Effects of the Booroola gene (Fec^B) on body weight, ovarian development and hormone concentrations during fetal life

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The aim of this study was to determine whether the Fec^B gene influenced some aspects of fetal development in sheep. Carrier (BB/B+) and non-carrier (++) female fetuses were recovered at specific times of gestation, namely, days 40, 55, 75, 90, 95 and 135. The results showed that the Fec^B gene influenced litter size, body weight and ovarian development during fetal life. The mean litter sizes were larger ($P < 0.05$) and body weights were lighter ($P < 0.05$) at most gestational ages in BB/B+ than in ++ fetuses. Morphometric studies of the ovary showed that the development of the BB/B+ ovaries was retarded: the ++ genotype had more oogonia at day 40 ($P < 0.01$), more germ cells entering meiosis at day 55, more primordial follicles developing at days 75, 90 and 95 ($P < 0.05$), a greater loss of germ cells by atresia at day 90 ($P < 0.01$) and more growing follicles ($P < 0.01$) and more antral follicles ($P < 0.05$) at day 135. Differences between the BB/B+ and ++ genotypes in the plasma concentrations of immunoreactive (i) inhibin, i-FSH, bioactive (b)-FSH or (i)-LH were not apparent at any age except for i-LH at day 75 (BB/B+ > ++; $P < 0.05$). Likewise no differences were noted in the contents of ovarian or adrenal oestradiol or i-inhibin except for i-inhibin in the adrenal at day 75 (++ > BB/B+, $P < 0.01$). No differences between the genotypes were noted in the i-inhibin contents of the mesonephros at day 40. In mid- to late but not early gestation (i.e. days 40 and 55) significant correlations (i.e. $P < 0.05$) were noted between litter size and body weight at days 75, 90 and 135, and between litter size and ovary weight, ovary volume, adrenal weight and pituitary weight at day 135. To eliminate the effect of litter size, equal numbers of BB/B+ and ++ embryos were transferred to respective recipient ewes, and fetuses were recovered at the equivalent of days 40 and 90 of gestation. The results showed that the genotypic difference in fetal body weight at day 40 (++ > BB, $P < 0.001$) and in number of oogonia at day 90 (++ > BB/B+, $P < 0.05$) were independent of litter size. We hypothesize that many of the differences between the Booroola genotypes in ovarian follicular development and pituitary function in neonatal and adult life may be a consequence of differences in the timing or rate of body weight or organ development in fetal life.

Introduction

Booroola ewes with the Fec^B gene have a higher ovulation rate than those without the gene (Bindon and Piper, 1982; Davis *et al.*, 1982). Previous studies with homozygous (BB), heterozygous (B+) and non-carriers (++) of the Fec^B gene have shown that there are differences in ovarian follicular development, the concentrations of pituitary hormones in plasma and the pituitary responses to exogenous GnRH between the genotypes both before and after puberty (see review by McNatty *et al.*, 1991). However, the question arises as to whether the pituitary differences are unrelated to or a cause of or a consequence of those in ovarian development. It is possible that this

question could be resolved by examining ovarian and pituitary function during fetal development. In sheep fetuses which have a gestation length of about 150 days, sexual differentiation occurs at about day 35 of gestation (Mauleon, 1969). In the ovaries of sheep fetuses, mitosis of germ cells begins before sexual differentiation and continues until about day 90, whereas meiosis begins at about day 55 (Mauleon, 1969; Smith *et al.*, 1991). Some time later, but before day 75, atresia of a proportion of the germ cells is evident with follicular formation and growth beginning at, or after, about day 75 and about day 90, respectively (Mauleon, 1969). With regard to gonadal hormones, immunoreactive (i)-inhibin has been detected in the plasma of sheep fetuses (of both sexes) from day 55 of gestation (Smith *et al.*, 1991; Phillips *et al.*, 1992) as well as in the testis at day 50 (Wongprasartsuk *et al.*, 1991). In female sheep fetuses, the concentrations of plasma i-inhibin were highest at day 55

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(e.g. about 80 iu ml^{-1}) and lowest at day 135 (e.g. about 25 iu ml^{-1}): in ewes the mean concentrations of plasma i-inhibin are about 12 iu ml^{-1} (McNatty *et al.*, 1992). At the level of the fetal ovary the contents or concentrations of inhibin have not been reported but those for oestradiol remain low throughout gestation (i.e. $\leq 10 \text{ pg per ovary}$; Smith *et al.*, 1991). While the above events are taking place, major changes are also occurring within the hypothalamic–pituitary system. The first i-GnRH-staining cells and fibres appear between day 40 and day 43 in the preoptic–hypothalamic area (Caldani and Batailler, 1991) and i-hypothalamic GnRH and pituitary i-FSH and i-LH have been detected at about days 55–60 (Gluckman, 1981; Smith *et al.*, 1991; Phillips *et al.*, 1992). From days 75 to 135 of gestation, the pituitary contents and concentrations of bioactive (b-) and i-FSH increase with increasing age but no change in b:i ratios have been noted (Phillips *et al.*, 1992). By contrast, the plasma concentrations of b-FSH remained essentially unchanged from days 75 to 135, whereas those for i-FSH reached a peak at day 90 and then declined towards term indicating that changes were occurring in the plasma b:i ratios for this hormone.

The aims of the present study were to identify whether the Fec^B gene influenced body weight, organ weight, ovarian development or reproductive hormones (i.e. i- and b-FSH, i-LH, i-inhibin and oestradiol) in the female at specific times during fetal life, namely days 40, 55, 75, 90, 95 and 135 of gestation. Moreover, some Booroola fetuses with or without the Fec^B gene were recovered at days 40 and 90 of gestation after embryo transfer to study some of the aforementioned characteristics after eliminating the possible effects of litter size.

Materials and Methods

The experimental procedures reported in this study were carried out in accordance with the 1987 Animal Protection (Codes of Ethical Conduct) Regulations of New Zealand after approval was granted by the Animal Ethics Committee of the Wallaceville Animal Research Centre.

Animals and treatments

Female fetuses (BB or B+, and ++) were recovered from pregnant ewes at days 40, 55, 75, 90, 95 and 135 after mating. The BB or B+ female fetuses ($n = 162$) were the result of matings between BB Booroola Romney rams ($n = 5$) and B+ Booroola Romney ewes so that all fetuses were either homozygous or heterozygous for the Fec^B gene. Since it was not possible to distinguish between the BB or B+ fetuses these are hereafter referred to as BB/B+ animals. The ++ female fetuses ($n = 111$) were the result of matings between ++ Booroola Romney rams ($n = 8$) and Booroola Romney ewes ($n = 56$) or Romney ewes ($n = 55$). Care was taken to ensure that all rams had an equal chance of contributing fetuses at each gestational age. There were no differences in the body weights of the BB and ++ rams. Fetuses were recovered after a barbiturate overdose (Euthatal; about $20 \text{ ml i.v. of } 500 \text{ mg ml}^{-1} \text{ w/v}$) was given to their mothers. The fetal blood samples were collected by cardiac puncture, centrifuged (4000 g , 20 min) at 4°C and the plasma was stored at -15°C until assayed. The

plasma samples were assayed for i- and b-FSH, i-LH and i-inhibin. The body weight and crown–rump lengths were measured and the ovaries, mesonephros (at days 40 and 55 only), adrenals and pituitary dissected and weighed. The left ovary was fixed in Bouin's fixative and serially sectioned at $4 \mu\text{m}$ for morphometric studies. The other organs (i.e. adrenals, mesonephros, right ovary and pituitary) were frozen to -70°C for subsequent homogenization and hormone assay.

