CHAPTER 5

ESTIMATION OF THE GENETIC RELATIONSHIP BETWEEN OVULATION RATE AND TESTICULAR DIAMETER IN THE TRANGIE D FLOCK

5.1 INTRODUCTION

The quantitative expression of male and female reproductive traits is genetically correlated, as has been clearly shown in studies with laboratory animal populations (Land, 1973; Islam et al., 1977 and Eisen and Johnson, 1981). Indications of genetic relationships have also been found in cattle (Brinks et al., 1979; Toelle and Robison, 1985), pigs (Proud et al., 1978) and sheep (Ricordeau et al., 1979; Burfening and Tulley, 1982 and Thorsteinsson et al., 1982), but the results of two sheep selection experiments with the latter species have been inconsistent. Hanrahan and Quirke (1982) reported that divergent selection, based on ovulation rate, in a Finnish Landrace population, has resulted in a significant correlated response in testicular diameter at 10 and 14 weeks of age. Genetic correlations between ovulation rate and testicular diameter at 10 and 14 weeks of age were estimated to be 0.41 and 0.35 respectively, and were not associated with significant differences in lamb liveweight. In contrast Land et al. (1982) who selected for liveweight-adjusted testicular diameter at 6 to 14 weeks of age in Finn-Dorset crossbreds, did not observe any change in ovulation rate or lambing performance, despite achieving a signficant divergence in the male trait. The disparity between the results of these two experiments may have been largely influenced by the difference in the actual male traits under selection.

Genetic improvement of the reproductive performance of the Australian Merino is restricted by the low level of prolificacy itself, which limits the selection pressure which can be applied, by the sex-limited nature of

the trait and by its low repeatability and heritability (Bindon and Piper, 1976).

In the Merino, therefore, there would be considerable value in defining an indirect selection criterion which does not suffer from the distribution related difficulties of prolificacy and its major determinant, ovulation rate, and which is measurable in males.

The aim of the study reported in this Chapter was to investigate, in a random-breeding flock representative of the NSW Merino breeding industry, the genetic relationships between ovulation rate (OR) at 4 ages and male gonadal size at 3 ages. Relationships between OR and serving capacity were not examined because of the absence of additive genetic variation in the measures of serving capacity in the males of this flock, as reported in Chapter 4.

5.2 MATERIALS AND METHODS

5.2.1 Animals and management

This study utilised records from animals of the Trangie D flock, the background and management of which, has been previously described in Chapter 2.

The male animals were those born in 1979-1981 and utilized in the study of male gonadal traits reported in Chapter 3. Also involved in this investigation were their dams, born during the period 1975-1980, and the D flock female progeny born in 1979-1981.

5.2.2 Experimental

5.2.2.1 Male Traits

Testicular diameter (TDM) measurements at 5, 8 and 12 months of age, which were chosen to be representative of gonadal size at pre-pubertal, pubertal and post-pubertal stages of sexual development respectively, were utilized in this study. The measurement technique and the gonadal and liveweight growth of the animals, have been described in Chapter 3.

5.2.2.2 Female Traits

Ewes born in 1975-1981, were examined by mid-ventral laparoscopy, according to the method pioneered for sheep by Thimonier and Mauleon (1969), to assess the total number of corpora lutea present on the surface of both ovaries (ovulation rate, OR). Each year with one exception ewes were grazed with vasectomised rams, and oestrus recorded, for 3-4 months prior to laparoscopy. The one exception was in 1978, when ewes born in 1975 had only a two day association with the teaser rams. These OR records have been excluded from the analyses presented in this Chapter.

Laparoscopy observations were first made on ewes at 17-18 months of age in early February of each year. On this occasion, all surviving female progeny were examined. Subsequent to laparoscopy, approximately half these young maiden ewes were randomly chosen for introduction into the breeding flock as replacements. The ewes were then annually assessed for OR in February of each year, until they were 53-54 months of age. These annual OR records will henceforth be referred to as records at 18, 30, 42, and 54 months of age. Each year, approximately 2 weeks after laparoscopy, ewes were allocated to mating groups of 30-40 mixed-age females and were single-sire joined for a period of 6 weeks. Specific details of the joining procedures, subsequent lambing management and recording have been reported in Chapter 2.

5.2.3. Data

5.2.3.1. Dam-son Covariance Analyses

TDM records of males born in the D flock in 1979-1981, and utilised in the study of male gonadal traits described in Chapter 3, and the OR records of their dams, formed the basis of the data set. Of the 606 possible dam-son combinations, for each male-female trait-pair, some were not included in the final data set, for one of the following reasons:

a) dam born before 1975, and hence no OR record;

b) dam examined by laparoscopy but ovary not fully visible;

c) zero value (no corpora lutea) recorded at laparoscopy.

The following numbers of dam-son combinations were included in the analyses:

18 month OR - 473

30 " " - 403

42 " " - 471

54 " " - 433

Within each age based OR classification, individual dams were able to be represented more than once, depending on their complement of entire male progeny, and also on their year of birth.

5.2.3.2. Half-sib Covariance Analyses

TDM records of the male and OR records of the female D flock animals born in 1979-1981, formed the basis of this analysis. As with the dam-son study, records of the females, where OR was not fully assessable or where no corpora lutea were found at laparoscopy, were excluded from relevant analyses.

In any analysis where a sire was not represented by at least 2 progeny of each sex, all progeny of that sire were excluded.

The structure of this data set, in terms of numbers of animals in each year-group, the average number of progeny per sire, and the numbers of sires represented for each male and female trait are presented in Table 5.1.

5.2.4 Statistical.

5.2.4.1 Dam-son Covariance Analyses

Estimates of the genetic relationship between dam ovulation rate and son TDM were calculated using the following stepwise procedure:

1. For both dam and son traits the following model was fitted to the data:

$$Y_{ijk} = \mu + L_i + G_{ij} + e_{ijk}$$

where:

Y_{ijk} = value of the dam or son trait for the k^{th} individual,

μ = the overall mean,

Table 5.1. Numbers of animals in each year-group, numbers of sires, and average number of progeny per sire for half-sib covariance analyses.

Dam age at OR record (months):	Year-of-birth group	Number of sires	Total progeny male	Total progeny female	Av. progeny per sire: male	Av. progeny per sire: female
18	1979	34	196	362	5.8	10.7
	1980	37	210	409	5.7	11.1
	1981	41	190	422	4.6	10.3
30	1979	34	194	204	5.7	6.0
	1980	37	210	214	5.7	5.8
	1981	40	189	282	4.7	7.1
42	1979	34	195	216	5.7	6.4
	1980	36	208	192	5.8	5.0
54	1979	34	195	205	5.7	6.0

L_i = the fixed effect of the i^{th} strain-line,

G_{ij} = the fixed effect of the j^{th} dam and son year of birth combination, and son litter size group in the i^{th} strain-line,

e_{ijk} = random errors, assumed $N(0,\sigma e^2)$.

2. Using least squares ANOVA procedures, variation due to the $u + L_i + G_{ij}$ was accounted for and the resulting residual covariance is an unbiassed estimate of phenotypic covariance between each pair of dam and son traits.

3. If the phenotype of the dam for trait 1 is:

$$D = G + E,$$

and that of the offspring for trait 2 is:

$$S = g + e,$$

where g and e represent genetic and environmental contributions to phenotype, and if the environmental components of the two models are uncorrelated with the genetic components or with each other, then the covariance between parent and offspring phenotypes is:

$$COV_p (D,S) = COV [(G + E), (g + e)]$$

$$= COV (G,g)$$

Further, since Cov(G,g) involves offspring and a single parent, then

$$Cov(G,g) = 1/2Cov_g(D,S) + \text{epistatic variation}$$

(Cunningham, 1969).

Twice the phenotypic covariance therefore estimates the additive genetic covariance (Cov_g) for the two traits plus some epistatic variation.

4. Using this covariance estimate, genetic correlations between dam and son traits were calculated as:

$$r_g = \frac{2\ Cov_p(D,S)}{\sqrt{(\sigma^2_D \times \sigma^2_S)}}$$

where $COV_p(D,S)$ = phenotypic covariance,

$\sigma^2 D$ = the genetic variance component for the dam trait,

$\sigma^2 S$ = the genetic variance component for the son trait,

(Falconer, 1981), whilst approximate standard errors were estimated using the method described by Mason (1964).

5. Estimates of the genetic variances were not able to be calculated from the above analyses of variance since variation due to sires was not included in the model. Appropriate estimates were therefore taken from the following sources:

   a) Male traits: i) the Least squares ANOVA sire component of variance used to calculate heritabilities of TDM in Chapter 3, and ii) the estimates from the Restricted Maximum Likelihood REML half-sib analyses which are described in the next section of this Chapter.

   b) Female traits: These were also taken from two sources: i)the Least squares ANOVA sire component of variance estimates used to calculate heritabilities in the report of Piper et al. (1984), and ii) the final estimates from the REML half-sib analyses, as described

above.The genetic variances are presented in Table 5.2.

Table 5.2. Genetic variances of OR and TDM used in the estimation of dam-son genetic correlations.

Age at trait measurement:	Source 1*	Source 2**	Source 3***
Male(TDM)			
5 months	0.03177520		0.07998276
8 "	0.43724272		0.38942640
12 "	0.20168844		0.15318178
Female(OR)			
18 months		0.00320792	0.00737390
30 "		0.01096448	0.01275677
42 "		0.03809168	0.02757119
54 "		0.00573160	0.02506284

* Source 1: Chapter 3, this Thesis.
** Source 2: from the analyses of Piper et al (1984).
*** Source 3: From the REML half-sib analyses in Section 5.3.2, this Thesis.

5.2.4.2. Half-sib covariance analyses

Estimates of the genetic relationship between TDM at 3 ages and OR at 4 ages in the female half-sibs, were calculated using the method of Schaeffer et al (1978). This procedure is designed to estimate variance and covariance components of a random effect (in this case, sires), when different variables are measured on different experimental units. The method involves solving Henderson's (1973) mixed model equations and then equating quadratic forms of these solutions to their expectations. The procedure is iterative and requires initial values to be assigned to the unknown components. Initial variance values were taken from Sources 1 and 2 (Table 5.2), while initial covariance values were taken from the dam-son covariance estimates. Convergence (if it occurs) is to a solution of the Restricted Maximum Likelihood (REML) estimating equations, described by Patterson and Thompson (1971). If the random effect is due to sires,

half-sib estimates of genetic parameters can be derived from the REML estimates of the variance components.

Using the model:

$$Yijklm = \mu + G_i + F_{ij} + S_{ijk} + B_{il} + e_{ijklm}$$

where

Y_{ijklm} = value of the dependent variable for the mth animal,

μ = value of the overall mean,

G_i = the fixed effect of the ith sex,

F_{ij} = the fixed effect of the jth strain-line in the ith sex,

S_{ijk} = the randon effect of the kth sire in the ijth strain-line,

B_{il} = the fixed effect of the lth birth type in the ith sex,

e_{ijklm} = random errors,

male and female trait-pairs were analysed separately for each year-of-birth group. Separate analyses for each year-of-birth group, as opposed to a single analysis incorporating all year-of-birth groups was necessitated by the extensive computational requirements of the algorithm. Standard formulae for the estimation of genetic correlations from paternal half-sib variance and covariance components were used (as described in Chapter 3), whilst standard errors were calculated by the method described by Hammond and Nicholas (1972).

