

Genes controlling ovulation rate in sheep

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Sheep provide a valuable model for studying the genetic control of ovulation rate. Recent progress includes the identification of mutations in BMP15 (bone morphogenetic protein 15) that increase ovulation rate in heterozygous carriers and block follicular development in homozygous carriers. The genes characterized to date appear to act principally within the ovary and result in earlier maturity of granulosa cells and reduced follicular size. There may also be other sites of action, and increased FSH concentrations appear to be important in the expression of the *FecB* phenotype. A new locus on the X chromosome in New Zealand Coopworth sheep increases ovulation rate by about 0.4 and is maternally imprinted. Results from studies in the Cambridge and Belclare breeds indicate that further genes remain to be characterized. Finding the first mutations leading directly to variation in ovulation rate is likely to speed up the identification and molecular analysis of these other genes. There is still much to learn about follicular development and the control of litter size from genetic models in sheep.

Multiple ovulation in mammals is a complex trait influenced by genetic and environmental factors. Primates and many ruminants typically release a single oocyte at each cycle whereas species such as mice and pigs, capable of rearing many offspring, have consistently high ovulation rates. Within species, the number of follicles that mature is tightly regulated to ensure optimal fertility and maximum survival of offspring. Current models of follicular selection indicate that multiple ovulation is controlled both by concentrations of FSH near the time of follicular selection and by intraovarian factors (Campbell *et al.*, 1995; Baird and Campbell, 1998; McNatty *et al.*, 1999).

One approach to understanding critical pathways in complex regulatory systems is to find natural and induced mutations that influence the target phenotype. Sheep have proved a valuable model for the study of follicular growth and selection. Most breeds have one or two ovulations but there is wide variation in ovulation rate among different strains influenced by genetic background and the effects of age, season and nutrition. An important step forward was made by Piper and Bindon (1982) when they presented the first evidence for segregation of a locus with a major effect on litter size in the Booroola strain of Merino sheep, subsequently shown in New Zealand Booroola flocks to result from its additive effect on ovulation rate (Davis *et al.*, 1982).

These observations generated a critical search for genes influencing ovulation rate in other strains of sheep and in other species. The best-characterized locus (*FecX*) was found in the New Zealand Romney breed (Inverdale, *FecX*^I) and maps to the sheep X chromosome (Davis *et al.*, 1991, 1992). Recent progress marks another milestone in our understanding of mechanisms controlling ovulation rate. The gene for the *FecX* locus has been identified and the first mutations that increase ovulation rate as part of the phenotype have been described (Galloway *et al.*, 2000). Here, we review genetic studies in sheep and new contributions to our understanding of the mechanisms controlling ovulation rate.

Follicle development

Folliculogenesis begins when follicles leave the resting pool of primordial follicles and enter the growth phase. Growing follicles undergo a complex process of development that includes proliferation and differentiation of several cell types in the follicle. At the same time, the oocyte is undergoing developmental changes necessary for resumption of meiosis after the preovulatory surge of gonadotrophins. In sheep, the stages of follicular development have been classified from the number of granulosa cells in the largest cross-section of follicles (McNatty *et al.*, 1999) to be types 1 (primordial), 1a (transitory), 2 (primary), 3 and 4 (preantral) and 5 (early antral; see Fig. 1). Type 1 refers to follicles with one layer of