The pituitary glands were homogenized into 2 ml buffer ($5 \text{ mmol potassium phosphate l}^{-1} + 0.1\% \text{ BSA (w/v)} + 1 \text{ mmol phenylmethylsulfonyl fluoride l}^{-1}$, $\text{pH } 7.4$) with $2 \times 25 \text{ s}$ bursts with an Ultraturrex homogenizer (Janke & Kunkel, IKA-Labortechnik, Staufen) on ice. The homogenates were centrifuged at 400 g for 5 min and then at $30\,000 \text{ g}$ for 30 min . Thereafter the supernatants were decanted (extract 1) and the pellets homogenized and centrifuged once more to obtain a second supernatant (extract 2). The supernatants (extracts 1 and 2) were stored at -20°C until assayed.

Embryo transfer experiment

Any effects of litter size at 40 or 90 days' gestation were eliminated by transferring equal numbers of embryos from BB rams mated to superovulated BB/B+ ewes and from ++ rams mated to superovulated ++ ewes to recipient Romney ewes. The BB/B+ and ++ donor ewes were superovulated using PMSG (Folligon, Intervet, Lane Cove) and Ovagen (Immuno-Chemical Products Ltd, Auckland). Briefly 13 BB/B+ and 16 Booroola Romney ewes were fitted with intravaginal progesterone devices (CIDR-s type G; AHI Plastic Moulding Co., Hamilton) for 10 days (day of CIDR-s implant, day 0). On day 10 the CIDR-s device was replaced and the ewes given an i.m. injection of PMSG (300 iu) and $8 \times 1 \text{ ml}$ injections (i.m.) of a 20 ml Ovagen ampoule starting from the time of PMSG injection (morning) and thereafter at intervals of 12 h and the second CIDR-s device was removed on the morning of day 13. On the evening of day 13 and on the morning of day 14 the BB/B+ ewes were mated as a flock with BB rams ($n = 6$) and likewise the ++ ewes were mated as a flock with ++ rams ($n = 6$). Four days after mating, 57 and 67 embryos were recovered from the BB/B+ and ++ ewes, respectively. Oestrous synchronized (i.e. with CIDR-s) Romney ewes each received three embryos (one ewe received four embryos) by non-surgical transfer (i.e. via a laparoscopic technique). Subsequently, 10 BB and 13 ++ female embryos were recovered from recipient ewes at the equivalent of day 40 of gestation and seven BB/B+ and nine ++ females at the equivalent of day 90 of gestation.

Morphometric studies

Ovarian volume and ovarian cortex volume $V_{(o)}$ were estimated by the Cavalieri principle (Gundersen and Jensen, 1987; see also Michel and Cruz-Orive, 1988). The ovarian cortex is defined as the outer region of ovary containing all the germ cell. The areas (a) in μm^2 of every 50th or 100th section ($4 \mu\text{m}$ each) were determined by point counting with $V_{(o)}$ calculated from $\sum ah$ where h is the distance in μm between the sections used to determine a . Germ cell number (N) was estimated by the disector method (Sterio, 1984) using section(s) pairs $h(s)$ apart. In this

instance adjacent sections were used thus $h(s) = 4 \mu\text{m}$. The tissues were sampled systematically with a minimum of 10 pairs of sections per organ (Gundersen, 1986). An unbiased counting frame of area $a_{(f)}$ was placed over the projected images of the sections and the numbers of germ cells exclusive to one section only (Q^-) were counted. The total germ cell count was derived:

$$N = \frac{\Sigma Q^-}{\Sigma a_{(f),h(s)}} \cdot V_{(o)}$$

During the estimation of number of germ cells the germ cells were classified into oogonia, oocytes in meiotic prophase but not surrounded by follicular cells (oocytes), primordial follicles (e.g. an oocyte surrounded by a flattened layer of granulosa cells), primary follicles (e.g. an oocyte with 1–2 layers of cuboidal granulosa cells) and larger follicles (secondary to antral follicles). Determination of the coefficients of error due to the stereological sampling procedures was estimated by the method described by Pakkenberg and Gundersen (1988), and West and Gundersen (1990). The coefficients of error for the germ cell numbers and ovarian volumes were $< 10\%$.

Hormone assays

i-FSH. The radioimmunoassay kit was supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, Maryland, USA. The ovine (o) FSH for iodination was NIAMDD-oFSH-I-1, the oFSH reference preparation was NIAMDD-oFSH-RP1 (biopotency $75 \times \text{NIH-FSH-S1}$) and the oFSH antiserum was NIAMDD-anti-oFSH-1 (AFP-C5288113). For assay, each pituitary supernatant was serially diluted and the results for both summed to obtain the total for each pituitary. The volume of plasma or pituitary extract used in the assay was 0.1 ml and each diluted sample was assayed in duplicate. The internal standards, standard curve samples and serially diluted extracts were prepared in FSH-free plasma from hypophysectomized ewes and the minimum detectable concentration was 0.2 ng ml^{-1} . The intra- and interassay coefficients of variation (CV) for the internal standards were both $< 8\%$.

b-FSH. The plasma and pituitary b-FSH were measured as described by Padmanabhan *et al.* (1987): the only modification was the use of a 0.5 ml substrate incubation volume instead of 1 ml. The validation of this assay for fetal sheep plasma has been reported by Phillips *et al.* (1992). Concentrations of b-FSH were measured using the production of oestradiol *in vitro* from Sertoli cells isolated from the immature male rats. The reference standard used was NIADDK-oFSH-RP-1. The sensitivity of the oestradiol radioimmunoassay (McNatty *et al.*, 1989) was 5 pg ml^{-1} and the intra- and interassay CVs were both $< 10\%$. The sensitivity of the FSH bioassay was $< 1 \text{ ng ml}^{-1}$ and the intra- and interassay CVs were < 12 and $< 17\%$, respectively.