5.2.4.3. Procedure for pooling genetic correlations

In the case of the genetic correlations estimated from the half-sib REML analyses, year-of-birth-group estimates were pooled to obtain one estimate for each male-female trait-pair. In addition, where appropriate, the r_g's calculated from both the dam-son analyses and the half-sib analyses, were pooled across ages. The pooling of r_g's was according to the method described by Ponzoni (1975):

$$\text{Pooled } r_g = \frac{\sum (1/V_i \times r_{gi})}{(\sum 1/V_i)}$$

where V_i = variance of the ith r_g estimate;

r_{gi} = i^{th} r_g estimate

The standard errors of pooled r_g estimates were calculated according to the following method:

$$\text{SE of pooled } r_g = \sqrt{\sum \left(\frac{1/V_i}{\sum 1/V_i} \right)^2 \cdot V_i}$$

where V_i = variance of the i^{th} r_g estimate (Snedecor and Cochran, 1967).

5.3. RESULTS

5.3.1 Genetic Correlations estimated from Dam-Son covariance analyses.

Mean values, SD's and CV's of OR at 18, 30, 42 and 54 months of age, for dams with sons born between 1979-1981 are presented in Table 5.3. Whilst means and SD's increased with age at OR measurement, CV's were relatively constant.

Table 5.3. Means, SD's and CV's of OR at 18, 30, 42 and 54 months of age of dams with sons born between 1979 and 1981.

Age at OR record (months):	Number of dams	OR		
		Mean	SD	CV(%)
18	473	1.28	0.46	35.9
30	403	1.34	0.50	37.3
42	471	1.38	0.50	36.2
54	433	1.46	0.56	38.4

The model used for the Least Squares Analysis of Variance was designed to permit unbiassed estimation of the dam-son covariances for each pair of dam and son traits. The fixed effects fitted, were strain-line and group,

the latter being a composite effect accounting for variation in year of birth and measurement of dam and son and also birth type of the son. The group effect was a highly significant source of variation ($P< 0.001$) for all male and female traits, whilst variation between strain-lines was of significance ($P< 0.05$) only for TDM at 5 and 12 months of age.

The phenotypic covariances for each pair of dam and son traits, estimated from the Analysis of Variance residual covariances are presented in Table 5.4.

Table 5.4. Covariances between dam OR at 18, 30, 42 and 54 months of age and son TDM at ages of 5, 8 and 12 months.

Age at OR record (months):	Son age (months) at TDM measurement: 5	8	12
18	0.00739652	0.02556761	0.01462863
30	0.01510356	0.00207232	0.00242678
42	-0.00066952	0.00118020	0.0014103
54	0.01043541	0.01996890	0.01864115

Utilising the covariance estimates presented in Table 5.4, genetic correlations between dam OR and son TDM were calculated using the two sets of dam and son genetic variances identified in Section 5.2.3. The resulting r_g estimates, for each of the four dam trait and three son trait combinations are presented in Table 5.5.

The genetic correlation between OR at 42 months and TDM at 5 months of age shows the smallest degree of association and this is consistent across both sets of genetic variances (-0.04 ± 0.54 and -0.03 ± 0.29, respectively for Source 1 and 2). Although several of the r_g values calculated using Source 1 genetic variances lie outside the upper theoretical value of +1, those calculated using Source 2 variance all lie within the theoretical boundaries.

Examination of the differences between r_g's calculated using Source

Table 5.5.Genetic correlations (±SE) between Dam OR and Son TDM at various ages and χ^2 tests of differences between correlations involving the same dam or son trait.

Dam OR	Source 1*							Source**						
at age (months):	Son TDM at age (months): 5		8		12		χ^2_{2df}	5		8		12		χ^2_{2df}
18	1.47	0.87	1.37	0.53	1.15	0.55	0.13^{NS}	0.61	0.44	0.95	0.31	0.87	0.34	0.41^{NS}
30	1.62	0.54	0.60	0.33	0.10	0.35	6.81*	0.95	0.47	0.06	0.33	0.11	0.37	2.69^{NS}
42	-0.04	0.31	0.03	0.19	0.00	0.20	0.03^{NS}	-0.03	0.29	0.02	0.21	0.00	0.23	0.02^{NS}
54	1.55	0.08	0.80	0.53	1.10	0.55	0.56^{NS}	0.47	0.30	0.40	0.21	0.60	0.23	0.42^{NS}
χ^2_{3df}	9.99**		8.63**		6.74^{NS}		-	3.32^{NS}		6.93^{NS}		6.24^{NS}		

* Source 1: Genetic variances of dam OR from Piper, et al.(1984) and of son TDM from Chapter 3, this Thesis.
**Source 2: Genetic variances from REML analyses described in Section 5.3.2 of this chapter.

1 genetic variances reveals that for the same female trait, values are heterogeneous only for r_g's involving OR at 30 months of age. When the male traits are considered, r_g's involving TDM at 5 and 8 months of age display heterogeneity when compared over the 4 ages of female OR measurement. In contrast, when r_g's calculated using Source 2 genetic variances are compared over the one trait, there are no significant differences between r_g values within any of the male or female traits.

Table 5.6 presents estimates of genetic correlations calculated by pooling, where appropriate, each of the four r_g values involving the one male trait.

The pooled estimates of r_g, calculated from those initially derived using Source 2 genetic variances, show close agreement ($\chi^2_{(2)}$= 0.159 NS) and were pooled again, to yield one overall estimate of r_g between OR at 18-54 months and TDM at 5-12 months. This procedure yields a value of 0.35 $\pm$ 0.08.

Table 5.6. Genetic correlations between TDM at 5, 8, and 12 months and OR at 18-54 months of age.

Age (months) at TDM measurement	Source 1*	Source 2*
5	N/A	0.38 $\pm$ 0.17
8	N/A	0.31 $\pm$ 0.12
12	0.21 $\pm$ 0.16	0.37 $\pm$ 0.14
Overall	0.21 $\pm$ 0.16	0.35 $\pm$ 0.08

* Sources of genetic variances as described for Table 5.5.

5.3.2 Genetic Correlations estimated from Half-Sib analyses

Mean values, SD's and CV's of OR at 18, 30, 42 and 54 months of age of the D flock ewes born in 1979-1981 are presented in Table 5.7.

REML analyses of variance were conducted for each male-female trait-pair within each year-of-birth group. The specific variance and

covariance estimates from each of the 27 individual analyses are presented in Appendix 1.

Table 5.7. Mean values, SD's and CV's of OR at 18, 30, 42 and 54 months of age of D flock ewes born 1979-1981.

Year of birth	OR at age (months): 18			30			42			54		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV
1979	1.08	0.31	28.7	1.36	0.55	40.0	1.17	0.39	33.3	1.66	0.61	36.7
1980	1.11	0.31	27.9	1.07	0.27	25.2	1.59	0.54	34.0	-	-	-
1981	1.08	0.29	26.9	1.54	0.53	34.4	-	-	-	-		

Table 5.8 details starting values of genetic variances which were used in the REML analyses, and lists the final estimates generated after 20 rounds of iteration for each of the 3 male and 4 female traits. The final variances as they appear in Table 5.8, have been calculated by pooling, over years and over traits of the opposite sex, the individual variance estimates weighted by the relevant sire degrees of freedom.

A similar pooling procedure was used to calculate the covariances and these are presented in Table 5.9, together with the starting values used in the REML analyses.

Table 5.8 Starting values and final pooled REML genetic variance estimates between TDM at 5, 8 and 12 months and OR at 18, 30, 42 and 54 months of age.

Trait	Starting values*	Final REML values
TDM at		
5 months	0.032	0.080
8 months	0.440	0.389
12 months	0.200	0.153
OR at		
18 months	0.003	0.007
30 months	0.011	0.013
42 months	0.038	0.028
54 months	0.006	0.025

* Starting values from Source 1 and Source 2 as detailed in Table 5.2.

Table 5.9. Starting values and final pooled REML genetic covariance estimates between TDM at 5, 8 and 12 months and OR at 18, 30, 42 and 54 months of age.

Age (months) at OR measurement:	Source	Age (months) at TDM measurement: 5	8	12
18	Starting value*	0.007	0.026	0.015
	Pooled REML	0.00686882	-0.00056567	0.00796319
30	Starting value*	0.015	0.002	0.002
	Pooled REML	0.01520039	0.00984823	0.00994774
42	Starting value*	-0.001	0.001	0.000
	Pooled REML	-0.00223613	0.00361462	0.03444505
54	Starting value*	0.010	0.020	0.019
	Pooled REML	0.03552839	0.02134742	0.035342631

*Starting values from dam-son covariance analysis, Section 5.3.1.,

The genetic correlations calculated from the pooled variances and covariances, described above, are presented in Table 5.10 together with χ^2 tests of significance of the differences between the r_g estimates common to each male and female trait. These r_g values range from -0.16 to 0.47 but within any one trait, the individual correlations with the traits of the other sex, do not differ significantly. Standard errors of these estimates, however, are large.

Table 5.10. Half-sib genetic correlations (±SE) between TDM at 5, 8 and 12 months and OR at 18, 30, 42 and 54 months of age and χ^2 tests of difference between correlations.

Age (months) at TDM measurement	Age (months) at OR measurement: 18	30	42	54	χ^2 (3df)
5	0.38 ± 0.38	0.47 ± 0.46	-0.16 ± 0.48	0.35 ± 0.54	1.097 NS
8	-0.03 ± 0.34	0.12 ± 0.42	-0.11 ± 0.36	0.19 ± 0.60	0.286 NS
12	0.17 ± 0.24	0.26 ± 0.48	0.21 ± 0.36	0.32 ± 0.51	0.293 NS
$\chi^2_{(2df)}$	0.649 NS	0.323 NS	0.544 NS	0.043 NS	

The genetic correlations, pooled over the 4 ages at OR measurement, between TDM at 5, 8 and 12 months and OR at 18-54 months of age are listed in Table 5.11..

Table 5.11 Half-sib genetic correlations (±SE) between TDM at 5, 8 and 12 months of age and OR at 18-54 months.

Trait	Age (months) at TDM measurement:			
	5	8	12	5 - 12
OR at age 18-54 months	0.28 ± 0.23	0.01 ± 0.20	0.21 ± 0.17	0.16 ± 0.11

Although the r_g's presented in Table 5.11 range from 0.01 to 0.28, they do not differ significantly ($\chi^2_{(2df)} = 0.920$,NS) from each other. The overall pooled estimate of the genetic correlation between TDM at 5-12 months and OR at 18-54 months of age, calculated from the r_g values in Table 5.11 is 0.16 ± 0.11.

5.4 DISCUSSION

5.4.1 Introductory

As indicated in the introduction to this Chapter, previous attempts at quantifying genetic relationships between male and female reproductive traits in domestic animals, have not provided animal breeders with firm evidence that improvement in female reproductive performance can be achieved by indirect selection on male physiological traits. This situation reflects the high cost of collecting appropriate sets of data, and also the evolution of the theory underlying estimation of variance and covariance components when different variables are measured on different experimental units. Whilst the theoretical optimum number of animals needed to estimate genetic correlations to a desired level of precision can be calculated (Robertson, 1959), the research worker or team is normally constrained by practical considerations to numbers well below the optimum, particularly for

traits of low heritability such as reproduction rate and its components. Likewise, although the statistical theory appropriate to the analysis of such data has been published (for example, Schaeffer et al., 1978), the computer software and/or computer space is not available to enable the optimal theory to be utilised in data analysis. The studies reported in this Chapter reflect, in both the experimental design and methods of statistical analysis used, the problems indicated above.

