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Development of the sheep ovary during fetal and early neonatal life and the effect of fecundity genes

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In female sheep fetuses, the mesonephros and genital ridge can be identified at days 20 and 23 of gestation (term = 145 days), respectively. Moreover oogonia can be observed at the genital ridge from as early as day 23. Around day 55 of gestation, some germ cells enter meiosis coincident with the arrival of mesonephric-derived somatic cells (i.e. the rete ovarii). From days 75, 100, 120 and 135 of gestation, primordial (one layer of flattened granulosa cells), primary (one complete layer of cuboidal granulosa cells; early preantral), secondary (preantral) and tertiary (antral) follicles, respectively, develop within the innermost regions of the ovarian cortex. During the early neonatal period highly variable numbers of antral follicles may be present. After examination of Booroola fetuses from day 28 of gestation, it seems that the $FecB^B$ gene is associated with retarded development of the heart (day 28) mesonephros (days 30–40) and from day 30 to early neonatal life, the ovary. With respect to the ovary, fewer oogonia (days 30–40), primordial follicles (day 75–90) and growing follicles (day 120 to 6 weeks after birth) have been observed in females carrying the $FecB^B$ gene. By contrast, the $FecB^B$ gene is not associated with differences in plasma gonadotrophin or immunoreactive inhibin until early neonatal life. In Inverdale (I) fetuses heterozygous for the $FecX^I$ gene (I+), retarded germ cell development was observed at days 40 and 90 of gestation. In putative homozygous carriers (II) of the Inverdale gene, germ cell development appeared normal until day 100, but thereafter from day 120 normal secondary follicles were not observed, although many abnormal follicular-like structures were present. In both I+ and II fetuses no obvious differences in gonadotrophin concentrations have been noted. Collectively, the evidence suggests that the fecundity genes $FecB^B$ and $FecX^I$, which affect ovulation rate in sexually mature females, are regulating organ differentiation or germ cell maturation or both processes during fetal life.

Introduction

Most breeds of domestic sheep have one or two lambs at each lambing; however, there are some breeds, including the Booroola Merino, Cambridge, D'Man, Finnish Landrace and Romanov, that consistently have litters of three or more (Bindon and Piper, 1986). Breeding experiments with high fecundity sheep have led to the discovery of two major genes (or mutations). The first of these major genes (the $FecB^B$ gene) was identified in Booroola Merinos (Piper and Bindon, 1982; Davis *et al.*, 1982). Booroola Merino

or Booroola Merino × Romney ewes which are homozygotes (BB), heterozygotes (B+) or non-carriers (++) of the $FecB^B$ gene have ovulation rates of ≥ 5, 3–4 or 1–2, respectively. The second major gene ($FecX^I$) was discovered in a strain of Romney ewes now referred to as Inverdales (Davis *et al.*, 1991, 1992). Heterozygotes (I+) of the $FecX^I$ gene have ovulation rates which average one or more than their non-carrier (++) controls, whereas homozygotes (II) have streak gonads and are sterile. With regard to Booroola sheep, two microsatellite markers have been reported linking the $FecB^B$ gene to human chromosome 4q and thus tentatively to sheep chromosome 6 (Montgomery *et al.*, 1993). In the case of Inverdales, extensive breeding experiments involving six male offspring of a known carrier ram and five of his maternal grandsons and an unrelated Romney control ram led to the finding that the $FecX^I$ gene was carried on the X chromosome (Davis *et al.*, 1991, 1992). At the time of preparing this paper no candidate gene for either Booroola or Inverdale has been reported and the mechanisms of action of these genes remains unclear.

The physiological effects of the $FecB^B$ and $FecX^I$ genes on hypothalamic, pituitary and ovarian function are the subject of extensive investigation and many of the results have been summarized elsewhere (see McNatty *et al.*, 1991a; Montgomery *et al.*, 1992). More recently, however, it has become evident that both the $FecB^B$ and $FecX^I$ genes influence gonadal development during fetal and early neonatal life. Thus it is possible that these so-called fecundity (i.e. Fec) genes are developmentally regulating organ differentiation or germ cell maturation or both processes (Smith *et al.*, 1993a, b, 1994). If this is correct, it might be that the influences of the Fec genes ($FecB^B$/$FecX^I$) on ovulation rates are a downstream consequence of developmental events that take place in fetal life.

The purpose of this paper is to review some aspects of ovarian development in sheep during fetal and early neonatal life and then to examine what is known about the effects of the $FecB^B$ and $FecX^I$ genes on these processes.

Gonadal Development in the Female Fetus

Some key events in gonadal development are summarized in Figs 1 and 2. The location and migration of primordial germ cells to the genital ridge from extragonadal sites with respect to day after mating has not to our knowledge been determined in sheep. However, germ cells can be identified in the genital ridge at day 23 after mating (Figs 1 and 2; see also Mauleon, 1978, for review). At day 30, streams of cells can be observed in the region between the mesonephric tissue proper and the gonads (Fig. 2). Our interpretation is that these cell streams are the rete ovarii or testis cells. Granulosa or Sertoli cell lineages are thought to be derived from either the upward migrating mesonephric-derived rete cells or from the surface epithelium of the gonad proliferating downwards or both (see Byskov and Hoyer, 1994 for review). In the ovary of the sheep fetus the first germ cells that begin meiosis are those localized to the innermost part of the cortex and coincident with the time of arrival of the rete ovarii (Figs 1 and 2). This interpretation is consistent with the view that the rete ovarii are essential for the initiation of germ cell meiosis (Byskov, 1974). We have not observed any involvement of the ovarian surface epithelial cells proliferating downwards into the innermost parts of the ovarian cortex. There is ample evidence from day 23 to day 120 for germ cell mitosis and from day 55 to day 120 for germ cell meiosis (Fig. 2; Mauleon, 1978; Smith *et al.*, 1993a). By day 75 of gestation, the first primordial follicles have formed in the innermost regions of the ovarian cortex, while germ cells in the immediately adjacent region are entering meiosis and those in the outermost regions of the cortex are undergoing mitosis. From day 75 to day 90 of gestation when follicles are forming, there is a concomitant massive loss (about 80%) of germ cells with an associated arrest or reduction in ovarian mass: no follicles beyond the primordial stage of development have been noted at this time. By day 100, the sheep ovary contains about 170 000 germ cells compared with the 900 000 present at day 75. Some of these germ cells are still present as oogonia (9%), but most are present as isolated oocytes (61%) or as oocytes enclosed in primordial follicles (28%). At day 100 about 1% of the germ cells are present in primary follicles (i.e. with one cuboidal layer of granulosa cells). By day 120, 51% of the germ cells (total population of 205 000 cells) is present in primordial follicles and 1% is present in growing follicles with up to three concentric layers of granulosa cells; the remaining germ cells are present mostly as isolated