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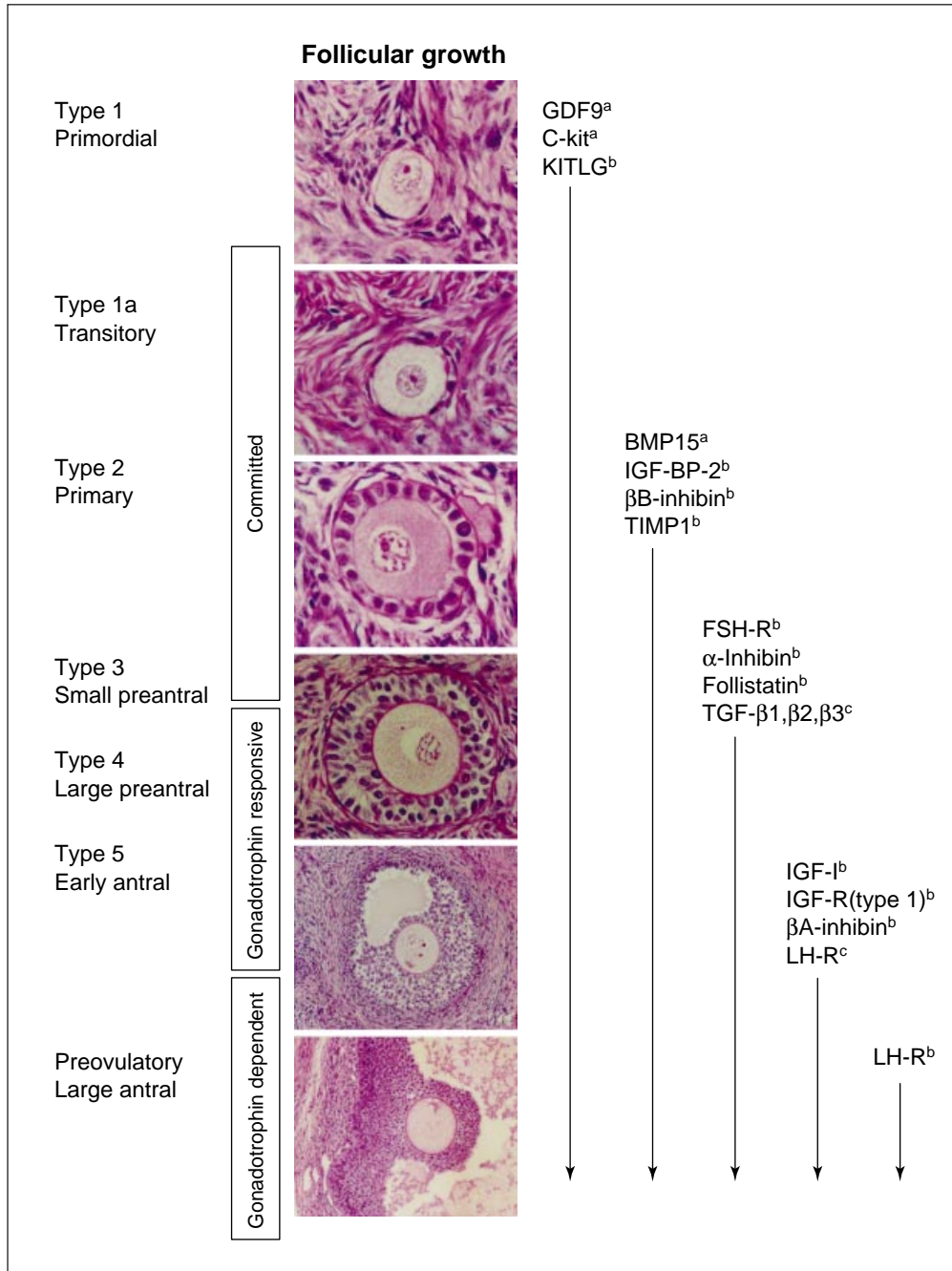


Fig. 1. Summary of the various stages of follicular growth in ewes together with the onset of expression of certain genes in (a) oocytes, (b) granulosa cells and (c) theca interna–externa (McNatty *et al.*, 1999; Galloway *et al.*, 2000; K. P. McNatty, unpublished). The solid vertical arrows indicate observations that all genes once expressed continue to be expressed at least until the time of the preovulatory LH surge. The Inverdale and Hanna gene mutations occur in the oocyte-derived BMP15 gene and thus block normal follicular growth from the type 2 stage of growth. The Booroola gene mutation, although unknown, affects follicular growth after the type 2 stage of growth but before the type 5 stage of development. Different classification systems have described the various stages of follicular growth as types 1–5, primordial to antral or as committed follicles to gonadotrophin-dependent follicles (McNatty, 1999; Scaramuzzi *et al.*, 1999). BMP15: bone morphogenetic protein 15; GDF9: growth differentiation factor 9; IGF: insulin-like growth factor; IGF-BP: IGF-binding protein; KITLG: KIT ligand; TGF: transforming growth factor; TIMP1: tissue inhibitor of metalloproteinase 1.

flattened granulosa cells; type 1a to follicles with one layer of cells that are a mixture of flattened and cuboidal granulosa cells; type 2 to one or two layers of cuboidal granulosa cells; type 3 to two to four layers of granulosa cells; type 4 to four to six layers of granulosa cells; and type 5 to more than five layers of granulosa cells with evidence of an antrum.

The classic endocrine control of ovarian function by FSH and LH forms one part of a complex regulatory network and interacts with systems of cell–cell interaction between different cell types in the follicle (Baird and Campbell, 1998; McNatty *et al.*, 1999). A range of molecules exerts both paracrine and autocrine actions at every stage of folliculogenesis (McNatty *et al.*, 1999). Genes expressed at different stages of follicular development in sheep are summarized (Fig. 1). Key questions in follicular development are: which signals control the exit of follicles from the resting pool; how are ovulatory follicles selected; and how is follicular dominance maintained? Genetic studies can address these questions where natural and induced mutations target key steps in these processes.

Loci affecting ovulation rate

In addition to genes in the Booroola (*FecB*) and Inverdale (*FecX*) sheep, there is evidence that major genes may be segregating in Icelandic (Johnmudsson and Adalsteinsson, 1985), Javanese (Bradford *et al.*, 1986), Olkuska (Radomska *et al.*, 1988), Cambridge (Owen *et al.*, 1990; Hanrahan, 1991), Belclare (Hanrahan, 1991), Lacaune (Bodin *et al.*, 1998) and Woodlands (Davis *et al.*, 2001) sheep breeds. Some genes in these breeds may represent alleles for a smaller subset of genes. In most cases, it is impossible to carry out suitable crossbreeding experiments because of restrictions on the movement of sheep between countries. Molecular characterization of the loci and gene mapping will provide ways to test whether mutations are present in the same genes or the loci map to the same chromosomes.