The animals of the Trangie D flock were chosen for this study because they were representative of the NSW Merino breeding industry, due to the origins of the foundation animals and new sires and to the policy of random breeding within the sub-flocks. When compared to closed random mating populations and those under selection, such a flock has the advantage that the genetic parameters estimated from it should be directly relevant to the existing Merino industry.

5.4.2 Genetic Correlations from Dam-Son covariance analyses

The model used to estimate the phenotypic covariance between dam and son was chosen with the aim of accounting for the major environmental sources of variation affecting male and female reproductive traits in the Trangie environment as shown by Piper et al. (1980), and Purvis, et al. (1984). Variation due to the composite effect of birth and measurement year (of dam and son) and birth type of son, was a highly significant source for all TDM and OR age combinations. The effect of strain-line (or bloodline) was of significance only for TDM at 5 and 12 months of age, which is in agreement with the findings in Chapter 3.

The mean OR of the D flock ewes included in these analyses increased with age, a finding which is in agreement with that of Piper et al. (1984) who studied a larger number of ewes from the same flock. An important feature of the ovulation rate of the ewes in this population is the very low percentage (over all ages - 1.3%) of ewes having 3 or more corpora lutea. This level of incidence is similar to that for triplets (LB/EL), quoted by

Piper and Bindon (1982), in Merino ewes under similar conditions and is perhaps lower than expected since, on average only 40% of lambing ewes shedding 3 eggs would give birth to 3 lambs (Hanrahan, 1980).

The covariance estimates between dam OR and son TDM ($Cov_p(D,S)$) presented in Table 5.4 show considerable variability and no pattern across ages is discernable. $Cov_p(D,S)$ values between OR at 42 months of age and the 3 TDM traits are uniformly small which is in contrast to values for combinations involving the three other OR records. There is no obvious explanation for this feature.

The two sets of genetic variances used to estimate genetic correlations, showed substantial disagreement for several of the male and female traits, despite being estimated from the same population. However, though the sampling variances of the genetic variance estimates were not calculated, they can be assumed to be large, particularly for the estimates taken from the half-sib REML analyses which were based on smaller numbers than those from Piper et al (1984).

These factors suggest that the genetic correlations calculated for the 12 male-female trait-pairs should be regarded simply as small sample estimates of the pooled r_g estimates presented in Table 5.6. This suggestion is reinforced by the large standard errors of the individual trait-pair r_g's presented in Table 5.5. Despite these large standard errors, however, significant differences between r_g's, calculated using Source 1 genetic variances, and involving TDM at 5 and 8 months and OR at 30 months, were found. Each of these sets of r_g estimates is characterised by individual values lying outside the upper theoretical limit of the distribution of r_g.

The r_g estimates, resulting from pooling over ages at OR measurement, where appropriate, ranged from 0.31 ± 0.12 to 0.38 ± 0.17 for those calculated from Source 2 genetic variances, whilst the single pooled r_g from Source 1 was 0.21 ± 0.16. Further pooling of the Source 2 estimates,

to obtain one estimate of the r_g between OR and TDM, gave a value of 0.35 $\pm$ 0.08. The practical implications of the magnitude of this estimate of r_g will be discussed later.

5.4.3 Genetic Correlations from Half-Sib REML analyses

The genetic correlations between OR and TDM, estimated using the paternal half-sib method, were calculated from data from animals born between 1979-1981. Whilst the male animals were the same as those represented in the dam-son covariance analyses, the females were a substantially different sub-set to those used in the dam-son analyses.

Due to the computational requirements of the REML procedure, analyses were conducted within year-of-birth groups and the estimates of r_g's were pooled over years to obtain the 12 individual trait-pair values (TDM at 5, 8 and 12 months x OR at 18, 30, 42 and 54 months of age). OR records at 42 and 54 months of age are represented by data from only 2 and 1 year-of-birth groups, respectively. This fact is reflected in the larger standard errors of the r_g's involving these traits.

When compared to starting values, the REML estimates of genetic variances (Table 5.8) and covariances (Table 5.9), obtained after 20 rounds of iteration, show a mixture of close agreement and substantial disagreement. In the case of the genetic variances of the male traits for example, the REML estimate of TDM at 5 months of age is more than double the starting value which was taken from the Least Squares analyses described in Chapter 3. This difference may be a reflection of the slightly different models used. Some of the environmental sources of variation identified in the Least Squares model were unable to be fitted in the model used for the REML analyses.

In comparison to the r_g's estimated by the parent-offspring covariance method, the 12 individual trait-pair r_g's resulting from the REML analyses, show a much smaller range of values (Table 5.10). Differences between r_g's involving particular male or female traits were

not significant, allowing an overall estimate of the r_g between TDM and OR to be calculated. This value of 0.16 $\pm$ 0.11 is not significantly different (X^2_{1df} = 1.95) from the estimate of 0.35 $\pm$ 0.08 from the dam-son covariance analyses.

5.4.4 General

The genetic relationship between OR and TDM in the random breeding Trangie D flock has been estimated from the covariance between dam and son using Least Squares methods, and from the covariance between paternal half-sibs using REML methods. Although the final estimates of the overall genetic correlation between TDM at 5-12 months and OR at 18-54 months, do not differ significantly, there are certain differences between the methods and in the structure of the data sets, which should be considered.

In the analyses of Piper et al. (1984) and also in the REML analyses discussed in this Chapter, genetic variances of OR at 18-54 months were calculated assuming that sire "families" consisted only of half-sibs. Likewise the genetic covariances from the REML analyses were assumed to estimate the covariance between half-sibs. However, a variable number of the animals were full-sibs, both sister-sister and brother-sister. The data sets used in the REML analyses, for example, contained approximately 5% of full-sib sisters and 6% of full-sib brother-sisters. Jackson (1983), has examined the bias, inherent in ignoring dams, in heritability estimates from sire-family variance components, and concluded that the method does not produce any appreciable bias for the range of conditions commonly encountered in sheep populations.

The genetic covariances estimated by the parent-offspring and half-sib methods, include components due to interaction variance arising from epistasis, with the coefficient of this source, in the covariance of parent-offspring, being four times that in half-sibs . The magnitude of the contribution from epistasis, however, can be identified only by special experimental designs (Falconer, 1981).

The estimates of the genetic correlation between OR and TDM in the Trangie D flock are similiar to the estimates reported by Hanrahan and Quirke (1982). In that study genetic correlations were calculated from the divergence between lines of Finn sheep, selected for one generation, on OR at 1.5 - 2.5 years of age. Using heritabilities of 0.6 for TDM and 0.5 for OR (the realized heritability calculated from the divergence between high and low selection lines), the r_g's between OR and 10- and 14-week TDM were estimated to be, respectively 0.41 and 0.35.

The similarity between the estimates of r_g from the present study and that of Hanrahan and Quirke (1982), is on first consideration surprising, given the substantially different heritability of OR (0.07 in the Trangie D flock; Piper et al., 1984) in the two populations. However, the difference is in the "observed" heritabilities and this may largely be a reflection of the two quite different distributions of OR in the Merino and Finn sheep. This effect of scale could also be expected to operate on the "observed" covariances involving OR, and result in ratios of genetic covariance and variance, being not different in the two populations, despite there being large differences in additive genetic variance.

The estimates of the r_g between OR and TDM, discussed above, are not at first sight supported by the results of the selection experiment reported by Land et al.

(1980) and Land et al. (1982). Selection for and against TDM at 6-14 weeks of age in Finn-Dorset cross lambs, resulted in significant differences between the selection lines in 10 week TDM (realized heritability 0.40 $\pm$ 0.08) but no correlated changes in OR were observed after 6 years of selection. The selection criterion, however, was TDM adjusted for liveweight, a trait which may have a different genetic relationship with OR to that of unadjusted TDM. The genetic relationship ($r_g^{\#}$) between liveweight (LW) adjusted TDM ($TDM^{\#}$) and OR can be derived as follows (B.P.Kinghorn and L.R. Piper, personal communication):

Let adjusted TDM be

$$TDM^{\#} = TDM - b_p.LW \qquad (1)$$

where b_p is the phenotypic regression of TDM on LW.

The genetic covariance between $TDM^{\#}$ and OR is

$$Cov_g(TDM^{\#},OR) = Cov_g(TDM - b_p.LW,OR)$$
$$= Cov_g(TDM,OR) - b_p Cov_g(OR,LW) \qquad (2)$$

and the genetic variance of $TDM^{\#}$ is

$$V_g TDM^{\#} = V_g(TDM - b_p.LW)$$
$$= V_g TDM + b_p^2.V_g LW - 2b_p.Cov_g(TDM,LW) \qquad (3)$$

Applying the standard genetic correlation formulae, we then obtain the expression for $r_g^{\#}$, the genetic relationship between $TDM^{\#}$ and OR:

$$rg\# = \frac{Cov_g(TDM,OR) - b_p.Cov_g(OR,LW)}{\sqrt{V_g OR \,.\, (V_g TDM + b_p^2.V_g LW - 2b_p.Cov_g(TDM,LW))}}$$

This formula may be rewritten as follows:

$$r_g^{\#} = \frac{r_g(OR,TDM).\sigma_g OR.\sigma_g TDM - b_p.r_g(OR,LW).\sigma_g OR.\sigma_g LW}{\sqrt{V_g OR \,.\, [V_g TDM + b_p^2.V_g LW - 2b_p.r_g(TDM,LW).\sigma_g TDM.\sigma_g LW}} \qquad (5)$$

In the case of the relationship between liveweight-adjusted testicular diameter($TDM^{\#}$) and ovulation rate, it can be seen from (5) above, that relative to the genetic relationship between unadjusted TDM and OR, the size of the correlation ($r_g^{\#}$) will depend upon:

i) the phenotypic regression of TDM on LW (b_p);

ii) the genetic covariance between LW and OR [$Cov_g(OR,LW)$];

iii) the genetic variance of LW ($V_g LW$); and

iv) the genetic covariance between unadjusted TDM and LW [$Cov_g(TDM,LW)$].

Utilising estimates of the various parameters from the sources indicated below, the "expected" genetic relationship between $TDM^{\#}$ and OR

can be calculated as follows:

Parameter	Value	Source
$r_g(OR,TDM)$	= 0.28	This Chapter
$V_g OR$	= 0.0145	Piper et al, 1984
$V_g TDM$	= 0.0318	Chapter 3
b_p	= 0.65	Chapter 3
$r_g(OR,LW)$	= 0.10	L.R. Piper (pers. comm.)
$V_g LW$	= 3.24	Chapter 3
$r_g(TDM,LW)$	= 0.84	Chapter 3

Then:

$$r_g^{\#} = \frac{0.28x\ 0.0145x0.032 - 0.65x0.10x0.0145x3.24}{0.0145x[0.032 + (0.65)^2x3.24 - 2x0.65x0.84x\ 0.032x3.24]}$$

$$= \frac{-0.0081}{0.1234}$$

$$= -0.065$$

Should the parameter values used in the above example be similar to the values in the population studied by Land , then the absence of a correlated response in OR, from selection on liveweight-adjusted testicular diameter, is not surprising. It should be noted, that if the genetic correlation between liveweight and ovulation rate is higher than the value of 0.1 used in the above example, the expected response in OR to selection for $TDM^{\#}$ is even more strongly negative.