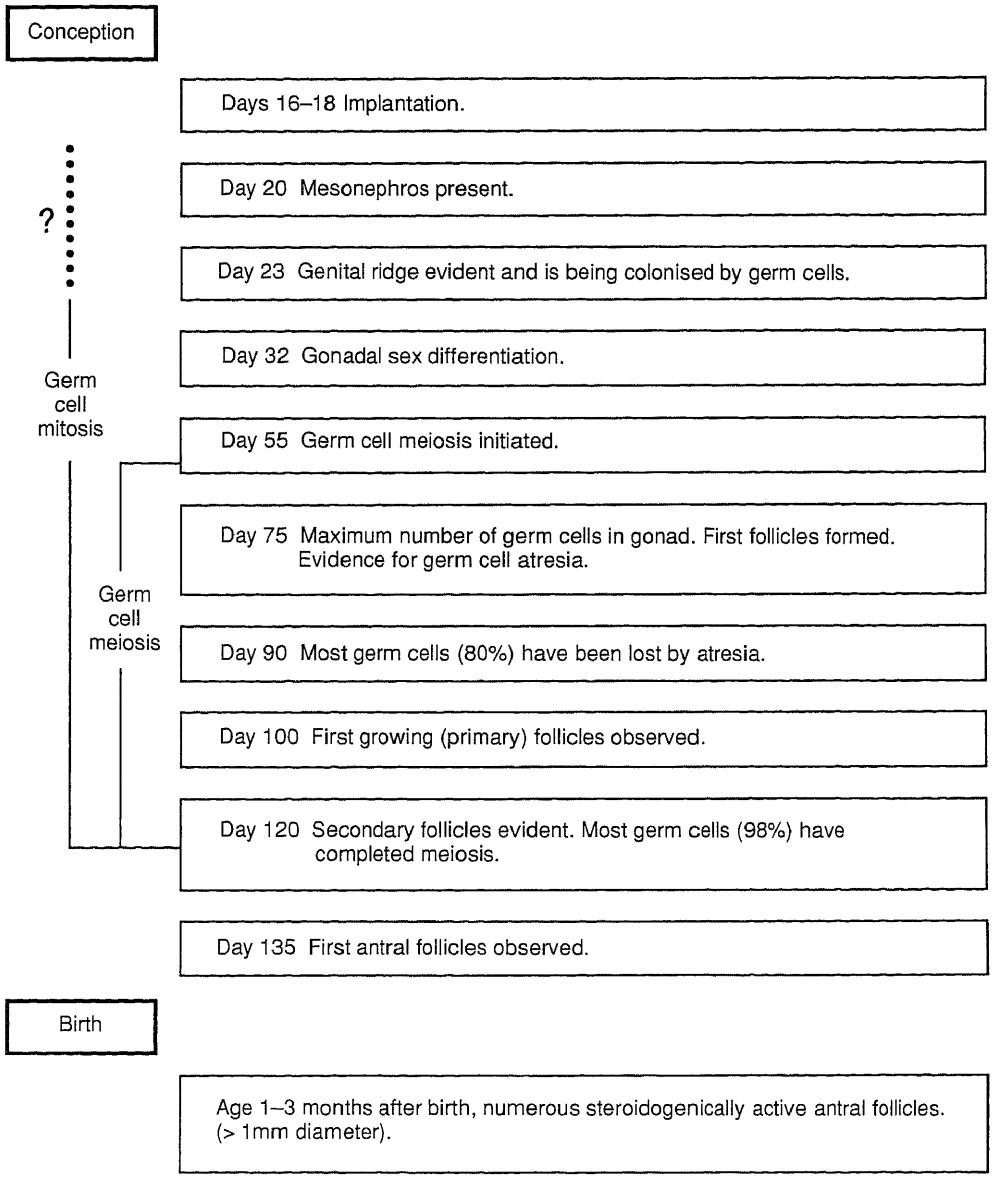

Fig. 1. Sequence of developmental events in the female sheep fetus and early neonate associated with gonadal formation.

oocytes (48%). By day 135, 91% of the germ cells ($n = 82\,000$) are in primordial follicles, 4% in growing follicles (i.e. up to early antral structures; 0.25–0.80 mm in diameter) and most of the remainder present as oocytes (5%). The medullary region of the ovary during days 100 to 135 is characterized by connective tissue, blood vessels, lymph and a prominent rete system (see Smith *et al.*, 1994).

In newborn ewe lambs the ovaries are very active both structurally and functionally and many preantral and antral follicles are present (Kennedy *et al.*, 1974; McNatty *et al.*, 1987; Sonjaya and Driancourt, 1987; Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993a; Braw-Tal, 1994). At 1–3 months after birth, ovarian mass increases markedly from about 30 mg at about 15 days before birth (i.e. at day

