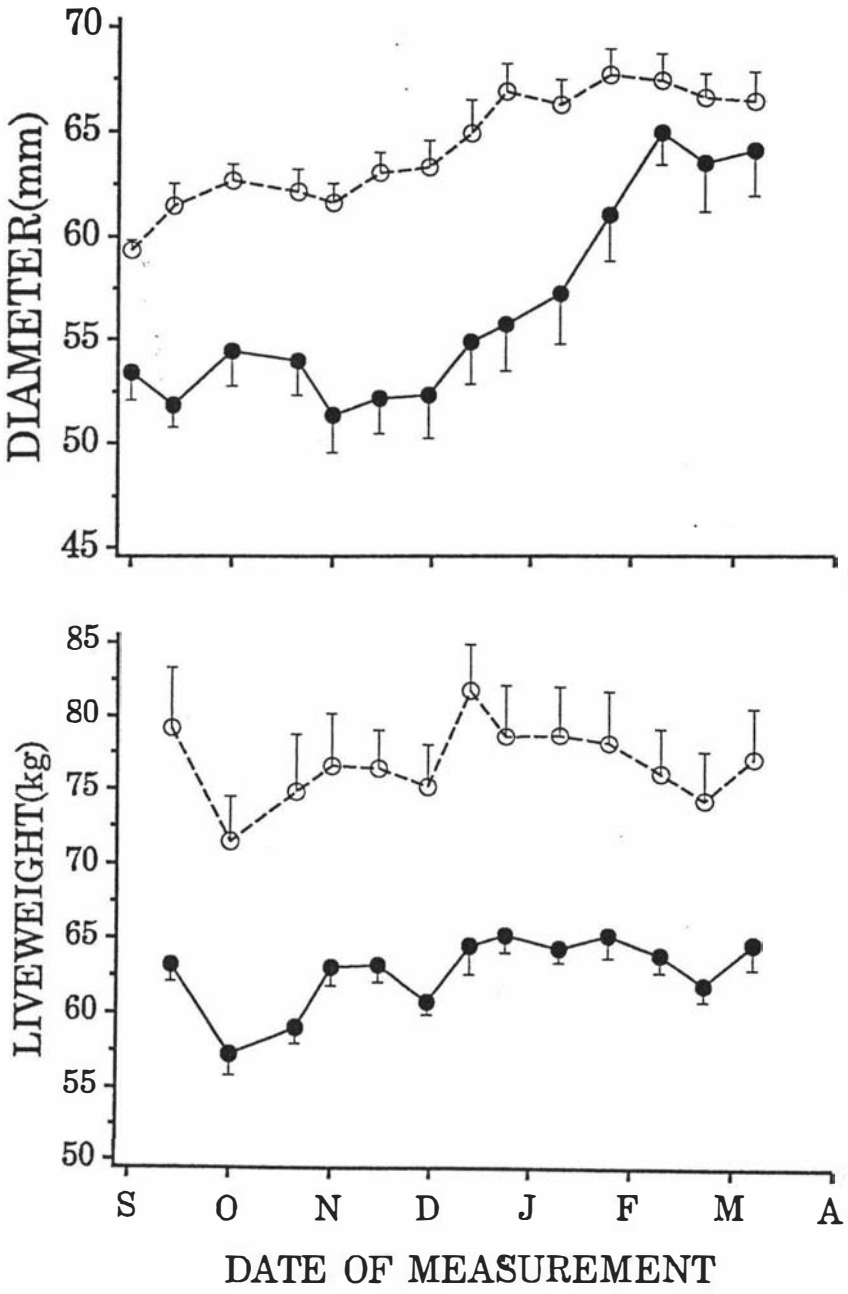


Figure 4.1. Variation in testis diameters (top panel) and liveweights (bottom panel) of Romney (n=6; ●——●) and Poll Dorset (n=6; o- - -o) rams during the period from September 1988 to March 1989. Vertical bars represent standard errors of the means.

4.2. Basal and Mean LH concentrations

No significant differences in basal LH concentrations were found. Over the whole trial period, mean LH concentrations were significantly ($P < 0.05$) higher in Romney (0.56±0.07 ng/ml) than in Poll Dorset (0.46±0.03 ng/ml) rams, with the major breed differences occurring in September, February and March (Table 4.1). The large increase in standard error for the mean LH concentration of Romney rams in February was due to the unusually high value of one ram (1.41 ng/ml, $P < 0.01$ for the outlier test based on kurtosis). When this value was excluded from the calculation, the mean LH concentration of Romney rams in February was 0.58±0.03 ng/ml, which was still significantly ($P < 0.01$) higher than that of Poll Dorset rams. The effects of time and breed x time interaction on mean LH concentrations were not significant.

Table 4.1. Effect of sampling time on LH secretion profile in Romney and Poll Dorset rams.[1,2]

Time	Breed (n=6)	Basal (ng/ml)	Mean (ng/ml)	Frequency (no./8h)	Amplitude (ng/ml)
22 Sept	Romney	0.43±0.01	0.53±0.02^a	1.25±0.31^a	1.26±0.08
	Dorset	0.42±0.01	0.44±0.02^c	0.42±0.20^b	1.08±0.10
09 Nov	Romney	0.44±0.01	0.46±0.02	0.75±0.17	0.96±0.19
	Dorset	0.42±0.01	0.49±0.05	0.50±0.18	2.64±1.21
21 Dec	Romney	0.41±0.01	0.51±0.03	0.92±0.27	1.77±0.25
	Dorset	0.43±0.02	0.51±0.03	0.83±0.17	1.54±0.19
02 Feb	Romney	0.45±0.02	0.72±0.14^a	2.25±0.44^a	1.62±0.30
	Dorset	0.41±0.01	0.45±0.02^b	0.75±0.25^b	1.00±0.20
15 Mar	Romney	0.44±0.01	0.56±0.02^a	2.42±0.39^a	0.91±0.09
	Dorset	0.41±0.02	0.43±0.03^c	0.75±0.31^c	0.92±0.08
Significance					
	Breed	NS	*	***	NS
	Time	NS	NS	**	NS
	Breed x Time	NS	NS	*	NS

[1] Values presented are means ± SEM.
[2] Values within each column and sampling time carrying different superscripts differ significantly (a vs b: $P < 0.05$; a vs c: $P < 0.01$).

4.3. Pulsatile LH secretion

Data for pulsatile LH secretion are presented in Table 4.1. Over the entire trial period, LH pulse frequency was significantly ($P < 0.001$) higher in Romney (1.52±0.41 pulses/8h) than in Poll Dorset (0.65±0.22 pulses/8h) rams, although the differences within sampling times reached significance only in September, February and March (Table 4.1). There were significant effects of sampling time ($P < 0.01$) and breed x time interaction ($P < 0.05$) on LH pulse frequency. In Romney rams, LH pulse frequency decreased slightly between September and November and then increased significantly ($P < 0.01$) between December and March to reach a value in March which was three times higher than that in November. In contrast, the seasonal variation in LH pulse frequency in Poll Dorset rams was less obvious and non-significant. No significant difference in LH pulse amplitude was detected.

4.4. Mean FSH concentrations

No FSH pulse was detected. The changes with sampling time in mean FSH concentrations of Romney and Poll Dorset rams are presented in Figure 4.2. There was a significant ($P < 0.01$) effect of time on plasma FSH concentrations, with a marginally significant ($P < 0.10$) breed x time interaction. In the Poll Dorset rams, the FSH concentrations increased significantly ($P < 0.01$) between September and February and then decreased ($P < 0.01$) in March. For the Romney rams, the FSH concentrations decreased slightly in November compared to September, and then increased ($P < 0.01$) to reach a maximum in February, before decreasing ($P < 0.01$) again in March. This resulted in a significant ($P < 0.05$) breed difference in mean FSH concentrations in November and a marginally significant ($P < 0.10$) breed difference in December. However, the overall breed difference in mean FSH concentrations was not significant (Romney, 1.03±0.21 ng/ml; Poll Dorset, 1.27±0.26 ng/ml; $P > 0.10$).

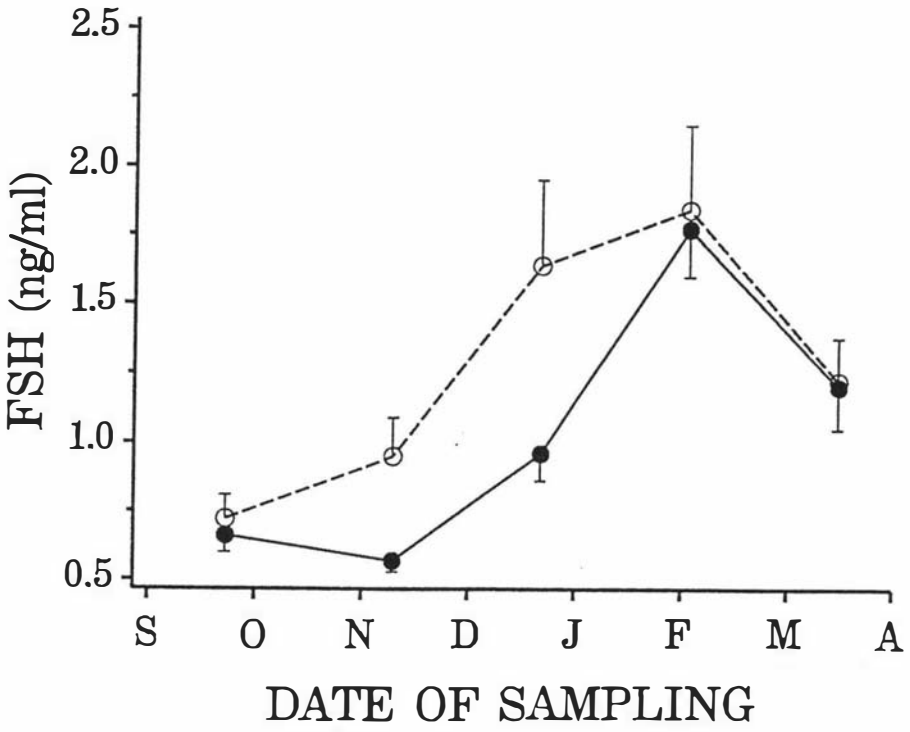


Figure 4.2. Mean plasma FSH concentrations of Romney (n=6, ●——●) and Poll Dorset (n=6, o- - -o) rams during the period from September 1988 to March 1989. Vertical bars represent standard errors of the means.

4.5. LH response to exogenous GnRH

The LH responses to a single intravenous injection of GnRH at various times are shown in Figure 4.3. There was a significant ($P < 0.01$) effect of time on the peak LH response to the GnRH challenge. The peak response decreased significantly ($P < 0.05$) in March compared to other times which did not differ significantly. No significant effects of breed or breed x time interaction on the peak LH response were apparent. The total LH response to the GnRH challenge, as measured by the area under the response curve above the basal concentration, varied significantly with sampling times ($P < 0.05$). In both breeds, the total responses increased over the summer and then decreased as the breeding season approached. There was a general trend towards Romney rams having greater total responses than Poll Dorset rams, but the overall effect of breed failed to reach significance (Romney, 632.5±198.5 min.ng/ml; Poll Dorset, 449.7±71.0 min.ng/ml; $P > 0.10$). This was due mainly to the large variability between rams within breeds. There was also no significant effect of breed x time interaction on the total LH response. The effects of breed, time and breed x time interaction accounted for only 30% of the variation in total LH response to GnRH.

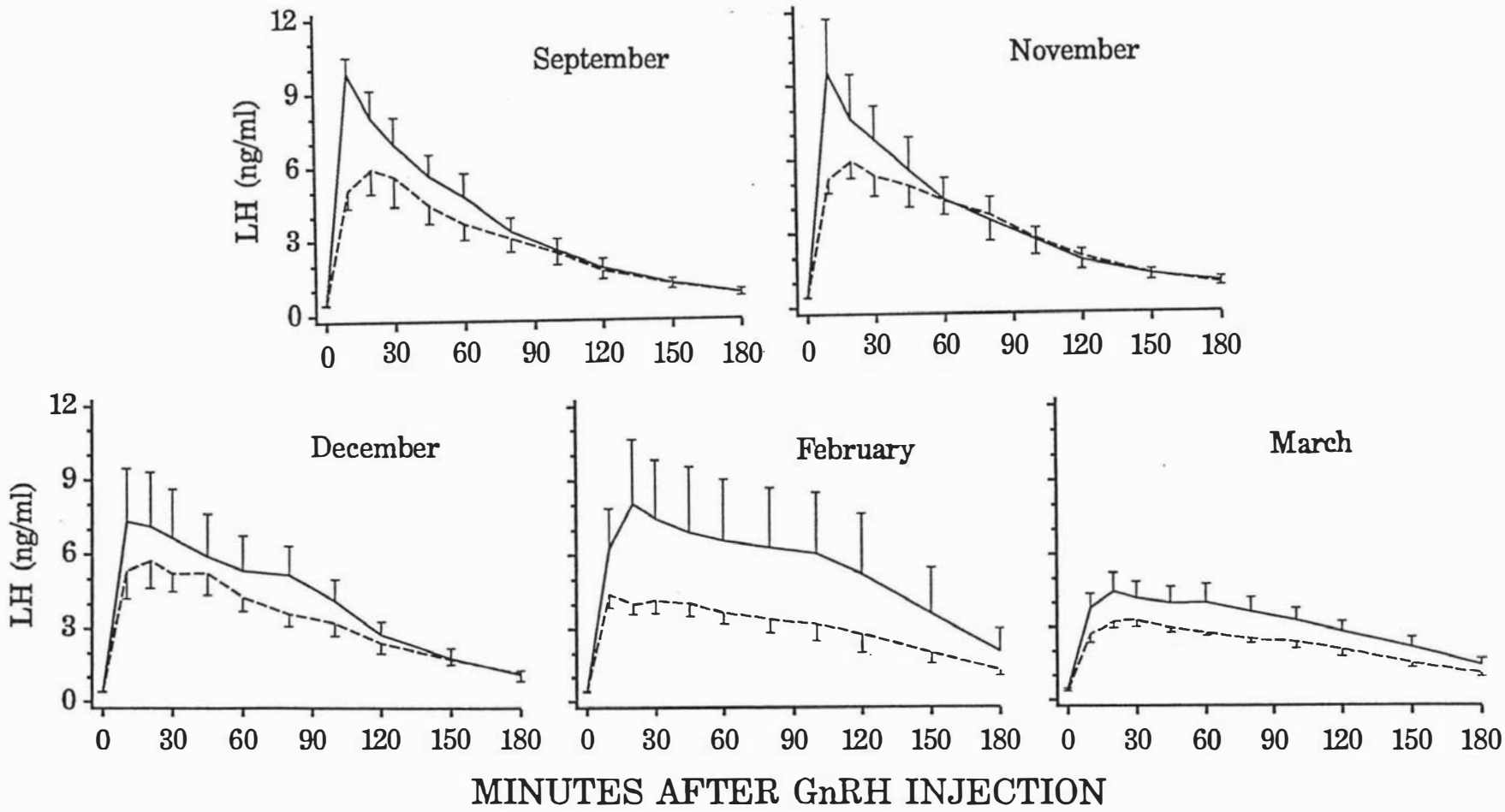


Figure 4.3. Plasma LH concentrations of Romney (n=6, ———) and Poll Dorset (n=6, - - - -) rams at different times (minutes) after a single intravenous injection of GnRH (50 ng/kg liveweight) on 22 September 1988, 9 November 1988, 21 December 1988, 2 February 1989 and 15 March 1989. Values at zero times are the basal concentrations obtained from samples taken during the 8 h period prior to GnRH injection. Vertical bars represent standard errors of the means.

5. Discussion

The present study compared the variation in testis size and gonadotrophin secretion between Poll Dorset and Romney rams during the transitional period from the non-breeding to the breeding season. Consistent with previous reports (Schanbacher and Ford, 1976; Islam and Land, 1977; Lincoln and Short, 1980; Pelletier et al., 1982; Sanford et al., 1984a), this study showed marked seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH in both Romney and Poll Dorset rams. However, the main objective of this trial was to identify some potential physiological and endocrinological characteristics in the ram which might be used as selection criteria for advancing the date of onset of the breeding season in their female progeny.