FecB locus

The Booroola Merino strain carries a single autosomal locus named *Fecundity Booroola* (*FecB*) (Davis *et al.*, 1982; Piper and Bindon, 1982). The effect of mutation is additive for ovulation rate with an increase of 1.65 for each copy (Piper *et al.*, 1985). The effect on litter size is semi-dominant because embryonic losses cause partial failure of multiple pregnancy (Piper *et al.*, 1985).

Phenotype. The most striking physiological effects of the *FecB* locus are on ovulation rate and the size and number of ovulatory follicles in the ovary. Follicles mature and ovulate at significantly smaller diameters in homozygous (*BB*) and heterozygous (*B+*) carrier ewes compared with non-carrier or wild-type (*++*) ewes (McNatty and Henderson, 1987; Montgomery *et al.*, 1992; Baird and Campbell, 1998). The smaller ovulatory follicles of *BB* ewes contain fewer granulosa cells than ovulatory follicles in *++* ewes (McNatty and Henderson, 1987; Montgomery *et al.*, 1992). The

increased number of ovulatory follicles offsets the reduced number of granulosa cells in individual follicles. Consequently, both the total number of granulosa cells from all ovulatory follicles and total oestradiol production from the ovaries of *B+/BB* ewes are similar to those of *++* ewes (Montgomery *et al.*, 1992; Souza *et al.*, 1997). Some differences in follicular characteristics persist after hypophysectomy, but data indicate the different functional characteristics of ovaries in the Booroola genotypes may not be entirely independent of pituitary hormones (Fig. 2) (Montgomery *et al.*, 1992). Despite the important differences in follicular development, oocytes from mature follicles in *BB* genotypes appear fully competent and produce viable offspring with no apparent differences in fertility or embryo viability among genotypes.

Effects of *FecB* in females during fetal and neonatal development. During fetal development, there are differences in carriers of the *FecB* locus in the heart and mesonephros at 28 days, and in body mass and crown–rump length between day 30 and day 40 (Fig. 2) (McNatty *et al.*, 1995a). For the remainder of fetal and neonatal life, differences among the genotypes were found only within the ovary (Fig. 2). For example, ovarian development was retarded in *BB* fetuses that had smaller mesonephri, smaller ovaries, fewer oogonia present at days 35–40, and fewer oocytes at day 55. However, a delay in loss of germ cells between day 75 and day 90 resulted in larger ovaries and a greater number of oogonia at day 90. Moreover, *BB* fetuses had fewer type 1 follicles at day 75 and day 90 and fewer type 2 and larger follicles at day 135 as well as during early neonatal life (Braw-Tal and Gootwine, 1989). However, by sexual maturity at ≥ 9 months of age, no genotype differences were noted in the number of types 1–4 or antral follicles (Fig. 1).

Hypothalamic–pituitary function in Booroola sheep. No differences in hypothalamic function have been noted in Booroola sheep, indicating that the principal effects of the *FecB* gene are likely to be downstream of the hypothalamus and possibly at the pituitary gland and the ovary (Montgomery *et al.*, 1992; McNatty *et al.*, 1993). The cumulative evidence for direct or indirect effects of the Booroola gene on pituitary function is substantial. The only gene-specific effect on pituitary hormones was observed for FSH (McNatty *et al.*, 1994). Moreover, in different ages and physiological states, some (Montgomery *et al.*, 1992; Braw-Tal *et al.*, 1993; McNatty *et al.*, 1993, 1994; Phillips *et al.*, 1993; Isaacs *et al.*, 1998) but not all studies (Driancourt *et al.*, 1991; Wheaton *et al.*, 1996; Souza *et al.*, 1997) found significantly higher FSH concentrations in *BB* compared with *++* animals. In most of these reports, gene-specific differences were not found for LH. Overall, the evidence indicates that the *BB* animals have a greater FSH output per cell relative to *++* animals (McNatty *et al.*, 1991; Heath *et al.*, 1996). For example, in studies with ovariectomized hypothalamic–pituitary disconnected (HPD) animals, the FSH concentration 10 min after an exogenous pulse of

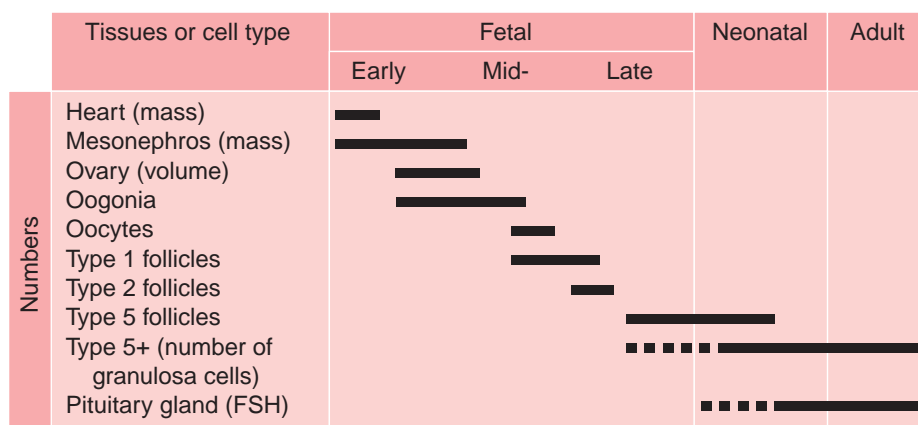


Fig. 2. Booroola genotype differences (*BB* versus *++*) at different stages of fetal life and neonatal or adult life with respect to specific tissues or cell type. The solid bars define periods during which there are significant differences between Booroola genotypes in the development or physiology of different tissues or cell types. The dashed lines indicate that the ontogeny of the difference is not known.