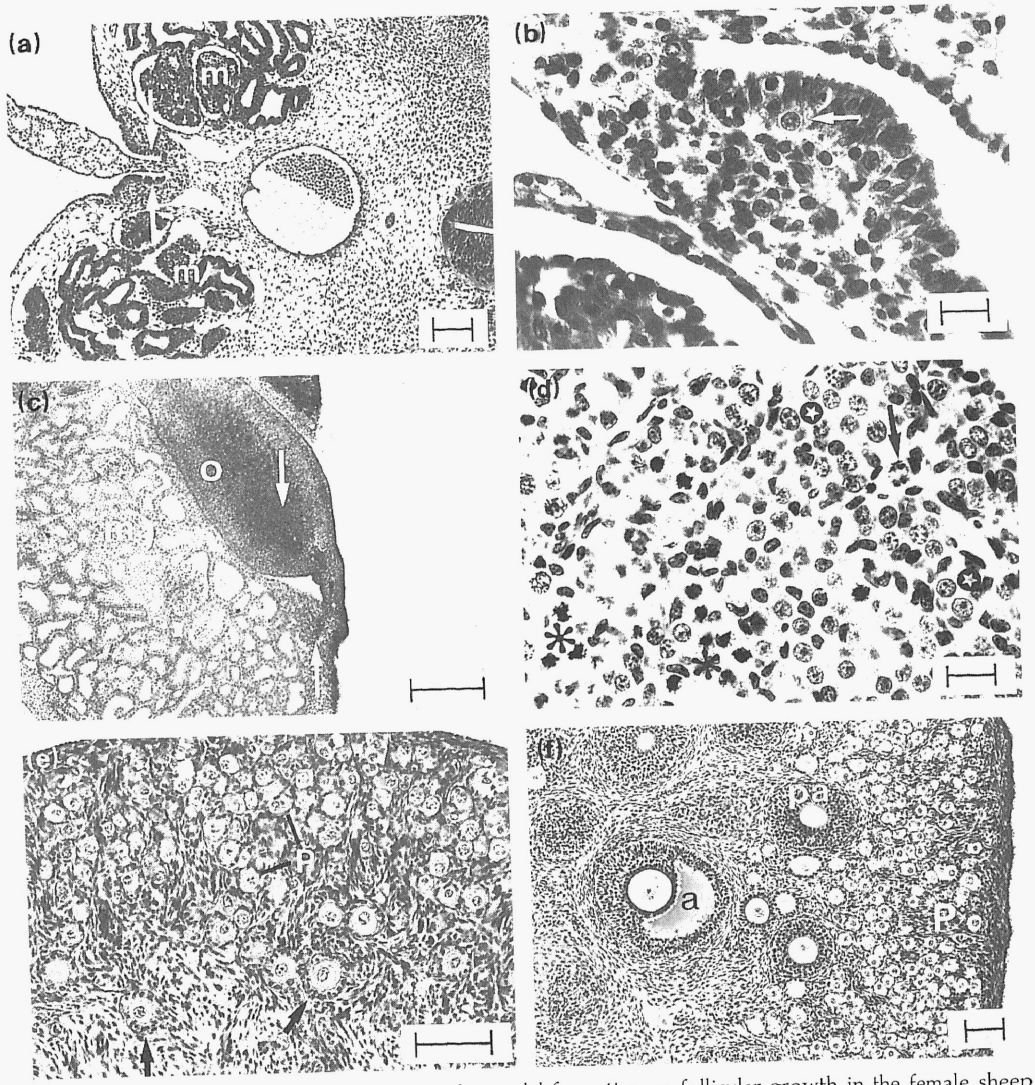

Fig. 2. Photomicrographs showing aspects of gonadal formation or follicular growth in the female sheep fetus. (a) The mesonephros (m) and gonadal ridge (arrows) at day 23 of gestation. Scale bar represents 100 μm. (b) A germ cell (arrow) located in the gonadal ridge at day 23 of gestation. Scale bar represents 20 μm. (c) Mesonephros (m) and ovary (o) at day 30 of gestation indicating cell streams (arrow) entering the gonad. Scale bar represents 500 μm. (d) Groups of oogonia (stars), meiotic germ cells (*) and a mitotic germ cell (arrow) at day 75 of gestation. Scale bar represents 20 μm. (e) Primordial (P) and early growing primary follicles (arrows) at day 100 of gestation. Scale bar represents 100 μm. (f) Primordial (P), large preantral (pa) and small antral (a) follicles at day 135 of gestation. Scale bar represents 100 μm.

135 of gestation) to about 300 mg; variable numbers of antral follicles (3–336) may be present with the largest at 3 months reaching preovulatory size (about 5 mm in diameter; McNatty *et al.*, 1987; Braw-Tal and Gootwine, 1989). Genetic background is known to affect ovarian development in newborn lambs; prolific breeds have smaller ovaries and fewer antral follicles than do non-prolific breeds early in life (Tassell *et al.*, 1983; Sonjaya and Driancourt, 1987; Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993a). This difference in numbers of antral follicles present between prolific and non-prolific breeds appears to decline with age (Sonjaya and Driancourt, 1989).

Table 1. Plasma concentrations of immunoreactive inhibin, FSH and LH in female fetuses and neonates, in sheep

Day of gestation or weeks after birth	Immunoreactive inhibin (i.u. ml^{-1})	FSH (ng ml^{-1})	LH (ng ml^{-1})	*n*
40 days	125^{a} (81, 192)	ND	ND	6
55 days	88^{a} (67, 116)	0.6^{a} (0.4, 0.8)	2.6^{a} (1.8, 3.8)	8
75 days	53^{b} (46, 59)	$1.3^{b,d}$ (0.9, 1.8)	2.9^{a} (2.4, 3.4)	11
90 days	$26^{c,d}$ (20, 33)	$2.5^{c,e}$ (1.8, 3.2)	$2.7^{a,b}$ (1.8, 3.8)	17
100 days	17^{c} (13, 23)	$2.5^{c,e}$ (1.9, 3.2)	$2.4^{a,b}$ (0.7, 5.8)	9
120 days	$21^{c,d}$ (10, 43)	$1.2^{a,b,d}$ (0.7, 1.9)	$1.1^{b,c}$ (0.4, 2.1)	6
135 days	20^{c} (15, 25)	$1.0^{a,b}$ (0.7, 1.4)	0.5^{c} (0.4, 0.7)	19
4 weeks	30^{d} (22, 40)	$1.8^{c,d}$ (1.3, 2.5)	0.5^{c} (0.2, 0.8)	25
12 weeks	8^{e} (6, 10)	4.3^{e} (2.6, 6.1)	0.6^{c} (0.3, 1.0)	20

Values are geometric means (and 95% confidence limits). For each column, numbers with different superscripts are significantly different from one another. Data from Smith *et al.* (1993a) and Isaacs *et al.* (1995). *n*: number of fetuses or neonates.

Inhibin and gonadotrophins

With respect to immunoreactive inhibin, the plasma concentrations in females decline from early to mid-gestation and thereafter during gestation remain at approximately twice the concentration observed in adults (Table 1). It is likely throughout most of gestation that the ovarian contribution of immunoreactive inhibin to the peripheral plasma concentration is very low, as there is little or no evidence for α and β_A inhibin mRNA from *in situ* hybridization studies or α and β_A inhibin peptide from immunocytochemical studies (Braw-Tal *et al.*, 1994, authors' unpublished data) or immunoreactive inhibin activity as assessed by radioimmunoassay of extracts from homogenized ovaries (Smith *et al.*, 1993a). Four weeks after birth, the plasma immunoreactive inhibin concentrations in females are increased once more compared with the concentrations during late gestation (Table 1; Braw-Tal *et al.*, 1993a). However by 12 weeks after birth, the immunoreactive inhibin concentrations have declined to values that are observed in adults. Evidence that the neonatal ovary synthesizes increased concentrations of inhibin relative to the fetal ovary has been confirmed by the presence of mRNA for both α and β_A inhibin (Braw-Tal, in press; Braw-Tal *et al.*, 1994) in granulosa cells of antral follicles and the presence of both peptide subunits by immunocytochemistry (R. Braw-Tal and D. A. Heath, unpublished).