Land et al (1982) have also reported that there has been a correlated response in liveweight in the selection flocks. The mature females of the line selected for increased liveweight-adjusted testicular size were lighter (approximately 10kg) than those of the line selected against this trait. Using the same approach as illustrated above, we can derive the equation which describes the expected genetic relationship between liveweight-adjusted testicular size and liveweight itself. Thus:

$$r_g^* = \frac{Cov_g(TDM^{\#}, LW)}{\sqrt{V_g TDM^{\#} \cdot V_g LW}} \qquad (6)$$

$$= \frac{Cov_g(TDM, LW) - b_p . V_g LW}{\sqrt{V_g TDM\# \cdot V_g LW}} \qquad (7)$$

It can be seen from (7) above, that the sign and size of r_g^* will depend largely upon the relative value of $Cov_g(TDM,LW)$ and $b_p . V_g LW$. It could be expected that the latter term would generally be larger than the former, which would result in a negative genetic correlation in line with the results of Land et al. (1982).

5.4.5 **Practical implications of the Genetic Correlation estimates between OR and TDM**

Walkley and Smith (1980) have examined the use of physiological traits as indirect selection criteria for the improvement of litter size in sheep. They compared genetic responses from direct selection for litter size, with those from indirect selection for sex-limited physiological traits and from combined selection using litter size and the physiological trait in a selection index. It was found that the value of a physiological trait as an indirect selection criterion depended primarily upon its genetic correlation with litter size.

In the study reported in this Chapter attention has focussed on ovulation rate as the female trait to be improved. Hanrahan (1980) has suggested that selection on ovulation rate would give greater gains in litter size than selection on litter size itself. Piper et al. (1984), whilst noting that the heritability of OR was not different to that of litter size in Merino sheep, suggested that, due to greater accuracy of measurement, lack of dependence upon the male for the expression of OR, and the ability to take several measurements in one year, OR would still be a

more efficient selection criterion for improving litter size in that breed.

Consideration of the merit of using TDM as an indirect selection criterion for the genetic improvement of OR in Merino sheep, involves estimation of the relative efficiency of such selection, when compared to direct selection on OR, and consideration of relative costs of the two procedures.

The correlated response in OR arising from selection on TDM, relative to the response from direct selection on OR can be calculated from:

$$\frac{CR_{OR}}{R_{OR}} = r_g \times \frac{i_{TDM} \times h_{TDM}}{i_{OR} \times h_{OR}}$$

where CR_{OR} = correlated response in OR from selection on TDM,

R_{OR} = direct response from selection on OR

r_g = genetic correlation between OR and TDM

i_{TDM} = selection intensity for rams

i_{OR} = selection intensity for ewes

h_{TDM} = square root of heritability of TDM

h_{OR} = square root of heritability of OR

Using selection intensities of 10% for rams and 25% for ewes, and heritabilities of 0.69 for TDM (TDM at 12 months of age, Chapter 3) and 0.07 for OR (Piper et al. 1984), the ratio of response in OR from indirect selection on TDM relative to direct selection on OR is 1.21 if r_g = 0.28 (the overall pooled r_g estimate from the dam-son and half-sib methods). In contrast, if the lower r_g estimate of 0.16 (half-sib method) is used, the ratio is 0.69, whilst it is 1.51 if the higher r_g estimate of 0.35 (dam-son method) is used.

Although the above calculations involve simplified assumptions and do not compare responses with schemes where selection is practiced in both sexes simultaneously, they reinforce the conclusions of Walkley and Smith (1980), that the size of the genetic correlation between the indirect

selection criterion and the trait to be improved is of primary importance in deciding the relative merit of indirect selection criteria.

CHAPTER 6

EFFECTS OF THE BOOROOLA F GENE ON GONADAL ACTIVITY AND PLASMA GONADOTROPHIN CONCENTRATIONS

6.1 INTRODUCTION

As outlined in the review of the literature in Chapter 1, the exceptional prolificacy of the Booroola Merino (mean OR of 4.2 and litter size of 2.5 in the CSIRO selection flock in 1982; Bindon, 1984) has been attributed largely to the action of a major gene, F, which operates, in an additive manner, to substantially increase OR above the those rates found in the Merino population in which it was first observed (Piper et al., 1985). The exact mechanism by which the F gene increases the number of follicles which mature and ovulate in each oestrous cycle, is not known. There is however, some evidence to suggest that the inhibin - FSH feedback regulation system is substantially different in female F gene carriers (Cummins et al., 1983; Bindon, 1984).

Although the expression of the F gene appears to be limited and confined to the female, it is of practical importance to be able to identify the F gene status (homozygous, heterozygous or non-carrier) of Booroola and Booroola crossbred males. Currently, this identification is dependent upon time-consuming progeny tests based on daughters' ovulation rate. Studies have, therefore, been carried out on male Booroola genotypes, aimed at finding "marker" traits which are indicative of F gene status.

Testicular growth and testicular size in Booroola rams and rams from Booroola crosses with other breeds, have been compared with non-

Booroola rams and crosses (Bindon and Piper, 1976; Curtis et al., 1980) but no differences between genotypes in these parameters were evident. More recently Purvis et al. (1983) have demonstrated that crossbred Booroola rams, with and without the F gene, have testes of similar size and growth rate, and Bindon et al. (1982) have shown that there is no difference in total daily sperm production between Booroola and control Merino rams.

The study of plasma gonadotrophin and steroid concentrations has also revealed that Booroola males are not different from other Merino genotypes during pre-pubertal (Findlay and Bindon, 1976) and post-pubertal (Stelmasiak et al., 1978; Bindon, et al., 1985) stages of development. However, differences between adult Booroola and control Merino rams in pituitary FSH content approached significance in the study of Robertson et al. (1983) and Courot (pers. comm.) report higher Sertoli cell numbers per gram of testicular tissue in adult Booroola rams.

Only in the study of Purvis et al. (1983) was the F gene status of male Booroola genotypes identified. In ewes with Merino and Romney backgrounds, the effect of the F gene on OR is additive, with each copy adding approximately 1.2 to 1.5 eggs (Piper et al., 1985). Therefore, comparisons aimed at identifying physiological and endocrinological traits correlated with the presence of the F gene, should be carried out with males whose F gene status is known. For practical and scientific reasons such studies should encompass both the pre- and post-pubertal periods. When this study began, the CSIRO Booroola flock was not homozygous at the F locus and progeny-tested FF Booroola rams were not available in Armidale. Performance tested Booroola females of

all three genotypes (FF, F+ and ++) were present in the flock and the design of these experiments was therefore restricted to comparisons of heterozygous F gene carriers (F+), non-carriers (++) of the same background genotype, and control Merino rams.

The experiments described in this Chapter utilized ram lambs from Booroola (FF and ++) and Peppin (Control) Merino ewes which had been crossed with Border Leicester rams, and they examined whether the F gene status or strain of Merino dam were correlated with measures of gonadal morphology, gonadal activity, or plasma gonadotrophin concentrations.

6.2 MATERIAL AND METHODS

6.2.1 Animals and management

Male progeny of the CSIRO AB42 flock, which is maintained at "Longford" Field Station, were studied. The background and management of this flock which contains Booroola and Control Merino ewes, and the environmental conditions at "Longford" have been described in Chapter 2.

6.2.1.1 Experiment 1

In June 1983, 21 rams were chosen from the surviving male progeny of the AB42 flock which were born in August 1982. The 21 rams were chosen to be representative of 3 groups which were categorised by being progeny of the following ewe genotypes:

(i) Group 1 - Booroola ewes classified on at least 2 OR records as being homozygous F gene carriers (i.e. at least one OR record > 5);

(ii) Group 2 - Booroola ewes classified on at least 2 OR records as being non-carriers of the F gene (i.e. no OR record > 2);

(iii) Group 3 - Control Merino ewes.

From the available progeny of the dams of each group, 7 rams were chosen so that the same Border Leicester sires were represented in each group, and as far as possible the use of full sibs was avoided. Other than these criteria, choice was on a random basis.

6.2.1.2 Experiment 2

From the AB42 male progeny born in August 1983, ram lambs were chosen at 1 week of age using the same criteria as used in Experiment 1. However, due to infertility in the Border Leicester sires, only 3 progeny of Booroola non-carrier (++) ewes were available. The numbers of ram lambs per group was as follows:

Group 1 - 10 ram lambs

Group 2 - 3 " "

Group 3 - 10 " "

Total - 23 " "

6.2.2 Experimental

6.2.2.1 Experiment 1

The 21 rams in this study were slaughtered on 8th May 1983 by intra-cardiac injection of 10 ml (3 g) of pentobarbitone sodium ("Valabarb", VR Laboratories, Syntex, Australia). Testes and epididymes were removed by opening the scrotal sac and severing the spermatic cord. After removal of the Tunica vaginalis, the epididymes were dissected free from each testis and both organs weighed. From each testis a wedge of testicular tissue (approximately 3 g) was removed, weighed and immediately frozen. Four additional cores of

tissue were removed and immersed in Bouin's fixative. Pituitary glands were also removed, trimmed of connective tissue and the neural lobe, and weighed.

Sperm reserves in the testes were determined from the frozen wedge of tissue by the homogenisation technique described by Amann and Almquist (1961). The cores of testis tissue previously placed in Bouin's fixative were dehydrated in an ascending series of ethanol solutions, cleared in xylene, embedded in paraffin, sectioned at 7 μ, with 140 μ between sections, and stained with haematoxylin and eosin. Seminiferous tubular diameter was calculated for each testis from the mean diameter of approximately 30 essentially circular tubules (10 tubules from each of 3 sections) which had clearly formed lumina. No corrections for contraction of the tissue due to the effect of fixation were made.

One day prior to slaughter, jugular venous blood was collected from each lamb at 20-minute intervals for 8 hours, beginning at 0830 h. The blood was centrifuged immediately at 2500 rpm, plasma recovered and stored at -20°C until assayed. FSH and LH were determined for each of the 24 samples by the methods described below.

On the day preceding blood collection, testicular diameter was measured using the technique described in Chapter 3, and unfasted live-weight recorded.

6.2.2.2 Experiment 2

Between 3 weeks and 8 months of age the 23 ram lambs in this experiment were subjected to the experimental schedule detailed in Table 6.1.

All procedures, except where indicated, were identical to those used in Experiment 1.

Table 6.1. Experimental schedule for the 23 AB42 ram lambs in Experiment 2

Date	Mean age (weeks)	Bleeding schedule*	Other procedures**
8- 9 Sept 1983	3	A	LW, TDM
25-26 Sept 1983	5	A	" "
2- 3 Nov 1983	10	B	" "
4- 5 Dec 1983	14	B	" "
7- 8 Jan 1984	18	A	" "
7- 8 Mar 1984	26	A	" "
1- 3 May 1984	33	B	" " , slaughter

* Bleeding schedule: A - Jugular venous blood collected at hourly intervals from 9 am.
B - Jugular venous blood collected using the same schedule as used in Experiment 1.

