Plasma FSH, LH and immunoreactive inhibin concentrations in FecB^B/FecB^B and FecB⁺/FecB⁺ Booroola ewes and rams from birth to 12 months of age

K. L. Isaacs¹, K. P. McNatty^{2*}, L. Condell², L. Shaw², D. A. Heath²,
N. L. Hudson¹, R. P. Littlejohn¹ and B. J. McLeod¹

¹Invermay Agricultural Centre, PO Box 50034, Mosgiel, New Zealand; and ²Wallaceville Animal Research Centre, PO Box 40063, Upper Hutt, New Zealand

Endocrine and developmental changes were examined in Booroola FecB^B/FecB^B (BB, $n = 16$) and FecB⁺/FecB⁺ (++, $n = 20$) ewe lambs, and BB ($n = 17$) and ++ ($n = 19$) ram lambs from 2 to 53 weeks of age. Blood samples were taken weekly for the measurement of plasma concentrations of FSH, LH, immunoreactive inhibin, progesterone (ewe lambs) and testosterone (ram lambs). Behavioural oestrus in the ewe lambs and testicular volume and the breakdown of foreskin adhesions in ram lambs were recorded. Blood samples were taken from another flock of BB ($n = 134$) and ++ ($n = 109$) ram lambs at 20 weeks of age for the analysis of immunoreactive inhibin. In ewe and ram lambs, there appeared to be genotype differences for FSH, LH and immunoreactive inhibin at specific times during the neonatal period. In BB and ++ ewe lambs, respectively, mean FSH concentrations were 4.3 and 2.0 ng ml⁻¹ (SED 0.54) between 4 and 6 weeks, 2.6 and 3.4 ng ml⁻¹ (SED 0.33) between 12 and 28 weeks, and 1.8 and 1.9 ng ml⁻¹ (SED 0.18) between 34 and 53 weeks of age. Mean plasma LH concentrations were lower in BB than in ++ ewe lambs from 26 to 53 weeks of age ($P < 0.05$) but not earlier. Mean concentrations of immunoreactive inhibin were also lower in BB than in ++ ewe lambs between 2 and 11 weeks (16.0 and 27.4 iu ml⁻¹, respectively; $P < 0.01$), but thereafter no differences were apparent. In BB ram lambs, FSH concentrations were high for 3–4 weeks longer than in the ++ animals during the first 10 weeks of life. Likewise there were periods between 11 and 20 weeks of age when the plasma LH concentrations were higher in BB than in ++ ram ($P < 0.05$) lambs. Subsequently, between 19 and 33 weeks of age, the immunoreactive inhibin concentrations were consistently higher ($P < 0.05$) in BB than in ++ rams and this difference between the genotypes was confirmed in the larger study of 243 ram lambs at 20 weeks of age (BB > ++; $P < 0.0005$). The endocrine differences, in males and females, could not be attributed to either litter size, livemass or sire. However, limited numbers of sires (two BB and two ++) were used in the present study, so potential sire effects cannot be ruled out. In ewe lambs, the time of onset of puberty did not differ between genotypes. In ram lambs, the onset of puberty was not determined but testicular development, assessed by changes in testosterone concentrations, did not differ between genotypes. Differences in penile development and changes in testicular volume between the genotypes were observed but these were confounded by differences in livemass or sire. The evidence suggests that there are FecB^B-related differences in pituitary and gonadal hormones in neonatal ewes and rams. It is hypothesized that these differences between genotypes are part of a sequence of developmental differences that begin in fetal life.

Introduction

The Booroola (FecB^B) gene is manifested in ewes as an increase in ovulation rate (Davis *et al.*, 1982). However, no overt effects

of the FecB^B gene on reproductive performance of rams have been observed. The physiological influences of the FecB^B gene on the endocrine system in Booroola sheep remain unclear. There is evidence that FecB^B differences in plasma FSH and immunoreactive inhibin concentrations may occur in ewe lambs during the first few weeks of life, but not during fetal life (Bindon *et al.*, 1985; Braw-Tal and Gootwine, 1989;

*Correspondence.
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Montgomery *et al.*, 1989; Phillips *et al.*, 1992; Braw-Tal *et al.*, 1993; Smith *et al.*, 1993, 1994). In ram lambs, the effects of the *FecB^B* gene on plasma hormone concentrations are equivocal (Seck *et al.*, 1988; Montgomery *et al.*, 1989; Purvis *et al.*, 1991) and, to our knowledge, no data are available on hormone concentrations in males during fetal life. In the above studies, only short-term sampling regimens were used and there are no detailed data on the patterns of hormone concentrations from birth to puberty.