GnRH (250 ng) was 1.6-fold higher in the *BB* than in the *++* genotype (McNatty *et al.*, 1991).

Results from studies of HPD ovary-intact ewes and animals treated with GnRH agonist (Deslorelin) (Hudson *et al.*, 1999) with comparable doses of gonadotrophins demonstrated that significantly more *++* than *BB* animals ovulated. When *BB* and *++* ewes were administered identical doses of FSH, the mean ovulation rate and plasma concentrations of FSH in those animals that ovulated was the same in both genotypes. The higher mean ovulation rate in intact *BB* compared with *++* ewes is probably due to effects of the *FecB* gene at the ovary as well as on pituitary FSH release.

Genetic mapping. The search for markers linked to the *FecB* locus began during the late 1980s in several laboratories (Montgomery *et al.*, 1992; Lanneluc *et al.*, 1994). Despite a threefold difference in mean ovulation rate between homozygous (*BB*) carriers and control (*++*) ewes, problems remain in assigning phenotypes to individuals on the basis of lifetime records of ovulation rate. Phenotypes have been assigned directly on the basis of records of maximum ovulation rate (Davis *et al.*, 1982; Montgomery *et al.*, 1993), maximum likelihood techniques (Elsen *et al.*, 1990) or treating the locus as a quantitative trait (Elsen *et al.*, 1990). Errors in phenotype assignment may influence the estimated position of the locus, and incorrect phenotype assignment of key individuals can seriously mislead the search for the gene by positional cloning.

Linkage to the *FecB* locus was first detected with an anonymous microsatellite marker also linked to secreted phosphoprotein 1 (SPP1) (Montgomery *et al.*, 1993). Additional genes from the same region were tested and the linkage group containing the *FecB* locus was mapped to sheep chromosome 6 (Montgomery *et al.*, 1994). An approach using DNA fingerprinting was also successful in identifying markers linked to the *FecB* locus in Booroola

flocks in France (Lanneluc *et al.*, 1994), although the chromosome location for the gene could not be determined using these markers.

Subsequent studies have sought to identify the gene responsible by candidate positional cloning. The critical region for the *FecB* locus lies between the genes for SPP1 and alcohol dehydrogenase 2 (ADH2; Fig. 3) (Lord *et al.*, 1998). Genes located on sheep chromosome 6 map to human chromosome 4 (Lanneluc *et al.*, 1996; Lord *et al.*, 1996). There is a large inversion in gene order between sheep chromosome 6 and human chromosome 4 near the region of the *FecB* locus (Lord *et al.*, 1996). The breakpoint for one end of the inversion has been mapped to a small region of 150 kb pairs between the genes for SPP1 (secreted phosphoprotein 1) and DMP1 (dentin-specific acidic phosphoprotein) (Lumsden *et al.*, 1999) outside the critical region for the *FecB* locus. Data on genes in the critical region from the human gene maps and the annotated human DNA sequence will provide information for positional cloning. Physical mapping and screening of candidate genes is in progress to try to locate the gene and mutation in carriers of the *FecB* locus.

Genes from chromosome 6 in sheep also map to pig chromosome 8 and a locus increasing ovulation rate maps to pig chromosome 8 (Rohrer, 1999; Wilkie *et al.*, 1999). It is not clear whether this is the homologue of the *FecB* locus in pigs and it will be necessary to clone the genes or complete detailed comparative studies to decide whether the same gene is responsible for effects on ovulation rate in the two species.

FecX locus

The Inverdale (*FecX*^l) locus was identified in a prolific family of Romney sheep (Davis *et al.*, 1995). A family line descended from a ewe (A281) with a history of 33 lambs