The first important finding from the present study was the observed difference between Romney and Poll Dorset rams in the timing and magnitude of seasonal variation in testis size. Although data from the present study did not allow statistical estimation of the time when testis size was at a minimum or maximum, it is apparent (see Figure 4.1) that the seasonal increase in testis size occurred earlier in Poll Dorset than in Romney rams. However, the magnitude of change was smaller in Poll Dorset (8%) than in Romney (28%) rams. Differences in seasonal variation in testis size have also been studied for other breeds. Islam and Land (1977) observed a difference of 49 days between Merino and Finnish Landrace rams in the time of minimum testis diameter. This difference was similar to that found between females of the same two breeds in time of onset of the breeding season (Wheeler and Land, 1977). Dufour et al. (1984) failed to show significant breed differences in time of seasonal increase in testis size. Rather they found that DLS rams (a "synthetic" population of 1/2 Dorset, 1/4 Leicester and 1/4 Suffolk selected for extended breeding season) had larger testes at the end of the breeding season than Suffolk rams. These differences between studies may be due to the different breeds used, because a breed difference in the length of the breeding season can be achieved by early onset and/or late cessation of the breeding season. Collectively, results from the present and previous studies suggest that, at least in the between-breed situation, time and/or magnitude of the seasonal increase in testis size of rams may be a good indicator of the timing of the breeding season in their female relatives.

Another important finding in the context of the present study was the marked breed differences in the pattern of seasonal variation in both FSH and LH secretion. For LH, the most prominent difference occurred in pulse frequency. In the more seasonal Romney rams, LH pulse frequency decreased slightly between September and November and then increased three-fold between November and March. In contrast, seasonal variation in LH pulse frequency between September and March was small and non-significant in the less seasonal Poll Dorset rams. For FSH, there was a breed difference in the time of the seasonal increase in circulating concentrations, which occurred earlier in Poll Dorset than in Romney rams. These results are in general agreement with those obtained from studies where rams of different breeds were exposed to artificial photoperiods of 6-month duration (D'Occhio et al., 1984). It has also been found that Poll Dorset rams reached a peak in testosterone concentrations earlier than other more seasonal breeds (D'Occhio and Brooks, 1983; Boland et al., 1985). Since the seasonal increase in testosterone secretion prior to the onset of the breeding season is dependent on increasing gonadotrophin stimulation (Lincoln and Short, 1980), a difference between breeds in the time of peak circulating testosterone concentrations would suggest a corresponding difference in timing of the seasonal increase in gonadotrophin secretion. In general, these results demonstrate that rams from breeds differing in seasonality of oestrous activity exhibit differences in time and magnitude of seasonal changes in gonadotrophin secretion.

The pattern of seasonal variation in the peak and total LH responses to a GnRH challenge was similar to that observed in other studies (Lincoln and Short, 1980; Sanford et al., 1984a). The present study failed to show any significant effect of breed or breed x time interaction on the characteristics of LH response to GnRH challenge. This is partly due to the large variation within breeds in the response, which was most noticeable for the Romney rams in December and February (see Figure 4.3). With respect to the identification of genetic markers in the ram for date of onset of the breeding season in the ewe, this large between-animal variation may be a potential resource to exploit.

In conclusion, results from the present trial show that breed differences in seasonality are associated with differences in the pattern of variation in testis size and gonadotrophin secretion during the transitional period from the non-breeding to the breeding season. Some of these differences could potentially be used to select rams within a breed for date of onset of the breeding season in their daughters.

CHAPTER V:

BREED AND SIRE EFFECTS ON PUBERTY AND TIMING OF THE BREEDING SEASON IN SHEEP

CHAPTER V:

BREED AND SIRE EFFECTS ON PUBERTY AND TIMING OF THE BREEDING SEASON IN SHEEP

1. Abstract

A progeny test was conducted to compare the effects of breed (Romney vs. Poll Dorset) and sire within breed (n=5) on pubertal oestrous activity and date of onset of the second (two-tooth) breeding season. Four hundred and ten mixed-age Romney ewes were randomly divided, with restriction to age, into 10 groups each of 41 ewes and each group was randomly assigned to be mated by one of the 10 rams. All female progeny were studied for pubertal oestrous activity and date of onset of the second breeding season. Compared with straightbred Romney hoggets, a significantly ($P < 0.05$) higher proportion of Poll Dorset cross hoggets reached puberty during the first breeding season (79.6 vs. 59.9%). Poll Dorset cross hoggets also reached puberty earlier (13 May±3 vs. 22 May±3, $P < 0.10$) and at a younger age (264±3 vs. 276±3 days, $P < 0.10$) and had significantly ($P < 0.05$) more pubertal oestrous cycles (2.7±0.2 vs. 2.0±0.2) than Romney hoggets. Sire within breed had a significant ($P < 0.05$) effect on the number of pubertal oestrous cycles but not on the date of, or age at, onset of puberty. Liveweight at the beginning of the breeding season had a significant effect on the percentage of hoggets reaching puberty during the first breeding season ($P < 0.01$) and on the number of pubertal oestrous cycles ($P < 0.05$). There were significant effects of breed ($P < 0.001$) and sire within breed ($P < 0.05$) on the date of onset of the second breeding season. The rams used in the progeny test had also been studied for seasonal variation in testis size and gonadotrophin secretion during the transitional period from the nonbreeding to the breeding season and significant breed differences in these parameters had been found (Chapter IV). However, pooled within-breed correlation coefficients between seasonal variation in testis size and gonadotrophin secretion in the sire, and date of onset of the second breeding season in the offspring, were low and not significantly different from zero.

2. Introduction

Between-breed variation in date of onset and duration of the breeding season has been reported in many studies (Hafez, 1952; Kelly et al., 1976; Knight et al., 1989). Within

a breed, there is also variation among individual animals in timing of the breeding season (Kelley & Shaw, 1943; Hanrahan & Quirke, 1986). However, the genetic basis of this within-breed variation is not clear. Hanrahan and Quirke (1986) summarized published results in this area and concluded that date of onset of the breeding season is moderately repeatable with some of this being attributable to a genetic component. No estimates of genetic parameters for duration of the breeding season have been reported. Baker et al. (1979) reported a heritability of 0.31 for the number of oestrous cycles during the pubertal breeding season.

The production of lambs outside the normal season has many advantages both to the sheep farmer and to the meat industry (Andrewes & Taylor, 1986). However, in New Zealand, the main sheep breeds normally used for lamb production have a distinct breeding season and can be induced to breed outside the normal breeding season only through the use of expensive hormones. This will remove much of the benefits sheep farmers expect to get by producing lambs out of season. Hence there is a need to develop a heavy-wool, fast-growing sheep breed that can breed at most times of the year. Selection for date of onset of the breeding season in the ewe will likely result in only limited genetic progress due both to the fact that oestrous activity is a sex-limited trait on which selection pressure cannot be applied prior to puberty and to the low selection pressure that can be applied on the ewe. As a result, it is desirable to develop techniques that will improve the rate of genetic gain in selection programmes for date of onset of the breeding season. One such technique would involve indirect selection in the ram (Walkley & Smith, 1980). However, the successful use of ram selection depends on identifying appropriate characters in the ram that can be used as selection criteria. The general approaches that can be taken to find useful genetic markers have been discussed by Blair et al. (1990). In the first instance, it may be advantageous to study breeds differing greatly in seasonality. Although differences found in the between-breed situation may not be the causes of differences in seasonality within breeds, they do highlight the possible parameters worth examining in a within-breed situation. The present progeny test was, therefore, conducted to test the breed (Romney vs. Poll Dorset) and sire within breed effects on pubertal oestrous activity and date of onset of the second breeding season. The sires used in this progeny test had also been studied for seasonal variation in testis size and gonadotrophin secretion. Therefore, an attempt was made to correlate these physiological and endocrinological parameters in the sires with oestrous activity in their female progeny with the objective of identifying possible genetic markers in the ram for date of onset of the breeding season in the ewe progeny.

3. Materials and methods

3.1. Animals and management

Five Poll Dorset and 5 Romney rams were used in this progeny test. The Poll Dorset rams were purchased from a ram breeding farm and the Romney rams were derived from a commercial flock on a Massey University farm. The rams were about 18 months of age at the time of mating. A flock of 410 mixed age Romney ewes (ranging from 1.5 to 6.5 years old) were involved in the progeny test. They were randomly divided, with restriction to age, into 10 mating groups each of 41 ewes and each group was randomly assigned to be mated by one of the 10 rams. Paddock mating commenced on 15 March 1988 and continued for 5 weeks. After mating, all ewes were managed as a single mob on mixed ryegrass and clover pastures. At lambing, during August-September, data were recorded for date of birth, birth rank, birth weight and identity of the dam which was used to determine a lamb's sire group. Each lamb was uniquely identified by a brass tag attached to its left ear. The lambs were weaned at 3-4 months of age. Thereafter, all ewe lambs were managed as a single unculled group until the end of the study. The rams used in the progeny test were retained for a further study, the detail of which has been described in Chapter IV.

3.2. Oestrous detection

On 1 March 1989, the ewe hoggets were joined with vasectomized teaser rams fitted with mating harnesses to detect the date of onset of puberty (defined as the first overt oestrus) and the number of pubertal oestrous cycles. The colour of marking crayons was changed every two weeks and tupping marks were recorded weekly until 15 July 1989 (three weeks after the winter solstice), when there were only less than 5% hoggets still cycling. The animals were weighed on 16 April when the first hogget was observed to be marked.

For detection of the date of onset of the second (two-tooth) breeding season, the ewes were weighed and joined with 5 entire rams fitted with mating harnesses on 1 November, 1989. Tupping marks were recorded weekly until the end of April 1990 when all the ewes had been mated.

3.3. Statistical analyses

Records relating to the incidence of puberty during the first breeding season were coded as binomial data describing whether a particular hogget had reached puberty or not. The coded data were then analysed using an iterative weighted least squares procedure after logit transformation of the data (Gilmour, 1985). All other analyses were performed using the general linear model procedure of the SAS statistical package (SAS Institute Inc., 1988). Data for number of pubertal oestrous cycles were subjected to the square root transformation (Steel & Torrie, 1980) before analysis in order to correct for lack of normality. For the purpose of statistical analyses, dates of onset of puberty and the second breeding season were coded as the number of day from a particular date. The general model fitted included, where appropriate, the non-genetic factors birth rank, date of birth, birth weight, and liveweight at the beginning of the breeding season. The genetic factors fitted in the model included breed as a fixed effect and sire within breed as a random effect.

4. Results

The breed means for date of birth, birth weight, weaning weight, April weight and November weight are summarized in Table 5.1. There were significant ($P < 0.01$) breed differences in all the parameters presented in Table 5.1.

Table 5.1. Date of birth (DOB), birth weight (BIRTHWT), weaning weight (WEANWT), April weight at the beginning of the first breeding season (APRILWT) and November weight prior to onset of the second breeding season (NOVWT) of animals sired by Romney or Poll Dorset rams.[1]

Sire breed	DOB	BIRTHWT (kg)	WEANWT (kg)	APRILWT (kg)	NOVWT (kg)
Romney	20 Aug±0.7	4.0±0.1	22.6±0.3	26.8±0.5	41.1±0.6
Poll Dorset	23 Aug±0.8	4.3±0.1	25.1±0.4	32.7±0.5	49.2±0.5

[1] Only data from ewe lambs were used in calculating the date of birth and birth weight.

Values presented are means±SEM.

4.1. Incidence of puberty

A total of 170 ewe hoggets (84 Romney and 86 Poll Dorset cross) were present throughout the first breeding season. Of these, 118 reached puberty during the first breeding season. The proportion of ewe hoggets that reached puberty during the first breeding season are presented in Table 5.2 for each sire. The first ewe hogget was observed to be marked on 16 April. There was a significantly ($P < 0.01$) higher proportion of Poll Dorset x Romney hoggets (79.6%) reaching puberty in the first breeding season than straightbred Romney hoggets (59.9%). The incidence of puberty was also affected by date of birth ($P < 0.05$) and April liveweight ($P < 0.01$). There was no significant effect of sire within breed on the incidence of puberty.

Table 5.2. Summary by breed and sire of the percentage of ewe hoggets reaching puberty during the first breeding season (%Pub), date of onset of puberty (Dof_1, 1 = 1 April), age at puberty (Age_Pub), number of pubertal oestrous cycles (No_Cycle) and date of onset of the second breeding season (Dof_2, 1 = 1 December).[1]

Sire breed	Sire	No.[2]	%Pub	Dof_1 (days)	Age_Pub (days)	No_Cycle	Dof_2 (days)
Romney	1	11(2)	63.6	65±5	284±9	1.3±0.2	93±10
	2	25(2)	68.0	49±5	272±6	2.2±0.3	76±8
	3	14(6)	50.0	45±7	273±6	1.9±0.5	100±15
	4	17(0)	47.1	47±7	274±7	2.4±0.4	98±9
	5	17(1)	70.6	56±5	280±5	1.9±0.3	102±9
Breed mean		84(11)	59.9	52±3	276±3	2.0±0.2	92±4
Dorset	1	18(1)	83.3	46±6	268±6	2.3±0.4	42±3
	2	14(0)	92.9	44±6	265±6	2.5±0.3	54±7
	3	19(0)	84.2	50±4	272±4	2.8±0.3	43±2
	4	17(1)	76.5	36±6	253±6	3.0±0.5	43±6
	5	18(0)	61.1	39±7	258±8	3.0±0.4	53±7
Breed mean		86(2)	79.6	43±3	264±3	2.7±0.2	47±3

[1] Values presented are means±SEM.

[2] Number of animals present during the first breeding season. Numbers in parentheses are the numbers of animals which died between the first and second breeding season.

4.2. Date and age at puberty

The mean date of, and age at, onset of puberty for animals born to each sire are shown in Table 5.2. The date of onset of puberty was earlier ($P < 0.10$) for Poll Dorset x Romney hoggets than for Romney hoggets. There was also a significant ($P < 0.05$) effect of birth rank on the date of onset of puberty. The age at puberty was significantly affected by birth rank ($P < 0.01$) and date of birth ($P < 0.001$). Animals born as singles reached puberty earlier in the year and were younger at the time of puberty than those born as twins, even after adjustment for liveweight. Lambs born earlier in the season reached puberty at an older age than those born later. The difference between breeds in the age at puberty was marginally significant ($P < 0.10$). No significant effects of sire within breed were found.

4.3. Number of pubertal oestrous cycles

Among those hoggets that reached puberty during the first breeding season, Romney hoggets had fewer ($P < 0.05$) oestrous cycles than Poll Dorset x Romney hoggets (Table 5.2). There was also a marginally significant ($P < 0.10$) effect of sire within breed on number of oestrous cycles. Liveweight in April ($P < 0.05$) and date of onset of puberty ($P < 0.001$) had significant effects on the number of oestrous cycles. Those hoggets reaching puberty earlier in the season had more oestrous cycles than those reaching puberty later.

4.4. Date of onset of the second breeding season

One hundred and fifty seven two-tooth (ca 18 months old) ewes (73 Romney and 84 Poll Dorset cross) were present at the second breeding season. The date of onset of the second breeding season was significantly ($P < 0.001$) earlier in Poll Dorset x Romney ewes than in straightbred Romney ewes (Table 5.2). The mean dates of onset of the second breeding season were 17 January for Poll Dorset x Romney ewes and 3 March for Romney ewes. There was also a significant ($P < 0.05$) sire within breed effect on date of onset of the second breeding season. Liveweight in November significantly ($P < 0.01$) affected date of onset of the breeding season in Romney ewes (regression coefficient = -2.65±0.90 days/kg liveweight), but not in Poll Dorset x Romney ewes (regression coefficient = 0.02±0.27 days/kg liveweight). The date of onset of the second breeding season was not influenced by whether the ewe had reached puberty during the first breeding season.