For FSH, plasma concentrations are first detectable at 55 days of gestation (Smith *et al.*, 1993a) about 12 days after the first cells in the preoptic–hypothalamic area are stained positively with a specific antibody to gonadotrophin-releasing hormone (Caldani and Batailler, 1991). Peak FSH concentrations were observed at days 90 and 100 of gestation and again at 4 and 12 weeks of postnatal life (Braw-Tal and Gootwine, 1989; Smith *et al.*, 1993a; Braw-Tal *et al.*, 1993a; authors' unpublished data). Although LH

Table 2. FecB^B gene-specific differences in female fetuses with respect to organ and gestational age in sheep

Organ	Gestational age (day) 28 (N_1/N_2)	30 (N_1/N_2)	35 (N_1/N_2)	40 (N_1/N_2)
Heart mass (g)	** (5/6)	NSD (7/9)	—	—
Mesonephros volume (mm^3)	NSD (3/3)	* (6/8)	** (7/10)	** (8/9)
Gonad volume (mm^3)	NSD (3/6)	NSD (6/8)	NSD (7/10)	NSD (8/9)
Liver mass (g)	NSD (4/6)	NSD (7/9)	—	—
Body mass (g)	NSD (5/6)	NSD (8/10)	* (8/10)	* (8/9)

Differences between the homozygous carriers (BB) and non-carriers (++) are indicated by an asterisk: *$P < 0.05$; **$P < 0.01$; NSD: not significantly different (analysis of variance), —: no data. In all cases where significant differences were noted the BB genotype was significantly lighter in mass or significantly smaller in volume relative to the ++ genotype.
N_1: number of BB fetuses; N_2: number of ++ fetuses.

could not be detected in plasma at day 40, high concentrations were noted from day 55 to day 100. Thereafter, at day 120 of gestation there was a decline in LH concentrations to low values by day 135. During early postnatal life mean LH concentrations were low. In the fetus, the peak concentrations of gonadotrophin at days 90–100 corresponded to the time when some follicles leave the primordial pool and develop into primary structures.

With respect to other hormones, the oestradiol content of the fetal ovary remains very low from day 55 until the end of gestation: the mean values are ≤ 12.5 pg per ovary. At day 135 of gestation, the mean ovarian content of oestradiol was about three times lower than that in the fetal adrenal. In the neonatal ovary at 12 weeks of life, granulosa cells in antral follicles have measurable aromatase activity and the follicular fluid concentrations of oestradiol are similar to those in adult ovaries (McNatty *et al.*, 1986, 1987). Of interest is the finding that cultures of sheep ovaries *in vitro*, but not of testis, at days 31 and 47 of gestation lead to the release of measurable concentrations of oestradiol in the media, suggesting that the ovary may actively produce oestradiol at about the time of sexual differentiation (Mauleon, 1978). The types of cell that produce this oestradiol are not known.

Effect of Booroola Genotype on Fetal Characteristics

Organ development from day 28 to day 40 of fetal life

Mass or volume measurements have been made on the developing heart, mesonephros, gonad, liver and on entire fetuses of the BB and ++ genotypes. The effects of litter size were eliminated by transfering equal numbers of BB and ++ embryos (8–16 cell embryos) to recipient ewes (3 embryos per recipient) and fetuses were recovered and karyotyped at the equivalent of days 28, 30, 35 and 40 of gestation: no effect between the genotypes on litter size or on the male:female ratios were noted in these studies. No significant effects of the FecB^B gene were noted on gonad volume, or liver mass (Table 2). The first significant gene effect was noted for the mass of the heart in female fetuses at day 28. The geometric mean (and 95% confidence limit) masses of the hearts in BB and ++ genotypes in females were 7.1 (4.8, 9.4) and 15.6 (11.4, 19.8) mg, respectively (BB < ++; $P < 0.01$; Student's t test). At day 30 of gestation, the difference between genotypes in mean mass of the heart was no longer apparent.

Table 3. Volumes of the mesonephros in developing female fetuses with respect to Booroola genotype and gestational age

Genotype	Gestational age (days)			
	28	30	35	40
BB	4.0 (1.6, 8.6) [3]	4.8 (4.2, 5.6)* [6]	7.5 (6.7, 8.4)** [7]	10.2 (7.6, 13.7)** [8]
++	6.8 (4.8, 9.3) [3]	6.2 (5.3, 7.2) [8]	9.5 (8.9, 10.8) [10]	15.9 (13.7, 18.6) [9]

Values are geometric means (and 95% confidence limits) in mm^3.
*$P < 0.05$; **$P < 0.01$ compared with ++ at each gestational age (Student's t test); [n]: number of fetuses.

However, differences in genotype were observed in the volumes of the mesonephros in females at days 30, 35 and 40 (Table 3). In previous studies the mesonephros has also been shown to be lighter in BB than in ++ ewes at day 40 (Smith *et al.*, 1993a). At days 35 and 40 in females, gene-specific differences were observed in body mass.

In summary, the most recent findings are that the Booroola Fec^B gene in the female appears to affect the development of the heart and subsequently the mesonephros. Later, at day 35 the Booroola $FecB^B$ gene affects body mass. However, by day 55 of gestation there are no differences between the genotypes with respect to the mesonephros and body mass and the major effects thereafter appear to be within the gonad (Smith *et al.*, 1993a, 1994; authors' unpublished data).

Ovarian development from day 30 to early neonatal life

In female fetuses, the most persistent effect of the $FecB^B$ gene appears to be on germ cell and follicle development (Fig. 3). The ++ genotype has more oogonia at days 30, 35 and 40, more germ cells entering meiosis at day 55, more primordial follicles developing at day 75 (and 90), a greater loss of germ cells by atresia at day 90, fewer primordial follicles at days 100 and 135 and more primary and secondary plus tertiary follicles at day 135 (Smith *et al.*, 1993a, 1994). In essence, it seems that, independent of litter size, the $FecB^B$ gene leads to a retardation in the timing of germ cell maturation and follicle development. It is likely that the retardation begins at or before day 30 of gestation when the germ cells are present as oogonia. The $FecB^B$ gene continues to affect follicular development during both prepubertal and adult life (McNatty *et al.*, 1986, 1987; Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993a; Isaacs *et al.*, 1995). For example, in ewe lambs at 1–1.5 months of age, the number of tertiary (antral) follicles ≥ 0.2 or > 1.0 mm diameter in the ++ genotype continues to exceed that in B+ animals, whereas at 3 months of age the number of antral follicles (> 1.0 mm diameter) in B+ exceeds that in the ++ genotype (Table 4). After animals have reached sexual maturity (i.e. > 9 months of age) no further differences between the genotypes in the total number of antral follicles have been noted even after chronic hypophysectomy (Table 4). However, during neonatal and adult life $FecB^B$ differences persist in the composition of granulosa cells and also in the diameters of the largest antral follicles (McNatty *et al.*, 1987).