In post-pubertal females, there is considerable evidence to suggest that the *FecB^B* gene influences the plasma concentrations of FSH (and to a lesser extent LH) but not those of immunoreactive inhibin (Bindon, 1984; McNatty *et al.*, 1991a, 1992; Montgomery *et al.*, 1992). The plasma concentrations of FSH are consistently higher in *FecB^B/FecB^B* (BB) than in the *FecB⁺/FecB⁺* (++) genotypes throughout the oestrous cycle and anoestrus, after ovariectomy, and also in ovariectomized or ovary-intact ewes treated with gonadotrophin-releasing hormone after hypothalamic–pituitary disconnection (McNatty *et al.*, 1987a, 1989, 1991b, 1993a). In rams, most studies have found no effect of the *FecB^B* gene on plasma FSH and LH concentrations that are independent of sire effects (Bindon *et al.*, 1985, 1989; Montgomery *et al.*, 1989; Hochereau-de Reviers and Seck, 1991; Price *et al.*, 1991a, b, c; Isaacs *et al.*, 1992).

The evidence suggests that the plasma concentrations of FSH and immunoreactive inhibin in females may diverge between the *FecB^B* genotypes after birth, whereas in males the evidence remains equivocal (see Seck *et al.*, 1991). If there are genotype differences, it remains uncertain as to whether they are sustained throughout the prepubertal period. The aims of the present study were to examine the plasma concentrations of FSH, LH and immunoreactive inhibin in BB and ++ Booroola ewe and ram lambs between birth and 12 months of age, to determine whether and when consistent gene-specific differences occur.

Materials and Methods

Animals

Booroola ewe lambs (16 BB and 20 ++) and Booroola ram lambs (17 BB and 19 ++) were from an interbred Booroola Merino × Romney flock at the Invermay Agricultural Centre, Mosgiel, New Zealand (latitude 45°S). All lambs were born in spring (during September–October) and were tagged at birth and identified according to their dam, and their birth mass, birth date, litter size (birth rank) and sex were recorded. Weaning took place in late November when lambs were 7–12 weeks of age.

These animals were the offspring of four sires (2 BB and 2 ++). The rams were the male offspring of progeny-tested rams that had been classified as either BB or ++ on the basis of four ovulation records from 31–36 female offspring, according to the criteria of Davis *et al.* (1982). Three ovulation rate observations had been used to classify the dams of these sires (Davis *et al.*, 1982). Within this study, there were 3–13 ewe lambs and 8–10 ram lambs within each of the four sire groups. The genotype (BB or ++) of the dams of these lambs had been

assigned on the basis of three ovulation rate observations (Davis *et al.*, 1982).

Blood samples were taken from a further 243 Booroola Merino ram lambs (109 ++ and 134 BB ram lambs) by venepuncture at 20 weeks of age to examine in more detail a potential *FecB^B* difference in plasma inhibin concentrations in prepubertal lambs. The ++ and BB ram lambs originated from 9 and 15 sires, respectively.

Experiments

Blood samples were collected from lambs weekly, and their live masses recorded every 2–3 weeks, from 2 to 53 weeks of age (age range at first sample was 2–3 weeks). Blood samples were collected by jugular venepuncture, centrifuged at 400 g for 15 min, and the plasma separated and stored at –20°C until assayed. Plasma FSH and LH concentrations were measured in all samples, whereas the concentration of immunoreactive inhibin was measured in all samples from a randomly selected subset of animals (9 BB and 10 ++ ewe lambs (3–6 animals per sire) and 10 BB and 7 ++ ram lambs (2–5 animals per sire)). Subsequently, immunoreactive inhibin concentrations in plasma were measured in a separate flock of ram lambs at 20 weeks of age.

To assess the onset of puberty, vasectomized rams fitted with mating harnesses were run with the ewe lambs when they were 15–20 weeks of age. Mating marks were recorded weekly to determine the occurrence of behavioural oestrus. Concentrations of progesterone in plasma were also measured in blood samples collected weekly from 21 to 26 weeks of age until 12 months of age. Ewe lambs were considered to be showing oestrous activity when concentrations of progesterone in plasma were $\geq 0.5 \text{ ng ml}^{-1}$ on two occasions within three consecutive samples, or $\geq 1 \text{ ng ml}^{-1}$ on one occasion.

Sexual development in ram lambs was assessed by measurements of testicular volume every 2 weeks, by the breakdown of adherence of the foreskin to the head of the penis, and from plasma testosterone concentrations in weekly blood samples. The volume of each testis was estimated according to the method of Walker *et al.* (1985) using a set of twelve volumetric beads that ranged from 20 to 435 ml. Measurements of testicular and penile development began when ram lambs were 5–10 weeks of age, and continued until they were 53 weeks of age. Breakdown of foreskin adhesion was scored on a scale of 1 (complete adhesion of foreskin to the head of the penis) to 5 (complete breakdown of this adhesion), according to the criteria described by Wiggins and Terrill (1953). Examinations of foreskin adhesion ceased for individuals when a score of 5 was recorded.