4.5. Correlation between the sire traits and date of onset of the second breeding season

Since there were few significant effects of sire within breed on pubertal oestrous activity, only correlations with date of onset of the second breeding season were calculated. The breed differences in seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH have been described in Chapter IV. Between breeds, the significant differences in date of onset of the second breeding season were associated with breed differences in seasonal variation in testis size and gonadotrophin secretion. Compared with Romney rams, Poll Dorset rams exhibited:

a) Smaller magnitude of seasonal variation in testis size.
b) Less marked seasonal variation in LH pulse frequency.
c) An earlier increase in plasma FSH concentrations.

There was no significant breed effect on either the peak or total LH response to the GnRH challenge. However, large variation between rams within breeds in the total LH response to the GnRH challenge was observed, this being most obvious in February.

Within breeds, the correlations between date of onset of the second breeding season in the ewe and the measured testis and endocrine parameters in the ram were generally low and not significantly different from zero. The pooled within-breed correlation coefficients between dates of onset of the second breeding season and the magnitude of seasonal variation in testis size (expressed as the percentage increase from minimum to maximum testis size), LH pulse frequency, plasma FSH concentrations and the total LH response to the GnRH challenge in February were 0.22, 0.12, -0.32 and 0.01 respectively ($P > 0.10$).

5. Discussion

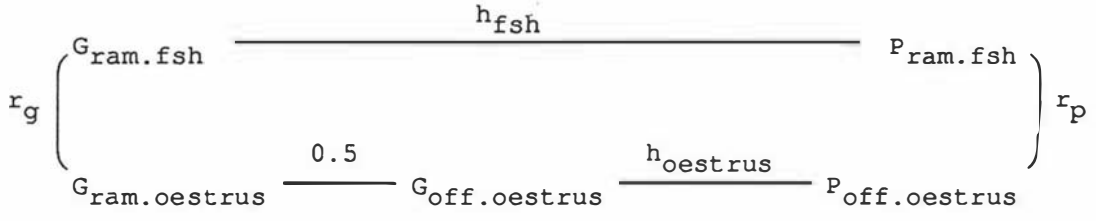
Results from this study showed that the proportion of ewe hoggets reaching puberty , the date and age at puberty and the number of oestrous cycles during the first breeding season, were all different between Romney and Poll Dorset x Romney ewe hoggets. Compared with Romney ewe hoggets, a higher proportion of Poll Dorset cross animals reached puberty during the first breeding season. The Poll Dorset crosses also reached puberty earlier and at a younger age, and had more oestrous cycles than the Romneys. Breed differences in pubertal oestrous traits have also been reported in other studies (Dyrmundsson, 1973; Quirke, 1978; Quirke et al., 1985). It is known that liveweight

has a determinative effect on the onset of puberty (Quirke, 1979; Foster et al., 1986). In the present study, the incidence of puberty and the number of pubertal oestrous cycles were significantly affected by liveweight at the beginning of the breeding season (fitted after breed in the model). Heavier hoggets were more likely to reach puberty during the first breeding season and had more oestrous cycles than lighter hoggets. Sire within breed had only a marginally significant effect on the number of pubertal oestrous cycles. In view of the small differences between sires within breed in date of onset of puberty and the second breeding season (see Table 5.2), the recording of tupping marks once a week may be less than adequate to identify the differences. In future studies, it will be desirable to record tupping marks more frequently than once a week, especially when the number of progeny per sire is small.

A large difference between Romney and Poll Dorset cross ewes in the date of onset of the second breeding season was observed. This agrees with other studies demonstrating the superior genetic merit of Poll Dorset animals for early onset of the breeding season (Kelly et al., 1976; Knight et al., 1989). The breed difference in date of onset of the second breeding season in the ewe was associated with breed differences in seasonal variation in testis size and gonadotrophin secretion in the ram. The early onset of the breeding season in Poll Dorset x Romney ewes was associated with early but small increases in testis size, less marked variation in LH pulse frequency and early increases in plasma FSH concentrations during the transitional period from the nonbreeding to the breeding season in their Poll Dorset sires (Chapter IV). These results indicate that, at least in the between breed situation, seasonal variation in testis size and gonadotrophin secretion during the transitional period from the nonbreeding to the breeding season are good indicators of seasonality. However, these between-breed differences offer no guarantee of a cause and effect relationship and have to be verified in a within-breed situation.

There were also significant sire within breed effects on date of onset of the second breeding season, indicating a within-breed genetic variation in this trait. This suggests that selection in the ram will lead to expected changes in the date of onset of the breeding season in their progeny. Unfortunately, unlike the between-breed situation where breed differences in date of onset of the breeding season were associated with significant differences in the seasonal variation in testis size and gonadotrophin secretion, the within-breed correlations between date of onset of the breeding season in the ewe and the seasonal variation in testis size and gonadotrophin secretion in the ram were very low. However, this situation does not mean that some of the physiological and endocrinological characteristics in the ram are not useful selection criteria for date of onset of the breeding season. Rams used in this progeny test were randomly selected

from a commercial flock. Therefore, within-breed variation in date of onset of the breeding season was likely to have been small. More importantly, because these are phenotypic correlations, they are expected to be small. Using plasma FSH concentrations as an example, the relationship between plasma FSH concentration in the ram and date of onset of the breeding season in the ram's progeny can be shown by the following diagram:



In the above diagram, the measured phenotypic value for plasma FSH in the ram, $P_{ram.fsh}$, is related to its genetic value, $G_{ram.fsh}$, via the square root of the heritability of plasma FSH, h_{fsh}. The relationship between the genetic value of plasma FSH, $G_{ram.fsh}$, and the ram's genetic merit for date of first oestrus, $G_{ram.oestrus}$, is measured by the genetic correlation coefficient r_g. Half of the sire's genetic value for date of first oestrus is passed onto its offspring, $G_{off.oestrus}$, which is related to the offspring's phenotypic value for date of first oestrus, $P_{off.oestrus}$, through the square root of the heritability of date of first oestrus, $h_{oestrus}$. Therefore, the calculated phenotypic correlation coefficient between the ram's plasma FSH trait and the date of first oestrus in its daughters, r_p, can be represented by the following equation:

$$r_p = h_{fsh} \cdot r_g \cdot 0.5 \cdot h_{oestrus}$$

Since h_{fsh}, r_g and $h_{oestrus}$ are all less than unity, r_p is expected to be less than 0.5. Rearranging the equation, we get:

$$r_g = 2 \cdot r_p / (h_{fsh} \cdot h_{oestrus}).$$

Because the product of $h_{fsh} \cdot h_{oestrus}$ is normally no more that 0.5, the above equation highlights the fact that a large genetic correlation coefficient is required for the exhibition of a small phenotypic correlation coefficient. Because of this intrinsic low phenotypic correlation between a ram's trait and its offspring's reproductive

performance, it follows that a large number of sires need to be tested before a significant phenotypic correlation can be obtained. One advantage of using the progeny test technique in searching for genetic markers is that a large number of traits can be tested simultaneously without prior knowledge of their importance. In practice, if some prior knowledge regarding the usefulness of one particular characteristic is available, it is possible to select rams at the two extremes based on this characteristic and to use these animals in the progeny test. Provided the right characteristic is used in the selection, the correlation coefficient is likely to be increased.

In conclusion, results from the present trial showed that there were breed differences in the proportion of ewe hoggets reaching puberty during the first breeding season, the date and age at puberty and the number of pubertal oestrous cycles. However, few sire within breed effects on pubertal oestrous activity were significant. There were significant effects of breed and sire within breed on the date of onset of the second breeding season. While differences between Romney and Poll Dorset x Romney ewes in date of onset of the second breeding season were associated with significant breed differences in the pattern of seasonal variation in testis size and gonadotrophin secretion in their sires, the within-breed correlations were very low.

C H A P T E R VI:

DIFFERENCES IN THE PATTERN OF SEASONAL VARIATION IN TESTIS SIZE, GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN ROMNEY RAMS AND THEIR RELATIONSHIPS WITH OESTROUS ACTIVITY IN THE RAMS' OFFSPRING

CHAPTER VI:

DIFFERENCES IN THE PATTERN OF SEASONAL VARIATION IN TESTIS SIZE, GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN ROMNEY RAMS AND THEIR RELATIONSHIPS WITH OESTROUS ACTIVITY IN THE RAMS' OFFSPRING

1. Abstract

An experiment was conducted to investigate the magnitude of differences between Romney rams in the pattern of seasonal variation in testis size, circulating gonadotrophin concentrations and the pituitary responsiveness to a GnRH challenge during the transitional period from the nonbreeding to the breeding season, and to correlate differences in testis size and endocrine parameters in the rams with the oestrous activity of the rams' progeny. Twelve Romney rams were selected from a group of 60 animals based on testis diameter measurements made during the 1988-1989 season to represent rams which had either an early (the "early" group) or a late (the "late" group) increase in testis size prior to the onset of the breeding season. Ten of the 12 rams were progeny tested in 1989 and their female progeny were studied for hogget and 2-tooth oestrous activity. During the period from 1 October 1989 to 20 March 1990, the selected rams were studied for seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to a GnRH challenge.

There were significant differences among Romney rams in the pattern of seasonal variation in testis size. Rams in the early group had an earlier and greater increase in testis size than rams in the late group during both the 1988-1989 and 1989-1990 seasons. Differences between the groups in the pattern of seasonal variation in testis size were associated with group differences in endocrine function. Thus rams in the early group had a significantly ($P < 0.01$) higher LH pulse frequency in March than those in the late group and the seasonal increase in plasma FSH concentrations occurred earlier ($P < 0.10$) in the early group than in the late group. There were also group differences in the pattern of seasonal variation in total LH response to the GnRH challenge. However, differences between groups in testis size and endocrine function were not associated with significant group differences in the date of onset of the breeding season in their daughters.

2. Introduction

In previous trials (described in Chapters IV & V), it was shown that differences between the Romney and Poll Dorset breeds in seasonality were associated with differences in the pattern of seasonal variation in testis size and gonadotrophin secretion during the transitional period from the nonbreeding to the breeding season. These results suggest that seasonal variation in testis size and gonadotrophin secretion prior to onset of the breeding season might be useful predictors of a ram's genetic merit for early onset of the breeding season. Thus in selection programmes for early onset of the breeding season, it might be possible to select rams based on these indicator traits instead of, or in combination with, direct selection for date of onset of the breeding season in the ewe. Because of the greater selection pressure that can be applied on the ram compared to the ewe, the rate of genetic progress could be greatly improved by indirect selection in the ram (Walkley & Smith, 1980).

While between-breed comparisons are useful in identifying parameters which might be examined as potential indicator traits in a within-breed situation, differences found between breeds do not necessarily guarantee a cause and effect relationship within a breed (Blair et al., 1990) and therefore have to be verified within breeds. Accordingly, the present study was conducted to investigate the extent to which within-breed variation exists, among Romney rams, in the seasonal changes in testis size, plasma gonadotrophin concentrations and the pituitary responsiveness to a GnRH challenge during the transitional period from the nonbreeding to the breeding season. The rams were also progeny tested and an attempt was made to correlate differences in testis size and endocrine function in the ram with the oestrous activity of their female offspring.

3. Materials and methods

3.1. Animals and selection procedures

Twelve Romney rams (aged 3 years) were used in this study. They were selected from a group of 60 animals based on testis diameter measurements made at 2-3 week intervals during the transitional period from the nonbreeding to the breeding season (from 1 October 1988 to 20 February 1989). Only the 7 measurements that were made prior to the summer solstice (22 December) were used for selection which was based on the following procedures. First, values for testis diameters of each ram were expressed as deviations from the mean of all rams at each measurement time (ie

individual values minus the mean). Next, a linear regression model of deviations on time was fitted to each ram. The regression coefficients from the above analysis were then ranked. Six rams with the greatest, and 6 with the smallest, coefficients were selected to represent rams with an early (the "early" group) and a late (the "late" group) increase in testis size. The rationale behind these selection procedures was that if a ram had an earlier increase in testis size than the others in the group, the deviations of testis diameters for that ram would increase with time during the early measurement period. This would result in a positive regression coefficient, the magnitude of which reflected the rate of increase in testis size relative to the group as a whole. The opposite was true if a ram had a late increase in testis size (Figure 6.1). The selected rams were used in the progeny test in 1989.

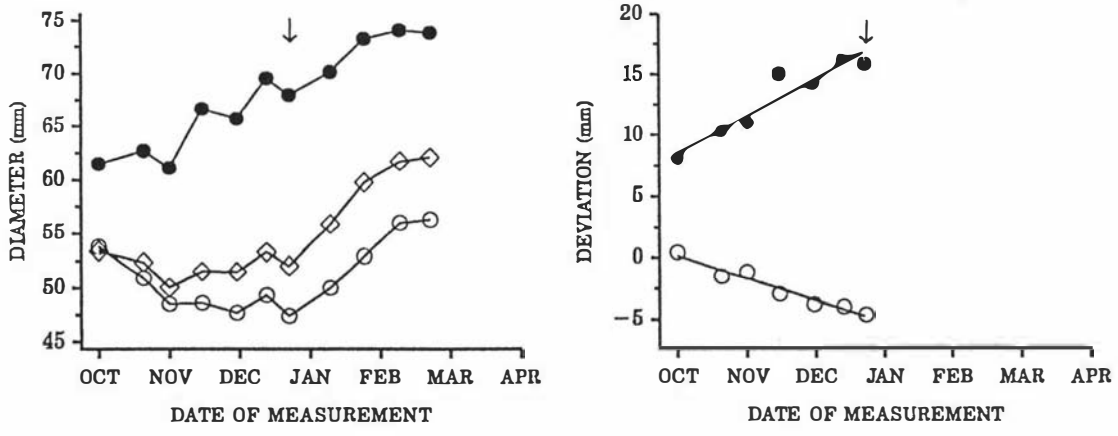


Figure 6.1. Diagrammatical illustration of the selection procedures. The left panel shows the seasonal changes in testis diameters of two typical rams (one from the early group ●——● and the other from the late group o- - -o) together with the mean testis diameters of the 60 rams (◇—◇). The right panel shows the deviations from the mean of testis diameters of rams pictured in the left panel and the regression lines fitted to each ram. Arrows above the diagrams indicate the summer solstice (22 December).

3.2. Progeny test and oestrus observation

Five rams from each of the early and late groups were progeny tested, with the sixth ram in each group acting as a reserve. A flock of 420 mixed age Romney ewes (ranging from 2.5 to 6.5 years old) were involved in the progeny test. They were randomly divided with restriction to age into 10 mating groups each of 42 ewes and each group was randomly assigned to be mated by one of the 10 rams. Paddock mating commenced on 21 March 1989 and continued for 5 weeks. After mating, all ewes were managed as a single mob on mixed ryegrass and clover pastures. At lambing, during August-September, data were recorded for date of birth, birth rank, birth weight and identity of the dam which was used to determine sire group. Each lamb was uniquely identified by a brass tag attached to its left ear. The lambs were weaned at 3-4 months of age. Thereafter, all ewe lambs were managed as a single unculled group until the end of the study.

On 1 March 1990, the ewe hoggets were joined with vasectomized teaser rams fitted with mating harnesses to detect the date of onset of puberty (defined as the first overt oestrus) and the number of pubertal oestrous cycles. The colour of marking crayons was changed every two weeks and tupping marks were recorded weekly until 21 July 1990 (one month after the winter solstice), when only 6% of the animals were still cycling. The animals were weighed on 12 April when the first hogget was marked.

For detection of the date of onset of the second (2-tooth) breeding season, the ewes were joined with vasectomized rams fitted with mating harnesses on 1 November 1990. Starting from 6 March 1991, when the first ewe was observed to be marked, tupping marks were recorded 3 times a week until all the ewes had been marked. Thereafter, the ewes were mated to entire rams. The ewes were weighed on 15 February 1991.