LH. The radioimmunoassay was identical to that described by McNatty *et al.* (1989). Briefly the iodination standard was NIDDK-oLH-I-3 (AFP-9598B), the LH antiserum was raised at the Wallaceville Animal Research Centre and is described by McNatty *et al.* (1987). The oLH reference preparation was

NIAMDD-oLH-23 (biopotency $2.3 \times \text{NIH-LH-S1}$). The volume of pituitary extract or plasma which was assayed in duplicate was 0.1 ml. The internal standard dilutions of pituitary extracts and standard curve samples were prepared in LH-free hypophysectomized ewe plasma. The overall intra- and interassay CVs for the internal standards which were included with every standard curve estimation were both $< 10\%$. The minimum detectable concentration of LH was 0.2 ng ml^{-1} .

i-Inhibin. The radioimmunoassay kit was purchased from D. M. Robertson (Monash University, Melbourne) and consisted of a highly purified 31 kDa bovine inhibin for iodination and a rabbit anti-31-kDa inhibin preparation (No. 1989). This assay using antiserum No. 1989 has previously been validated for the measurement of inhibin in peripheral plasma of sheep (Findlay *et al.*, 1990) and the procedure used was identical to that described by McNatty *et al.* (1992). The antiserum has less than 1% crossreactivity with ovine FSH, LH, growth hormone, prolactin, bovine activin-A, porcine transforming growth factor- β , Mullerian inhibiting substance, the reduced and alkylated α - and β -subunits of bovine inhibin and follistatin. However, the antiserum has a 288% crossreaction with the bovine pro- α C-subunit (Robertson *et al.*, 1989). The reference standard was a pool of charcoal-treated ovine follicular fluid (batch 2). The assay buffer was 0.1 mol phosphate buffer l^{-1} with 0.15 mol sodium chloride l^{-1} and 5% (w/v) BSA, pH 7.4. Standards or unknowns were diluted in either assay buffer or ovariectomized ovine plasma (OOP; previously shown to be free of immunoreactive inhibin-like activity) to a volume of 100 μl . All homogenized tissue samples were assayed using assay buffer, whereas all plasma samples were assayed using OOP. The efficiency of recovery of inhibin from homogenized ovarian tissues was not determined.

The sensitivity of the assay (i.e. at 10% displacement of the tracer) was $< 0.5 \text{ iu}$ per tube both in the presence and absence of OOP. The mean intra- and interassay coefficients of variation were both $< 16\%$.

Oestradiol. The radioimmunoassay procedures were as described by McNatty *et al.* (1989). The gonads were homogenized in up to 3 ml of 70% (v/v) methanol, frozen to -20°C overnight, centrifuged at -10°C at 500 g for 10 min, and the supernatant decanted into a tube and evaporated to dryness under dry nitrogen gas. Thereafter the residue was dissolved in 400 μl of assay buffer (0.1 mol phosphate buffer l^{-1} with 0.15 mol NaCl l^{-1} and 0.1% (w/v) gelatin, pH 7.4). The plasma samples were subjected to the affinity chromatography step described by Webb *et al.* (1985). The limit of detection was $\leq 3 \text{ pg}$ per tube and the intra- and interassay CVs were 12 and 16%, respectively.

Statistical analysis. Data from individual fetuses were log-transformed and subjected to analysis of variance to test for differences between genotypes. When the variances between the data sets were unequal, genotype differences were examined using the Kruskal–Wallis test. Differences between genotypes were confirmed using partial correlation analysis controlled for litter size. The possibility of differences arising from the use of Booroola Romney and Romney control animals (i.e. as the ++ animals) was tested by analysis of variance using breed (Booroola

Table 1. Effect of gestational age and Booroola genotype on litter size, crown–rump length and body weight in female fetuses

Gestational age (days) [Booroola genotype]	n	N _f	Litter size	Female fetuses	
				Crown–rump length (cm)	Body weight (g)
40 [BB/B+]	13	23	2.7 (2.2, 3.4)	3.6 (3.5, 3.7)	3.8 (3.6, 4.1)
40 [++]	12	12	1.3** (1.0, 1.5)	3.7 (3.6, 3.9)	4.4* (4.0, 4.8)
55 [BB/B+]	22	33	2.4 (2.0, 2.8)	8.6 (8.4, 8.8)	25.8 (24.4, 27.3)
55 [++]	20	24	1.4** (1.2, 1.6)	8.9 (8.6, 9.2)	28.5 (26.9, 31.1)
75 [BB/B+]	29	34	2.4 (2.0, 2.8)	15.8 (15.3, 17.3)	150.7 (138.2, 165.0)
75 [++]	22	24	1.2** (1.1, 1.4)	16.9* (16.6, 18.2)	189.4** (179.1, 200.6)
90 [BB/B+]	22	28	2.5 (2.1, 2.9)	21.8 (21.3, 22.3)	443.5 (415.6, 473.3)
90 [++]	20	21	1.2** (1.0, 1.4)	22.9* (22.4, 23.4)	517.5** (486.9, 549.3)
95 [BB/B+]	13	19	2.4 (1.9, 2.9)	24.4 (24.0, 24.8)	591.8 (554.2, 631.5)
95 [++]	10	11	1.8 (1.5, 2.3)	24.5 (23.8, 25.2)	630.1 (563.8, 704.3)
135 [BB/B+]	17	25	2.4 (2.0, 2.8)	36.4 (34.7, 38.1)	2288 (1969, 2659)
135 [++]	17	19	1.3** (1.1, 1.6)	40.9** (39.3, 42.6)	3653** (3344, 3989)

Values are geometric means (and 95% confidence limits).

n = number of ewes; N_f = number of female fetuses; litter size refers to male and female fetuses.

*P < 0.05; **P < 0.01 Student's *t* test across genotype with respect to gestational age.

Romney or Romney), gestational age and ovarian characteristic or hormone concentrations as the variables; no differences were noted. The effect of litter size on each data set was tested by linear regression analysis within each genotype for possible interactions: no interactions were found. Partial correlation analyses controlled for genotype were performed to determine the effects of litter size. These effects of litter size were also confirmed by multiple regression analysis. The data are presented as geometric means (and 95% confidence limits).

Results

Effect of gestational age and Booroola genotype on litter size, crown–rump length, body weight and organ weights

Irrespective of genotype, there were increases in crown–rump length, body weight, pituitary weight and adrenal weight during gestation similar to those reported previously for sheep (Tables 1 and 2) (Thurley *et al.*, 1972; Noden and De Lahunta, 1985). Irrespective of genotype, mean ovarian weight increased from day 40 to day 75 but then declined at day 90 (day 75, 18.4 (16.2, 20.6) mg; day 90, 15.0 (13.8, 16.2) mg; *P* < 0.02; geometric mean (95% confidence limits)). By day 95, the mean

ovarian weight had increased once more to values similar to those found at day 75 (day 95; 18.3 (16.3, 20.2) mg). By day 135, the mean ovarian weight had increased still further from that found at day 95 (e.g. day 135; 28.6 (25.6, 31.6) mg; *P* < 0.01 compared with day 95).