** Other procedures: LW - unfasted live weight
TDM - testicular diameter
Slaughter - same procedure as for Experiment 1 except rams killed by severence of the jugular vein.

6.2.3 Radioimmunoassay methods

6.2.3.1 Luteinising hormone

LH was measured by double antibody RIA using the rabbit anti-ovine LH anti-serum previously characterised by Gidley-Baird and Bindon (1976). For the experiments described in this Chapter, ovine LH (LER 1374-A - a generous gift from Dr L E Reichert) was used as tracer (specific activity 90-95 μCi/ g) after radio-iodination by the lactoperoxidase method of Thorell and Johansson (1971). NIH-LH-S16 was used as the LH standard, run in quadruplicate at log-2 dose intervals from 126 pg to 32 ng/ml. The method involved 24 hour simultaneous addition procedure, with incubation at 27^{o}C, followed by 16 hour precipitation with donkey anti-rabbit gamma globulin as

second antibody. Under the conditions of the assay (e.g. depending upon sensitivity requirements, anti-LH used at an initial dilution of 1/160,000 tracer mass equivalent to 60-65 pg [10^4 cpm] per tube; duplicate 100 µl aliquots of ovine plasma unknowns), the sensitivity of the assay (defined here as the plasma LH concentration equivalent to a B/BO value of 0.9) was 0.3 - 0.4 ng NIH-LH-S16/ml. Six ovine reference samples were run in each assay. These were undiluted ram plasma and five serial dilutions of castrate male plasma (1/1 ... 1/16). For the six reference preparations between and the within-assay CV estimates are shown in Tables 6.2 and 6.3.

6.2.3.2 Follicle-stimulating hormone

FSH was measured in a double antibody system using a rabbit anti-ovine FSH antiserum provided by NIAMDD (NIAMDD anti-FSH01, AFP-C528812). The tracer FSH preparation was ovine-FSH, also supplied by NIAMDD (NIAMDD-OFSH-1). This was radioiodinated by the lactoperoxidase method of Thorell and Johansson (1971) to a specific activity of 90-95 µCi/ g. The reference preparation for standard curves was ovine-FSH supplied by NIAMDD (NIAMDD OFSH-RP-1, approximate biopotency 75 times NIH-FSH-S1). The protocol for the ovine-FSH RIA was as described by NIAMDD specifications supplied with the reagents.

Six ovine reference samples were run in each assay. These were undiluted ram plasma and five serial dilutions of ovariectomised (OVX) ewe plasma (1/5 ... 1/80). For these reference preparations mean plasma FSH concentrations and CV% were:

Table 6.2. Between-assay coefficients of variation for plasma LH concentrations of six plasma reference preparations

	Reference preparation					
	Ram	Castrate male				
Assay No.		1/16	1/8	1/4	1/2	1/1
1	2.0	0.8	1.5	3.0	5.7	10.6
2	1.9	0.7	1.2	2.6	5.0	9.9
3	1.7	0.7	1.3	2.7	5.2	11.1
4	2.0	0.8	1.5	2.9	5.2	11.3
5	2.0	0.7	1.2	2.6	4.7	10.6
6	2.1	0.7	1.6	2.7	5.3	11.1
7	1.8	0.7	1.2	2.6	5.2	9.8
8	1.9	0.7	1.2	2.2	4.8	8.6
Mean	1.93	0.73	1.34	2.66	5.14	10.38
CV %	6.6	6.3	12.6	9.0	6.1	8.7

Table 6.3. Within-assay (i.e. between replicate) coefficients of variation for LH estimates of six plasma reference preparations

	Reference preparation					
	Ram	castrate male				
Replicate (cpm)		1/16	1/8	1/4	1/2	1/1
1	2575	3142	2881	2435	1819	1400
2	2545	3236	2817	2531	1815	1418
3	2663	3080	2819	2470	1921	1418
4	2524	3140	2933	2338	1882	1456
Mean	2576.75	3149.50	2862.50	2443.50	1859.25	1423.0
CV%	2.4	2.0	1.9	3.3	2.8	1.7

Reference prep.	Mean (ng/ml)	CV%
Ram	0.48	8.8
OVX 1/80	1.03	6.5
1/40	1.54	7.0
1/20	2.59	6.2
1/10	4.41	5.0
1/5	8.09	6.5

For the seven separate assays the between-assay CV% for three reference preparations was:

OVX ewe plasma @	Mean (ng/ml)	CV%
1/16 dilution	2.4	6.6
1/8 "	4.7	6.3%
1/4 "	9.2	5.1%

All reagents were added simultaneously, incubated for 24 hours at 27°C, followed by 16 hour precipitation at 4°C using donkey anti-rabbit gamma globulin. Under the conditions used for the experiments in this Chapter (i.e. NIAMDD anti-OFSH-1 used at an initial dilution of 1:8000, tracer mass equivalent to 60-65 pg (10^4 cpm)/tube, duplicate 100 µl aliquots of ovine plasma unknowns) the sensitivity of the method was 0.25 - 0.5 ng NIAMDD OFSH-RP-1/ml of plasma.

6.2.4 Statistical

Data were analysed using Least Squares ANOVA procedures. The following models were fitted to describe the different sets of data, as indicated:

(i) Model 1

This model was used to describe the sperm production and seminiferous tubule diameter data of the rams in Experiment 1, and the TDM and mean plasma FSH and LH of the animals in Experiment 2 for each of the 7 times of measurement. The principal questions being asked in this analysis were whether strain of dam (Booroola or Control Merino),

Booroola dam genotype (FF or ++) and hence ram lamb genotype (F+ or ++), sire and birth type exerted significant effects on any of the traits.

The model was described by the following equation:

$$Yi_{jklm} = \mu + M_i + D_{ij} + S_k + B_l + e_{ijklm}$$

where Y_{ijklm} = dependent variable,

μ = overall mean,

M_i = fixed effect of the i^{th} Merino dam strain, (i = 1,2),

D_{ij} = fixed effect of the j^{th} dam genotype within the Booroola strain, (i=1; j=1,2),

S_k = random effect of the k^{th} Border Leicester sire, (k=1,7 or 1,5),

B_l = fixed effect of the 1^{th} birth type class, (l=1,2,3),

e_{ijklm} = random errors, assumed N (0, δ^2_e)

(ii) Model 2

This model was used to describe the data collected at slaughter which was common to Experiments 1 and 2. The model is essentially the same as Model 1 except the contrasts of interest are now combined over two years' data. The model was described by the following equation:

$$Y_{ijklmn} = \mu + P_i + S_{ij} + M_k + D_{il} + B_m + e_{ijklmn}$$

where Y_{ijklmn} = dependent variable,

μ = overall mean,

P_i = fixed effect of the i^{th} experiment, (i=1,2),

S_{ij} = random effect of the j^{th} sire used in the i^{th} experiment (j=1,7 or 1,5),

M_k = fixed effect of the k^{th} Merino strain, (k=1,2),

D_{il} = fixed effect of the l^{th} dam genotype within the Booroola strain, (l=1,2),

B_{ijm} = fixed effect of the m^{th} animal within the Booroola strain,

e_{ijlkmn} = random errors, assumed $N(0,\delta^2_e)$

(iii) Model 3

This model was used to carry out repeated measure ANOVA's on the TDM, FSH and LH data, collected at 7 ages, from the animals in Experiment 2. Birth type was initially included in this model but due to confounding with dam strain and genotype in the presence of animal terms, it was excluded from the final model. The model was described by the following equation:

$$Y_{ijklmn} = \mu + M_i + D_{ij} + A_{ijl} + P_k + (DP)_{ijk} + e_{ijklmn}$$

where Y_{ijklmn} = dependent variable,

μ = overall mean,

M_i = fixed effect of the i^{th} Merino dam strain, (i=1,2),

D_{ij} = fixed effect of the j^{th} dam genotype within the Booroola strain, (i=1, j=1,2),

A_{ijl} = random effect of the l^{th} animal within strain and genotype (l=1,10;1,3;1,10)

P_k = fixed effect of the k^{th} time of measurement, (k=1,7 or 1,3),

$(DP)_{ijk}$ = interaction between dam genotype within the Booroola strain and time of measurement, (i=1; j=1,2; k=1,7 or 1,3),

e_{ijklmn} = random errors, assumed $N(0, \delta^2_e)$.

6.3 RESULTS

6.3.1 Spermatozoa production and seminiferous diameter

The mean values and measures of dispersion of spermatozoa per gram of testicular tissue (SPG), total spermatozoa reserves (TSR) and seminiferous tubule diameter (STD) of the 21 rams in Experiment 1, at 9.5 months of age are presented in Table 6.4.

Table 6.4 Mean, SD, CV% and range of spermatozoa per gram of testis (SPG), total testicular spermatozoa reserves (TSR) and seminiferous tubule diameter (STD) at 9.5 months of age for the 21 rams in Experiment 1.

Trait	Mean	SD	CV%	Range
SPG (x 10^6)	59.6	21.6	36.3	28.6 - 116.8
TSR (x 10^9)	14.9	7.2	48.3	4.1 - 32.9
STD (μ)	211.2	46.3	21.9	136.0 - 304.0

Analysis of the sources of variation in SPG, TSR and STD, using Model 1 as described in Section 6.2.4, revealed that none of the effects, including dam strain and genotype, was significant. The comparative values of STD, TSR and SPG for the crossbred progeny of homozygous Booroola ewes (FF), and of non-carrier (C) ewes are presented in Table 6.5.

Table 6.5. Least Squares mean (± SE) sperm per gram of testis (SPG), total testicular sperm reserves (TSR) and seminiferous tubule diameter (STD) for the crossbred Booroola heterozygotes (F+), crossbred Booroola non-carriers (++) and Control Merino (C) rams at 9.5 months of age (n = 7 per group).

Trait	Genotype: F+		++		C	
SPG (x 10^6)	56.7	13.9	53.8	17.2	66.1	14.2
TSR (x 10^9)	14.9	4.2	12.7	5.2	16.0	4.3
STD (u)*	227.0	21.0	203.0	25.0	192.0	21.0

* Rounded to the nearest micron.

6.3.2 Morphological and hormonal traits at 8.5-9.5 months of age of rams in Experiments 1 and 2.

Mean whole body (LW), anterior pituitary (PW), testicular (TW) and epididymal (EW) weights, testicular diameter (TDM), and plasma FSH and LH concentrations of the 21 rams in Experiment 1, and 23 rams in Experiment 2, when aged 9.5 and 8.5 months of age respectively, are presented in Table 6.6.

Examination of the sources of variation in these morphological and hormonal traits, using Model 2 as described in Section 6.2.4, revealed that Experiment (1 or 2) was a highly significant source of variation ($P < 0.01$) for all traits except for EW ($P = 0.10$) and for FSH. The effects of strain, sire, dam genotype (within the Booroola strain) and birth type were not significant. (ANOVA mean squares for these 7 traits are presented in Appendix 2). The least Squares means (± SE) appropriate to the rams in Experiments 1 and 2 are presented in Table 6.7 for each of the 7 traits.