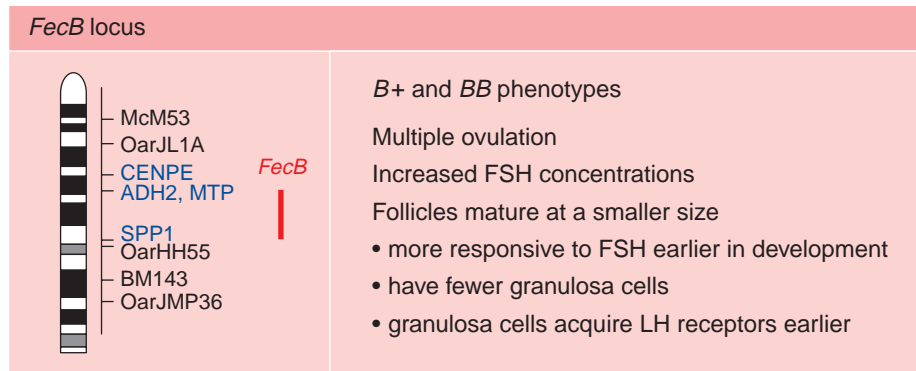


Fig. 3. A partial linkage map of sheep chromosome 6 (Lord *et al.*, 1996; Lumsden *et al.*, 1999) showing the location for the *FecB* locus together with the major phenotype for heterozygous (*B+*) and homozygous (*BB*) carriers of the mutation. Gene loci are shown in blue and microsatellite markers in black. The solid red bar shows the location of the *FecB* locus between the genes for alcohol dehydrogenase 2 (*ADH2*) and secreted phosphoprotein 1 (*SPP1*). Other genes shown on the map are those for centromere protein E (*CENPE*) and microsomal triglyceride transfer protein (*MTP*). Sheep and cattle microsatellite markers shown on the map are *McM53*, *OarJL1A*, *OarHH55*, *BM143*, and *OarJMP36*.

from 11 lambings was identified in a flock screened for exceptional litter size (Davis *et al.*, 1995). High ovulation rates were observed in female descendants of A281 within the flock (Davis *et al.*, 1995). Segregation studies in the sons and grandsons of putative carriers demonstrated X linkage (the locus was carried on the X chromosome; Davis *et al.*, 1991, 1995). Subsequently, it was called the Inverdale fecundity locus (*FecX^I*). Mating rams with one copy of the gene (*IY*) with heterozygous (*I+*) daughters demonstrated that homozygous (*II*) carriers have 'streak' ovaries and are infertile (Davis *et al.*, 1992).

Fortunately for the subsequent gene search, a second strain of Romney sheep on the Hanna property (*FecX^H*), apparently unrelated to Inverdale sheep, was also found to carry the same X-linked phenotype (Davis *et al.*, 1995). Complementation from crossing two strains is the classical method to determine whether two loci are the same. Crossing *FecX^I* with *FecX^H* animals produces *FecX^I/FecX^H* infertile females with streak ovaries indistinguishable from *FecX^I/FecX^I* females (Davis *et al.*, 1995) showing that the two strains carried mutations at the same locus.

Phenotype. The effect of the mutation in heterozygous carrier females is to increase ovulation rate by about 1.0 and litter size by about 0.6 (Davis *et al.*, 1995). Homozygous females have small, flattened streak ovaries that show no sign of follicular activity (Davis *et al.*, 1992). The streak ovaries contain primordial follicles, but follicles do not develop beyond the primary (type 2) stage.

Effects of *FecX^I* in females during fetal development. No effects of *FecX^I* genotype were detected in crown-rump length, weight of fetus, mass of the mesonephros or ovarian volume at 40 days of age (Smith *et al.*, 1997). The number of germ cells present in the ovary of *I+* carriers was significantly

lower at 40 days and significantly higher at 90 days compared with non-carriers (Smith *et al.*, 1997). At day 90 of gestation, mean diameters of the follicles in *I+* ovaries were smaller than for those in *++* or *II* ovaries, due in part to differences in mean diameter of oocytes. However, from day 105 of gestation, follicular development in *I+* carriers was similar to that in controls.

Germ cell populations in homozygous (*II*) carriers were similar to those in controls during early fetal development (Smith *et al.*, 1997), but differences become apparent by day 105 of gestation. At this stage, the ovaries of *II* fetuses are devoid of normal type 3 follicles and contain abnormal structures including oocytes devoid of follicular cells, follicles with degenerating oocytes or oocyte-free follicles (Braw-Tal *et al.*, 1993; Smith *et al.*, 1997). Comparisons of oocyte diameter and the number of granulosa cells show that as oocytes enlarge in *II* carriers, there is no parallel increase in the number granulosa cells or evidence of organized granulosa cell development. Results demonstrate that the normal transformation of a type 2 follicle into a type 3 follicle is blocked in homozygous carriers of the *FecX* mutation.

Effects of *FecX^I* on ovarian function in neonatal and adult female sheep. In neonatal and adult ewes, there were no differences between the three genotypes (*++*, *I+* or *II*) in the total numbers of type 1, 1a or 2 follicles in the ovary (Braw-Tal *et al.*, 1993; Smith *et al.*, 1997). Thus the process of follicular formation and factors involved in the initiation of follicular growth are probably the same for all genotypes. However, the total number of antral follicles (≥ 1 mm diameter) was found to be greater in adult *I+* ewes than in *++* ewes (Shackell *et al.*, 1993; McNatty *et al.*, 1995b). This difference was due to the presence of more small antral