3.3. Endocrine study

All 12 selected rams were used in the endocrine study. Beginning in early October 1989 (the nonbreeding season), testis diameters and liveweights of the selected rams were measured at 2-3 week intervals until March 1990. On four occasions during this period (1 November 1989, 13 December 1989, 31 January 1990 and 20 March 1990), the rams were brought indoors for intensive blood sampling and GnRH challenge studies. At each sampling period, 6 ml of blood was collected at 15 min intervals for 8 hours commencing at 0900 h. After the last sample was taken (1700h), each animal

received an intravenous injection of synthetic GnRH (NIDDK, NIH) at a dose of 50 ng/kg liveweight. Further blood samples were collected at 10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min after GnRH injection. All samples were analysed for FSH and LH except for those taken after GnRH injection which were analysed only for LH. Sensitivity of the LH assay was 0.09±0.01 ng/ml. The intra-assay coefficients of variation (c.v.) were 4.8, 3.6 and 5.2%, and the inter-assay c.v. 13.9, 10.4 and 7.0%, at mean LH concentrations of 0.5, 1.3 and 3.0 ng/ml, respectively. The FSH assay sensitivity was 0.13±0.03 ng/ml, while the intra-assay c.v. were 10.5, 11.0 and 5.1%, and the inter-assay c.v. 13.4, 12.5 and 9.8%, at mean FSH concentrations of 0.8, 2.2 and 4.4 ng/ml, respectively.

3.4. Statistical analyses

The testis size and endocrine parameters were subjected to analysis of variance for repeated measures with time being the repeated factor (SAS Institute Inc., 1988). In order to apply the repeated measures analysis to the testis diameter data, measurements taken on individual animals during each month were averaged to obtain a monthly mean testis diameter. This parameter was then used in the analysis. Comparisons between means at different sampling times were carried out using orthogonal contrasts. Records relating to incidence of puberty during the first breeding season were coded as binomial data describing whether a particular hogget had reached puberty or not. The coded data were then analysed using an iterative weighted least squares procedure after logit transformation of the data (Gilmour, 1985). All other oestrous data were analysed using the general linear model (GLM) procedure of the SAS statistical package. Data for number of pubertal oestrous cycles were subjected to the square root transformation (Steel & Torrie, 1980) before analysis in order to correct for lack of normality.

4. Results

4.1. Testis diameters and liveweights

Testis diameters and liveweights of the selected rams during the 1988-1989 season are shown in Figure 6.2. The overall mean testis diameter of rams in the early group (59.5±0.9 mm) was significantly ($P < 0.01$) greater than that of rams in the late group (53.7±0.7 mm). There were also significant group differences in the pattern of seasonal variation in testis size (group x time interaction, $P < 0.01$). For rams in the early group, testis size increased 25% from a minimum of 53.4 mm in late October to a maximum of 66.6 mm in February. For rams in the late group, the corresponding value was 16 %

from 50.1 mm in late December to 58.5 mm in February. The liveweight of rams in the early group (72.0±0.7 kg) was significantly ($P < 0.01$) greater than that of rams in the late group (59.5±0.5 kg).

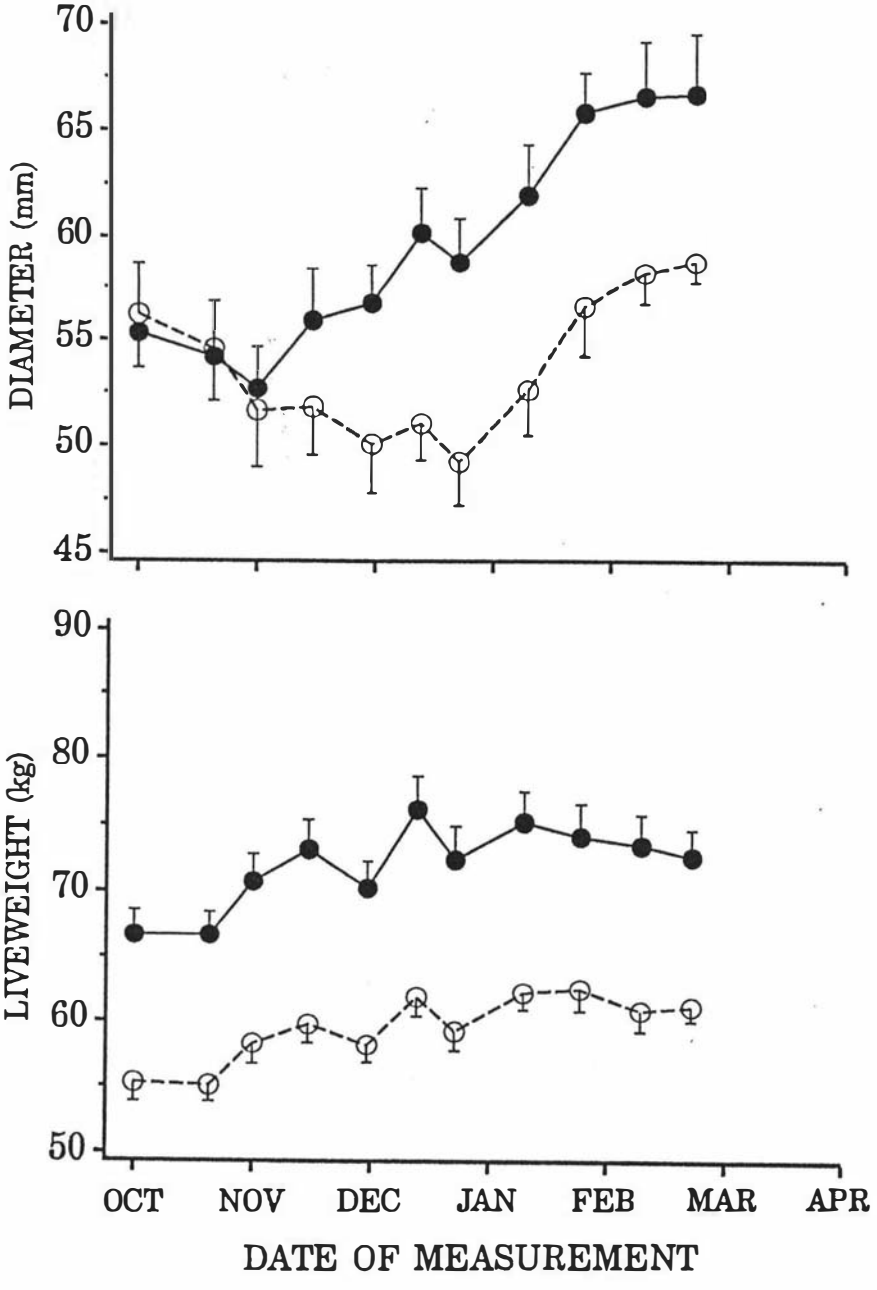


Figure 6.2. Seasonal variation in testis diameter (top panel) and liveweight (bottom panel) for rams in the early (n=6; ●——●) and late (n=6; o- - -o) groups during the 1988-1989 season. Vertical bars represent standard errors of the means.

The classification of rams into two groups based on the method described previously was repeatable from year to year (Figure 6.3). Thus the correlation between ranks of the regression coefficients during the 1988-1989 and 1989-1990 seasons was 0.80 (Spearman rank correlation, $P < 0.01$). In the 1988-1989 season, the regression coefficients for rams in the early group were all positive, whereas those of rams in the late group were all negative (since this was the basis on which assignment to groups was made). During the 1989-1990 season, the regression coefficients of 3 rams (1 from the early group and 2 from the late group, of which 2 were coincidentally the reserve rams) changed sign (see Figure 6.3). Consequently, the rams were reclassified based on the regression coefficients during the 1989-1990 season. Rams with positive regression coefficients (n=7) were classified into the early group and those with negative regression coefficients (n=5) were classified into the late group. Because the endocrine study was carried out during the 1989-1990 season, the data were analysed using the newly classified groups. This resulted in the number of progeny-tested rams being 6 and 4 for the early and late groups, respectively.

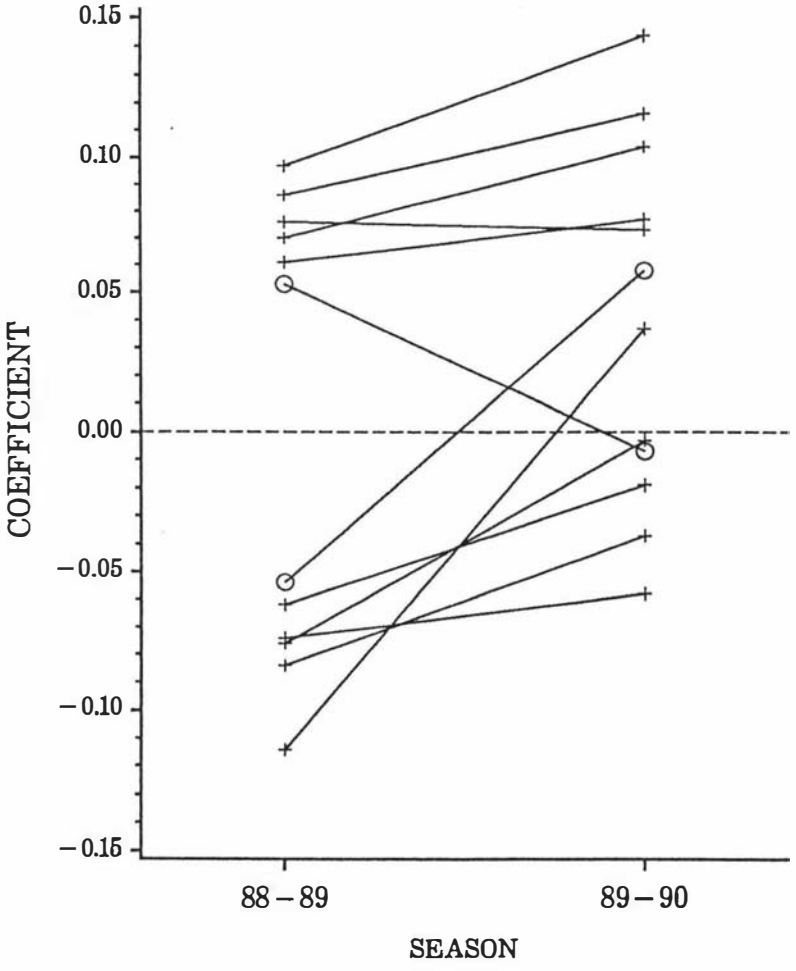


Figure 6.3. Changes in the regression coefficients of testis diameter deviations on time for the selected rams during the 1988-1989 and the 1989-1990 seasons. The two reserve rams are indicated by the circles.

Testis diameters and liveweights during the 1989-1990 season are presented in Figure 6.4. The overall mean testis diameter of rams in the (reclassified) early group (59.2±0.8 mm) was significantly ($P < 0.01$) greater than that of rams in the late group (54.8±0.5 mm). There were significant ($P < 0.01$) differences between groups in the pattern of seasonal variation in testis diameter. Testis diameter increased 14% for rams in the early group from a minimum of 55.1 mm in late October to a maximum of 62.8 mm in February. The corresponding increase for rams in the late group was 10% from 52.5 mm in December to 57.7 mm in February. The liveweight of rams in the early group (75.6±1.1 kg) was significantly ($P < 0.01$) greater than that of rams in the late group (66.9±1.1 kg). The liveweight varied significantly ($P < 0.01$) with season, but there was no apparent association between seasonal changes in testis size and in liveweight.

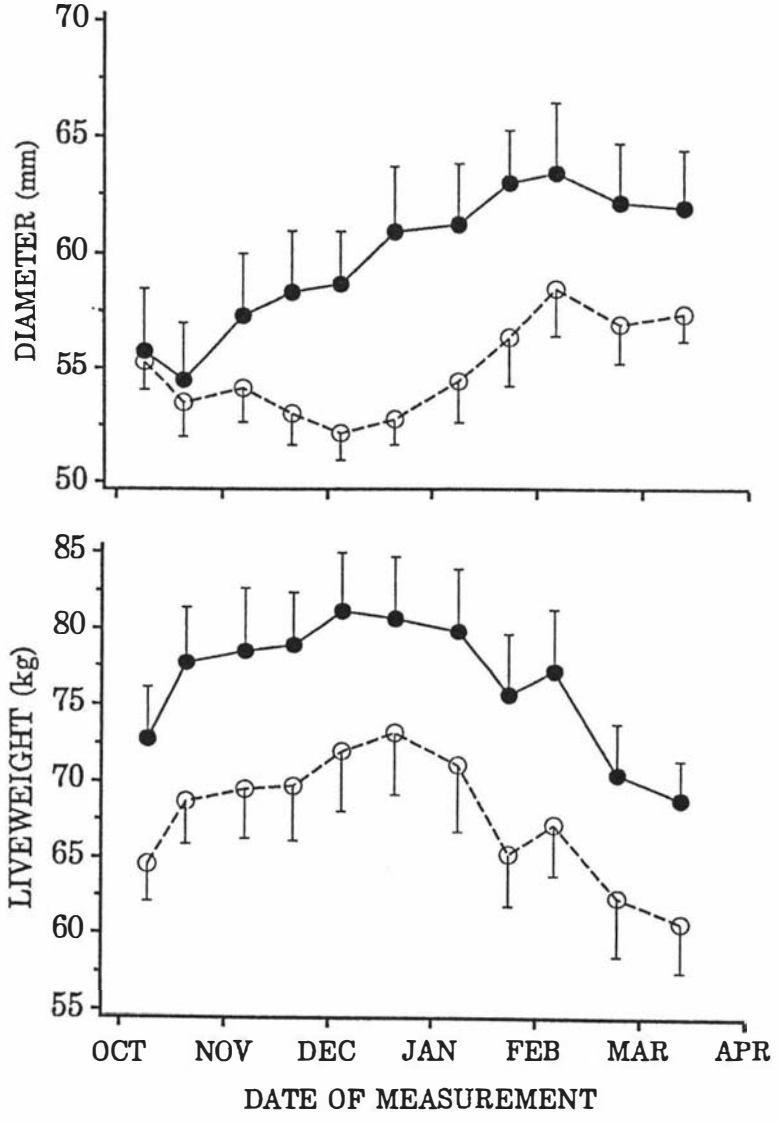


Figure 6.4. Seasonal variation in testis diameter (top panel) and liveweight (bottom panel) for rams in the (reclassified) early (n=7; ●——●) and late (n=5; o- - -o) groups during the 1989-1990 season. Vertical bars represent standard errors of the means.

4.2. LH

The effects of (reclassified) group and sampling time on circulating LH concentrations are shown in Table 6.1. There were no significant differences between the early and late groups in the overall basal (early, 0.33±0.02; late, 0.29±0.01 ng/ml) and mean (early, 0.39±0.03; late, 0.34±0.02 ng/ml) LH concentrations. Both the basal and mean LH concentrations varied significantly ($P < 0.01$) with sampling time, but there was no significant difference between groups in the pattern of this variation (group x time interactions, $P > 0.10$). The mean LH concentration of rams in the early group was significantly ($P < 0.05$) higher than that of rams in the late group in March (Table 6.1).

Over the entire trial period, rams in the early group had a significantly ($P < 0.05$) higher LH pulse frequency than rams in the late group (2.2±0.2 vs. 1.4±0.2 pulses/8h), mainly as a result of the significant ($P < 0.001$) group difference in LH pulse frequency in March. LH pulse frequency varied significantly ($P < 0.001$) with sampling time. There was also a significant ($P < 0.01$) group difference in the pattern of variation in LH pulse frequency. Changes in LH pulse frequency were similar between November and January for both groups, but LH pulse frequency of rams in the early group increased significantly ($P < 0.01$) between January and March, while that of rams in the late group decreased slightly (Table 6.1). LH pulse amplitude varied significantly ($P < 0.05$) with sampling time, but no significant effects of group or group x time interaction were found.

4.3. FSH

FSH concentrations varied significantly ($P < 0.01$) with sampling time and the group difference in the pattern of variation was marginally significant (group x time interaction, $P < 0.10$, Table 6.1). The increase in FSH concentrations appeared to occur earlier in the early group than in the late group. Although there was a general trend towards rams in the early group having higher FSH concentrations than those in the late group, the group difference in mean FSH concentrations over the entire sampling period was not significant (early 2.08±0.32, late 1.43±0.10 ng/ml, $P > 0.10$). Rams in the early group had marginally higher FSH concentrations in December than rams in the late group ($P < 0.10$).