Table 6.6. Means, SDs, CV%s and range of values for morphological and hormonal traits of rams from Experiments 1 (n=21) and 2 (n=23), when aged 8.5 and 9.5 months, respectively.

Trait	Mean	SD	CV%
Anterior pituitary weight (g)	0.42	0.08	19.0
Testicular weight (g)	152.6	46.0	30.1
Epididymal weight (g)	26.4	5.4	20.5
Live weight (kg)	40.3	8.8	21.8
Testicular diameter (cm)	4.70	0.66	14.0
Plasma FSH (ng/ml)	0.34	0.23	67.6
Plasma LH (ng/ml)	0.77	0.48	62.3

Table 6.7. Least Squares mean values (± SE) for morphological and hormonal traits of rams from Experiment 1 (n = 21) at 8.5 months of age, and Experiment 2 (n = 23) at 9.5 months of age.

Trait	Experiment 1		Experiment 2	
	Mean	SE	Mean	SE
Anterior pituitary weight (g)	0.38	0.02	0.47	0.02
Testicular weight (g)	126.6	11.2	182.4	10.2
Epididymal weight (g)	24.2	1.7	27.3	1.6
Live weight (kg)	32.3	1.5	47.9	1.3
Testicular diameter (cm)	4.30	0.19	4.92	0.07
Plasma FSH (ng/ml)	0.23	0.09	0.27	0.08
Plasma LH (ng/ml)	0.35	0.12	1.08	0.11

The rams in Experiment 2, which were 1 month older than those in Experiment 1 when slaughtered, showed markedly higher values for all traits except epididymal weights and plasma FSH.

Although differences between the crossbred Booroola F gene carriers (F+) and non-carriers (++) were not significant for any of the 7 traits, the F+ group ranked above the ++ rams for all traits except pituitary weight and live weight.

Table 6.8. Least Squares means (± SE) for morphological and hormonal traits of crossbred Booroola rams with (F+) and without (++) the F gene. Overall means for Experiments 1 and 2.

Trait	F+ (n = 17) Mean	SE	++ (n = 10) Mean	SE
Anterior pituitary weight (g)	0.40	0.02	0.42	0.03
Testicular weight (g)	153.8	9.9	144.7	15.2
Epydidymal weight (g)	26.8	1.5	23.0	2.3
Live weight (kg)	39.8	1.3	39.6	2.0
Testicular diameter (cm)	4.76	0.17	4.35	0.25
Plasma FSH (ng/ml)	0.38	0.08	0.23	0.12
Plasma LH (ng/ml)	0.84	0.11	0.62	0.16

6.3.3 Testicular diameter and plasma gonadotrophin concentrations in rams from Experiment 2

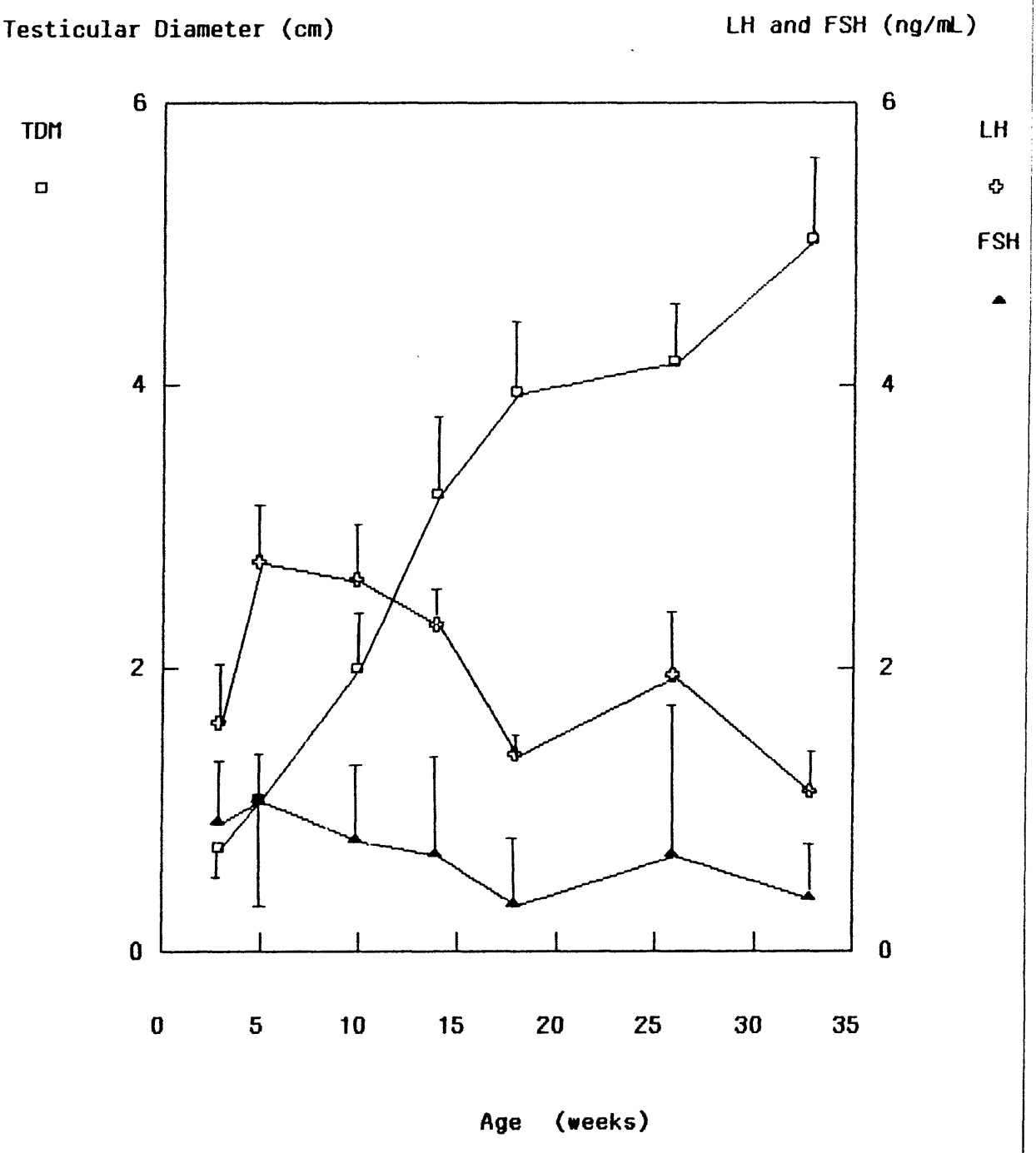
6.3.3.1 TDM and mean plasma FSH and LH concentrations from 3 - 33 weeks of age

Figure 6.1 illustrates the changes in TDM and mean plasma FSH and LH which occurred between 3 and 33 weeeks of age in the animals in Experiment 2.

Testicular diameter showed a curvilinear pattern of increase with age but mean plasma FSH and LH concentrations showed an increase from 3 weeks of age to maximum values at 5 weeks. These maxima were followed by gradual decreases until 18 weeks of age at which time a transitory smaller/increase in both hormones occurred.

Examination of the sources of variation in TDM and mean plasma FSH and LH at the 7 measurements, was carried out using two different approaches. In the first instance each trait, at each measurement, was analysed as a separate dependent variable using Model 1 as

Figure 6.1 Overall changes with age in testicular diameter, FSH and LH in the ram lambs in Experiment 2.



described previously in Section 6.2.3. This model included effects due to strain of Merino dam, sire, Booroola dam genotype (within the Booroola strain) and birth type. (Tables presenting ANOVA mean squares are contained in Appendix 3).

Variation between the two Merino strains was not significant for TDM, plasma FSH or LH concentrations at any of the 7 measurements, whilst significant variation due to sires was found only for FSH at 14 weeks of age ($P < 0.05$). Birth type effects were significant ($P < 0.05$) for TDM at 3, 5 and 14 weeks of age, with single-born rams having larger testes than those born as multiples (Table 6.9).

Table 6.9 Least Squares mean (±SE) TDM at 3, 5 and 14 weeks of age for single, twin and triplet-born rams in Experiment 2

Age at TDM measurement	Birth type*					
	Single (n=7)		Twin (n=10)		Triplet** (n=6)	
3 weeks	0.89	0.06[a]	0.67	0.05[b]	0.53	0.10[b]
5 weeks	1.26	0.07[a]	0.94	0.06[b]	0.87	0.12[b]
14 weeks	3.60	0.17[a]	3.08	0.15[b]	2.58	0.29[b]

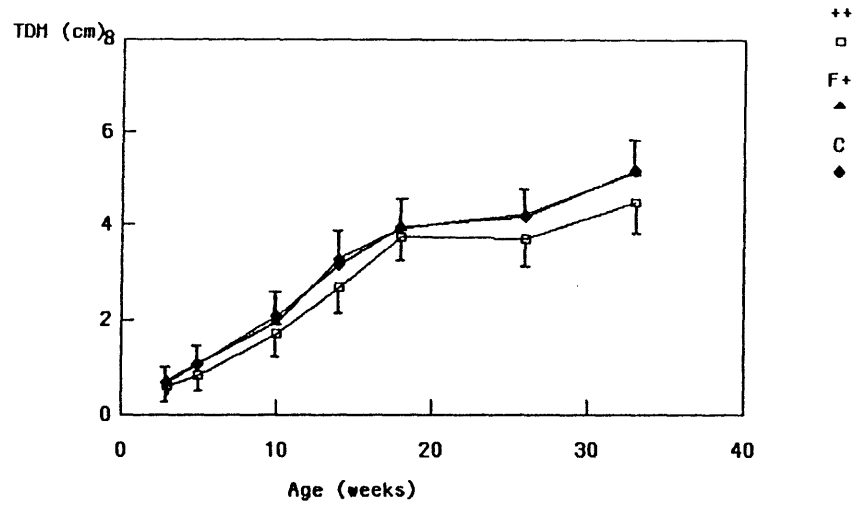
* Means with different superscripts within the same row significantly different ($P < 0.05$);
** Triplet classification includes 3 rams born as quadruplets.

The Least Squares mean TDM and mean plasma FSH and LH concentrations of crossbred Booroola rams, with (F+) and without (++) the F gene and of the control Merino rams (C) are illustrated in Figure 6.2 for each of the 7 occasions on which measurements were taken.

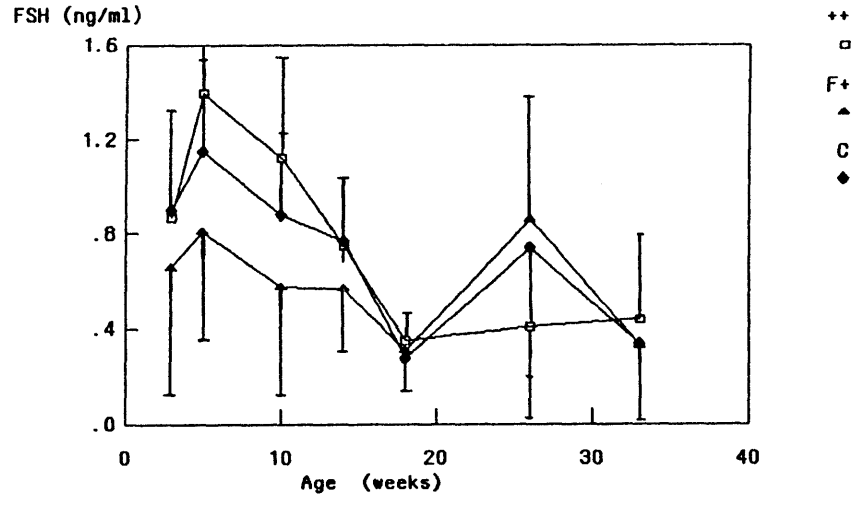
Differences between the F+ and ++ groups were significant only for the mean LH concentration at 10 weeks of age. The F+ ram lambs at this

Figure 6.2 Testicular diameter, FSH and LH changes with age in the F+, ++ and C groups in Experiment 2.