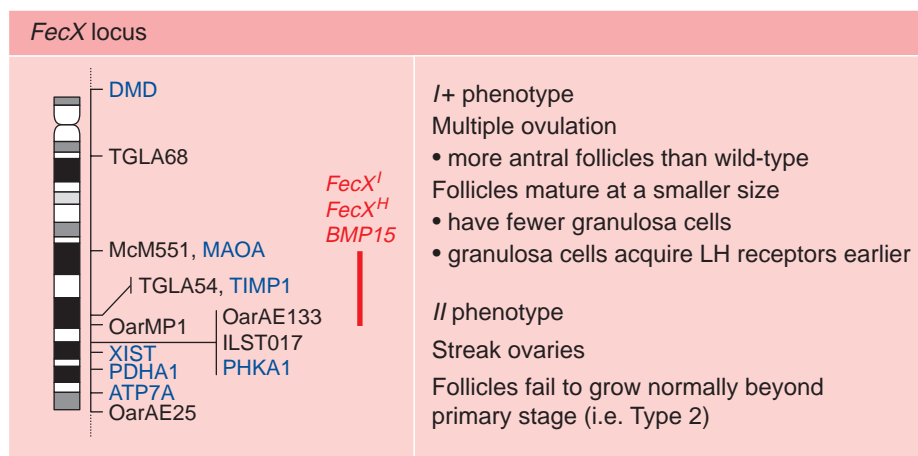


Fig. 4. A partial linkage map of the sheep X chromosome (Galloway *et al.*, 2000) showing the location for the *FecX* locus together with the phenotypes for heterozygous (*I+*) and homozygous (*II*) female carriers of the mutation. Gene loci are shown in blue and microsatellite markers in black. The solid red bar shows the location of the *BMP15/FecX* locus (Galloway *et al.*, 2000) between the markers *McM551* and *OarMP1*. Gene loci shown on the map are *ATP7A* (ATPase, Cu²⁺ transporting α -polypeptide), *DMD* (Duchenne muscular dystrophy), *BMP15* (bone morphogenetic protein 15, also known as growth differentiation factor 9B), *MAOA* (monoamine oxidase A), *PDHA1* (pyruvate dehydrogenase E1 α), *PHKA1* (phosphorylase kinase α -1), *TIMP1* (tissue inhibitor of metalloproteinase 1) and *XIST* (inactive X-specific transcript). Sheep and cattle microsatellite markers are *ILSTS017*, *McM551*, *OarAE25*, *OarAE133*, *OarMP1*, *TGLA54* and *TGLA68*.

follicles and fewer granulosa cells in each of the small and large antral follicles of *I+* compared with *++* ewes. Preovulatory follicles in *I+* ewes also appear to mature at a smaller diameter and this earlier maturation may be attributable to greater sensitivity to FSH and an earlier acquisition of receptors for LH (Shackell *et al.*, 1993).

Ovarian volumes in adult *II* ewes are about 25% of those in non-carrier ewes, and the difference in ovarian volume occurs some time during the first 6 months after birth. This difference is probably due to the absence of normal follicular growth beyond type 2 follicles in the *II* ewes (Braw-Tal *et al.*, 1993; Smith *et al.*, 1997). Abnormal structures first observed in fetal ovaries are a common feature in the ovaries of neonatal and adult *II* ewes (Braw-Tal *et al.*, 1993; McNatty *et al.*, 1995b). These abnormal structures include oocyte-free follicles, a coalesced oocyte-free structure and even larger abnormal tumour-like structures (Juengel *et al.*, 2000).

Genetic mapping. The *FecX* locus was assigned to the sheep X chromosome by classical segregation analysis (Davis *et al.*, 1991) and therefore mapping studies to locate the gene were concentrated on the X chromosome. Carrier (*IY*) rams were mated to heterozygous (*I+*) carrier ewes to generate pedigrees for marker studies (Galloway *et al.*, 2000). Homozygous carrier ewes have streak ovaries and *II* daughters can be identified by direct observation of the ovaries before puberty (Davis *et al.*, 1995) without the errors associated with phenotype assignment on the basis of ovulation rate in Booroola families.

A linkage map was constructed for the sheep X chromosome (Galloway *et al.*, 1996) and markers from the X chromosome were screened in a three-generation flock in which the Inverdale gene was segregating. The *FecX* locus was mapped to a 10 cM region at the centre of the X chromosome (Fig. 4) (Galloway *et al.*, 2000) in a region containing the genes for *TIMP1* (tissue inhibitor of metalloproteinase 1) and *MAOA* (monoamine oxidase A). The equivalent region of the human X chromosome is Xp11.2–p11.4 (Galloway *et al.*, 2000).

BMP15 (bone morphogenetic protein 15), also known as *GDF9B* (growth differentiating factor 9B), is a member of the transforming growth factor superfamily, expressed specifically in the oocyte in several species (Dube *et al.*, 1998; Laitinen *et al.*, 1998). The gene mapped to the human X chromosome Xp11.2 (Dube *et al.*, 1998) and is a strong candidate for the *FecX^I* phenotype. The gene was shown to map to the sheep X chromosome within the critical region and no recombinants were observed between the *FecX* locus and *BMP15* in 78 coinformative meioses (Galloway *et al.*, 2000). The ovine gene was sequenced and shown to be similar to the gene in humans, mice and rats, with a full length sequence of 1179 bp containing two exons. Mutations were found in *BMP15* in both *FecX^I* and *FecX^H* carriers. In *FecX^H* carriers a single C→T transition at nucleotide 67 of the coding region for the mature peptide introduces a premature stop codon into the sequence, which probably results in complete loss of *BMP15* function in the homozygous carriers of the *FecX^H* variant.

In *FecX^I* carriers, a single T→A transversion at nucleotide

position 92 results in the substitution of a valine with aspartic acid in a highly conserved region of the protein (Galloway *et al.*, 2000). It appears likely that the amino acid change impairs the ability of BMP15 to form dimers and interferes with the biological action of BMP15 in ewes homozygous for the *FecX¹* variant.