Statistically significant effects of genotype were noted for all fetal characteristics at some, but not necessarily all, gestational ages. Genotypic differences in litter size and body weight were noted at all gestational ages except for body weight at days 55 and 95 and litter size at day 95; at day 55 the body weight differences approached significance (*P* = 0.08, Table 1). The earliest genotypic difference in organ weight was that noted for the mesonephros at day 40 gestation; this organ, which is about 10 times heavier than the ovary at day 40, is no longer present as a distinct entity at day 75 or thereafter. The weights of the other organs (e.g. pituitary, ovary, adrenal) were not different between the genotypes until day 90 or 95, from which time the mean weights were consistently, but not always significantly, heavier in the ++ than in the BB/B+ fetuses (Table 2).

Significant correlations between litter size and the above-mentioned fetal characteristics were established at some but not all gestational ages. Partial correlation analyses controlled for genotype showed no significant correlations at days 40 and 55 between litter size and crown–rump length, body weight,

Table 2. Effect of gestational age and Booroola genotype on weights of pituitary, ovary, mesonephros and adrenal

Gestational age (days) [Booroola genotype]	n	N ₁	Fetal organ weight			
			Pituitary (mg)	Ovary (mg)	Mesonephros (mg)	Adrenal (mg)
40 [BB/B+]	13	23	—	1.4 (1.3, 1.6)	13.1 (11.3, 14.7)	2.4 (1.0, 3.6)
40 [++]	12	12	—	1.5 (1.2, 1.7)	17.3** (14.9, 20.6)	2.3 (1.8, 2.4)
55 [BB/B+]	22	33	4.8 (2.7, 7.0)	9.3 (6.6, 12.1)	10.6 (8.4, 12.8)	10.7 (9.4, 12.0)
55 [++]	22	24	3.6 (2.9, 4.2)	9.0 (6.2, 11.9)	12.2 (9.8, 14.6)	11.5 (10, 12.9)
75 [BB/B+]	29	34	13.5 (11.7, 15.3)	16.8 (14.1, 19.5)	—	33.8 (30.3, 37.3)
75 [++]	22	24	13.1 (11.1, 15.1)	20.4 (17.6, 23.2)	—	35.0 (31.5, 39.4)
90 [BB/B+]	22	28	24.9 (23.1, 26.8)	15.3 (13.8, 16.7)	—	53.1 (48.4, 57.7)
90 [++]	20	21	29.3* (26.0, 32.4)	14.7 (12.6, 16.8)	—	57.9 (50.7, 65.1)
95 [BB/B+]	13	19	33.5 (30.7, 36.3)	16.4 (15, 17.9)	—	52.2 (47.2, 57.2)
95 [++]	10	11	36.2 (32.5, 40.0)	22.2** (18.1, 27.2)	—	71** (65.3, 77.0)
135 [BB/B+]	17	25	77 (68, 86)	25 (21, 28)	—	159 (146, 172)
135 [++]	17	19	89 (78, 99)	33 (28, 38)	—	184 (161, 207)

Values are geometric means (and 95% confidence limits).

n = number of ewes; N₁ = number of female fetuses. The values for the ovary, mesonephros and adrenal refer to the means of the left and right hand organs.

*P < 0.05; **P < 0.01 Student's *t* test across genotype with respect to gestational age.

pituitary, ovary, mesonephros or adrenal weights. At day 75 there were significant partial correlations between litter size and body weight ($R = -0.31$, $P < 0.05$), and between litter size and adrenal weight ($R = -0.42$, $P < 0.01$). At day 95, litter size was correlated with crown-rump length only ($R = -0.34$, $P < 0.05$). At day 135 significant partial correlations were noted between litter size and body weight, pituitary, ovary and adrenal weights. The partial correlation coefficients for body weight, pituitary, ovary and adrenal weights were $R = -0.63$ ($P < 0.01$), -0.41 ($P < 0.05$), -0.57 ($P < 0.01$) and -0.45 ($P < 0.05$), respectively.

Effect of gestational age and Booroola genotype on ovarian characteristics during fetal development

Irrespective of genotype the mean ovarian weight in this subset of animals, in which other ovarian characteristics were measured, increased from day 40 to day 75, remained essentially unchanged around day 90 and then increased once more by days 95 and 135 (day 135 > day 95, $P < 0.05$; day 95 > day 90, $P < 0.05$) (Table 3). The mean volume of the ovary and ovarian cortex increased from day 40 to day 95 (all $P < 0.01$) and thereafter remained unchanged at day 135 (ovarian volume) or declined

($P < 0.05$) in the case of the ovarian cortex, irrespective of genotype.

The changes in the total population of germ cells followed the same pattern in both genotypes. Between days 40 and 55 of gestation, the mean number of germ cells for both genotypes combined increased by about 18-fold (irrespective of genotype), and was followed by about a 1.2-fold increase between days 55 and 75. A rapid decline (of about 83%) was noted between day 75 and day 90: irrespective of genotype on day 75, the geometric mean (95% confidence level) germ cell population was 902 288 (804 354, 1 012 128) whereas at day 90 the population was 154 167 (127 296, 186 716). Between days 90 and 95, the overall mean population remained essentially unchanged, i.e. day 95 population was 189 991 (158 999, 224 642). From day 95 to day 135, the mean number had declined once more by about 60%, with 63 841 (51 149, 79 682) germ cells being found on day 135.

Significant effects of genotype were noted for all ovarian characteristics at some gestational ages. For ovarian weight, ovarian volume and ovarian cortex volume, differences between the genotypes were noted at days 95 and 135. At day 135, but not day 95, ovarian volume and weight were correlated with litter size ($R > -0.4$, $P < 0.05$ for both characteristics). For

Table 3. Effect of gestational age and Booroola genotype on ovarian weight (mg), volume (mm³), cortical volume (mm³) and number of germ cells ($\times 10^{-3}$)

Gestational age (days) [Genotype]	Number of female fetuses	Ovarian characteristic			
		Ovarian weight	Ovarian volume	Cortex volume	Germ cell number
40	18	1.5	0.60	0.26	31
[BB/B+]		(1.3, 1.7)	(0.54, 0.66)	(0.22, 0.29)	(27, 35)
40	10	1.5	0.69	0.31	50**
[++]		(1.2, 1.8)	(0.59, 0.79)	(0.25, 0.38)	(38, 66)
55	13	5.7	1.79	1.58	763
[BB/B+]		(4.7, 6.8)	(1.21, 2.52)	(1.07, 2.23)	(652, 892)
55	9	5.6	1.67	1.53	677
[++]		(4.7, 6.5)	(1.12, 2.37)	(1.00, 2.19)	(511, 896)
75	15	15.5	3.52	2.90	982
[BB/B+]		(13.6, 17.5)	(3.02, 4.08)	(2.50, 3.34)	(540, 1147)
75	11	15.8	4.70	3.87	805
[++]		(13.6, 18.1)	(3.39, 6.39)	(2.79, 5.25)	(676, 957)
90	11	15.8	7.05	5.25	198
[BB/B+]		(13.6, 18.1)	(5.56, 8.86)	(5.00, 7.82)	(147, 267)
90	12	14.8	5.05	3.81	123**
[++]		(13.6, 18.1)	(3.55, 7.05)	(2.68, 5.28)	(101, 149)
95	16	16.2	9.3	6.69	162
[BB/B+]		(15.0, 18.1)	(8.47, 10.29)	(6.02, 7.42)	(135, 194)
95	11	22.0*	12.36**	8.54*	237
[++]		(18.1, 27.2)	(10.62, 14.37)	(7.25, 10.02)	(173, 325)
135	17	23	9.35	4.13	68
[BB/B+]		(20, 26)	(7.92, 11.01)	(3.60, 4.72)	(48, 96)
135	13	33**	16.71**	6.41**	59
[++]		(27, 39)	(14.20, 19.64)	(5.63, 7.27)	(32, 79)

Values are geometric means (and 95% confidence limits).