Hormone assays

Plasma concentrations of FSH were measured in duplicate using a homologous radioimmunoassay kit supplied by The National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (McNatty *et al.*, 1989). The oFSH used for iodination was NIDDK-oFSH-I-1, the reference preparation was NIDDK-oFSH-RP-1, and the oFSH antiserum was NIDDK-anti-oFSH-I.

The intra- and interassay coefficients of variation were less than 10% and the limit of detection was 0.2 ng ml^{-1} plasma.

Plasma concentrations of LH were assayed in duplicate by the radioimmunoassay method described by McNatty *et al.* (1989). The oLH used for iodination was NIDDK-oLH-I-3 and the reference preparation was NIAMDD-oLH-S23. The intra- and interassay coefficients of variation were less than 10% and the limit of detection 0.2 ng ml^{-1} .

Inhibin was measured by a radioimmunoassay procedure validated by Findlay *et al.* (1990) and identical to that described by McNatty *et al.* (1992). The inhibin antiserum was a rabbit antiovine 31 kDa inhibin preparation (antiserum number 1989) and the iodination standard was a purified 31 kDa bovine inhibin preparation. The specificity of the antiserum is reported by Robertson *et al.* (1989); the major crossreaction of this antibody (288%) is with bovine pro- α C-subunit. The reference standard was a pool of charcoal-treated follicular fluid (oFF2) which was calibrated against the WHO porcine standard 86/690 (National Institute of Biological Standards and Control, Potters Bar). Relative to the WHO 86/690 Standard, oFF2 contained $534\,000 \text{ iu ml}^{-1}$. The mean intra- and interassay coefficients of variation were both $< 16\%$ and the sensitivity of the assay was 3 iu ml^{-1} .

Progesterone and testosterone were both measured by radioimmunoassay procedures described by McNatty *et al.* (1981, 1982). Progesterone was extracted from $200 \mu\text{l}$ plasma with 2×5 volumes of petroleum ether (b.p. $40\text{--}60^\circ\text{C}$). The limit of detection for progesterone was 0.1 ng ml^{-1} , and the intra- and interassay coefficients of variation were both $\leq 10\%$. Testosterone from $200 \mu\text{l}$ plasma was extracted with freshly distilled ether, with a mean extraction efficiency of 80%. The minimum detectable concentration was 0.1 ng ml^{-1} , and the intra- and interassay coefficients of variation were 11% and 14%, respectively.

Statistical analyses

The LH and testosterone concentrations were log transformed to stabilize their variation. Thereafter, a univariate analysis of variance (ANOVA) for each variable was performed at each sample time for both genders, with sire as the block structure and genotype as the treatment structure (GENSTAT, 1987). This showed no evidence ($P > 0.05$) that between-sire variation (adjusted for genotype) exceeded within-sire variation, so the sire effect was not considered in any subsequent analyses.

FSH, log LH, inhibin and livemass data for each sex, and log testosterone and testicular volume data for ram lambs, at each age from 2 to 53 weeks, were analysed by univariate ANOVA, with genotype as the treatment structure. Means or maxima of the hormone data over periods within this age range, chosen from the data, and specified in each corresponding results section, were analysed by ANOVA with genotype as the treatment structure (GENSTAT, 1987). Geometric means are significantly different when the ratio of one to another is greater than the square of the standard error associated with that ratio (SER). Possible relationships between FSH and inhibin with respect to genotype were tested by linear regression analysis (GENSTAT, 1987). To examine the variation of hormone

measurements, mean genotype-adjusted autocorrelations for time lags 1–52 weeks were calculated for FSH, LH and inhibin for each sex. For log LH there was no evidence of any autocorrelation.

Mating times and the presence of high progesterone concentrations were analysed using a binomial generalized linear model with logit link formation, fitting genotype, to assess the onset of puberty in ewe lambs.

Results

Ewe lambs

FSH. The mean FSH concentrations in plasma of the BB and ++ ewe lambs are summarized (Fig. 1a). The overall mean FSH concentrations for BB and ++ ewe lambs were 4.3 and 2.0 (SED 0.54) ng ml^{-1} , respectively, between 4 and 6 weeks of age; and 2.6 and 3.4 (SED 0.33) ng ml^{-1} , respectively, between 12 and 28 weeks of age. These differences between the genotypes were significant over both periods ($P < 0.001$ and $P < 0.05$, respectively). The overall means for BB and ++ ewe lambs between 34 and 53 weeks of age were 1.8 and 1.9 (SED 0.18) ng ml^{-1} , respectively, and these values were not significantly different. The mean genotype-adjusted autocorrelations for FSH in females declined from 0.54 at a lag of 1 week, to a stable value of 0.31 after a lag of 20 weeks or greater.