At 1–1.5 months of age, the diameters of the largest antral follicles in BB/B+ or ++ genotypes were similar (about 1.7 mm diameter). However, by 3 months of age the largest in BB/B+ animals were ≤ 4.5 mm in diameter, whereas in ++ animals they exceeded 5 mm in diameter. By 3 months of age, the granulosa cells in 3–4.5 mm diameter follicles from the BB/B+ genotype had acquired functional LH receptors and peak aromatase activity, while those in ++ animals did not acquire these characteristics until follicles had reached > 5.0 mm diameter (McNatty *et al.*, 1987). In BB/B+ ewes it seems that both before and after puberty the number of granulosa cells for a given follicular diameter (> 1.0 mm) have fewer granulosa cells than the number present in ++ ewes. These data suggest that in BB/B+ ewes,

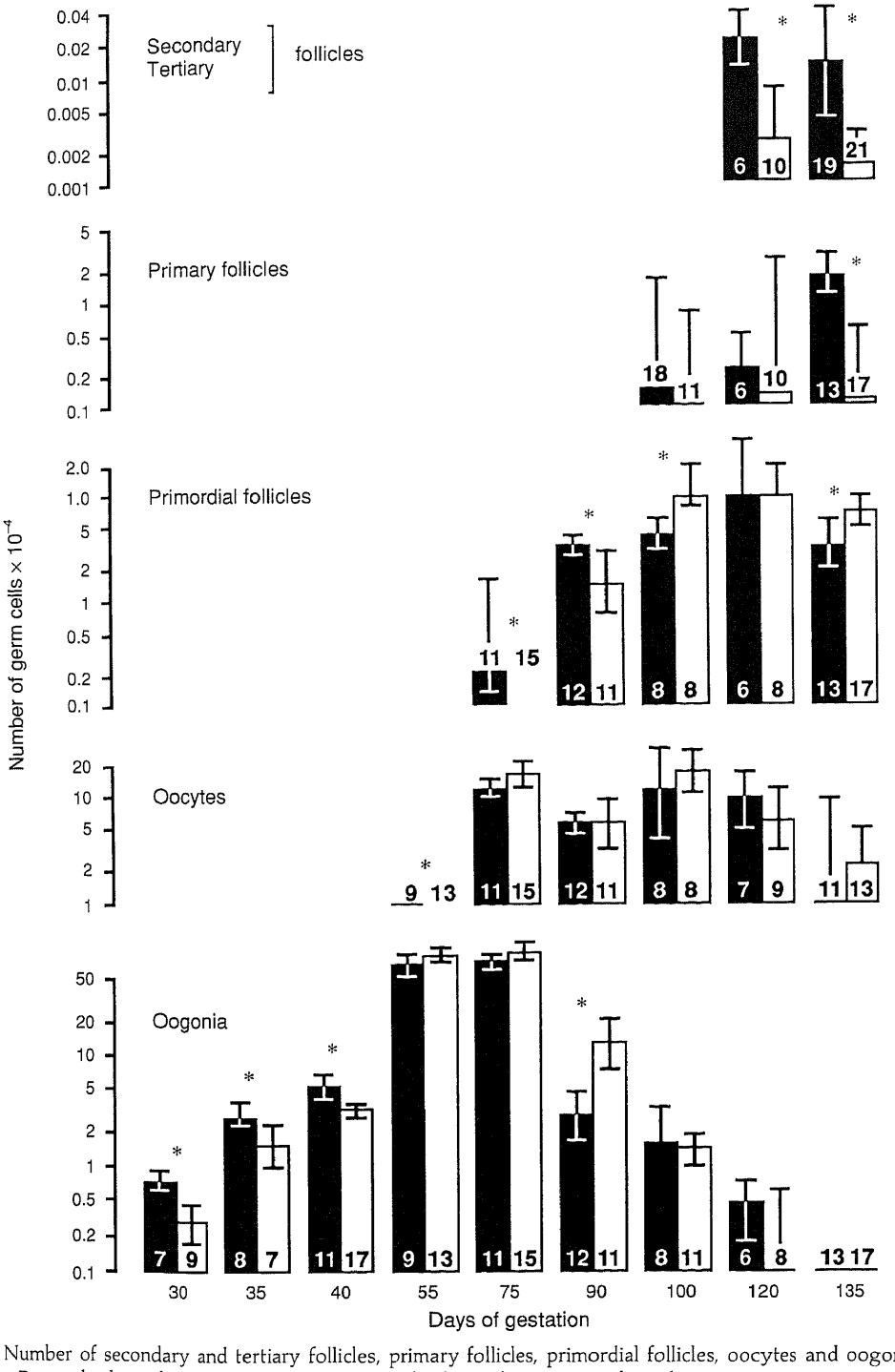

Fig. 3. Number of secondary and tertiary follicles, primary follicles, primordial follicles, oocytes and oogonia per ovary in Booroola sheep fetuses with respect to specific days of gestation. The values given are geometric means plotted on a log scale and the vertical bars are the 95% confidence limits. Tertiary and secondary follicles, respectively, refer to antral and preantral follicles with more than one layer of cuboidal granulosa cells. Primary follicles refer to follicles with one complete layer of cuboidal granulosa cells, whereas primordial follicles are those with a flattened layer of pregranulosa cells. (■): Numbers in homozygous non-carriers of the $FecB^B$ gene; (□): numbers in homozygous carriers of the $FecB^B$ gene. The numbers associated with each histogram refer to the number of fetuses; * indicates significant differences between the genotypes. Data redrawn from Smith *et al.* (1993a, 1994).

Table 4. Number of antral follicles with respect to age, hypophysectomy (HPX) and Booroola genotype

Booroola genotype	Age			
	1–1.5 months[a]	3 months[b]	5–6 years[c]	HPX 5–6 years[c]
BB or B+	1 (0, 13)	29 (24, 35)	87 (53, 140)	68 (49, 94)
++	49 (6, 367)*	18 (14, 22)*	84 (57, 124)	74 (59, 92)

Values are geometric means (and 95% confidence limits). *Significantly different from BB or B+. Data from [a]Braw-Tal and Gootwine (1989): follicles ≥0.2 mm in diameter; [b]McNatty *et al.* (1987): follicles > 1.0 mm in diameter; [c]McNatty *et al.* (1990): follicles > 0.1 mm in diameter.

granulosa cells reach a similar overall stage of differentiation to those in ++ ewes, but with one less cell division (McNatty *et al.*, 1986).