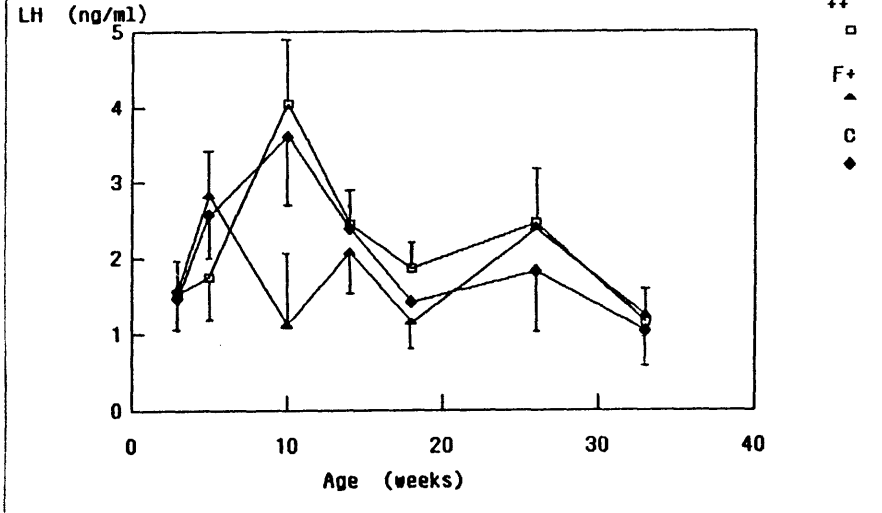
A)Testicular diameter (TDM)



B) Plasma FSH concentration



C) Plasma LH concentrations



age had significantly lower ($P < 0.05$) mean LH concentrations (1.11 ± 0.52 ng/ml) than ++ ram lambs (4.05 ± 0.82 ng/ml).

The data characterising the TDM and mean plasma FSH and LH concentrations were also subjected to repeated measures analysis of variance, using Model 3 as described in Section 6.2.3. This model simultaneously examined the variation between animals over the 7 measurements and allowed assessment of whether differences between the two Booroola genotypes were consistent over the time course of the experiment and whether the genotypes were on average different over the 7 measurements. In Table 6.10 the ANOVA mean squares for these analyses, for each of the 3 traits, are presented.

Table 6.10. ANOVA mean squares for repeated measures of TDM and mean plasma FSH and LH concentrations of rams from Experiment 2

Source of variation*	df	Trait		
		FSH (ng/ml)	LH (ng/ml)	TDM (cm)
Strain	1	0.023132	0.000417	5.129922***
Period	6	1.445040***	9.088661***	48.327643***
Booroola dam genotype	1	0.152739	4.299471*	0.162617
Animals	20	0.403888***	0.992419	0.497399***
Period x Booroola dam genotype	6	0.013989	1.850420*	0.023137
Remainder	126	0.108892	0.636933	0.123941

* Strain and Booroola dam genotype tested against animals; other effects against remainder. Where between-animal, within-group data varied the next appropriate error line was used.

These analyses reveal highly significant effects of the age at measurement (period) on the 3 traits and of variation within animals for FSH and TDM but not for LH. In the analyses of TDM at each of the

separate measurements, strain effects were not significant, However, when averaged over all 7 measurements in this analysis, the Control Merinos had larger testes (3.02 ± 0.04 cm) than the Booroola crossbred rams (2.63 ± 0.04 cm). This apparent inconsistency may be due to the fact that birth type could not be included in the model for the repeated measures analysis. Birth type had significant effects on TDM (see Table 6.9).

Differences between the F+ and ++ rams were significant ($P < 0.05$) only for mean plasma LH. On average, over the 7 measurements, the F+ group had lower mean plasma LH concentrations than did the ++ group (1.75 ± 0.10 vs 2.27 ± 0.17 ng/ml). There was also a significant ($P < 0.05$) interaction between LH concentrations in these groups over the 7 measurements. The relevant Least Squares means for the components of this interaction are presented in Table 6.11.

Table 6.11. Least Squares mean (± SE) plasma LH concentrations illustrating the interaction of Booroola dam genotype and age at measurement (Period) from the ANOVA presented in Table 6.8.

Age at measurement (weeks)*	Booroola Dam Genotype F+ (n = 10)	++ (n = 3)
3	1.55 (0.24)	1.66 (0.37)
5	2.61	2.82
10	1.80	4.13
14	2.15	2.55
18	1.19	1.69
26	1.86	1.91
33	1.12	1.12

* At 3, 5, 18 and 26 weeks of age, plasma LH concentrations were determined on 4 x 1-hour samples. At

10, 14 and 33 weeks of age, determinations were made from 24 x 20-minute samples.

6.3.3.2 Plasma LH profiles at 10, 14 and 33 weeks of age

Basal LH (BLH), the number of pulses of LH (NLHP) and the maximum pulse height (MPH) were determined for the animals in Experiment 2 at the 3 ages (10, 14 and 33 weeks) when they were bled every 20 minutes for 8 hours. Overall means and standard deviations of these parameters are presented in Table 6.12.

Table 6.12. Mean (and standard deviations) of LH profile characteristics of all rams in Experiment 2, at 10, 14 and 33 weeks of age

Age at measurement	LH profile characteristic			
	Basal LH (ng/ml)	LH pulses		Max. height (ng/ml)
		Proportion of animals with pulses	Number of pulses/8h*	
10 weeks	0.93 (0.20)	19/23	1.26	12.39
14 "	0.97 (0.22)	23/23	1.96	5.34
33 "	0.68 (0.20)	11/23	1.18	1.85

* of animals with pulses

Although mean BLH was relatively similar at 10 and 14 weeks of age, and had declined by 33 weeks of age, the proportion of animals exhibiting pulses and number of LH pulses (NLHP) per animal reached a maximum at 14 weeks. The maximum height of the pulses, however, was greatest at 10 weeks of age.

Variation between the animals in BLH and NLHP was examined by least squares ANOVA using the same repeated measures model as used for the data in Section 6.3.3.1. The ANOVA mean squares from these analyses are presented in Table 6.13. For both BLH and NLHP, variation

between ages at measurement (period) and between animals within genotypes (animals) were the only significant ($P < 0.05$) sources of variation. Neither strain of Merino or Booroola dam genotype had any significant influence on the magnitude of BLH nor NLHP.

It was not possible to examine variation between animals in the maximum pulse height at 10, 14 and 33 weeks of age using the repeated measures ANOVA procedure which was used for BLH and NLHP, due to the different number of animals exhibiting pulses at the different ages. Comparisons, at each age, between the 3 genotype groups, were therefore conducted using the Fisher Exact Probability Test (Siegel, 1956) for the proportion of animals within each genotype (++, F+ and C) exhibiting pulses, and using the t-test (Steel and Torrie, 1981) for the group mean maximum LH pulse height. The results of these comparisons are presented in Table 6.14.

Table 6.13. ANOVA mean squares for repeated measures analysis of LH profile characteristics of rams in Experiment 2

Source*	df	Parameter	
		Basal LH (ng/ml)	No. of LH pulses (/8 h)
Strain	1	0.025671	0.427111
Period	2	0.576698***	10.887111***
Booroola dam genotype	1	0.066188	0.534188
Animals	20	0.2583112*	0.726111*
Booroola dam genotype x period	2	0.011034	0.782906
Remainder	42	0.035258	0.341514

The proportion of animals within each genotype group which exhibited pulses did not significantly differ at any of the 3 ages. The maximum LH pulse height (MPH), was similar at 14 and 33 weeks of age for the 3 genotypes, but was significantly lower at 10 weeks of age in the F+ group (4.8 ± 0.4 ng/ml) when compared to the ++ (20.2 ± 6.0 ng/ml) and c (15.6 ± 1.2 ng/ml) groups. The range of MPH's within the F+ rams, however, showed substantial overlap with values of MPH from rams of the other two groups.

Table 6.14. Proportion of rams exhibiting LH pulses and means, standard derivations and ranges of maximum pulse heights (ng/ml) at 10, 14 and 33 weeks of age in the ++, F+ and C groups

Age and genotype	Proportion showing LH pulses	Maximum LH pulse height (ng/ml) Mean*	SD	Range
10 weeks:				
++	3/3	20.2[a]	18.0	3.9 - 39.5
F+	8/10	4.8[b]	3.0	1.9 - 10.5
C	9/10	15.6[a]	11.1	3.8 - 24.2
14 weeks:				
++	3/3	7.6a	5.4	2.8 - 13.4
F+	10/10	4.5a	2.3	1.3 - 9.3
C	10/10	5.7a	2.9	3.3 - 12.4
33 weeks:				
++	1/3	1.6a	-	-
F+	6/10	2.2a	1.5	1.4 - 5.2
C	4/10	1.6a	0.7	1.2 - 2.7

* Means with different superscripts, within the same column, significantly different ($P < 0.05$).

6.4 DISCUSSION

6.4.1 GENERAL

The studies described in this Chapter were primarily designed to compare gonadal activity and plasma gonadotrophin concentrations in crossbred Booroola rams with and without a copy of the F gene. Of secondary interest was the contrast between crossbred males derived from Booroola or Control Merino ewes.

The CSIRO's AB42 flock was chosen as the source of animals for this study because it generates crossbred Booroola ram progeny which, on the basis of their dams' ovulation rate and litter size records, can be classified as being single copy F gene carriers (F+) or non-carriers (++). After adjustment for environmental factors and the influence of sire, differences between groups of F+ and ++ rams could therefore be interpreted as being due to the effect of the F gene.

The numbers of animals included in the 2 experiments reflected a compromise between maximising group size and the labour requirements of the analytical techniques, especially hormone assays, used in this study. In Experiment 2, however, only 3 male progeny of ++ Booroola ewes were available for inclusion in the study. This feature of the experimental design limits the precision of contrasts between the F+ and ++ crossbred rams.

6.4.2 GONADAL SIZE

The results from Experiments 1 and 2 reaffirm the findings of Purvis et al. (1983) that testicular size at 8 to 12 months of age does not differ between crossbred Booroola rams, with and without a

copy of the Booroola F gene, nor does this trait differ between crossbred Booroola and Control Merino rams. In addition, the comparisons made in Experiment 2 extend this conclusion to the neonatal, prepubertal and pubertal stages of sexual development. At no age between 3 weeks and 8 months was testicular size found to differ between the 3 genotypes. This is in agreement with data comparing testicular size in Booroola and Control Merino rams (Bindon and Piper, 1976), but is at variance with several between-breed studies, where positive associations between prolificacy and testicular size have been found (Land, 1973; Land and Carr, 1975; Ricordeau et al., 1979).