LH. The geometric mean LH concentrations were low ($< 0.7 \text{ ng ml}^{-1}$) but variable throughout the first year of life (Fig. 1b). Geometric mean concentrations of LH were not significantly different between genotypes when averaged between 2 and 9 weeks of age; they were 0.4 ng ml^{-1} for both BB and ++ (SER 1.14), or when averaged between 10 and 25 weeks of age, when the values in both genotypes were 0.3 ng ml^{-1} (SER 1.15). However, the mean LH concentrations were significantly lower in BB than in ++ ewe lambs when averaged over 26–53 weeks of age (0.3 and 0.5 ng ml^{-1} , respectively, SER 1.14 , $P < 0.05$).

Immunoreactive inhibin. In both genotypes, immunoreactive inhibin concentrations in plasma declined steadily between 2 and 11 weeks of age and BB ewe lambs showed significantly lower ($P < 0.001$) mean concentrations than did ++ ewe lambs (16.0 and 27.4 iu ml^{-1} , respectively, SED 2.80) (Fig. 1c). Between 12 and 53 weeks of age there were no differences in mean concentrations between genotypes. However, there was a significant increase in the mean immunoreactive inhibin concentrations of 2.3 iu ml^{-1} (SED 0.28 , $P < 0.001$) for both genotypes from respective mean values of 7.0 and 6.8 iu ml^{-1} for BB and ++ ewe lambs when averaged from 12 to 32 weeks of age (SED 0.61) to 9.4 and 9.1 iu ml^{-1} for BB and ++ ewe lambs, respectively, when averaged from 33 to 53 weeks of age (SED 0.81). The mean, genotype-adjusted autocorrelations for immunoreactive inhibin declined from 0.45 at a lag of 1 week, to a stable value of 0.09 at 20 weeks or greater.

Relationship between FSH and immunoreactive inhibin. Results for the subset of ewe lambs were analysed by linear

Fig. 1. Mean plasma (a, e) FSH, (b, f) LH and immunoreactive inhibin (c, g) and mean livemass (d, h) for BB (●) and ++ (○) Booroola ewe (a, b, c, d) and ram (e, f, g, h) lambs from 2 to 53 weeks of age. For LH, the values represent geometric means, and values on the y-axis are back-transformed; the y-axis is on a linear scale so the value of each minor increment on the y-axis is the difference between the successive major increments divided by the number in between, for example $(1.00 - 0.37)/9 = 0.07 \text{ ng ml}^{-1}$. Standard errors of the difference (SED) are shown as bars on the x-axis. Arrows denote significant differences at $P < 0.05$. (---): Mean age at first mating.

regression analysis over selected ages (2, 4, 15, 30 and 50 weeks). Results were also examined with respect to the mean values for 4–6, 12–28, 29–53 weeks or overall. No significant correlations were found at any selected age for either genotype or for the ++ ewes at weeks 4–6, 12–28 or 29–53. The only significant correlation found for ++ ewe lambs was when all the data were combined ($P < 0.05$; $R = -0.678$). For the BB ewe lambs a significant correlation was noted

over weeks 12–28 ($P < 0.05$; $R = -0.619$) but not over all the data.

Puberty. One BB ewe for which blood samples were not assayed for progesterone was excluded from the following analyses. Mating marks indicated that 12 of 15 BB and 17 of 20 ++ ewe lambs (80% and 85%, respectively, SED 13) exhibited behavioural oestrus. Of those that mated, the mean age at first

mating was 36.6 and 36.5 weeks of age (SED 1.2) for BB and ++ genotypes, respectively. High progesterone concentrations, indicative of oestrus, were found in 10 of 15 BB and 12 of 20 ++ ewe lambs (67% and 65%, respectively, SED 18). Only 9 BB and 11 ++ ewe lambs (60% and 55%, respectively, SED 17) were both marked by rams, and exhibited high progesterone concentrations. Two ewe lambs per genotype were neither marked by the ram nor showed high progesterone concentrations. There were no significant gene-specific differences in any of these parameters.

Birth rank and livemass. The mean birth ranks for BB and ++ ewe lambs were 2.63 and 1.65, respectively. For the BB genotype, there were 0, 4, 5, 6 and 1 ewe lambs born to ewes with litter sizes of single, twins, triplets, quadruplets and quintuplets, respectively. For the ++ genotype there were 7 and 13 ewe lambs born to ewes with single or twin litters, respectively. BB ewe lambs had a significantly lighter body-mass than did ++ ewe lambs at all times throughout the study (Fig. 1d). However, BB twins were not significantly lighter than ++ twins at birth, or at any subsequent time. Livemass generally decreased with increasing birth rank up to triplet litters, but the relationship between livemass and birth rank became less apparent with increasing age. The mean livemass difference between genotypes was 1.8 kg at 2 weeks of age (mean livemass 6.5 kg and 8.3 kg for BB and ++ ewe lambs, respectively, SED 0.60) and 4.2 kg at 53 weeks of age (mean livemass 34.3 kg and 38.5 kg for BB and ++ ewe lambs, respectively, SED 1.39). The maximum difference was 4.5 kg and this occurred at 18 weeks of age. There was no evidence that birth rank influenced gene-specific differences in FSH, LH or inhibin secretion.