Gonadotrophin and inhibin concentrations during fetal and neonatal life

In female fetuses, no consistent differences between BB/B+ and ++ fetuses have been observed for the plasma concentrations of FSH, LH or immunoreactive inhibin from day 40 through to day 135 of gestation (Smith *et al.*, 1993a, 1994). The concentrations of these hormones are similar to those described in Table 1. However, during early neonatal life $FecB^B$ differences have been found with respect to FSH and immunoreactive inhibin but not to LH in ewe-lambs (Braw-Tal and Gootwine, 1989; Montgomery *et al.*, 1989; Braw-Tal *et al.*, 1993a; Isaacs *et al.*, 1995). In four separate studies a consistent feature has been that the plasma FSH concentrations are significantly higher in BB or B+ than in ++ ewe lambs at 4–6 weeks of age. Thereafter, from 12 to 28 weeks of age, the plasma FSH concentrations were higher in the ++ than in the BB or B+ genotypes (Braw-Tal and Gootwine, 1989; Braw-Tal *et al.*, 1993a; Isaacs *et al.*, 1995). After puberty, BB/B+ ewes have consistently higher concentrations of FSH during both the breeding season and anoestrus compared with ++ controls (McNatty *et al.*, 1992).

In contrast to FSH, plasma concentrations of immunoreactive inhibin were significantly lower in BB or B+ than in ++ ewe-lambs at 4 weeks of age. The differences between the genotypes persisted until about 9–10 weeks after birth, but thereafter for the remainder of the prepubertal period and adulthood no genotype differences were observed (McNatty *et al.*, 1992; Braw-Tal *et al.*, 1993a; Isaacs *et al.*, 1995).

It is possible that the differences in FSH and immunoreactive inhibin between BB and ++ in ewe lambs are a consequence of the $FecB^B$ differences in ovarian follicular development. During late gestation and during the first few days after birth, most of the immunoreactive inhibin in peripheral plasma is probably of extraovarian origin (R. Braw-Tal, unpublished). It is hypothesized that after birth, the secretion of immunoreactive inhibin from extraovarian sources declines, but that the rate at which the concentrations fall may be offset by the extent of ovarian follicular development and thus ovarian inhibin synthesis. As follicular development is more advanced in non-carriers than in carriers of the $FecB^B$ gene (Braw-Tal *et al.*, 1993a), it might be that the decline in plasma inhibin is slower in ++ than in BB ewe lambs thereby accounting for the higher FSH concentrations in the BB genotype during the first few weeks after birth (Braw-Tal *et al.*, 1993a; Isaacs *et al.*, 1995). In turn, the higher FSH concentrations in BB/B+ ewes in the first weeks may account for the subsequently higher follicular activity at 12 weeks of age (Table 4). The potential effects of litter size and sire cannot be entirely excluded from postnatal effects of the $FecB^B$ gene on plasma gonadotrophin and inhibin concentrations. However, when newborn animals are matched for litter size, the genotypic differences in the plasma concentrations of inhibin in early neonatal life and plasma FSH in both neonatal and adult life still persist; this suggests that litter size *per se* cannot account for all the differences observed. The underlying reasons why animals with the $FecB^B$ gene have a different pituitary FSH response to gonadotrophin-releasing hormone (Braw-Tal and Gootwine, 1989; McNatty *et al.*, 1991b, 1992) but not a different growth hormone or

Table 5. $FecX^I$ gene-specific differences with respect to germ cell characteristics with respect to genotype and gestational age

	Gestational age (days)					
	40		90			
Genotype	Oogonia ($\times 10^{-3}$)	n	Oogonia ($\times 10^{-3}$)	Oocytes ($\times 10^{-3}$)	Primordial follicles ($\times 10^{-3}$)	n
++	47^a (38, 58)	15	29^a (18, 48)	74^a (46, 118)	33^a (26, 42)	14
Authentic I+	24^b (19, 31)	11	42^a (21, 82)	168^b (100, 280)	36^a (21, 61)	12
Putative I+	27^b (20, 36)	4	22^a (14, 35)	176^b (106, 293)	21^a (8, 53)	8
Putative II	43^a (31, 58)	5	2^b (0, 67)	75^a (39, 141)	25^a (20, 30)	7

Values are geometric means (and 95% confidence limits). The data are numbers of germ cells for the left ovary only. For each column, values with different superscripts are significantly different ($P < 0.05$; Duncan's multiple-range test).

thyroid-stimulating hormone response to growth hormone-releasing hormone or thyroid-releasing hormone, respectively (McNatty *et al.*, 1994) remains unclear. The link between fetal development and pituitary sensitivity to GnRH in postnatal life remains to be established.

Fetal Characteristics of Inverdale Genotypes

Inverdale fetuses have been recovered from pregnant ewes at days 40, 90, 120 and 135 after mating. Matings of carrier rams (I) with control ewes (++) produce female fetuses that are all heterozygous carriers of the $FecX^I$ gene. Matings of control rams (++) with ++ ewes produce ++ fetuses, while matings of I rams with I+ ewes produce a mixture of homozygous (II) and heterozygous (I+) fetuses; the putative II and I+ fetuses cannot be distinguished by any overt characteristics.

At days 40 and 90, no differences were noted between ++ ($n = 15$), authentic I+ ($n = 11$) or putative II or I+ fetuses ($n = 9$) with respect to body mass, mass of mesonephros (day 40 only) or ovarian volume. However, when the total numbers of germ cells were compared in ++ and authentic I+ fetuses, the numbers in the I+ genotype were significantly lower at day 40 and significantly higher at day 90 (Table 5; Smith *et al.*, 1993b). At day 90, most of the germ cells were present as oocytes and significantly more oocytes were found in authentic I+ fetuses than in the controls. At days 40 and 90, the putative II and I+ fetuses were segregated based on two distinct germ cell populations in the ovaries at those times. One subpopulation of fetuses contained a germ cell population that was not different from the authentic I+ animals and these were referred to as putative I+. The remainder had mean germ cell populations that were significantly different from the authentic I+ genotype and were thus referred to as putative II (see Table 5). The number of germ cells in the putative II genotype were in most instances similar to that in the controls. At day 90, the mean diameters of oocytes in the I+ genotype differed from that in the ++ and putative II genotype. For example, the geometric mean (and 95% confidence limits) diameters for isolated oocytes in the ++, I+ and putative II genotypes were 20.5 (19.6, 21.3), 17.9 (17.2, 18.5) and 19.8 (18.3, 21.5) μm, respectively. Likewise at day 90, the geometric mean diameters for oocytes enclosed in primordial follicles were 27.2 (26.2, 28.3), 25.8 (25.4, 26.3) and 27.3 (26.7, 27.9) μm, respectively. The mean diameters of the oocytes in the I+ genotypes were significantly smaller than in the other two genotypes in both instances (i.e. isolated oocytes, follicle enclosed oocytes; both $P < 0.05$; analysis of variance). The reasons why the diameters of the oocytes but not oogonia in the I+ genotype are smaller than in the II or ++ genotypes remains to be resolved.