Ovarian characteristics are those of the left ovary.

* $P < 0.05$; ** $P < 0.01$ Student's *t* test across genotype with respect to gestational age.

germ cell number, there were no significant correlations with litter size at any gestational age.

Effect of gestational age and Booroola genotype on the number of germ cells present as oogonia, oocytes or oocytes enclosed in primordial follicles, primary follicles and secondary/tertiary follicles

Although the changes in the germ cell population followed the same pattern in both genotypes, there was evidence of developmental retardation in the ovaries of the BB/B+ genotype relative to the ++ genotype, especially when the germ cells were measured at different developmental stages (Fig. 1). All the germ cells at day 40 gestation were present as oogonia. At day 40 (see also Table 3), a significant difference was found in the number of germ cells between the genotypes (++ > BB/B+; $P < 0.01$). In both genotypes, the rapid increase between days 40 and 55 in the number of germ cells identified as oogonia indicated that this was the period of highest mitotic activity. Oocytes were observed on day 55 in ++ but not BB/B+ ovaries indicating that meiosis of germ cells had started in the ++ but not in the BB/B+ animals. In both groups, between days 55 and 75, the increase in number of oogonia was not significant, whereas there was a marked increase (>tenfold) in the number of germ cells entering meiosis with no genotypic difference noted. However, by day 75, primordial follicles were observed in ++ but not BB/B+

ovaries. Between days 75 and 90, there was a highly significant loss of germ cells in both genotypes, although the loss was greater in ++ than in BB/B+ ovaries resulting in a significantly lower number of oogonia in ++ than in BB/B+ at day 90 ($P < 0.01$). At day 95, the decrease in the number of oogonia continued in BB/B+ but not in the ++ genotype. By day 135, all oocytes were in the diplotene stage of meiotic prophase and were present in either primordial or growing (primary or secondary/tertiary) follicles. In the ++ genotype at day 135 there were significantly fewer primordial follicles than in the BB/B+ ovaries ($P < 0.01$), but significantly more primary follicles ($P < 0.01$). Moreover, the mean diameters of the developing follicles were significantly greater in the ++ than those in the BB/B+ ovaries; the mean diameters (mm) in the ++ and BB/B+ ovaries were 0.0872 (0.0758, 0.1004) and 0.0651 (0.0595, 0.0713), respectively ($P < 0.01$; geometric means (and 95% confidence limits)). At 135 days' gestation antral follicles were more common in ++ than in BB/B+ ovaries; 12 of 13 ++, whereas only 2 of 17 BB/B+ animals contained antral follicles.

Effect of Booroola genotype on fetal characteristics in embryo-transferred ewes at day 40 or 90 of gestation

The purpose of the embryo transfer experiment at day 40 was to establish whether the effect of Booroola genotype on

Fig. 1. Number of oogonia, oocytes, primordial follicles, primary follicles and secondary or tertiary follicles in ++ (■) and BB/B+ (□) female Booroola fetuses at different days of gestation. The histograms refer to geometric means and vertical bars represent the 95% confidence limits. The numbers by the histograms refer to the number of fetuses * $P < 0.05$; ** $P < 0.01$ for BB/B+ versus ++ at each gestational age.

body weight was independent of litter size. At day 40 the geometric means (and 95% confidence limits) for litter size for the BB and ++ embryos were 2.4 (2.0, 2.8) and 2.4 (2.2, 2.6), respectively. The geometric means (and 95% confidence limits) for the body weights of the BB and ++ embryos were 3.9 (3.6, 4.2) and 4.6 (4.4, 4.9) g, respectively; these values were significantly different ($P < 0.001$).

The purpose of the experiment at day 90 was to examine the effect of genotype on a wide range of fetal characteristics including body weight and germ cell maturation. No genotype differences were noted in mean litter size, crown-rump length, body weight, or mean weight of the pituitary or adrenal (Table 4). However, differences between the genotypes were noted in

Table 4. Effect of Booroola genotype on fetal characteristics in embryo-transferred Booroola fetuses at day 90 of gestation. Values are geometric means (and 95% confidence limits)

Fetal characteristics	Booroola genotype	
	BB/B+	++
Litter size	1.9 (1.4, 2.5)	2.2 (1.7, 2.7)
Crown-rump length (cm)	22.0 (21.2, 22.8)	22.7 (22.2, 23.3)
Body weight (g)	461.2 (418.1, 508.8)	479.6 (444.3, 517.7)
Pituitary weight (mg)	36.3 (31.5, 41.2)	35.6 (30.2, 41.0)
Adrenal weight (mg)	55.8 (41.3, 70.5)	51.6 (45.9, 57.3)
Ovary weight (mg)	18.7 (14.6, 22.9)	14.3* (12.0, 16.7)
Ovarian volume (mm ³)	8.32 (7.15, 9.66)	5.98* (5.09, 6.00)
Ovarian cortex volume (mm ³)	6.22 (5.11, 7.53)	4.51* (3.77, 5.35)
Number of germ cells ($\times 10^{-3}$)	432 (304, 612)	264† (176, 397)
n	7	9

n = number of female fetuses. The mean weights of the adrenal and ovary are the average of the left and right hand organs, whereas the ovarian volume, ovarian cortex volume and numbers of germ cells refer to the left hand ovary. * $P < 0.05$, † $P = 0.056$, compared with BB/B+ (ANOVA).

Table 5. Effect of genotype on number of oogonia, oocytes and primordial follicles at day 90 of gestation in embryo-transferred Booroola fetuses

Germ cell	Booroola genotypes	
	BB/B+	++
Oogonia	192 548 (124 617, 297 509)	95 273* (54 814, 165 595)
Oocytes	223 933 (133 586, 375 382)	128 940 (76 092, 218 491)
Primordial follicles	38 334 (27 427, 53 578)	20 406 (10 447, 39 858)
Number of fetuses	7	9

Values are geometric means (and 95% confidence limits). The numbers refer to those of the left ovary.