Ram lambs

FSH. BB ram lambs had significantly higher FSH concentrations than did ++ rams at 8, 9, 10 and 29 weeks of age, and significantly lower concentrations at 22 and 23 weeks of age (Fig. 1e). The FSH concentrations fell from peak values at 5 (++) rams and 9 (BB rams) weeks to reach 0.4 ng ml^{-1} by 10 (++) genotype) and 13 (BB genotype) weeks of age, respectively: the overall means, when averaged from 2 to 12 weeks of age, were 0.7 and 0.6 ng ml^{-1} , respectively (SED 0.08, Fig. 1e). Between 13 and 31 weeks of age there was a further small decline in FSH concentration from 0.4 to 0.3 ng ml^{-1} for BB and ++ genotypes (SED 0.09). Thereafter, the concentrations remained constant, between 32 and 53 weeks of age, at 0.3 ng ml^{-1} (SED 0.01) for both genotypes. The mean genotype-adjusted autocorrelations for FSH in males declined from 0.70 at a lag of one week, to a stable value of -0.08 at a lag of 26 weeks or greater.

LH. Geometric mean LH concentrations in rams tended to be higher between 2 and 23 weeks, than from 24 to 53 weeks of age (Fig. 1f). There were no gene-specific differences in geometric mean LH concentrations when averaged between 2 and 23 weeks of age (means 0.3 ng ml^{-1} (SER 1.08) for both BB and ++ ram lambs) or when averaged between 24 and 53 weeks of age (means 0.2 ng ml^{-1} (SER 1.04) for both BB and ++ ram lambs).

Plasma immunoreactive inhibin concentrations. The plasma concentrations of immunoreactive inhibin in ram lambs are summarized in Fig. 1g. While there were no gene-specific differences in immunoreactive inhibin concentrations between 2 and 15 weeks of age (overall means were 428 and 424 iu ml^{-1} for BB and ++ rams, respectively, SED 23.1), the concentrations fell at a slower rate and were significantly higher for BB than for ++ rams between 16 and 37 weeks of age (means 294 and 231 iu ml^{-1} , respectively, SED 21.7, $P < 0.01$). From 37 to 53 weeks of age, the concentrations of immunoreactive inhibin were at the lowest recorded values, were relatively constant, and no significant effect of genotype was noted (140 and 132 iu ml^{-1} for BB and ++ rams, respectively, SED 15.6). The mean genotype-adjusted autocorrelations for immunoreactive inhibin in males declined from 0.75 at a lag of 1 week, to a stable value of -0.07 after a lag of 26 weeks or greater.

For comparison, the respective geometric mean (and 95% confidence limits) immunoreactive inhibin concentrations in the large flock of BB ($n = 134$) and ++ ($n = 109$) ram lambs were 343 (330, 357) and 303 (279, 330) iu ml^{-1} at 20.2 (19.7, 20.7) week of age. A highly significant effect of genotype ($P < 0.0005$), but no sire effect, was noted.

Relationship between the plasma concentrations of FSH and immunoreactive inhibin. With one exception, no significant correlations between FSH and immunoreactive inhibin were noted at any of the selected ages described previously for the ewe lambs (i.e., 2, 4, 15, 30 and 50 weeks of age). At 50 weeks of age, a relationship was noted (++; $R = 0.795$, $P < 0.001$; BB; $R = 0.831$, $P < 0.05$). When both genotypes were analysed separately, no significant relationships were noted at 4–6, 12–28 or 29–53 weeks of age or in the mean values overall.

Testicular development. In most ram lambs, the relationship between changes in testicular volume and age followed a cuboidal pattern (Fig. 2), increasing to peak volumes in all but four ($n = 2$ per genotype) individuals, then falling to a nadir in all but two (++) genotype) other lambs. BB ram lambs had significantly smaller testicular volumes than did ++ animals between 12 and 33 weeks of age, but not thereafter. In BB ram lambs, compared with ++ animals, peak testicular volume was significantly lower (208 ml and 249 ml, respectively, SED 14.2, $P < 0.01$), and was attained significantly later (at 32.7 and 30.1 weeks of age, respectively, SED 1.05, $P < 0.05$) and at a significantly lighter livemass (30.5 kg and 34.1 kg respectively, SED 1.03, $P < 0.001$). There were no gene-specific differences in minimum testicular volume recorded (158 ml and 168 ml for BB and ++ lambs, respectively, SED 10.5), or in the age at which this was reached (48.6 and 48.0 weeks for BB and ++ lambs, respectively, SED 1.76), but BB ram lambs had a significantly lighter bodymass (37.2 kg versus 41.7 kg, SED 1.56, $P < 0.01$) when testis volume was at its minimum. However, when adjusted for livemass, the genotype differences for testicular volume were not significant ($P > 0.05$) except at week 30 (++) > BB, $P < 0.03$).