Table 6. Differences in ovarian development during fetal and neonatal life in $FecB^B$, $FecX^I/FecX^+$ and $FecX^I/FecX^I$ ewes compared with controls

Day of gestation or weeks after birth	Difference relative to control genotype		
	$FecB^B$	$FecX^I/FecX^+$	$FecX^I/FecX^I$
28 days	Lower heart mass		
30–40 days	Smaller mesonephros Lower number of oogonia		
40 days	Lower body mass	Lower number of oogonia	
75 days	Lower number of primordial follicles		
90 days	Higher number of oogonia Lower number of primordial follicles	Higher number of oogonia	
	Lower level of germ cell atresia	Smaller diameter oocyte	
100–135 days	Higher number of primordial follicles		No secondary follicles Abnormal follicular structures
135 days	Lower numbers of primary and secondary follicles		
4–6 weeks	Lower numbers of tertiary follicles		
	Higher plasma FSH Lower plasma inhibin		No secondary or tertiary follicles Inhibin undetectable in plasma concentrations
12–28 weeks	Higher numbers of tertiary follicles		Gonadotrophin similar to castrate animals
	Higher plasma inhibin/lower plasma FSH		

At days 120 and 135 of gestation, both ++ and authentic I+ fetuses have ovaries with most germ cells in primordial follicles and some in either primary or secondary structures. However, in a proportion of putative II/I+ fetuses some ovaries contained germ cells in primordial follicles in the cortical region, numerous isolated oocytes devoid of follicular cells in the medullary region and degenerating oocytes in follicles or oocyte-free follicles in the innermost regions of the cortex. In those ovaries, no normal growing primary or secondary follicles were noted. On the basis of finding that adult II 'streak' ovaries contain numerous oocyte-free follicles (Braw-Tal *et al.*, 1993b), we have provisionally classified fetuses at days 120 and 135 of gestation as putative II if oocyte-free follicles or abnormal follicular-like structures were present. Oocyte-free follicles have only rarely been observed in control or in authentic I+ fetuses.

No $FecX^I$ effects were noted in the plasma concentrations of immunoreactive inhibin at days 40 and 90 or in the plasma concentrations of FSH at days 90, 100, 120 and 135 of gestation. To our knowledge, no data are yet available for the neonatal period with respect to immunoreactive inhibin or gonadotrophins in peripheral plasma. In sexually mature I+ animals, the immunoreactive inhibin or gonadotrophin concentrations in plasma are not different from those of ++ animals (Shackell *et al.*, 1993), whereas in the II animals, inhibin is undetectable, while the gonadotrophins are in the range observed for castrate animals (Braw-Tal *et al.*, 1993b).

Conclusion

There is clear evidence that the $FecB^B$ and $FecX^I$ genes influence ovarian development during fetal and early neonatal life as well as during adult life (Table 6). Moreover, these differences in ovarian

development during fetal life are not accompanied by changes in the plasma or pituitary concentrations of gonadotrophin or plasma immunoreactive inhibin or ovarian contents/concentrations of inhibin or oestradiol. In Booroola ewes, differences in ovarian development are preceded by temporal differences in the mass of the heart and in the volume of the mesonephros. The heart, mesonephros and gonad are all of mesodermal origin. It remains to be established whether organs derived from other germ layers are affected. Perhaps the $FecB^B$ mutation affects the timing or rate of tissue growth or differentiation including that of germ cell development, follicle formation and growth. Unlike the Booroola gene, there are no observable effects of the Inverdale gene on somatic cells of mesodermal origin (for example, the mesonephros) or on body mass at day 40 of gestation. However, like the Booroola gene, the heterozygous but not homozygous sheep fetus contains more germ cells at day 40 and less at day 90 of development. For the homozygous Inverdale, no genotype effects are observed until day 120 of gestation when follicular development beyond the primordial phase is impaired. Further studies are required to elucidate why the Inverdale gene influences gonadal development in the I+ and II animals in a different way.

References

Bindon BM and Piper LR (1986) The reproductive biology of prolific sheep breeds *Oxford Reviews of Reproductive Biology* **8** 414–451

Braw-Tal R Expression of mRNA for follistatin and inhibin/activin subunits during follicular growth and atresia *Journal of Molecular Endocrinology* (in press)

Braw-Tal R and Gootwine E (1989) Pituitary response to GnRH and ovariectomy in Booroola–Awassi and Awassi ewe lambs *Journal of Reproduction and Fertility* **87** 73–80

Braw-Tal R, Bor A and Gootwine E (1993a) Plasma immunoreactive inhibin and FSH in prepubertal Assaf and Booroola–Assaf ewe lambs *Domestic Animal Endocrinology* **10** 87–94

Braw-Tal R, McNatty KP, Smith P, Heath DA, Hudson NL, Phillips DJ, McLeod BJ and Davis GH (1993b) Ovaries of ewes homozygous for the X-linked Inverdale gene ($FecX^I$) are devoid of secondary and tertiary follicles but contain many abnormal structures *Biology of Reproduction* **49** 895–907

Braw-Tal R, Tisdall DJ, Hudson NL, Smith P and McNatty KP (1994) Follistatin but not α or β_A inhibin subunit mRNA is expressed in ovine fetal ovaries in late gestation *Journal of Molecular Endocrinology* **13** 1–9

Byskov AG (1974) Does the rete ovarii act as a trigger for the onset of meiosis? *Nature* **252** 396–397

Byskov AG and Hoyer PE (1994) Embryology of mammalian gonads and ducts. In *The Physiology of Reproduction* (2nd Edn) pp 487–540. Eds E Knobil and JD Neill. Raven Press, New York

Caldani M and Batailler M (1991) Localization of LHRH-IR structures in the sheep fetus brain *Proceedings of the 11th Annual Meeting of the European Neuroscience Association*, Abstract 8 Ed. RD Guiller. Oxford University Press, Oxford