Table 6.1. Basal and mean LH concentrations, LH pulse frequency, LH pulse amplitude and mean FSH concentrations for rams in the early (E) and late (L) groups at different sampling times.[1,2]

Time	Group	LH				FSH
		Basal (ng/ml)	Mean (ng/ml)	Frequency (no./8h)	Amplitude[3] (ng/ml)	Mean (ng/ml)
01 Nov	E	0.29±0.02	0.30±0.02	0.3±0.2	0.94	1.29±0.24
	L	0.27±0.02	0.29±0.01	0.5±0.2	0.44±0.05	0.97±0.10
13 Dec	E	0.32±0.02	0.43±0.02	1.1±0.1	1.37±0.21	2.48±0.49
	L	0.29±0.01	0.37±0.03	0.9±0.2	1.19±0.08	1.24±0.16
31 Jan	E	0.34±0.02	0.40±0.03	2.8±0.4	0.63±0.03	2.59±0.39
	L	0.29±0.01	0.38±0.02	2.3±0.2	0.66±0.06	1.91±0.16
20 Mar	E	0.36±0.02	0.43±0.03[a]	4.4±0.4[a]	0.56±0.04	1.97±0.28
	L	0.30±0.01	0.33±0.02[b]	1.7±0.3[c]	0.53±0.04	1.60±0.12
Significance						
Group		NS	NS	*	NS	NS
Time		**	**	***	*	**
Group x Time		NS	NS	**	NS	+

[1] Early group n = 7; late group n = 5.

[2] Values presented are means±SEM.

[3] Only one complete LH pulse was present and used for calculating pulse amplitude for rams in the early group on 1 November. This mean therefore has no variance.

[a,b,c] Means within each column and sampling time carrying different superscripts differed significantly (a vs b, $P < 0.05$; a vs c, $P < 0.01$)

4.4. LH responses to GnRH challenge

Both the peak and total LH responses to GnRH were significantly ($P < 0.01$) affected by sampling time (Table 6.2). Generally speaking, both the peak and total LH responses decreased as the breeding season approached in March. There was a significant ($P < 0.05$) group difference in the pattern of seasonal variation in the total LH response to GnRH. Among rams in the early group, the total LH response increased slightly between November and December, and then decreased in January. For rams in the late group, there was a continuous decrease over the whole sampling period. No significant group differences in either the peak or the total LH response to GnRH were apparent.

Table 6.2. Peak and total LH responses to an intravenous injection of exogenous GnRH (50 ng/kg liveweight) for rams in the early (E) and late (L) groups at different sampling times.[1,2]

Time	Group	Peak (ng/ml)	Total (min.ng/ml)
01 Nov	E	8.36±0.99	503.32± 68.47
	L	7.67±1.69	465.55±116.87
13 Dec	E	6.71±0.82	550.12± 72.18
	L	5.53±1.11	400.03± 95.63
31 Jan	E	3.40±0.61	339.44± 51.43
	L	4.25±0.83	377.00± 96.51
20 Mar	E	4.03±1.35	290.63± 42.67
	L	3.15±0.62	263.67± 65.81
Significance	Group	NS	NS
	Time	**	**
	Group x Time	NS	*

[1] Early group n = 7; late group n = 5. Values presented are means±SEM.
[2] There were no significant group difference in either the peak or the total LH response to GnRH at any sampling time.

4.5. Oestrous activity

The proportion of ewe hoggets reaching puberty in the first breeding season, date of onset of the first oestrus, age at puberty, number of pubertal oestrous cycles, and the date of onset of the second breeding season, are summarized by sire and group in Table 6.3. There were no significant group or sire effects on the proportion of ewe hoggets reaching puberty during the first breeding season, the date of onset of the first oestrus, age at puberty or the number of pubertal oestrous cycles. Nor was there a significant group effect on the date of onset of the second breeding season, although there was a significant ($P < 0.05$) sire effect on this trait. The correlation coefficients (across groups) between date of onset of the second breeding season and the magnitude of variation in testis size, LH pulse frequency and FSH concentrations were -0.01, 0.44 and 0.25 respectively ($P > 0.10$).

Table 6.3. Summary by sire and group of the percentage of ewe hoggets reaching puberty during the first breeding season (%Pub), date of onset of the first oestrus (Dof_1, 1 = 1 April), age at puberty (Age_Pub), the number of pubertal oestrous cycles (No_cycle) and the date of onset of the second breeding season (Dof_2, 1 = 1 March).[1]

Sire	Group	No.[a]	%Pub	Dof_1	Age_Pub	No_cycle	Dof_2
1	Early	17(1)	82.4	48±7	267±7	3.1±0.4	19±2^{cde}
2	Early	15(0)	93.3	53±6	273±6	2.2±0.3	26±1^{b}
3	Early	10(0)	70.0	46±8	265±9	2.7±0.4	18±2de
4	Early	13(1)	92.3	58±5	273±3	2.6±0.4	26±2^{b}
5	Early	15(0)	86.7	50±7	263±7	3.2±0.4	19±1^{cde}
6	Early	10(1)	90.0	52±5	272±4	2.3±0.3	16±3^{e}
Mean	Early	80(3)	85.0	51±3	269±3	2.7±0.2	21±1
7	Late	18(0)	77.8	54±5	271±5	2.4±0.4	21±2^{bcde}
8	Late	9(0)	33.3	46±0	270±2	2.7±0.9	24±5^{bcd}
9	Late	15(2)	73.3	47±5	266±4	3.0±0.4	21±2^{bcde}
10	Late	12(1)	75.0	51±8	263±8	3.1±0.5	18±2de
Mean	Late	54(3)	68.5	50±3	268±3	2.8±0.2	21±1

[1] Values presented are means±SEM.

[a] Number of ewe hoggets present during the first breeding season. Numbers in parentheses are the numbers of animals which died between the first and second breeding season.

[b,c,d,e] Means within each column carrying the same superscript do not differ significantly ($P > 0.05$).

5. Discussion

The objectives of the present trial were to investigate the extent to which within-breed variation in the seasonal changes in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH existed in Romney rams, and to relate these differences to the date of onset of the breeding season in their progeny. To achieve this, two groups of rams differing in timing of the seasonal increase in testis size were selected. The purpose of the selection procedure was to objectively select two extreme groups of rams that had either an early or a late increase in testis size during the transitional period from the nonbreeding season to the breeding season. Since the increase in testis size prior to the onset of the breeding season occurs before the summer solstice in Romney rams (Pelletier & Almeida, 1987; see also Chapter IV), the use of measurements made prior to the summer solstice should allow separation of rams which had an early or a late increase in testis size. In the present trial, the regression coefficients of 3 rams changed sign during the 1989-1990 season and these animals were reclassified according to the regression coefficients during the second season. This did not affect the progeny test because its main aim was to search for a relationship between the measured testis and endocrine parameters in the rams and date of onset of the breeding season in the rams' female progeny. Since the endocrine study was carried out during the 1989-1990 season, the endocrine data were analysed based on the new classification. However, the data were also analysed according to the original classification (Appendix I). Taking into account the annual variation in the environmental conditions under which the rams were reared and the error associated with testis diameter measurements, the selection procedure appeared to be reasonably repeatable, objective and easy to implement.

In both seasons, the seasonal increase in testis size occurred earlier in the early group than in the late group, but reached maximum values at about similar times of the year (see Figures 6.2 & 6.4). The magnitude of seasonal variation in testis size was greater for rams in the early group than for rams in the late group during both seasons, although this effect was less pronounced during the 1989-1990 season than during the 1988-1989 season. Within-breed variation in the magnitude of seasonal variation in testis size has been reported previously (Ringwall et al., 1989). Collectively, these results show that there are differences among rams of the same breed in the time and magnitude of seasonal variation in testis size and these differences appear to be repeatable from year to year. The liveweight of rams in the early group was significantly greater than that of rams in the late group. This is unexpected and it is not clear why the time of seasonal increase in testis size is related to liveweight. This relationship does not appear to have been reported previously.

The classification of rams into groups, based on timing of the seasonal increase in testis size prior to the onset of the breeding season, reflected differences in the endocrine function between rams in these groups. Rams in the early group had a significantly higher LH pulse frequency in March than those in the late group. Thus the larger magnitude of seasonal variation in testis size for rams in the early group was associated with greater seasonal changes in LH pulse frequency. The time of the seasonal increase in plasma FSH concentrations was also earlier for the early group than for the late group and this appeared to be closely related to the earlier increase in testis size of rams in the early group.

Unfortunately, the differences between groups in testis size and endocrine function were not associated with significant group differences in the date of onset of the breeding season. This situation seems to argue against the usefulness of any of the testis size and endocrine parameters as predictors of a ram's genetic merit for early onset of the breeding season. However, the lack of a significant group difference in the date of onset of the second breeding season could have been caused by some unknown environmental factors. In the second breeding season, the first ewe was observed to be marked on 5 March, which was later than when ewes of this breed normally start their breeding season (Knight et al., 1989; see also Chapter V). When the breeding season did start, 95% of the ewes came into oestrus within a period of 25 days. This degree of synchrony in oestrous activity was unexpected. It could not have been caused by the ram effect as the ewes were run with two teaser rams from 1 November 1990.

The lack of group differences in date of onset of the breeding season might also have been caused by the use of inappropriate criteria for the selection procedures. In the present study, rams were selected for an early or late increase in testis size. Coincidentally, the selection procedures resulted in rams with an early increase in testis size having a greater magnitude of seasonal variation in testis size than those with a late increase in testis size. This contrasts with differences found between Romney and Poll Dorset rams where the less seasonal Poll Dorset rams had an earlier but smaller seasonal increase in testis size (Chapter IV). If the timing and magnitude of seasonal variation in testis size are antagonistic to one another, this would result in their effects being cancelled by each other and no significant group differences would be found. Apparently, studies are needed to investigate the relationship between the two components (timing and magnitude) of the pattern of seasonal variation in testis size and the date of onset of the breeding season in their female relatives.

In conclusion, results from the present experiment showed that there were large differences between Romney rams in the pattern of seasonal variation in testis size. These differences were correlated with differences in endocrine function, but not with date of onset of the breeding season in their female offspring.

C H A P T E R VII:

EFFECTS OF SEASON AND TESTOSTERONE TREATMENT ON GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN CASTRATED ROMNEY AND POLL DORSET RAMS

CHAPTER VII:

EFFECTS OF SEASON AND TESTOSTERONE TREATMENT ON GONADOTROPHIN SECRETION AND PITUITARY RESPONSIVENESS TO GnRH IN CASTRATED ROMNEY AND POLL DORSET RAMS

1. Abstract

The effects of season and testosterone treatment (administered via Silastic capsules) on gonadotrophin secretion and pituitary responsiveness to GnRH challenge were studied in castrated Romney (n=8) and Poll Dorset (n=8) rams, two breeds which differ markedly in seasonality. Inhibition by testosterone treatment of LH pulse frequency, basal and mean LH concentrations, mean FSH concentration, and the peak and total LH responses to exogenous GnRH was significantly ($P < 0.01$) greater during the nonbreeding than during the breeding season. Poll Dorset rams were less sensitive to testosterone treatment than Romney rams. In rams not receiving testosterone treatment, LH pulse frequency was significantly ($P < 0.05$) lower during the nonbreeding season than during the breeding season in the Romneys (15.8±0.9 vs. 12.0±0.4 pulses/8h), but not in the Poll Dorsets (13.6±1.2 vs. 12.8±0.8 pulses/8h). It is concluded that, in rams, season influences gonadotrophin secretion through a steroid-independent effect (directly on hypothalamic GnRH secretion) and a steroid-dependent effect (indirectly on the sensitivity of the hypothalamo-pituitary axis to the negative feedback of testosterone). The magnitude of these effects appears to be related to the seasonality of the breed.

2. Introduction

It has been clearly demonstrated that, in rams and ewes, photoperiod influences reproductive activities through its effects on gonadotrophin secretion (Lincoln & Short, 1980; Karsch et al., 1984). Studies in ovariectomized ewes have shown variation in gonadotrophin secretion at different times of the year or when ewes are exposed to artificial photoperiods of different lengths. In addition, a marked effect of photoperiod on the sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of oestradiol has been demonstrated in ovariectomized ewes bearing constant-release implants of oestradiol (see review by Karsch et al., 1984). These results suggest that, in the ewe, photoperiod affects pituitary gonadotrophin secretion through both a direct

effect on hypothalamic GnRH secretion (the steroid-independent effect) and an indirect effect on the sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of oestradiol (the steroid-dependent effect, Karsch et al., 1984).

While studies in castrated rams have shown a pattern of seasonal changes in gonadotrophin secretion similar to those observed in intact rams (Pelletier & Ortavant, 1975a; Lincoln & Short, 1980; Sanford et al., 1984b), there have been few detailed studies of seasonal changes in hypothalamo-pituitary sensitivity to the negative feedback effects of testosterone in the ram. Pelletier & Ortavant (1975b) found that injection of testosterone propionate had a greater inhibitory effect on LH release in castrated rams exposed to an increasing photoperiod than in those exposed to a decreasing photoperiod. More recently, variation in the sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of testosterone has been demonstrated in castrated Soay rams exposed to artificial photoperiods (Lincoln & Ebling, 1985). However, in another study with castrated rams bearing testosterone or oestradiol implants, Schanbacher (1980a) did not find any significant interactions between the effects of photoperiod and steroid treatment on gonadotrophin secretion and pituitary responsiveness to GnRH. Clearly further studies are needed to investigate the mechanisms by which photoperiod affects gonadotrophin secretion in the ram.

It is well documented that large breed differences exist in length of the breeding season (Hafez, 1952). In the ram these are manifested in differences in the timing and magnitude of seasonal variation in testis size, semen production and libido (Haynes & Schanbacher, 1983). Studies in the ewe show that breed differences in seasonality may be related to the sensitivity of the hypothalamo-pituitary axis to both the direct and indirect effects of photoperiod (Martin et al., 1983; Webster & Haresign, 1983; Karsch et al., 1984; Thomas et al., 1988). However, there has been no comparative study, conducted in the ram, of breed differences in sensitivity to the steroid-independent and steroid-dependent effects of photoperiod. Therefore, the present study was conducted to investigate the effect of season on gonadotrophin secretion in castrated Poll Dorset and Romney rams, half of which had been treated with testosterone administered via Silastic capsules, in order to: (1) confirm the existence of both steroid-dependent and steroid-independent effects of season on gonadotrophin secretion in the ram; and (2) compare differences between breeds of short (Romney) vs. long (Poll Dorset) breeding season in their sensitivity to seasonal effects with respect to gonadotrophin secretion. Such information is relevant to the problem of identifying indirect predictors of genetic merit for aseasonality because a better understanding of endocrine factors regulating seasonality in the ram should allow a more targeted approach to the search for such predictors.

3. Materials and methods

3.1. Animals and experimental procedure

The experiment was a balanced 2 x 2 factorial design involving 2 breeds (Romney vs. Poll Dorset) and 2 testosterone treatments (control vs. testosterone-treated) repeated during 2 seasons (breeding and nonbreeding season). Sixteen castrated mature rams (8 Romney and 8 Poll Dorset) were used in this trial. These rams were castrated under general anaesthesia in late August 1989. Immediately following castration, half of the animals in each breed were each implanted with 8 testosterone-filled Silastic capsules (testosterone-treated group) and the remaining animals (control group) each received 8 empty capsules. Details of the capsules are provided below. Immediately before implantation, the capsules were sterilized by rinsing in 70% ethanol. Capsules were implanted with a trocar on one side of the body, over the ribs just behind the shoulder, under local anaesthesia. On two occasions (20 April and 7 November, 1990) corresponding to the breeding and nonbreeding seasons respectively at this latitude (40.1°S), the animals were subjected to intensive blood sampling and GnRH challenge studies. Eight weeks before each planned date of blood sampling, the old testosterone capsules were removed and new ones implanted on the opposite side of the body. The empty capsules in control animals were also taken out and reimplanted on the opposite side of the body. This procedure was undertaken to ensure that the testosterone concentrations maintained by the capsules were the same for both seasons.