Complete breakdown of foreskin adhesion occurred later in BB than in ++ rams (23.6 and 21.6 weeks of age, respectively, SED 0.72, $P < 0.01$), and at a significantly lighter livemass (26.9 kg and 30.1 kg, respectively, SED 0.97, $P < 0.001$). There

Fig. 2. Mean testicular volume (a) and testosterone concentrations (b) for BB (●) and ++ (○) Booroola ram lambs from 2 to 53 weeks of age. For testosterone, the values represent geometric means; and values on the y-axis are back-transformed; the y-axis is on a linear scale so the value of each minor increment is the difference between the successive major increments divided by the number in between, for example $(1.00 - 0.37)/9 = 0.07 \text{ ng ml}^{-1}$. Standard errors of the difference (SED) are shown as bars on the x-axis. Arrows denote significant differences at $P < 0.05$.

was no evidence that livemass or testicular volume was related to age at complete breakdown of foreskin adhesions, when adjusted for genotype.

In both genotypes, the plasma testosterone concentrations increased from about 0.3 ng ml^{-1} at 2 weeks of age, to reach a peak of 4.5 ng ml^{-1} between 30 and 35 weeks of age (Fig. 2b). Thereafter, the testosterone concentrations fell to a nadir of 0.3 ng ml^{-1} at 41 weeks of age. Geometric mean testosterone concentrations for BB and ++ rams at 2–25, 26–40 and 41–50 weeks of age were 0.4 and 0.6 ng ml^{-1} (SER 1.25), 1.2 and 1.5 ng ml^{-1} (SER 1.26) and 0.8 and 0.7 ng ml^{-1} (SER 1.25),

respectively, increasing again thereafter. There were no gene-specific differences in concentrations of testosterone.

Birth rank and livemass. The mean birth rank for BB and ++ ram lambs was comparable to those of ewe lambs of the same genotype (2.82 and 1.79 lambs born per lambing ewe, respectively). For the BB genotype, there were 1, 3, 8, 4 and 1 ram lambs born to ewes with litter sizes of singletons, twins, triplets, quadruplets and quintuplets, respectively. For the ++ genotype, there were 4 and 15 ram lambs born to ewes with single or twin litters, respectively. There were no gene-specific

differences in birthmass or subsequent livemasses between single or between twin born ram lambs, and livemass generally decreased with increasing birth rank, particularly in younger rams. BB ram lambs were significantly lighter than ++ ram lambs on 44 of the 52 livemass observations (Fig. 1h). The livemass differences were 1.3 kg at 2 weeks of age (7.5 kg and 8.8 kg, respectively, SED 0.55; $P < 0.05$), a maximum of 5 kg at 22 weeks of age (25.5 kg and 30.5 kg; SED 1.21; $P < 0.001$), and 2.6 kg at 53 weeks of age (44.3 kg and 46.9 kg for BB and ++ ram lambs, respectively, SED 1.56; $P > 0.05$).

There were no apparent sire effects on FSH, LH or immunoreactive inhibin secretion or on livemass within sexes. As with ewe lambs, there was no evidence that birth rank influenced gene-specific differences in FSH, LH or immunoreactive inhibin secretion.

Discussion

During the first year of life, the patterns of plasma FSH in ewe lambs were markedly different between Booroola genotypes. BB ewe lambs had higher FSH concentrations than did ++ ewe lambs at 4–6 weeks of age, lower concentrations at 12–28 weeks of age, and similar FSH concentrations at 34–53 weeks of age. Similar patterns of FSH concentrations in ewe lambs, together with gene-specific differences at 4–6 weeks of age, are reported by others (Bindon *et al.*, 1985; Montgomery *et al.*, 1989; Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993). However, in all these studies, with the exception of Braw-Tal and Gootwine (1989), the FSH concentrations in ++ ewe lambs are not found to increase above those of BB ewe lambs by 12 weeks of age, as was found in the present study. An explanation for this discrepancy may be that the sampling frequency undertaken after 12 weeks of age in previous studies was insufficient for the genotype difference to become apparent.