Davis GH, Montgomery GW, Allison AJ, Kelly RW and Bray AR (1982) Segregation of a major gene influencing fecundity in progeny of Booroola sheep *New Zealand Journal of Agricultural Research* **25** 525–529

Davis GH, McEwan JC, Fennessy PF, Dodds KG and Farquhar PA (1991) Evidence for the presence of a major gene influencing ovulation rate on the X chromosome of sheep *Biology of Reproduction* **44** 620–624

Davis GH, McEwan JC, Fennessy PF, Dodds KG, McNatty KP and O, W-S (1992) Infertility due to bilateral ovarian hypoplasia in sheep homozygous ($FecX^I$ $FecX^I$) for the Inverdale prolificacy gene located on the X chromosome *Biology of Reproduction* **46** 636–640

Isaacs KL, McNatty KP, Condell L, Shaw L, Heath DA, Hudson NL, Littlejohn RP and McLeod BJ (1995) Plasma FSH, LH and inhibin concentrations in $FecB^B/FecB^B$ and $FecB^+/FecB^+$ Booroola ewes and rams from birth to 12 months of age *Journal of Reproduction and Fertility* **103** 89–97

Kennedy JP, Worthington CA and Cole ER (1974) The post-natal development of the ovary and uterus of the Merino lamb *Journal of Reproduction and Fertility* **36** 275–282

McNatty KP, Lun S, Heath DA, Ball K, Smith P, Hudson NL, McDiarmid J, Gibb M and Henderson KM (1986) Differences in ovarian activity between Booroola × Merino ewes which were homozygous, heterozygous and non-carriers of a major gene influencing their ovulation rate *Journal of Reproduction and Fertility* **77** 193–205

McNatty KP, Lun S, Heath DA and O'Keeffe LE (1987) Ovarian follicular activity in Booroola lambs with and without a fecundity gene *Journal of Reproduction and Fertility* **79** 57–66

McNatty KP, Heath DA, Hudson N and Clarke IJ (1990) Effect of long-term hypophysectomy on ovarian follicle populations and gonadotrophin-induced adenosine cyclic 3′,5′-monophosphate output by follicles from Booroola ewes with or without the F gene *Journal of Reproduction and Fertility* **90** 515–522

McNatty KP, Henderson KM, Fleming JS, Clarke IJ, Bindon BM, O'Shea J, Hillard MA and Findlay JK (1991a) The physiology of the Booroola ewe. In *Major Genes for Reproduction in Sheep* pp 105–124 Eds JM Elsen, L Bodin and J Thimonier. INRA, Toulouse

McNatty KP, Hudson NL, Shaw L, Condell LA, Ball K, Seah S-L and Clarke IJ (1991b) GnRH-induced gonadotrophin secretion in ovariectomized Booroola ewes with hypothalamic–pituitary disconnection *Journal of Reproduction and Fertility* **91** 583–592

McNatty KP, Heath DA, Hudson NL, Ball K and Condell L (1992) Concentrations of immunoreactive inhibin in ovarian and

peripheral venous plasma and follicular fluid of Booroola ewes that are homozygous carriers or non-carriers of the Fec^B gene *Journal of Reproduction and Fertility* **95** 489–502

McNatty KP, Hudson NL, Shaw L and Moore L (1994) Plasma concentrations of FSH, LH, thyroid-stimulating hormone and growth hormone after exogenous stimulation with GnRH, TRH and GHRH in Booroola ewes that are homozygous carriers or non-carriers of the Fec^B gene *Journal of Reproduction and Fertility* **102** 177–183

Mauleon P (1978) Ovarian development in young mammals. In *Control of Ovulation* pp 141–158 Eds DB Crighton, NB Haynes, GR Foxcroft and GE Lamming. Butterworths, London

Montgomery GW, Scott IC, Littlejohn RP, Davis GH and Peterson AJ (1989) Concentrations of FSH are elevated in newborn ewe lambs carrying the Booroola F gene but not in lambs from a prolific Romney strain *Reproduction, Fertility and Development* **1** 289–308

Montgomery GW, McNatty KP and Davis GH (1992) Physiology and molecular genetics of mutations that increase ovulation rate in sheep *Endocrine Reviews* **13** 309–328

Montgomery GW, Crawford AM, Penty JM, Dodds KG, Ede AJ, Henry HM, Pierson CA, Lord EA, Galloway SM, Schmack AE, Sise JA, Swarbrick PA, Hanrahan V, Buchanan FC and Hill DF (1993) The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q *Nature Genetics* **4** 620–624

Piper LR and Bindon BM (1982) The Booroola Merino and the performance of medium non-Peppin crosses at Armidale. In *The Booroola Merino* pp 9–20 Eds LR Piper, BM Bindon and RD Nethery CSIRO, Melbourne

Shackell GH, Hudson NL, Heath DA, Lun S, Shaw L, Condell L, Blay LR and McNatty KP (1993) Plasma gonadotrophin concentrations and ovarian characteristics in Inverdale ewes that are heterozygous for a major gene ($FecX^I$) on the X chromosome that influences ovulation rate *Biology of Reproduction* **48** 1150–1156

Smith P, O W-S, Hudson NL, Shaw L, Heath DA, Condell L, Phillips DJ and McNatty KP (1993a) Effects of the Booroola gene (Fec^B) on body weight, ovarian development and hormone concentrations during fetal life *Journal of Reproduction and Fertility* **98** 41–54

Smith P, O W-S, Heath DA, Hudson NL, Davis GH and McNatty KP (1993b) The Booroola (Fec^B) and Inverdale ($FecX^I$) genes influence ovarian development in early foetal life *Proceedings of the New Zealand Society of Animal Production* **53** 281–285

Smith P, Braw-Tal R, Corrigan K, Hudson NL, Heath DA and McNatty KP (1994) Ontogeny of ovarian follicle development in Booroola sheep fetuses that are homozygous carriers or non-carriers of the Fec^B gene *Journal of Reproduction and Fertility* **100** 485–490

Sonjaya H and Driancourt MA (1987) Ovarian follicles during infancy in Romanov and Ile-de-France ewe lambs *Journal of Reproduction and Fertility* **81** 241–248

Sonjaya H and Driancourt MA (1989) Ovarian morphology and function in 12-week-old lambs from breeds with different ovulation rates as adults *Reproduction, Fertility and Development* **1** 265–272

Tassell P, Kennedy JP and Bindon BM (1983) Ovarian follicles of newborn Merino lambs from genetic lines which differ in fecundity *Australian Journal of Biological Sciences* **36** 351–355