At each sampling period, 6 ml of blood was collected via indwelling cannulae at 12 min intervals for 8 hours commencing at 0830 h. After the last sample was taken (1630h), each animal received an intravenous injection of synthetic GnRH (NIDDK, NIH) at a dose of 50 ng/kg liveweight. Further blood samples were collected at 10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min after GnRH injection. All samples were analysed for FSH and LH (Chapter II) except for samples collected after the GnRH challenge which were analysed only for LH. The sensitivity of the LH assay was 0.10±0.01 ng/ml. The intra-assay coefficients of variation (c.v.) were 7.1, 3.5 and 5.1%, and the inter-assay c.v. 8.6, 3.5 and 8.5%, at mean LH concentrations of 1.0, 2.5 and 9.9 ng/ml, respectively. For the FSH assay, the sensitivity was 0.10±0.03 ng/ml. The intra-assay c.v. were 5.3 and 4.8%, and inter-assay c.v. 6.5, and 5.0%, at mean FSH concentrations of 2.2 and 4.4 ng/ml, respectively.

3.2. Testosterone assay

Since it has been shown that Silastic capsules similar to those used in the present study maintain constant plasma testosterone concentrations over a 24 h sampling period (D'Occhio et al., 1982), plasma samples taken from each ram during each 8 h basal sampling period were pooled. They were assayed for testosterone by radioimmunoassay based on that described by Smith and Hafs (1973), as modified and validated by Wilson and Lapwood (1978). All samples from the study were analysed in one assay run. The assay sensitivity was 0.12 ng/ml and the intra-assay c.v. was 14.5% at a testosterone concentration of 1.6 ng/ml. Plasma pools from intact rams of the same breeds which had been sampled during the breeding season (15 March) and nonbreeding season (9 November) in another study (Chapter IV) were also assayed for testosterone, in order to determine whether the testosterone concentrations maintained by the capsules were in the normal physiological range for rams of these breeds.

3.3. Testosterone capsules

The testosterone capsules were prepared according to Schanbacher (1980b). Medical grade Silastic tubing (3.35 mm inner diameter, 4.65 mm outer diameter; Dow Corning, Midland, MI, USA) was cut into 15 cm lengths and sealed at one end with Silastic Adhesive Type A (Dow Corning, Midland, MI, USA). The tubes were packed with crystalline testosterone (Cat. No. T-1500, Sigma Chemical Company, St. Louis, MO, USA) 24 hours later and the open end sealed with adhesive. Empty capsules were made by sealing both ends of the tubes with adhesive. The capsules were left in the air to dry for a week and then stored in a desiccator until being used.

3.4. Statistical analyses

The data were subjected to analysis of variance for repeated measures with season being the repeated factor (SAS Institute Inc., 1988). Since there was a significant breed difference in plasma testosterone concentrations maintained by the implants (see Results), breed effects on other parameters were adjusted (within treatment) to a common mean testosterone concentration by covariance analysis to remove possible confounding of breed differences in plasma testosterone concentrations with the real breed effect. Comparisons of multiple means were performed using Duncan's multiple-range test.

4. Results

4.1. Plasma testosterone concentration and liveweight

The testosterone capsules maintained plasma testosterone concentrations in castrated rams which were slightly higher than (Romney), or similar to (Poll Dorset), those of intact rams during the nonbreeding season (Table 7.1). The testosterone capsules maintained similar plasma testosterone concentrations during both seasons, but Romney rams had significantly ($P < 0.01$) higher testosterone concentrations than Poll Dorset rams. The Romney rams were also significantly ($P < 0.01$) lighter than the Poll Dorset rams. Adjustment to a common liveweight eliminated the significant breed effect on plasma testosterone concentrations. In rams implanted with empty capsules, plasma testosterone concentrations were low and close to the assay sensitivity (0.12 ng/ml).

4.2. Basal and mean LH

These values are presented in Table 7.2. There were significant seasonal differences in the effects of testosterone treatment on the basal and mean LH concentrations ($P < 0.01$ for season x treatment interaction). During the breeding season, testosterone treatment had no significant effect on the basal or mean LH concentrations in either breed. However, during the nonbreeding season, testosterone treatment significantly ($P < 0.01$) inhibited the basal and mean LH concentrations in both breeds.

4.3. LH pulse frequency

Values for LH pulse frequency are presented in Table 7.2. The effect of testosterone treatment on LH pulse frequency was significantly influenced by season and breed ($P < 0.01$ for season x treatment interaction, $P < 0.05$ for breed x treatment interaction). In Romney rams, testosterone treatment significantly ($P < 0.01$) suppressed LH pulse frequency during both seasons, but the suppression was greater during the nonbreeding season (from 12.0 to 0 pulses/8h) than during the breeding season (from 15.8 to 7.6 pulses/8h). In Poll Dorset rams, testosterone treatment had no significant effect on LH pulse frequency during the breeding season (13.6 vs. 12.4 pulses/8h). However, during the nonbreeding season, LH pulse frequency was significantly ($P < 0.01$) reduced by testosterone treatment (from 12.8 to 2.1 pulses/8h). There were also breed differences in their sensitivity to the direct effect of season. The reduction in LH pulse frequency from the breeding to the nonbreeding season in control rams (bearing empty capsules) was significant for the Romneys (15.8 vs. 12.0 pulses/8h, $P < 0.05$), but non-significant for the Poll Dorsets (13.6 vs. 12.8 pulses/8h, $P > 0.10$).

Table 7.1. Plasma testosterone concentration and liveweight in control and testosterone-treated Romney and Poll Dorset castrated rams during the breeding (B) and nonbreeding (NB) seasons, together with data for intact rams.[1]

Breed	Season	Testosterone (ng/ml)	Liveweight (kg)
Control, n=4			
Romney	B	0.12±0.05[a]	61.5±3.4[a]
Romney	NB	0.15±0.04[a]	63.3±3.7[a]
Poll Dorset	B	0.11±0.03[a]	71.4±1.9[b]
Poll Dorset	NB	0.14±0.03[a]	72.0±1.0[b]
Testosterone-treated, n=4			
Romney	B	2.01±0.33[b]	62.4±1.0[a]
Romney	NB	1.91±0.36[b]	64.0±2.1[a]
Poll Dorset	B	1.38±0.15[c]	73.9±2.1[b]
Poll Dorset	NB	1.30±0.13[c]	73.5±2.8[b]
Intact, n=6			
Romney	B	6.15±0.73[d]	63.9±1.7[a]
Romney	NB	1.01±0.28[c]	63.0±1.2[a]
Poll Dorset	B	3.10±1.06[c]	77.1±3.5[b]
Poll Dorset	NB	1.28±0.45[c]	76.6±3.6[b]

[1] Values presented are means±SEM.

[a,b,c,d] Means within each column carrying the same superscript do not differ significantly ($P > 0.05$).

Table 7.2. Basal and mean luteinizing hormone (LH) concentrations, LH pulse frequency, LH pulse amplitude and mean follicle stimulating hormone (FSH) concentration in control and testosterone-treated Romney and Poll Dorset castrated rams during the breeding (B) and non-breeding (NB) seasons.[1]

Season	Treatment	Basal (ng/ml)	Mean LH (ng/ml)	Frequency (no./8h)	Amplitude (ng/ml)	Mean FSH (ng/ml)
Romney, n=4						
B	Control	2.12±0.56^{ac}	2.45±0.61^{ac}	15.8±0.9^{b}	0.73±0.11^{a}	13.75±1.17^{ab}
B	Testosterone	1.30±0.45^{ab}	1.59±0.50^{ab}	7.6±2.1^{a}	0.76±0.16^{a}	13.13±1.48^{ab}
NB	Control	2.03±0.45^{ac}	2.56±0.54^{ac}	12.0±0.4^{c}	1.20±0.18^{ab}	11.87±0.70^{ab}
NB	Testosterone	0.25±0.01^{b}	0.25±0.01^{b}	0^{d}	-	0.47±0.04^{d}
Poll Dorset, n=4						
B	Control	2.72±0.29^{c}	3.38±0.31^{c}	13.6±1.2^{bc}	1.37±0.12^{ab}	21.28±3.26^{ac}
B	Testosterone	2.75±0.25^{c}	3.50±0.41^{c}	12.4±0.9^{bc}	1.91±0.56^{b}	23.40±2.12^{c}
NB	Control	2.87±0.47^{c}	3.44±0.56^{c}	12.8±0.8^{bc}	1.25±0.30^{ab}	20.83±2.86^{ac}
NB	Testosterone	0.79±0.38^{b}	1.40±0.68^{ab}	2.1±1.3^{d}	4.43±0.66^{c}	9.45±5.88^{b}

[1] Values presented are means±SEM.

^{a,b,c,d} Means within each column carrying the same superscript do not differ significantly ($P > 0.05$).

4.4. LH pulse amplitude

The effect of testosterone treatment on LH pulse amplitude (pooled across both breeds) differed significantly between seasons ($P < 0.01$ for season x treatment interaction; Table 7.2). Testosterone treatment had no significant effect on LH pulse amplitude during the breeding season, but it significantly ($P < 0.01$) increased LH pulse amplitude during the nonbreeding season.

4.5. FSH

Mean FSH concentrations are shown in Table 7.2. The effects of testosterone treatment on FSH concentrations differed significantly between the two seasons ($P < 0.01$ for season x treatment interaction). During the breeding season, testosterone treatment had no significant effect on FSH concentrations, whereas it significantly ($P < 0.01$) reduced FSH concentrations during the nonbreeding season in both breeds. There were significant ($P < 0.05$) breed effects independent of season and treatment on FSH concentrations which were higher in Poll Dorset rams than in Romney rams (18.7 vs. 9.6 ng/ml).

4.6. LH response to GnRH challenge

The effects of breed, season and testosterone treatment on the peak and total LH responses to the GnRH challenge are presented in Table 7.3. The effects of testosterone treatment on both the peak and total LH responses to the GnRH challenge differed significantly between seasons ($P < 0.01$ for season x treatment interaction). During the breeding season, testosterone treatment had no significant effects on either the peak or the total LH responses to GnRH, whereas during the nonbreeding season it significantly ($P < 0.01$) reduced both the peak and total responses. Poll Dorset rams had significantly ($P < 0.05$) greater peak responses to the GnRH challenge than Romney rams. No other effects on either the peak or total LH responses to the GnRH challenge were significant.

Table 7.3. Peak and total luteinizing hormone (LH) response to gonadotrophin releasing hormone challenge in control and testosterone-treated Romney and Poll Dorset castrated rams during the breeding (B) and nonbreeding (NB) seasons.[1]

Season	Treatment	Peak response (ng/ml)	Total response (min.ng/ml)
Romeny, n=4			
B	Control	25.77±6.60^{ac}	1621.0±426.5^{ab}
B	Testosterone	19.05±5.99^{ab}	1424.6±385.9^{ab}
NB	Control	21.54±4.82^{ab}	1277.7±321.0^{ab}
NB	Testosterone	1.88±0.41^{b}	157.1± 45.9^{c}
Poll Dorset, n=4			
B	Control	45.54±10.8^{c}	2139.7±399.6^{a}
B	Testosterone	35.86±4.11^{ac}	1927.3±222.8^{ab}
NB	Control	43.54±8.64^{c}	2160.8±317.7^{a}
NB	Testosterone	16.76±8.32^{ab}	994.3±464.9^{bc}

[1] Values presented are means±SEM.

^{a,b,c} Means within a column carrying the same superscript do not differ significantly ($P > 0.05$).

5. Discussion

The present study investigated the effects of testosterone treatment on gonadotrophin secretion during both the breeding season and the nonbreeding season in castrated rams of the Romney and Poll Dorset breeds, these being breeds whose ewes differ greatly in seasonality (Hafez, 1952; Kelly et al., 1976; Knight et al., 1989). Recently-castrated rams were used because there is evidence that rams castrated for long periods may lose their responsiveness to testosterone (Thieulant & Pelletier, 1979). The first blood sampling took place about 7 months after castration, a period which should have been sufficient for gonadotrophin secretion to reach equilibrium after castration (Caraty, 1983; Montgomery et al., 1985). Therefore, differences between the two

sampling periods would reflect a true seasonal effect. The testosterone treatment used in the present study was effective in maintaining plasma testosterone concentrations at the lower end of the normal physiological range observed in intact rams of the same breeds. For the purposes of the present study, it was desirable to have plasma testosterone concentrations at the lower end of the normal physiological range to avoid masking the breed and seasonal differences due to over-inhibition.

The first important finding from the present study was that the effects of testosterone treatment on gonadotrophin secretion and pituitary responses to the GnRH challenge were markedly affected by season. During the nonbreeding season, testosterone treatment significantly suppressed LH pulse frequency, mean and basal LH concentrations, FSH concentrations, and the peak and total LH responses to the GnRH challenge. However, during the breeding season, most of the inhibitory effects of testosterone were lost and only the inhibition of LH pulse frequency in Romney rams remained. Even with LH pulse frequency, the reduction was smaller during the breeding season than during the nonbreeding season (Table 7.2). The greater inhibitory effects of testosterone on gonadotrophin secretion during the nonbreeding season as compared to the breeding season confirm and extend the earlier observation by Pelletier & Ortavant (1975b) that a single injection of testosterone propionate exerted a greater suppression of LH release in animals exposed to 16 h photoperiod than in animals exposed to 8 h photoperiod. Thus, in the ram, one mechanism by which season affects gonadotrophin secretion is via its effect on the sensitivity of the hypothalamo-pituitary axis to the negative feedback effects of gonadal steroids, a feature that has been extensively investigated in the ewe (Karsch et al., 1984) and the male hamster (Turek & Ellis, 1981). Because of this seasonal variation in sensitivity of the hypothalamo-pituitary axis to the negative feedback of testosterone, intact rams are able to maintain greater gonadotrophin secretion during the breeding season in the face of an increased negative feedback from the testis.

There are also breed differences in sensitivity to testosterone treatment. The LH pulse frequency of Romney rams was inhibited to a greater extent by the testosterone treatment than that of Poll Dorset rams. Testosterone significantly inhibited LH pulse frequency only during the nonbreeding season in Poll Dorset rams, whereas it significantly inhibited LH pulse frequency during both seasons in Romney rams. Thus the more seasonal Romney rams appeared to be more sensitive to testosterone inhibition than the less seasonal Poll Dorset rams. Similar differences in the sensitivity of the hypothalamo-pituitary axis to the negative feedback effect of oestradiol between ewes of breeds of long vs. short breeding season have also been reported (Thomas et al., 1988). These results suggest that one way by which some breeds achieve a longer

breeding season is by becoming less sensitive to the negative feedback effects of steroids, especially during long days. Some caution is, however, needed when interpreting the breed differences in sensitivity to testosterone treatment observed in the present study because of the breed differences in plasma testosterone concentrations maintained by the capsules. An attempt was made to remove this confounding effect by covariance analysis, but it is not known whether this procedure completely removed the effect of breed differences in plasma testosterone concentrations from the physiological, rather than the statistical, point of view. Further studies are required to confirm this.

Another finding from the present study was the observed breed difference in the seasonal variation in LH pulse frequency in rams not treated with testosterone. LH pulse frequency was significantly lower during the nonbreeding season than during the breeding season in Romney, but not in Poll Dorset, rams. This suggests that there exists a direct, steroid-independent effect of season on hypothalamic GnRH secretion in the more seasonal Romney rams but not in the less seasonal Poll Dorset rams. This agrees with findings from studies with ovariectomized ewes. In a study comparing ovariectomized Suffolk and Merino ewes, Thomas et al., (1988) showed that the LH pulse frequency was significantly lower during seasonal anoestrus than during the breeding season in Suffolk (8.2±0.5 vs. 10.8±0.8 pulses/8h; $P < 0.05$), but not in Merino (9.0±0.7 vs. 10.2±0.8 pulses/8h), ewes. The proportional reduction in LH pulse frequency from the nonbreeding to the breeding season in the Suffolk ewes (24%) is similar to that obtained in the present study for Romney rams (a 24% reduction from 15.8 to 12.0 pulses/8h). A direct effect of season on LH pulse frequency in castrated rams has also been observed by Sanford et al. (1984b). In general, these studies suggest that there exists, in both the ram and the ewe, a steroid-independent effect of season which influences directly hypothalamic GnRH secretion. However, the magnitude of this direct effect of season appears to be related to the seasonality of the breed concerned. This could be another way by which breed differences in length of the breeding season are achieved.