Foster *et al.* (1975) found a pattern of FSH concentrations from birth to 28 weeks of age in Shropshire and Shropshire crossbred ewe lambs similar to that recorded for ++ lambs in the present study. In BB and ++ ewe lambs, the pattern of LH concentrations was erratic up to 26 weeks of age. There was no consistent effect of genotype over this period, confirming the findings of Bindon *et al.* (1985). However, between 26 and 53 weeks of age, ++ ewe lambs had significantly higher mean LH concentrations than did BB ewe lambs. In the present study, the patterns of LH concentration in ++ ewe lambs were not consistent with those reported by Bindon *et al.* (1985) or Foster *et al.* (1975). This probably reflects the pulsatile pattern of LH and the low sampling frequency undertaken in the present study.

BB ewe lambs had significantly lower mean concentrations of immunoreactive inhibin than did ++ ewe lambs between 2 and 12 weeks of age, but not thereafter. After 12 weeks of age, the plasma concentrations of immunoreactive inhibin were comparable to those observed in adult females. It is likely that most of the immunoreactive inhibin in ewe lambs during the first weeks of life is not of ovarian origin, as ovariectomy at 2 weeks of age leads to the loss of < 40% of the immunoreactive inhibin from plasma (R. Braw-Tal, unpublished data). In contrast, ovariectomy at 8 weeks of age, or after puberty, leads to more than 85% of the immunoreactive inhibin being removed from the circulation (R. Braw-Tal, unpublished data; McNatty

et al., 1992). In the ovaries of newborn lambs (2–14 weeks of age) or adult ewes, ovarian granulosa cells are a major site of mRNA synthesis for α , β_A and β_B inhibin (Engelhardt *et al.*, 1993; Tisdall *et al.*, 1993; Braw-Tal, 1994). Moreover, using an identical inhibin assay procedure to that used in the present study, McNatty *et al.* (1993b) showed that there is a highly significant linear relationship between the number of granulosa cells present in follicles ≥ 1 mm diameter and plasma concentrations of immunoreactive inhibin in ewes ($R = 0.68$; $P < 0.001$). It is therefore reasonable to suggest that, from about 12 weeks of age, the assay for immunoreactive inhibin was recognizing inhibin-like peptides, mainly from granulosa cells. If this assumption is correct, then despite possible genotype differences in the pattern of follicle development after 12 weeks of age (McNatty *et al.*, 1987b; Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993), the total number of granulosa cells in the developing antral follicles in BB and ++ ewe lambs is likely to be similar, as is the case in adult Booroola ewes (McNatty *et al.*, 1991b). The poor correlations between FSH and immunoreactive inhibin in either genotype suggest that the assay is not detecting only biologically active dimeric inhibin, but also other crossreacting forms of inhibin-related peptide. At present, there is no acceptable assay available to measure only biologically active inhibin in the peripheral plasma of sheep, and the contributions of intra- or extraovarian inhibins to the genotypic differences in plasma FSH secretion in Booroola lambs and ewes cannot therefore be resolved.

Ewe lambs first showed behavioural oestrus at approximately 37 weeks of age in both genotypes, and this was coincident with the fall in FSH and the rise in immunoreactive inhibin. However, the plasma progesterone data suggest that most animals did not continue to cycle regularly. These findings for FSH, progesterone and the onset of behavioural oestrus are in agreement with those of Foster *et al.* (1975). In general, the concentrations of FSH around puberty were similar to those recorded in adult ewes, whereas concentrations of LH tended to be higher than in adult ewes (Foster *et al.*, 1975; McNatty *et al.*, 1987a).

In Booroola ram lambs, there appeared to be developmental differences for FSH, LH and immunoreactive inhibin at specific times during the neonatal period. With respect to FSH, it seems that between 6 and 10 weeks of life, peak secretion of FSH in BB ram lambs was increased for 3–4 weeks more than it was in ++ animals. Although not consistently different, there was also a tendency for LH concentrations to be higher in BB than in ++ ram lambs from 11 to 20 weeks of age: for example at 12, 16 and 17 weeks, the BB animals had significantly higher LH values than did the ++ genotype. Subsequently, between 19 and 33 weeks, the concentrations of immunoreactive inhibin were higher in BB than in ++ ram lambs. The differences between the genotypes for FSH, LH and immunoreactive inhibin could not be attributed to litter size, livemass or sire. In the case of immunoreactive inhibin, it is unlikely that the apparent genotype effect is due to sire as the larger study of ram lambs derived from a large number of sires ($n = 9$ –14 per genotype) demonstrated a specific genotype, but no sire, effect at 20 weeks of age. However, for the longitudinal study of FSH and LH, the confounding effects of sire cannot be ruled out (see Purvis *et al.*, 1991). Notwithstanding this reservation, the findings for FSH and LH in the present study are consistent

with those of Seck *et al.* (1991), who showed that B gene carriers had higher concentrations of FSH, but not of LH, compared with ++ animals up to 10 weeks of age, and that the changes in FSH occurred before the onset of testicular growth.