*Significantly different from ($P < 0.05$) BB/B+ animals.

ovarian weight, ovarian volume and ovarian cortex volume. The differences between the genotypes in total number of germ cells approached significance ($P = 0.056$). The effect of Booroola genotype on the different stages of germ cell maturation at day 90 in the above transferred embryos are summarized in Table 5. Differences between the genotypes were noted

Fig. 2. Plasma concentrations of (a) i-FSH, (b) b-FSH and pituitary contents of (c) i-FSH and (d) b-FSH in ++ (■) and BB/B+ (□) female Booroola fetuses at different days of gestation. The histograms refer to geometric means and vertical bars represent the 95% confidence limits. The numbers by the histograms refer to the number of fetuses. The values above the b-FSH histograms refer to the b:i FSH ratios for the subset of fetuses in which both b-FSH and i-FSH were measured. The number of fetuses are represented by the numbers in brackets for the b-FSH data. * $P < 0.05$ for BB/B+ versus ++ at each gestational age. ND: not detected.

in the number of oögonia (BB > ++, $P < 0.05$), but not in the number of oocytes or primordial follicles.

Changes in the plasma concentrations and pituitary contents of b-FSH, i-FSH and i-LH with respect to day of gestation and genotype

In plasma, i-FSH was not measured at day 40 but at day 55 i-FSH was detectable in all animals; b-FSH was not measured at days 40 and 55 (Fig. 2). For i-FSH, the mean concentrations in both genotypes increased from day 55 to peak values at day 90 (about 3 ng ml⁻¹) and thereafter at days 95 and 135 declined to between 1.8 and about 1.4 ng ml⁻¹. No genotype effects were noted for i-FSH at any gestational age. For b-FSH compared with i-FSH, the same overall pattern was observed at days 75, 90 and 135 (b-FSH was not measured at day 95) except that the b-FSH concentrations at days 75 and 135, but not at day 90, were higher than those for i-FSH. Thus the b:i FSH ratios at days 75 and 135 varied between 1.5 and 1.9, whereas at day 90 the b:i ratio was 0.9 to

1.0 (Fig. 2). No genotype effects were noted for b-FSH at any gestational age. No effects of litter size on plasma b-FSH were noted. For i-FSH, however, there was a significant partial correlation with litter size at day 90 ($R = -0.57$; $P < 0.01$).

The pituitary contents of i-FSH and b-FSH was not detectable at days 40 or 55 of gestation. For both i-FSH and b-FSH the pituitary contents increased progressively from undetectable values at day 55 to the highest recorded values at day 135 (i.e. 1.7–10.0 μg FSH per pituitary). When the b:i ratios in pituitary content were compared in the subset of animals where both b-FSH and i-FSH were measured they matched those observed in plasma: at days 75 and 135 the b:i ratios were between 1.5 and 1.9 whereas at day 90 they were at unity. Differences in pituitary content between the genotypes were noted for i-FSH at days 75 and 135 and for b-FSH at day 75 (all $P < 0.05$; Fig. 2). No correlations were noted between litter size and pituitary b-FSH or i-FSH content at any gestational age.

The mean plasma concentrations of i-LH at day 40 were not measured but at days 55, 75 and 90 they were about

Fig. 3. Plasma concentrations of (a) i-LH and pituitary contents of (b) i-LH in ++ (■) and BB/B+ (□) female Booroola fetuses at different days of gestation. The histograms refer to geometric means and vertical bars represent the 95% confidence limits. The numbers within the histograms refer to the number of fetuses. ** $P < 0.01$ for BB/B+ versus ++ at each gestational age. ND: not detected.

Fig. 4. Plasma concentrations of inhibin in ++ (■) and BB/B+ (□) female Booroola fetuses at different days of gestation. The histograms refer to geometric means and vertical bars represent the 95% confidence limits. The dotted line represents the mean concentration in adult female sheep. The numbers within the histogram refer to the number of fetuses.

Table 6. Effect of gestational age and Booroola genotype on the ovarian and adrenal contents of oestradiol (pg per gland)

Organ	Gestational age (days)							
	55		75		90		135	
	BB/B+	++	BB/B+	++	BB/B+	++	BB/B+	++
Ovary	6.7 (3.5, 12.2) [13]	5.9 (3.2, 10.4) [10]	9.4 (5.6, 15.4) [16]	6.7 (4.3, 10.4) [13]	9.8 (6.7, 13.9) [21]	9.6 (6.2, 14.6) [18]	12.5 (9.4, 16.6) [23]	10.5 (7.2, 15.2) [19]
Adrenal	6.2 (2.8, 12.7) [13]	3.4 (1.4, 7.2) [10]	8.6 (5.6, 13.0) [15]	8.6 (5.6, 13.0) [13]	7.1 (5.2, 9.5) [22]	12.1 (7.9, 18.2) [18]	38.4 (32.4, 45.3) [24]	36.9 (27.7, 49.0) [19]

Values are geometric means (and 95% confidence limits).

The values for the ovary and adrenal refer to the right hand organ.

[n] = Number of fetuses.

		Gestational age (days)									
		40		55		75		90		95	
Organ		BB/B+	++	BB/B+	++	BB/B+	++	BB/B+	++	BB/B+	++
Ovary		≤2	≤2	2.2 (1.7, 2.7)	≤2	5.3 (2.0, 12.3)	≤2	12.9 (6.4, 25.0)	16.0	12.2 (6.4, 22.4)	9.4 (5.4, 16.3)
<i>n</i>		4	2	6	1	7	2	3	2	13	10
Mesonephros		2.0 (1.9, 2.1)	2.3 (1.7, 2.9)	2.7 (1.9, 3.6)	≤2.0	—	—	—	—	—	—
<i>n</i>		5	5	8	2	—	—	—	—	—	—
Adrenal		2.1 (1.9, 2.3)	3.9 (1.3, 9.3)	13.4 (4.8, 34.5)	17.3	34.4 (24.3, 45.8)	94.1** (63.3, 136.9)	83.6 (26.4, 261.2)	120.0 (71.5, 201.1)	76.4 (55.1, 105.7)	97.7 (71.7, 132.9)
<i>n</i>		5	5	8	2	7	5	3	3	19	11

The right ovary, right mesonephros and left adrenal gland were measured for inhibin content. The only exception to this was for this adrenal day 40 when both adrenals were pooled for assay. n = number of ovaries, mesonephros or adrenals tested. Detection limit for inhibin was 2 iu per gland.

3–4 ng ml⁻¹ (Fig. 3). At days 95 and 135 the concentrations were between 0.6 and 1.2 ng ml⁻¹. Irrespective of genotype the concentrations of i-LH at days 95 and 135 were each significantly lower than those at days 55, 75 and 90 ($P < 0.05$). No differences between the genotypes were noted at any gestational age except at day 75 when the mean concentration in the BB/B+ animals was significantly ($P < 0.01$) higher than in the ++ genotype. No effects of litter size on plasma i-LH were noted except for LH at days 90 ($R = -0.45$; $P < 0.05$) and 95 ($R = -0.60$; $P < 0.01$).