In contrast to other studies in the ram (Pelletier & Ortavant, 1975a; Lincoln & Short, 1980; Sanford et al., 1984b), the present study did not find any significant seasonal difference in the mean LH and FSH concentrations of control rams (ie those implanted with empty capsules). The reasons for these discrepancies are not clear but could be due to differences in photoperiod (natural vs. artificial), breeds (Romney and Poll Dorset vs. Ile-de-France and Soay) and location. In the present study, the reduction in LH pulse frequency during the nonbreeding as compared with the breeding season in

Romney rams was compensated for by an increase in LH pulse amplitude. The net effect was that no seasonal difference existed in mean LH concentrations.

In conclusion, the present study showed that, in the ram, season influences gonadotrophin secretion through both a steroid-dependent mechanism (indirectly affecting the sensitivity of the hypothalamo-pituitary axis to the negative feedback effect of testosterone) and a steroid-independent mechanism (directly affecting GnRH secretion from the hypothalamus). The sensitivity of the hypothalamo-pituitary axis to these seasonal effects varies among breeds differing in seasonality and may be the means by which breed differences in seasonality are achieved. The same difference may well exist within a breed but, if so, could not be readily utilized as a selection criterion because its measurement requires the removal of the testes. Nevertheless, the net result of differences in the sensitivity of the hypothalamo-pituitary axis to the seasonal effects in entire animals is a difference in the pattern of seasonal variation in gonadotrophin secretion, the timing of which appears to dictate breeding season length in the ewe and the seasonal growth and regression of testes in the ram. Thus results from the present trial support the potential usefulness of seasonal variation in testis size and circulating gonadotrophin concentrations as selection criteria for early onset of the breeding season.

CHAPTER VIII:

GENERAL DISCUSSION AND CONCLUSIONS

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The main objective of the present study was to investigate the potential usefulness of some physiological and endocrinological characteristics of rams as predictors of genetic merit for early onset of the breeding season in their daughters. Thus in selection programmes for early onset of the breeding season, it might be possible to select rams based on these indicator traits instead of, or in combination with, direct selection for date of onset of the breeding season in the ewe. Because of the greater selection pressure that can be applied to rams compared to ewes, the rate of genetic progress could be greatly improved by indirect selection in the ram (Walkley & Smith, 1980). Characteristics of rams examined in the present study included testis size, gonadotrophin secretion and pituitary responsiveness to a GnRH challenge. The choice of these characters was based on current understanding of the mechanisms controlling seasonal breeding in both rams and ewes. Although some other characters (such as libido and semen production) might also be useful, they were not considered in the present study because of the time frame of the study and also because of the difficulties associated with making repeatable and accurate measurements of these characters (Dufour et al., 1984; Boland et al., 1985). In addition, these characters are largely regulated by the same endocrine pathways that control seasonal variation in testis size.

Throughout this study, the emphasis was on the *pattern* of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to GnRH. The pattern of variation was considered to be more important than the absolute values of these parameters at a given time because, although the onset of the breeding season in the ewe is normally regarded as a discrete phenomenon, changes in the endocrine mechanisms leading to onset of the breeding season are gradual (Martin, 1984). It is these seasonal changes in the endocrine mechanisms controlling reproductive function that drive the seasonal variation in breeding activity in both the ram and the ewe (Lincoln & Short, 1980; Karsch et al., 1984).

The general approaches that can be used to find useful predictors of genetic merit have been discussed by Blair et al. (1990). Briefly, these approaches involve either studying animals differing in the desired trait to search for differences in some potential indicator traits, or studying animals differing in some potential indicator trait(s) for differences in the desired trait. As a first approach in the present study, differences between rams of breeds with short (Romney) and long (Poll Dorset) breeding seasons

in the pattern of seasonal variation in testis size, gonadotrophin secretion and the pituitary responsiveness to a GnRH challenge were compared. The rationale was that, if patterns of seasonal variation in testis size, gonadotrophin secretion and/or pituitary responsiveness to a GnRH challenge were good indicators of a ram's genetic merit for early onset of the breeding season, breeds that differed in time of onset of the breeding season could exhibit differences in the pattern of seasonal variation in these parameters. Because of the large differences between the two breeds in date of onset of the breeding season (Kelly et al., 1976; Knight et al., 1989), it would be expected that any between-breed difference in these characters was likely to be greater than that which would normally be found in a within-breed situation (particularly where it was not possible to examine very large numbers of rams within a breed). Results from this trial (Chapter IV) showed that, compared with Romney rams, Poll Dorset rams had an earlier but smaller seasonal change in testis size, less marked seasonal variation in LH pulse frequency and an earlier increase in plasma FSH concentrations but no difference in LH responses to the GnRH challenge.

Having found large between-breed differences in the pattern of seasonal variation in testis size and gonadotrophin secretion, another trial (Chapter VII) was initiated to investigate the differences between Romney and Poll Dorset rams in the mechanisms by which season affects gonadotrophin secretion. Results from this trial demonstrated that season affected gonadotrophin secretion through both a steroid-independent mechanism (directly on the hypothalamic GnRH pulse generator) and a steroid-dependent mechanism (indirectly on the sensitivity of the hypothalamo-pituitary axis to the negative feedback effect of testosterone). Of most relevance to the present study was the finding that there were significant breed differences in the sensitivity to both the steroid-dependent and steroid-independent effects of season, with Poll Dorset rams being less sensitive than Romney rams in each case. It is presumably this difference in sensitivity to the seasonal effects that determines the seasonality of a breed. The same difference may well exist within a breed but, if so, could not be readily utilized as a selection criterion because its measurement requires the removal of the testes. Nevertheless, the net result of differences in the sensitivity of the hypothalamo-pituitary axis to the seasonal effects in entire animals is a difference in the pattern of seasonal variation in gonadotrophin secretion, the timing of which appears to dictate breeding season length in the ewe and the seasonal growth and regression of testes in the ram. Thus results from this trial supported the potential usefulness of seasonal variation in testis size and circulating gonadotrophin concentrations as selection criteria for early onset of the breeding season.

The results from between-breed comparison trials were very promising. They indicated that, in the between-breed situation, early onset of the breeding season was associated with early but small seasonal changes in testis size and gonadotrophin secretion. These were, in turn, caused by breed differences in sensitivity to the environmental signals regulating seasonality (primarily, it is presumed, photoperiod). However, while between-breed studies are useful in providing clues regarding what parameters to search for in the within-breed situation, differences found between breeds do not necessarily guarantee a cause-and-effect relationship within a breed (Blair et al., 1990) and therefore have to be verified within breeds. Accordingly, differences among rams of the Romney breed in the pattern of seasonal variation in testis size, gonadotrophin secretion and pituitary responsiveness to GnRH were studied. To increase the likelihood of detecting differences among rams of the same breed, 60 Romney rams were screened on the basis of seasonal changes in testis diameters. Those with the earliest or latest increases in testis diameter were then selected and used in the trial (Chapter VI). Results from this trial showed that there were large differences among rams of the same breed in the pattern of seasonal variation in testis size and gonadotrophin secretion. However, there were some differences between the results obtained from this trial and those from the trial comparing Poll Dorset and Romney rams (Chapter IV). In the within-breed trial, an early increase in testis size was associated with a greater seasonal change in absolute testis size. Conversely, in the between-breed comparison, the early increase in testis size of Poll Dorset rams was associated with a smaller absolute change. Since the relationships between these two components (timing and magnitude) of the pattern of seasonal variation in testis size and date of onset of the breeding season are not known, the significance of these differences between the two studies cannot be determined. Nevertheless, the important point was that there were large differences in the pattern of seasonal variation in testis size and gonadotrophin secretion among rams of the same breeds which could potentially be exploited in selection programmes. Clearly, further studies are needed to establish the relationships between the timing and the magnitude of seasonal changes in testis size and the date of onset of the breeding season. While it would be possible to investigate all combinations of timing and magnitude of the seasonal change in testis size, this would be very expensive. Given a situation of limited resources, the alternative would be to select rams at the extremes of timing and magnitude and use these animals in the study. To achieve this, rams would need to be classified with respect to timing (early(E) and late(L)) and magnitude (great(G) and small(S)) of seasonal variation in testis size into four groups (EG, ES, LG and LS) and differences between ewes sired by rams from these groups in date of onset of the breeding season studied. The immediate problem that would need to be solved is how to classify rams into the above-mentioned four groups. One method would be to first classify all rams,

based on the procedures described in Chapter VI, into two groups with positive and negative regression coefficients. Within each group, the rams would then be ranked according to both the absolute values of their regression coefficients and the magnitude of seasonal change in testis diameter. From these ranks some delineations (based on extremes of timing and magnitude) could be chosen and rams assigned to each of the four groups using these delineations. Progeny testing of these rams would then be required to establish these relationships.

After the existence of some potential indicator traits has been demonstrated, the next step is to find out which of these traits are genetically correlated with the desired trait, in this case date of onset of the breeding season. This is a very complex and expensive operation and was definitely beyond the time frame of the present study. However, an attempt was made in the present study to gain some insights into the potential usefulness of the parameters measured. For this purpose, two progeny tests were initiated. The first involved rams of the Romney and Poll Dorset breeds (Chapter V), and the second involved rams only of the Romney breed (Chapter VI). As expected, Poll Dorset-cross ewe hoggets were more likely to reach puberty during their first breeding season, reached puberty earlier in the year and at a younger age, and had more oestrous cycles in their first breeding season than straightbred Romney hoggets. However, there were few significant effects of sire within breed on pubertal oestrous activity. During the second breeding season, Poll Dorset cross ewes came into the breeding season significantly earlier than straightbred Romney ewes. Heterosis might also have contributed to the observed between-breed differences in pubertal oestrous activity and date of onset of the second breeding season (Hanrahan & Quirke, 1986), but this effect could not be quantified in the present study. There was also a significant effect of sire within breed on date of onset of the second breeding season. However, the pooled within-breed correlations between date of onset of the second breeding season and the testis and endocrine parameters measured in the sires were very low for the reasons discussed in Chapter V. This is the major disadvantage of using progeny tests in the search for genetic markers. In order to establish a significant relationship, a large number of sires need to be tested. The correlation might be increased by selecting rams at the extremes and using these animals in the progeny test, provided the appropriate criteria were used in the selection process. Therefore, in the second progeny test, the rams used in the test were first selected for extremely early or late increases in testis size prior to onset of the breeding season. Despite this effort, differences in the pattern of seasonal variation in testis size and gonadotrophin secretion between Romney rams in the early and late groups were not associated with significant group differences in date of onset of the breeding season in their daughters. Again, correlations between date of onset of the breeding season in the female

offspring and the magnitude of seasonal variation in testis size and other endocrine parameters in the sires were low and non-significant. As was noted in Chapter VI, some unknown environmental factors during the two-tooth breeding season may have compromised the results. In the second breeding season, the first ewe was observed to be marked on 5 March, which was later than when ewes of this breed normally start their breeding season (Knight et al., 1989; see also Chapter V). When the breeding season did start, 95% of the ewes came into oestrus within a period of 25 days. This degree of synchrony in oestrous activity was unexpected. It could not have been caused by the ram effect as the ewes were run with two teaser rams from 1 November 1990. The lack of group differences in date of onset of the breeding season might also have been caused by the use of inappropriate criteria in the screening of rams. In the present study, rams were selected for an early or late increase in testis size. This selection procedure also resulted in rams with an early increase in testis size having a greater magnitude of seasonal variation in testis size than those with a late increase in testis size. If the timing and magnitude of seasonal variation in testis size are antagonistic to one another with respect to their relationship with date of onset of the breeding season (as was suggested by the Dorset vs Romney comparison), it could be expected that no significant differences would be found.

It is known that nutrition and liveweight have a determinative effect on the onset of puberty (Foster et al., 1986). Therefore, pubertal oestrous activity would not be an accurate measure of genetic merit for early onset of the breeding season in adult animals unless all factors affecting liveweight, such as date of birth, birth rank and growth rate, were eliminated. In the present study, date of onset of the second breeding season was not significantly affected by the date of, or age at, onset of puberty. Thus in future studies, more attention should be directed towards date of onset of the second breeding season.

The inability of the present study to demonstrate significant within-breed relationships between date of onset of the breeding season and the measured ram characters precludes any definite conclusions regarding the usefulness of these characters as selection criteria for early onset of the breeding season. Nevertheless, the results did show marked differences among rams of the same breed in the pattern of seasonal variation in testis size and gonadotrophin secretion. Furthermore, significant sire differences in date of onset of the breeding season were found (Chapters V & VI). The question remaining is how these differences are related. That is, how can the between-sire differences in genetic merit for early onset of the breeding season be identified by some measurable characters, such as those identified in the present study?

While the comparison of physiological characteristics between Romney vs Poll Dorset rams and between Romney rams is a useful first approach, the definitive assessment of whether these characteristics can be used as indirect predictors of genetic merit requires further studies. In the present series of experiments, several characteristics of the rams (including the timing and magnitude of seasonal variation in testis size, LH pulse frequency, mean FSH concentrations and LH responsiveness to a GnRH challenge) were found to vary both between and within breeds. The between-breed differences imply that they are under genetic control which is one factor determining potential usefulness as indicator traits for date of onset of the breeding season. However, before any indicator traits can be incorporated into selection programmes, their genetic correlations with date of onset of the breeding season must be established. Because it was not possible to examine large numbers of rams in this study, the magnitude of these genetic relationships remains to be established through the use of the progeny test or by other means (eg paternal half-sib correlations). However, a large number of sires need to be tested before a significant relationship can be established. For a phenotypic correlation coefficient of 0.25 to be statistically significant at the 5% level, approximately 60 observations (ie sires) would be required. The advantage of the progeny test technique is that, while it requires many sires, a large number of characters can be tested simultaneously without prior knowledge of their importance, providing opportunities for collaborative studies to reduce the cost.

Not all the parameters identified in the present study would be equally suitable as selection criteria. For example, measurement of the seasonal variation in LH pulse frequency requires intensive blood sampling over long periods. This may prove to be too costly for it to be of much practical value unless its use will greatly increase the selection differential and/or selection accuracy, and/or reduce the generation interval. Therefore, studies need to be carried out to elucidate the relationships between these potential indicator traits. If, as seems very likely (see Chapter VI), strong relationships exist among these parameters, it should be possible to reduce the number of parameters which must be measured in order to accurately predict the genetic merit of a ram. This would reduce the cost of prediction accordingly.

After the appropriate indicator trait(s) have been identified, selection programmes based on the indicator trait(s), alone or in combination with direct selection for date of onset of the breeding season, may be initiated. The level of enhanced genetic progress will be the ultimate test for the usefulness of these indicator trait(s).

In future studies, some other questions will also require attention. The first is the time of the year when the indicator traits should be measured. To answer this question, it

needs to be determined which of the components (timing or magnitude) of the pattern of seasonal variation is more important. The present study did not provide answers to this question. If the time of seasonal increase is important, measurements made during the period from October to December should be sufficient because the seasonal increase in testis size and gonadotrophin secretion takes place before the summer solstice (22 December). However, if the magnitude of seasonal variation is important, measurements have to be made over a longer period (probably from October to March).

The other aspect requiring attention is the effect of liveweight and nutrition on the pattern of seasonal changes in these parameters. In the present study, a significant relationship was not found between seasonal variation in liveweight and seasonal variation in testis size. However, significant effects of nutrition and liveweight on testis size and gonadotrophin secretion have been reported (Islam & Land, 1977; Masters & Fels, 1984; Xu, 1987). In the present study, the effect of variation in liveweight on testis size might be masked by the overriding effect of photoperiod. This is clearly demonstrated in Figure 6.4, which shows that testis size continued to increase during the period from January to March despite the concurrent decrease in liveweight. The effect of variation in liveweight is a major problem for studies in which animals have be to be maintained on pastures over a long period. Therefore, in order to estimate accurately the profile of seasonal variation in testis size and gonadotrophin secretion, it is important to reduce the variation in liveweight over time. In addition, it was found in Chapter VI that rams in the early group were significantly heavier than those in the late group. The exact nature of the relationship between liveweight and the pattern of seasonal variation in testis size is not known but could be important in the context of the present study. Further study is needed to investigate the relationship between liveweights of rams from a homogeneous flock and the pattern of seasonal variation in testis size. If it turns out that liveweight affects of the pattern of seasonal variation in testis size, it may be necessary to perform selection among rams of similar liveweight or to simply use liveweight as a covariate.