In the present study, the genotype difference in the plasma concentrations of immunoreactive inhibin between 19 and 33 weeks of age was observed in the absence of comparable changes in testosterone. The testicular output of testosterone, which paralleled the changes in testicular volume, is likely to be of Leydig cell origin, whereas the immunoreactive forms of the α subunit of inhibin or intact inhibin recognized by the antibody could originate from a number of sources, such as Leydig cells, Sertoli cells and possibly peritubular cells (Franchimont *et al.*, 1981; Risbridger *et al.*, 1989; DeWinter *et al.*, 1992). The lack of any significant correlations between FSH and immunoreactive inhibin suggest that biologically active dimeric forms of inhibin are unlikely to be the principal products. At present there is no evidence to suggest that the cellular composition of the testes are different between the Booroola genotypes, notwithstanding differences in testes mass or volume (Hochereau-de Reviers and Seck, 1991). Thus, the reasons for the apparent FecB^B differences in the plasma concentrations of immunoreactive inhibin in ram lambs during neonatal life cannot be established from this study.

Plasma FSH and LH concentrations were consistently lower in males than in females, as observed by Savoie *et al.* (1981) and Ricordeau *et al.* (1984, 1990). In contrast, the plasma concentrations of immunoreactive inhibin were up to ten times higher in ram lambs than in ewe lambs, possibly reflecting the cellular composition of the ovary and the testis at this time. For example, in ram lambs, estimates of the number of Sertoli cells are of the order of 22×10^8 cells, whereas equivalent estimates of granulosa cell numbers in ewe lambs would be about 1×10^8 (Hochereau-de Reviers and Seck, 1991; K. P. McNatty, unpublished). The plasma concentrations of testosterone in females from birth to puberty are not known; equivalent comparisons for this steroid cannot therefore be made. Autocorrelation analysis revealed that, in both females and males, there was a relatively high correlation for FSH and immunoreactive inhibin between weeks, and that for females, but not males, the relationship for FSH remained relatively high (0.31), even over time lags of 20 or more weeks.

In males, puberty has been defined as the age at which the first spermatozoa appear in the epididymis, when the first spermatozoa appear in the ejaculate, or the time of the first fertile mating (Hochereau-de Reviers and Seck, 1991). None of these criteria were recorded in the present study. Instead we chose to assess sexual development using measurements of testicular volume, breakdown of adherence of the foreskin to the head of the penis, and plasma testosterone concentration. Booroola–Merino sheep are seasonal breeders under New Zealand farm conditions; the onset of puberty can therefore be compromised by time of year at birth. The ram lambs in the present study were between 20 and 36 weeks of age during the normal breeding season for sexually mature sheep. At the time these animals were reaching 40 weeks of age (late August or September), both rams and ewes would be likely to be entering seasonal anoestrus (Price *et al.*, 1991c). In ram lambs, peak testosterone and testicular volumes were reached between 28 and 35 weeks of age. The adherence of the foreskin to the head

of the penis was completely broken down by 23.6 and 21.7 weeks of age in BB and ++ rams, respectively ($P < 0.01$). The decline in testis volume after 32–35 weeks, and the corresponding fall in testosterone, is consistent with the changes observed in sexually mature Booroola Merino $\times$ Romney rams during the breeding season, with the subsequent nadir and increase in testis volume consistent with that observed at the onset of the following non-breeding season (Price *et al.*, 1991c). Thus, although the precise timing of puberty in the genotypes was not determined, the time at which it was likely to have occurred was between 28 and 35 weeks of age. During this time there were no consistent differences between the genotypes in FSH, LH or testosterone concentration, although the plasma concentration of immunoreactive inhibin in BB rams may still have been greater. Around the apparent onset of puberty, the ++ rams were consistently heavier and the volumes of their testes consistently larger; however, these effects were probably a consequence of litter size. The only overt effect that appeared to be related to genotype was the age of breakdown of foreskin adhesions, although the difference was small (within the sampling interval) and may have been influenced by sire.

In conclusion, we hypothesize that the apparent FecB^B-related development differences in FSH and immunoreactive inhibin, and to a lesser extent LH, recorded in both ewe and ram lambs during the first year of life, may be part of a process that begins during fetal life (Smith *et al.*, 1993). We suggest that this reflects a retardation of fetal development imposed by the presence of the Booroola gene. Whether the FecB^B differences in plasma FSH in adult females arise from developmental changes in neonatal life remains to be determined. Moreover, the cellular origins, and forms of immunoreactive inhibin that contribute to the FecB^B differences in both male and female lambs require further study.

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