FecX2 locus

The screened flock within which the *FecX* locus was identified has proved a valuable resource. Ewes were screened from the three major sheep breeds in New Zealand, Romney, Coopworth and Perendale. In 1984, high ovulation rates were recorded among daughters of one of the foundation Coopworth rams. This family was designated as the Woodlands line and an extensive progeny-testing programme was initiated in 1987 to determine whether a gene for ovulation rate segregated in this family.

Initial segregation studies indicated that high ovulation rates in this family did not follow simple Mendelian segregation (Davis *et al.*, 2001). However, on the basis of experience with the X-linked inheritance of the *FecX* locus, careful progeny test records were kept over many years and different models of inheritance were tested. Records from 50 progeny-tested rams revealed that an imprinted gene on the X chromosome (Woodlands gene; *FecX2*) that increased ovulation rate by 0.39 was segregating in this Woodlands family (Davis *et al.*, 2001). The Woodlands gene is maternally imprinted, as only females inheriting the gene from their sire display increased ovulation rates. Furthermore, the Woodlands gene is expressed only upon paternal inheritance from carrier males that are the progeny of dams in which the gene is silenced. Hence, the Woodlands gene is not expressed in ewes that receive it from either carrier dams (expressers or silenced) or from carrier males that were the progeny of expresser dams. The Woodlands gene is the first imprinted gene reported on the X chromosome of sheep and is the only imprinted gene shown to increase ovulation rate. There is no evidence of the infertility that occurs in homozygous ewes carrying the Inverdale gene. Molecular and physiological studies have recently commenced to determine the location of *FecX2* on the X chromosome and the biochemical pathways involved.

Cambridge and Belclare sheep

Two other prolific sheep populations were developed as composite breeds using prolific sheep screened from a variety of genetic backgrounds (Hanrahan, 1991). The first was the Cambridge breed established in Britain in 1964. Litter size and ovulation rate in the Cambridge breed vary considerably, with ovulation rates ranging from 1 to 13 eggs shed and 2.5% of litters ≥ 6 (Hanrahan, 1991). An analysis of ovulation rate records in Cambridge ewes at Bangor indicated the presence of a major gene with an effect size of 1.72 for ovulation rate in 2- and 3-year-old ewes (Owen *et al.*, 1990). Results were consistent with a gene frequency of 0.3–0.4 and the absence of dominance.

An association has been reported between ovulation rate and HBB (haemoglobin) genotypes in Cambridge sheep (Glazko *et al.*, 1997). HBB maps close to the FSHB locus on sheep chromosome 15, indicating FSH as a candidate gene. However, it is possible that the association with the HBB locus is due to inclusion of the Finn breed since this breed differs in the frequency of HBB alleles compared with other European breeds (Sise *et al.*, 1991). Segregation studies are necessary to demonstrate linkage to the HBB locus in the Cambridge breed.

In Ireland, the Belclare breed was developed in the late 1970s by reciprocal crossing of Fingalway and High Fertility prolific lines (Hanrahan, 1991). Ewes with exceptionally high ovulation rate have been recorded in the Belclare breed, and the repeatability of ovulation rate is high in the daughters of these ewes, indicating possible segregation of a major gene or genes (Hanrahan, 1991; Reynaud *et al.*, 1999). Studies of ovaries in putative heterozygous carriers or non-carriers in the Belclare breed demonstrated differences in the number and size of ovulatory follicles (Reynaud *et al.*, 1999). Carrier ewes had significantly more ovulatory follicles, but these were smaller and contained fewer granulosa cells. Segregation studies support the presence of major genes in the Belclare breed (Webb *et al.*, 1998; Reynaud *et al.*, 1999), but the situation is complicated since more than one gene may be present.

Ewes with infertility and non-functional ovaries have been observed in both the Cambridge and Belclare breeds (Hanrahan, 1991; Owen, 1996; Webb *et al.*, 1998). In some prolific breeds, abnormalities of the reproductive tract can occur through XX/XY chimaerism (Hanrahan, 1991). No XX/XY chimaerism was detected in a small number of infertile female Cambridge sheep with hypoplastic ovaries and a small uterus (Hanrahan, 1991). Unlike the homozygous carriers of the *FecX* locus, some sterile ewes of the Belclare and Cambridge breeds do contain small growing follicles, and follicles are occasionally visible on the surface of the ovary (Hanrahan, 1991; Webb *et al.*, 1998). Evidence from breeding studies indicates that a recessive gene that appears to follow autosomal rather than X-linked inheritance causes ovarian hypoplasia in the Belclare breed (Webb *et al.*, 1998; Reynaud *et al.*, 1999). Ewes known to be carriers of the gene for ovarian hypoplasia do not always have high ovulation rates themselves, and carrier rams can be classified as non-carriers of a gene for ovulation rate (Reynaud *et al.*, 1999). However, ewes with exceptionally high ovulation rates have descendants with ovarian sterility, indicating some interaction between the loci involved in ovulation rate and ovarian hypoplasia, or that the genes are linked. Both breeds received genetic contributions from the Lleyne breed and, since infertile ewes have been reported in the Lleyne breed (Vaughan *et al.*, 1997), a gene influencing ovarian hypoplasia may have come from this source.