The pituitary contents of i-LH increased progressively from undetectable values at day 40 to the highest recorded values at day 135 (i.e. 2–10 µg LH per pituitary). No differences between the genotypes were noted at any gestational age. Moreover no significant effects of litter size were noted at any gestational age.

Effect of gestational age and Booroola genotype on the ovarian and adrenal content of oestradiol in female fetuses

In the ovary the mean oestradiol content was consistently low throughout gestation being two- to fourfold above the detection limit (i.e. ≤ 3 pg per gland) (Table 6). With respect to the adrenal, the oestradiol contents were also consistently low on days 55, 75 and 90 and not different from those in the ovary. However on day 135 the mean adrenal content (irrespective of genotype) was significantly higher ($P < 0.01$) than that on day 90 and also higher than that in the ovary at either days 90 or 135.

For both the ovary and adrenal no differences were noted between the genotypes at any gestational age. Moreover there was no effect of litter size on oestradiol content in either the ovary or adrenal.

Effect of gestational age and Booroola genotype on the ovarian, mesonephros and adrenal contents of inhibin in female fetuses

The inhibin contents in the gonad and mesonephros were close to the limits of detection of the assay at days 40 and 55 (Table 7). The adrenal content was low at day 40, but by day 55 the content was at least four times higher than those of the gonad and mesonephros. Thereafter the mean content of adrenal inhibin increased to between 76 and 120 iu per gland at days 90 and 95, whereas that for ovarian inhibin increased to between 9 and 16 iu per gland. The ovarian and adrenal contents of inhibin were not measured at day 135.

No differences were noted between the genotypes in the inhibin contents of the ovary or mesonephros at any gestational age, however, at most gestational ages the number of observations for the ++ genotype was insufficient for meaningful comparisons. For the adrenal gland a difference between the genotypes was noted at day 75 but not at any other gestational age. When the effect of litter size was tested against the hormone content per organ, the only difference noted was at day 75 when a significant partial correlation was found between litter size and adrenal inhibin content ($R = -0.78$, $P < 0.01$).

Effect of gestational age and Booroola genotype on the immunoreactive concentrations of plasma inhibin in female fetuses

The highest mean concentrations of inhibin in plasma were observed at day 40 of gestation (i.e. 116–118 iu ml⁻¹) (Fig. 4). Thereafter, at days 55, 75, 90 and 95, the mean concentrations declined progressively to reach values (about 20–26 iu inhibin ml⁻¹) that were approximately twofold more than those observed in adult ewes (McNatty *et al.*, 1992); at day 135 the inhibin concentrations were not different from those at day 95. No differences between the genotypes were noted at any gestational age. No effects of litter size on the inhibin concentrations were noted except at day 75 ($R = -0.58$, $P < 0.01$).

Discussion

The major findings from this study are that there are differences in litter size, body weight, and ovarian development during fetal life between female fetuses with and without the *Fec^B* gene. Moreover, there were also differences between the genotypes in the mesonephros, pituitary and adrenal. A key issue in elucidating the role of the *Fec^B* gene in fetal development is to determine whether the above differences are due to a direct effect of genotype or are a consequence of litter size or other factors such as the maternal intrauterine environment. At all gestational ages, the mean body weights of the ++ fetuses were heavier than those of the BB/B+ fetuses, however these differences were not always statistically significant. For example, no statistical differences between the BB/B+ and ++ fetuses were noted at days 55 or 95. Moreover, at days 75 and 135 the genotype differences in body weight were compromised by a significant association with litter size. From these results it could be argued that the genotype differences in body weight might be indirect and due to the effects of litter size. However, the embryo transfer experiments confirmed the genotype effect on body weight at day 40 but not at day 90. Thus, the present experiments demonstrate a real effect of genotype on body weight in early gestation (i.e. day 40) but it is uncertain whether this persists throughout pregnancy or thereafter. There is some preliminary evidence to suggest an association between the *Fec^B* gene and postnatal growth. Montgomery *et al.* (1991) compared the growth rates of embryo-transferred BB ($n = 8$) and ++ ($n = 8$) rams born as singletons and found that BB rams grew more slowly from 12 to 64 weeks of age; the mean growth rate difference was 13 g per day. In a second study G. Davis (personal communication) compared the body weights of full sibling ++ ($n = 32$) and B+ ($n = 34$) ewe lambs and noted that the mean body weight differences at birth and 12 weeks of age were 185 g and 1010 g heavier in the ++ than in the B+ animals; the difference at 12 weeks approached statistical significance ($P = 0.08$).

The most consistent effect of the *Fec^B* gene during fetal life was on ovarian development, a finding supported by the results of the embryo transfer experiment at day 90 of gestation. Collectively these data suggest that ovarian development is retarded in BB/B+ animals compared with ++ animals. For example, at day 40 during the mitotic proliferative phase more

germ cells were found in ++ than in BB/B+ ovaries; at day 55, a small number of germ cells had begun to enter meiosis in ++ but none was found in BB/B+; at day 75, primordial follicles were observed in ++ but not in BB/B+; at day 90 more germ cells had been lost in ++ than in BB/B+ and at day 95 the loss in germ cells continued in BB/B+ but not in ++; and at day 135 there were more secondary or tertiary follicles in ++ than in BB/B+ and antral follicles were more often present in the former genotype. In the embryo-transfer study at day 90 where the litter sizes were balanced, the ++ group had significantly fewer oogonia associated with significantly smaller and lighter ovaries than in the BB/B+ genotype, suggesting that developmental retardation within the ovary may be directly related to the *Fec^B* gene. However, some caution is needed with the above interpretation. For example, the weight of the mesonephros at day 40 gestation was significantly lighter in the BB/B+ than in the ++ animals. The mesonephros has a marked influence on the ovarian cellular composition and on follicle formation (Zamboni *et al.*, 1979; Byskov, 1986). Thus it is possible that the differences between the genotypes in ovarian weight, ovarian volume, ovarian cortex volume, or time of onset of germ cell or follicle development are not a direct action of the gene but an indirect one due to differences in the mesonephros induced by *Fec^B*. Alternatively, development of the ovary and mesonephros and perhaps even the pituitary might be influenced by the same (genetic) factor(s) that affect body weight.

The finding that BB/B+ fetuses have retarded ovarian development relative to non-carriers is a phenomenon that has previously been observed in comparisons between Booroola and non-Booroola controls and between high and low prolific breeds during neonatal life (Tassel *et al.*, 1983; Sonjaya and Driancourt, 1987; Braw-Tal and Gootwine, 1989). It is not known for these animals whether the mechanisms effecting the differential patterns of ovarian development are the same or different from those in animals that differ mainly with respect to the Booroola gene.

In rats it has been shown that male neighbours may alter sexual development of female fetuses (Vom Saal *et al.*, 1981). In prolific breeds of sheep, it is possible that the presence or absence of a male fetus might influence body weight or ovarian development in adjacent females. However no effects of male fetuses were noted on either body weight or ovarian characteristics in the females in early to mid-gestation (data not shown). Thus the genotypic differences in body weight or mesonephric or germ cell development are unlikely to be attributable to variability in the sex of littermates.