However, a recent study (Walker et al., 1984) has examined testicular size in progeny of (i) Booroola (Bo) rams crossed with Bungaree South Australian strong wool (Bu) ewes, (ii) Trangie Fertility (TF) rams crossed with Bu ewes and (iii) purebred Bu progeny. Between 5 and 15 months of age the purebred Bu progeny had smaller testes than the half-bred Bo and TF rams which themselves did not differ. This latter finding (1/2 Bo = 1/2 TF) was in contrast to the ranking of equivalent female progeny for reproductive performance (Bu < 1/2 TF < 1/2 Bo). The authors suggested that the inconsistency could be explained by the effect of a gene of major effect influencing female performance but having no effect on testicular size in the 1/2 Bo. This suggestion is supported further by the report of Oldham et al. (1984) that F gene carrier and non-carrier Booroola-cross progeny, from non-Booroola ewes, did not differ in testicular size between 14 and 32 weeks of age.

In the repeated measures analysis of testicular size in

Experiment 2, the Control Merino and the Booroola genotypes differed significantly. However, in these analyses no adjustments were made for the effects of birth-type and it is likely that the difference between strains was largely due to the difference between them in the distribution of litter size.

6.4.2 GONADAL MORPHOLOGY

Examination of testicular activity, as measured by sperm per gram of testis tissue, total testicular sperm reserves and seminiferous tubule diameter, (Experiment 1, rams aged 9.5 months) established that the 3 genotypes also did not differ in these parameters (Table 6.3). Both total sperm reserves and seminiferous tubule diameter have been shown to be closely correlated with testicular size (Knight, 1973; Raadsma, 1981). However, the number of sperm per gram of testis tissue is not closely correlated and could be expected to reflect more closely the efficiency of germ cell production. As such, this parameter might be expected to reflect any major de-regulation of the process of spermatogenesis if this occurs in rams carrying the F gene. However, the similarity in the above measures of gonadal morphology and activity, suggests that such de-regulation either does not occur in male carriers or that the level of investigation which characterised this study was not sufficiently close to the site of action of the F gene to be able to detect it. This contrasts sharply with the effects of the F gene in the ewe, where the feedback regulation of ovulation rate is clearly disrupted.

The maturation of germ cells within the ewe's gonads is integrated over the oestrous cycle and ovulation is synchronised both

within and between ovaries, to occur at a precise time within this cycle. In the male there is a similar cyclical maturation and release of spermatozoa, which occurs at the level of the Sertoli cell. Integration of activity between adjacent Sertoli cell-germ cell compartments ensures a continuous production of spermatozoa from the gonad (Courot et al., 1970). Further investigation of gonadal morphology and activity of F gene carriers may be more rewarding if they are focused on the Sertoli cells and associated germ cells. The localised environment associated with individual Sertoli cell-germ cell compartments may reflect more closely the environment of the entire female gonad.

6.4.4 PLASMA GONADOTROPHIN CONCENTRATIONS

6.4.4.1 Plasma FSH

The age-related changes in plasma FSH concentrations of the Booroola and Peppin Merino crossbreds agree well with those found in previous studies of other breeds and crosses(Lee et al., 1976a; Blanc and Terqui, 1976; Walton et al., 1978; Ricordeau et al., 1984). Concentrations increased to a maximum (1.06 ng/ml) at the fifth postnatal week and thereafter declined gradually until, at 26 weeks of age, a small and transitory increase was observed. Examination of the variation in mean FSH levels between animals revealed that the only effect, other than age, which significantly influenced the variation, was for the sire effect at 14 weeks of age. Given the extremely small number of sires represented in the design, it may be that this effect was due to sampling variation.

These findings reinforce the results of previous studies of FSH

in Booroola and control Merino genotypes where no FSH concentration or profile differences were found(Findlay and Bindon, 1976; Robertson *et al.*, 1983; Bindon *et al.*, 1984). In addition, they suggest that neither the *F* gene nor the remainder of the Booroola genotype, *per se*, exert significant effects on FSH profiles when compared with Control Merino rams. A recent report by Ricordeau *et al.* (1984) of prepubertal FSH concentrations in crossbred Lacaune x Romanov ram lambs, which were the progeny of sires selected on a prolificacy index, showed that FSH levels at 3-11 weeks of age were independent of birth type, genotype and the prolificacy index of their sire. These authors did note, however, that pure bred Romanov ram lambs at 4-10 weeks of age had significantly higher FSH concentrations than similarly-aged *F*1 crossbred ram lambs. Sanford *et al.* (1982) have also demonstrated breed differences in FSH in prepubertal males. In that study, Finnish Landrace ram lambs had significantly higher ($P < 0.01$) FSH levels than low prolificacy genotypes at approximately 85 days of age. When compared as adults, however, these differences were not apparent. These findings suggest the need for comparative studies of Booroola genotypes and other prolific breeds in the one environment to enable further investigations of possible differences in the mechanism or pathways of control of prolificacy.

6.4.4.2 Plasma LH concentrations

Several between-breed studies also have demonstrated a positive association between pre-pubertal (4-14 weeks of age) plasma LH concentrations in ram lambs and prolificacy or ovulation rate (Thimonier *et al.*, 1972; Blanc *et al.*, 1975; Carr and Land, 1975).

However Echternkamp and Laster (1976) and Hanrahan et al. (1977) did not find a similar association. Within breeds, Hanrahan et al. (1977) found a positive effect in ram lambs of selection for litter size in the Galway breed on plasma LH at 8 weeks of age, whilst Bindon and Turner (1974) found a similar association between selection lines of Merinos. However, in a further study of pre-pubertal ewe lambs from the same lines, Bindon et al. (1985) found that when adjustments were made for litter size at birth, basal LH was not different in these lines. No similar study to that has been reported for male lambs.

In the present study, plasma LH concentrations were measured on the rams in Experiment 1 at 9 months of age and on 7 occasions from 3 weeks to 8 months of age on the rams in Experiment 2. On 3 occasions in the latter experiment (10, 14 and 33 weeks of age) the rams were characterised for plasma LH every 20 minutes for 8 hours, so that LH profile characteristics of LH could be identified.

As with FSH, the changes in mean plasma LH concentrations with age agree closely with those found in previous studies (Lee et al., 1976a; Foster et al., 1978). Pooled data for all animals showed that maximum values of mean plasma LH occurred at 5 weeks of age, after which a gradual decline in levels occurred, until at 26 weeks a small and transitory increase was apparent. Examination of the factors influencing variation in mean LH concentration established that, other than age at measurement, which was highly significant, there was a significant difference between the 2 Booroola genotypes (F+ or ++) at 10 weeks of age. This also appeared as a significant age at measurement x Booroola genotype interaction when LH at the

different ages was examined by repeated measures ANOVA. The F+ group attained maximum mean LH values at 5 weeks of age, had substantially lower values at 10 weeks of age, and returned to levels just below maximum values at 14 weeks of age. In contrast, the ++ group attained maximum mean LH levels at 10 weeks of age, after which a steady decline was observed until 26 weeks of age.

Examination of the components of mean LH concentrations at the 3 ages at which LH profiles were characterised, revealed differences between F gene carriers and non-carriers at 10 weeks of age, but then only in maximum LH peak height (MPH). The F+ group ram lambs at 10 weeks of age had significantly lower MPH (4.8 ± 0.4 ng/ml) than either the ++ group (20.2 ± 6.0 ng/ml) or the C group (15.6 ± 1.2 ng/ml). This difference in MPH was the main cause of the difference in mean LH concentration at 10 weeks of age. The increase in mean LH levels between 10 and 14 weeks of age in the F+ group was not due to any change in MPH (4.8 ± 0.4 ng/ml and 4.5 ± 0.2 ng/ml respectively), but was largely due to an increase in the number of LH pulses per 8 hours (1.29 to 1.90). In contrast, the ++ and C groups showed substantial reduction in MPH (20.2 ± 6.0 to 7.6 ± 1.8 and 15.6 ± 1.2 to 5.7 ± 0.3 ng/ml, respectively), between 10 and 14 weeks of age. They also displayed a similar increase to the F+ group in the number of LH pulses (1.25 to 2.00; pooled value for the ++ and C groups).

In comparison with crossbred non-carriers (++) and Control Merinos, crossbred Booroola F+ ram lambs do not differ in the magnitude of basal LH release from the pituitary, nor in the frequency of pulsatile release. These data therefore suggest that the difference in mean LH concentration occurs because of a more

rapid onset in the reduction of the size of bolus releases of LH from the pituitary or, more likely (due to the similar mean LH levels at 5 weeks of age) because of smaller bolus releases from the beginning of the pulsatile release of LH.

Lee et al. (1976b, 1981) have suggested that the rising levels of peripheral LH in the male neonate may reflect an increased sensitivity of the pituitary to GnRH, although clearance of maternal steroids has not been ruled out as a possible contribution (Bremner et al., 1981). At 5-7 weeks of age, besides the increase in pituitary responsiveness to GnRH, rapid increases in testosterone levels follow GnRH-stimulated increases in LH levels (Lee et al., 1976b). However Blanc and Terqui (1976) have shown that surgical cryptorchidism, which does not affect testosterone or oestradiol plasma levels, considerably elevates LH levels at this stage of development, and this is thought to be due to the absence of negative feedback inhibition of LH release by inhibin. In the context of the present study, the above findings suggest that males carrying the F gene may have a greater pituitary sensitivity to gonadal feedback or that the gonads of these animals begin to exert negative feedback effects on the pituitary at an earlier age. Clarification of the mechanism which results in differential pituitary release of LH at 10 weeks of age in F gene carrier and non-carrier ram lambs will require further and more detailed studies of these genotypes at this stage of development. Characteristation of testosterone and inhibin levels would be an important component of such studies. An important first step will be to confirm that there are differences in MPH at 10 weeks of age, and that the present results are not simply a sampling

abberation. This possibility cannot be ruled out, given the small numbers of animals in this study and the considerable overlap in the MPH between individual animals of the 3 genotypes.

As indicated earlier, the results of genetic studies of plasma LH in pre-pubertal rams have not shown consistent differences associated with genotype prolificacy. Hanrahan et al. (1977) concluded that there was "no single or simple expectation tenable for the effects of genetic group on plasma LH". The results of the study of LH profiles reported in this Chapter suggest that part of the reason for the contrasting results of such studies is the complex relationship between age, LH pulse frequency, and the magnitude of such pulses. In addition, relative to the length of time between birth and puberty, the changes in the characteristics which determine mean LH concentrations occur extremely rapidly. Therefore the identification only of mean LH levels may result in erroneous conclusions being drawn.

6.5 CONCLUSIONS

The studies described in this Chapter generally reinforce the conclusion of Bindon (1984) that the Booroola F gene appears to be sex-limited in its expression. Apart from the mean LH concentration difference at 10 weeks of age, none of the measures or indicators of male gonadal activity used in this study, was different for the two F locus genotypes. Although the inhibin-FSH system has been shown to be different in female F gene carriers, there is no evidence from the investigations described here that the F gene has any effect on plasma FSH concentrations in the young male. Preliminary evidence

was found, that the presence of the F gene may result in a different rate of maturity of the hypothalmic-pituitary-gonad feedback mechanism, but further study is required to substantiate this finding.