In conclusion, the present study has identified several physiological and endocrinological parameters in rams that might potentially be used as predictors of genetic merit for date of onset of the breeding season in ewes. Further, and more extensive, studies designed specifically to establish the genetic correlations between these potential parameters and date of onset of the breeding season are needed before these parameters can be incorporated into selection programmes. Finally, this study has also demonstrated that seasonality of rams is regulated by steroid-dependent and steroid-independent mechanisms similar to those operating in the ewe.

APPENDIX I

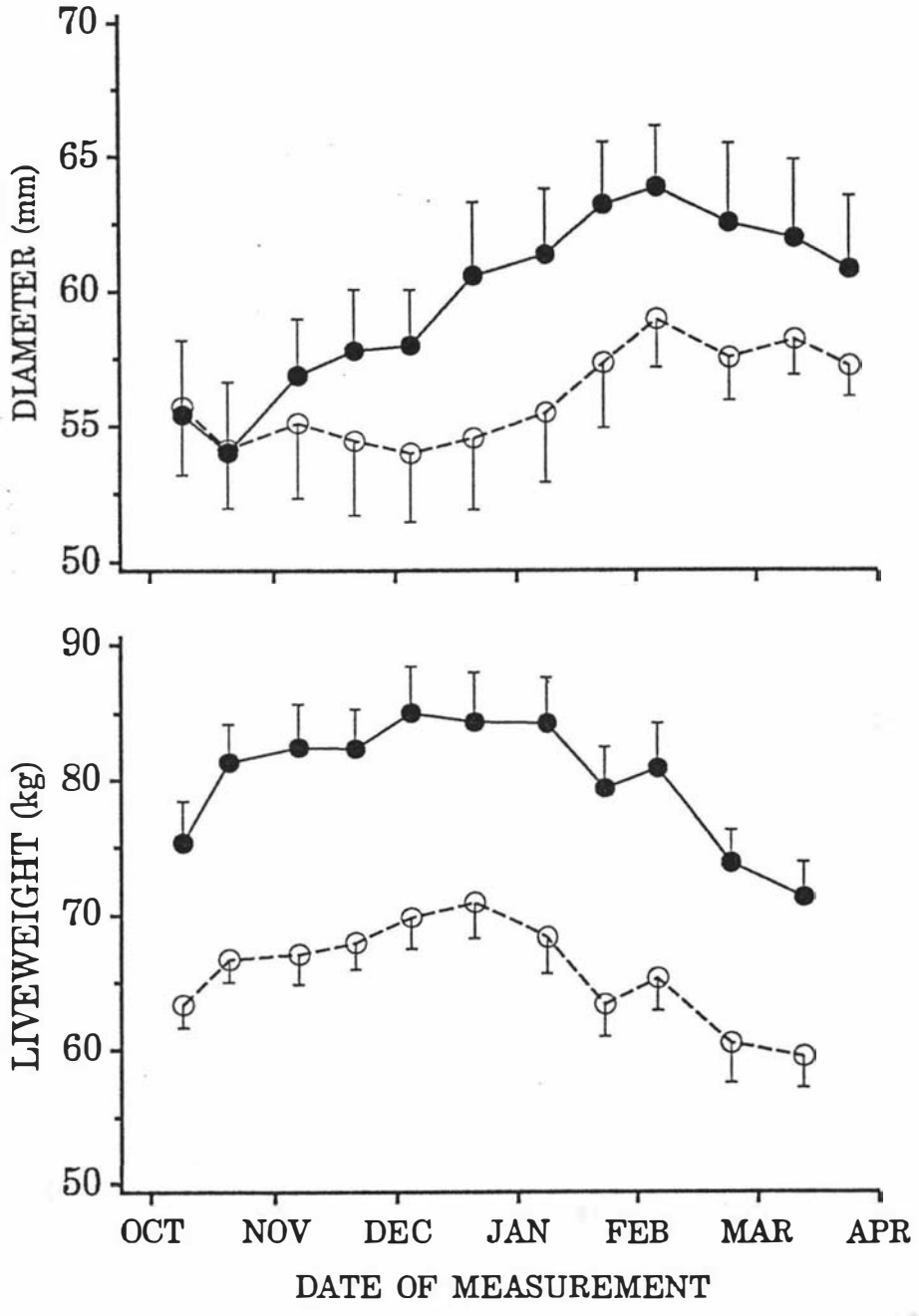
APPENDIX I

This appendix present some of the data from Chapter VI (Figure 6.4, Tables 6.1, 6.2 & 6.3), which have been reanalysed according to groups as originally classified based on testis diameter measurements made during the 1988-1989 season.

Appendix Figure 1.1 shows the seasonal variation in testis diameter and liveweight for rams in the original early and late groups during the 1989-1990 season. Essentially, there are no differences in the inferences drawn between the comparisons in the original and reclassified groups, although the magnitude of group differences varies slightly between the two methods of classification. Based on the original groups, the overall mean testis diameter of rams in the early group (59.6±1.0 mm) was significantly ($P < 0.01$) greater than that of rams in the late group (55.9±0.5 mm). There were significant ($P < 0.01$) differences between groups in the pattern of seasonal variation in testis diameter. Testis diameter of rams in the early group increased by 18% from a minimum of 55.1 mm in late October to a maximum of 62.8 mm in February. The corresponding increase for rams in the late group was 9% from 54.0 mm in early December to 58.9 mm in February. The liveweight of rams in the early group (80.0±1.4 kg) was significantly ($P < 0.01$) greater than that of rams in the late group (65.6±1.1 kg). The liveweight varied significantly ($P < 0.01$) with season but, as in the analysis of reclassified groups, there was no apparent association between seasonal changes in testis size and in liveweight.

Basal and mean LH concentrations, LH pulse frequency, LH pulse amplitude and mean FSH concentrations for rams in the early and late groups (based on the original classification) at different sampling times are presented in Appendix Table 1.1. There are several differences between results presented in Appendix Table 1.1 and those for the reclassified groups in Table 6.1. The major differences occurred in LH pulse frequency. Based on the original groups, no significant effects of group or group x time interactions on LH pulse frequency were apparent. However, both the effects of group and group x time interaction were significant with respect to LH pulse frequency when the data were analysed using the reclassified groups. In addition, the group differences in mean LH concentration and LH pulse frequency in March were significant for the reclassified groups but nonsignificant for the original groups. These differences in inferences drawn between comparisons in the original and reclassified groups reflected the existence of environmental effects on the pattern of seasonal variation in testis size. As there is likely a cause and effect relationship between seasonal variation in gonadotrophin secretion and seasonal variation in testis size, it is

more appropriate to relate these parameters in the same season (ie under the same environmental conditions). This supports using the reclassified groups in the analyses.



Appendix Figure 1.1. Seasonal variation in testis diameter (top panel) and liveweight (bottom panel) for rams in the early (n=6; ●——●) and late (n=6; o- - -o) groups (based on the original classification) during the 1989-1990 season. Vertical bars represent standard errors of the means. See Figure 6.4 for comparison with reclassified groups.

Appendix Table 1.1. Basal and mean LH concentrations, LH pulse frequency, LH pulse amplitude and mean FSH concentrations for rams in the early (E) and late (L) groups (based on the original classification) at different sampling times.[1,2,3] See Table 6.1 for comparison with reclassified groups.

Time	Group	LH				FSH
		Basal (ng/ml)	Mean (ng/ml)	Frequency (no./8h)	Amplitude (ng/ml)	Mean (ng/ml)
01 Nov	E	0.29±0.02	0.31±0.02	0.5±0.2	0.61±0.17	1.05±0.12
	L	0.28±0.02	0.29±0.01	0.3±0.1	NA[4]	1.26±0.28
13 Dec	E	0.30±0.02	0.39±0.03	1.0±0.2	1.10±0.01	2.19±0.34
	L	0.31±0.02	0.42±0.02	1.1±0.1	1.48±0.23	1.74±0.60
31 Jan	E	0.30±0.02	0.40±0.02	2.6±0.2	0.70±0.02	2.49±0.37
	L	0.23±0.03	0.38±0.03	2.6±0.5	0.59±0.04	2.13±0.36
20 Mar	E	0.32±0.01	0.36±0.02	2.8±0.7	0.52±0.02	1.95±0.29
	L	0.36±0.03	0.41±0.04	3.8±0.3	0.57±0.05	1.68±0.20
Significance						
Group		NS	NS	NS	NS	NS
Time		**	***	***	*	**
Group x Time		NS	NS	NS	NS	NS

[1] Number of rams in each group is 6.

[2] Values presented are means±SEM.

[3] There were no significant group difference in any parameters at any sampling time.

[4] No complete LH pulses were present for rams in the late group on 1 November.

Appendix Table 1.2 presents peak and total LH responses to the intravenous injection of exogenous GnRH (50 ng/kg liveweight) for rams in the early and late groups (based on the original classification) at different sampling times. There were no differences in conclusions drawn from comparisons between the original and the reclassified groups, except that the level of significance for group x time interactions on total LH response to GnRH changed from 5% for the reclassified groups to 10% for the original groups.

Appendix Table 1.2. Peak and total LH responses to an intravenous injection of exogenous GnRH (50 ng/kg liveweight) for rams in the early (E) and late (L) groups (based on the original classification) at different sampling times.[1,2] See Table 6.2 for comparison with reclassified groups.

Time	Group	Peak (ng/ml)	Total (min.ng/ml)
01 Nov	E	8.00±1.33	470.31± 96.06
	L	8.15±1.24	504.85± 80.38
13 Dec	E	6.34±1.25	514.01±111.12
	L	6.10±0.57	461.16± 54.34
31 Jan	E	3.01±0.72	309.89± 71.37
	L	4.50±0.56	400.29± 64.34
20 Mar	E	3.90±1.63	274.69± 57.30
	L	3.42±0.45	284.11± 47.06
Significance	Group	NS	NS
	Time	**	**
	Group x Time	NS	+

[1] Number of rams in each group is 6.

[2] There were no significant group difference in either the peak or the total LH response to GnRH at any sampling time.

Appendix Table 1.3 shows the proportion of ewe hoggets reaching puberty during the first breeding season, date of onset of the first oestrus, age at puberty, the number of pubertal oestrous cycles and the date of onset of the second breeding season. There were no differences in the inferences drawn from comparisons between the original and the reclassified groups.

Appendix Table 1.3. Summary by sire and group (based on the original classification) of the percentage of ewe hoggets reaching puberty during the first breeding season (%Pub), date of onset of the first oestrus (Dof_1, 1 = 1 April), age at puberty (Age_Pub), the number of pubertal oestrous cycles (No_cycle) and the date of onset of the second breeding season (Dof_2, 1 = 1 March). See Table 6.3 for comparison with reclassified groups.

Sire	Group	No.[a]	%Pub	Dof_1	Age_Pub	No_cycle	Dof_2
1	Early	17(1)	82.4	48±7	267±7	3.1±0.4	19 ± 2^{cde}
2	Early	15(0)	93.3	53±6	273±6	2.2±0.3	26 ± 1^{b}
3	Early	10(0)	70.0	46±8	265±9	2.7±0.4	18 ± 2^{de}
4	Early	15(0)	86.7	50±7	263±7	3.2±0.4	19 ± 1^{cde}
5	Early	10(1)	90.0	52±5	272±4	2.3±0.3	16 ± 3^{e}
Mean	Early	67(2)	84.5	50±3	268±3	2.7±0.2	20±1
6	Late	18(0)	77.8	54±5	271±5	2.4±0.4	21 ± 2^{bcde}
7	Late	9(0)	33.3	46±0	270±2	2.7±0.9	24 ± 5^{bcd}
8	Late	15(2)	73.3	47±5	266±4	3.0±0.4	21 ± 2^{bcde}
9	Late	13(1)	92.3	58±5	273±3	2.6±0.4	26 ± 2^{b}
10	Late	12(1)	75.0	51±8	263±8	3.1±0.5	18 ± 2^{de}
Mean	Late	67(4)	70.3	51±3	269±3	2.8±0.2	22±1

[a] Number of ewe hoggets present during the first breeding season. Numbers in parentheses are the numbers of animals which died between the first and second breeding season.

[b,c,d,e] Means within each column carrying the same superscript do not differ significantly ($P > 0.05$).

In conclusion, data presented in this Appendix and those in Chapter VI indicate that reclassification did not greatly affect inferences except with respect to LH pulse frequency. As previously noted, the use of reclassified groups is appropriate in this case because it compares the parameters under a similar environment (ie in the same season). Reclassification did not affect inferences drawn regarding the progeny test data, at least partly because 2 of the 3 reclassified animals were coincidentally reserves and therefore did not contribute to the progeny test.

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LIST OF ABBREVIATED JOURNAL NAMES

Acta Endocrinol. (Copenh)	Acta Endocrinologica (Copenhagen)
Anim. Breed. Abstr.	Animal Breeding Abstracts
Anim. Prod.	Animal Production
Anim. Reprod. Sci.	Animal Reproduction Science
Ann. Biol. anim. Bioch. Biophys.	Annales de Biologie animale Biochimie Biophysique
Ann. N.Y. Acad. Sci.	Annals of the New York Academy of Science
Aust. J. Agric. Res.	Australian Journal of Agricultural Research
Aust. J. Biol. Sci.	Australian Journal of Biological Sciences
Aust. J. Exp. Agric. Anim. Husb.	Australian Journal of Experimental Agriculture and Animal Husbandry
Biochem. J.	Biochemical Journal
Biol. Reprod.	Biology of Reproduction
Biol. Rev.	Biological Reviews
Can. J. Anim. Sci.	Canadian Journal of Animal Science
Can. J. Physiol. Pharmacol.	Canadian Journal of Physiology and Pharmacology
Clin. Chem.	Clinical Chemistry
Domestic Anim. Endocr.	Domestic Animal Endocrinology
Fertil. Steril.	Fertility and Sterility
Int. J. Androl.	International Journal of Andrology
Ir. J. Agric. Res.	Irish Journal of Agricultural Research
Ir. Vet. J.	Irish Veterinary Journal
J. Agric. Sci. Camb.	Journal of Agricultural Science, Cambridge
J. Anim. Sci.	Journal of Animal Science
J. Endocr.	Journal of Endocrinology
J. Reprod. Fert.	Journal of Reproduction and Fertility
J. Reprod. Fert. Suppl.	Journal of Reproduction and Fertility, Supplement
J. Steroid Biochem.	Journal of Steroid Biochemistry
Livest. Prod. Sci.	Livestock Production Science
Mol. Cell. Endocr.	Molecular and Cellular Endocrinology
N.Z. Agric. Sci.	New Zealand Agricultural Science
N.Z. J. Agric. Res.	New Zealand Journal of Agricultural Research
N.Z. J. Exp. Agric.	New Zealand Journal of Experimental Agriculture
N.Z. J. Sci. Technol.	New Zealand Journal of Science and Technology
Oxford Rev. Reprod. Biol.	Oxford Reviews of Reproductive Biology
Proc. Aust. Soc. Anim. Prod.	Proceedings of the Australian Society of Animal Production
Proc. Br. Soc. Anim. Prod.	Proceedings of the British Society of Animal Production
Proc. N.Z. Grassland Assoc.	Proceedings of the New Zealand Grassland Association

Proc. N.Z. Soc. Anim. Prod.	Proceedings of the New Zealand Society of Animal Production
Proc. Ruakura Farmers' Conf.	Proceedings of Ruakura Farmers' Conference
Proc. Soc. Exp. Biol. Med.	Proceedings of the Society for Experimental Biology and Medicine
Recent Prog. Horm. Res.	Recent Progress in Hormone Research
Reprod. Nutr. Develop.	Reproduction, Nutrition, Development
Sheep Res. J.	Sheep Research Journal
Wool Technol. Sheep Breed.	Wool Technology and Sheep Breeding