These studies show that genotypic differences in body weight and in development of germ cells and mesonephros precede any of those observed for hormones in peripheral plasma or in the pituitary or adrenal; no differences in oestradiol or i-inhibin content were noted in the ovary or mesonephros. No differences between the genotypes were noted in plasma b-FSH or i-FSH concentrations at any stage of fetal life, whereas after birth and in adult females consistently higher i-FSH concentrations are found in BB/B+ animals compared with ++ animals (see McNatty *et al.*, 1991 for review): no data for b-FSH in adult animals have been reported. The plasma b-FSH results for female fetuses were similar to those described by Phillips *et al.* (1992). For plasma i-LH, there was a significant difference

between the genotypes at day 75 (BB/B+ > ++), but not at any other age; the lower concentrations of plasma i-LH in the ++ were independent of litter size and occurred at the time coincident with the formation of the first primordial follicles in the ++ but not in the BB/B+ genotype. It is not known whether any types of ovarian cell have LH receptors at this time or whether any importance can be attached to this finding. Differences between the genotypes were noted in the pituitary contents of i-LH at day 135 (++ > BB/B+), i-FSH and b-FSH at days 75 and 135 i-FSH at (++ > BB/B+); it is unlikely that these differences were due to the effects of litter size. The changing b:i FSH ratios in both plasma and pituitary at days 75, 90 and 135 are consistent with the hypothesis that there are changes in the forms of FSH throughout gestation in the ovine fetus. The lack of any genotypic difference in b:i FSH ratios suggests that different forms of FSH are unlikely to be the cause of any putative *Fec^B* gene effect on gonadal development. For i-inhibin the only difference between the genotypes was noted in the adrenal at day 75 and this difference was also associated with a litter size effect. It has been suggested that inhibin might be associated with the *Fec^B* gene in neonatal or sexually mature female sheep (see McNatty *et al.*, 1991 for review). The present studies report no *Fec^B* differences at any stage from day 40 of gestation in the plasma concentrations of i-inhibin. Moreover, results in BB and ++ Booroola ewes show no *Fec^B* differences in the production of b-inhibin by granulosa cells *in vitro* (Henderson *et al.*, 1991) or in ovarian i-inhibin secretion rates or i-inhibin concentrations in peripheral plasma using the same radioimmunoassay methodology described here (McNatty *et al.*, 1992). As the α subunit of inhibin has been excluded from genetic linkage to the *Fec^B* gene (G. W. Montgomery, unpublished data) and since no gene differences in the mRNA for the α inhibin subunit have been noted (Fleming *et al.*, 1992), it seems unlikely that inhibin is responsible for the developmental differences noted in Booroola fetuses, despite the extraordinarily high concentrations at days 40 and 55 of fetal life. Although it is reasonable to exclude inhibin as a hormone influencing growth in Booroola sheep, our results do not exclude a possible role for activin. It is worth noting that Fleming *et al.* (1992) reported a higher total amount of β_A inhibin mRNA from follicular extracts of BB than from ++ ovaries. Moreover, since there is evidence of widespread gene expression for activin during early fetal development and a role for activin in ovarian and pituitary function (Ling *et al.*, 1986; Bilezikjian *et al.*, 1990; Woodruff *et al.*, 1990; Xiao *et al.*, 1990; Roberts *et al.*, 1991), it is possible that differing amounts/forms of this hormone are produced in Booroola fetuses.

Although this study has focused principally on testing for differences in Booroola sheep in fetal life, it also provides new information on ovarian function and hormone concentrations/contents during fetal development. The present studies on ovarian development enlarge and expand upon those of Mauleon (1961, 1969). The pattern of oogenesis conforms to that reported in rats (Beaumont and Mandl, 1962), humans (Baker, 1963), guinea-pigs (Ioannou, 1964), rhesus monkeys (Baker, 1966), mice (Burgoyne and Baker, 1985) and buffaloes (El-Ghannam and El-Naggar, 1974). Our results show that the increase in the germ cell population is due to proliferation of oogonia followed by a dramatic decrease due largely to atresia during meiotic prophase. The present results also confirm those

of Mauleon (1969) who reported that mitosis of oogonia continues long after the initiation of meiosis. The present findings show that during the period when massive germ cell atresia occurs (i.e. between days 75 and 95) there is no increase in ovarian weight, whereas in other organs growth continues unabated.

With regard to hormones and fetal development, the present results support the notion that the fetal ovary is steroidogenically inactive throughout gestation (Rabinovici and Jaffe, 1990). The lack of increase in oestradiol content in the ovary is consistent with the absence of large antral follicles which are the major source of this steroid in sheep (McNatty, 1981). It was of interest to note the high plasma concentrations of i-inhibin at 40 and 55 days' gestation and the low or undetectable contents of this hormone in the ovary, mesonephros and adrenal at this time and the very high adrenal contents of i-inhibin at days 75 to 95 relative to those in the ovary. Since the mesonephros up to day 55 contains pregranulosa cells and the ovary up to day 95 contains pregranulosa cells or granulosa cells in primordial follicles with few, if any, developing follicles, the present findings are consistent with the notion that ovarian inhibin output increases with increasing follicular size (Findlay *et al.*, 1990; McNatty *et al.*, 1992). It seems reasonable to suggest that from days 55 to 95 the adrenal is a source of at least some of the circulating inhibin. However, it is likely that tissue(s) other than the ovary, mesonephros and adrenal contribute to the plasma concentrations at days 40 to 55. It is not known whether the i-inhibin is biologically active inhibin or inactive forms of the α subunit. The high concentrations of i-inhibin at days 40 and 55 and the low or undetectable concentrations/contents of pituitary FSH at days 40 and 55 could mean that either pituitary FSH synthesis is almost completely suppressed or that the pituitary is not yet capable of FSH synthesis. At day 55 the latter seems unlikely since the plasma concentrations of i-LH are similar to those in ovariectomized ewes.

A comparison of body weights, crown-rump lengths and organ weights or volumes between the embryo-transferred embryos at day 90 and the embryos derived from the control animals at day 90 reveals that these are similar. However the numbers of germ cells in the BB/B+ and ++ genotypes suggest that the germ cells in the embryo-transferred embryos have not undergone the same degree of atresia as those in the controls. The difference between the two treatment groups is small (i.e. <1 mitotic division); however, the data suggest that the embryo-transferred embryos were slightly retarded compared with the controls.

In summary, the results from this study suggest that the Fec^B gene influences litter size, body weight and organ weight during fetal development. The results also suggest that germ cells and follicle formation develop later in BB/B+ fetal ovaries than in the controls. We hypothesize that many of the Fec^B differences in follicular development and pituitary function in neonatal and adult life may be a consequence of differences in the timing or rate of body weight or organ development in fetal life.

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