The segregation patterns for putative major genes in the Belclare and Cambridge breeds are complex, but offer valuable models for further study of genes affecting ovulation rate. Further segregation data together with genetic

analysis for the presence of mutations in BMP15 and linkage to markers close to the *FecX* locus may help clarify the nature of the genes and the relationship (if any) to known loci influencing ovulation rate. In view of the recent segregation data for the *FecX2* locus indicating imprinting of an X-linked gene and the reported segregation patterns in the Belclare breed, the possibility of an imprinted gene should be considered.

Mechanisms controlling follicle selection and ovulation rate

The search for genes in the pathway controlling ovulation rate in sheep has been very productive. Identifying individual genes has contributed significantly to our understanding and changed our view of inherited variation for this important phenotype. Chromosome locations are known for three genes. Two of these are on the X chromosome, with one gene subject to imprinting. The discovery of new loci, combined with complex patterns of inheritance and the difficulty of diagnosis based on ovulation rate records, indicates that genes of moderate-to-large effect may be common in different sheep populations.

The genes all act at different points along the pathway of follicular development and once follicles have left the primordial follicle pool. The *FecX^l/BMP15* knockout acts early in the growth phase (type 2 follicles; Fig. 1). Failure of the BMP15 signal from the early developing oocyte in *ll* ewes means that granulosa cells fail to divide and support the oocyte (Braw-Tal *et al.*, 1993; McNatty *et al.*, 1995b). Consequently, the oocyte enlarges, but then degenerates. The gene or genes causing infertility in the Cambridge and Belclare breeds appear to act later than the *FecX^l/BMP15* knockout mutation since small growing follicles are seen in the ovaries of these sheep (Hanrahan, 1991; Webb *et al.*, 1998). Alternatively, the gene acts at a similar stage, but the mutation responsible is less severe than the BMP15 knockout so some follicles can progress further. Segregation data indicate that the gene is inherited as an autosomal recessive (Webb *et al.*, 1998; Reynaud *et al.*, 1999) and is therefore not *BMP15*. Given the similarity in the phenotype, the closely related growth factor GDF9 (McGrath *et al.*, 1995) is a candidate gene for the infertility observed in Cambridge sheep.

Inactivation of one copy of BMP15 in heterozygous (*I+*) carriers of the *FecX^l* results in increased ovulation rate from a larger pool of antral follicles with granulosa cells responsive to LH (Shackell *et al.*, 1993). Reduced concentrations of active BMP15 may reduce the number of mitotic divisions in the granulosa cells and lead to a reduced amount of steroid and inhibin release by each follicle, thereby delaying the suppressive effects on plasma FSH and allowing selection of additional follicles. Another possibility is that reduced concentrations of BMP15 affect the actions of other growth factors from the oocyte on proliferation and differentiation of granulosa cells. Further work with the heterozygous BMP15 knockout should help

to determine the interactions of BMP15 with other growth factors and how this mutation affects ovulation rate.

The *FecB* locus acts later in follicular development. Functional differences among genotypes are apparent in ovarian follicles near the time of antrum formation (type 4–5 follicles; Fig. 1). It is still not known why ovarian follicles in *B+* and *BB* ewes undergo one fewer doubling of their population of granulosa cells than those in *++* ewes (that is, 17 versus 18 doublings; Fig. 2). It may be that just before or near the time of antrum formation, more granulosa cells in follicles in *BB* ewes are induced to enter a differentiation pathway rather than to undergo another round of mitosis. The data also support an effect of the *FecB* gene/mutation at the pituitary gland. The reason for variation in FSH differences among studies is not known, but could relate to genetic background. Mutations in the FSH receptor can result in higher concentrations of FSH and reduced response to exogenous FSH administration (Perez Mayorga *et al.*, 2000), although FSHR is excluded as the site for the *FecB* mutation (Montgomery *et al.*, 1995). It is likely that the *FecB* gene product is expressed in the ovary and the pituitary gland, or ovarian expression indirectly influences circulating FSH by some mechanism yet to be determined.

In the only study comparing the effects of *FecB* and *FecX* alone and in combination, Davis *et al.* (1999) concluded that the effect of both genes on ovulation rate was multiplicative by demonstrating no suppressive effects of either locus. The effect of *FecB* in either the presence or absence of *FecX* was to increase ovulation rate by about 90% and the effect of *FecX* in the presence or absence of *FecB* was to increase ovulation rate by 44%. These results contrast with previous studies of *FecB* (Piper *et al.*, 1985) that indicated that the effect of *FecB* on ovulation rate in flocks at different levels of prolificacy was additive rather than multiplicative. The effect of the two genes in combination was 34% higher than expected from an additive model, indicating that there may be some complimentary gene action between the loci.

In carriers of the *FecB* locus, there are gene-specific differences in development that extend beyond the reproductive axis. There is evidence for a quantitative trait locus (QTL) with a significant effect on growth from birth to weaning 20 cM distal to the *FecB* locus (Walling *et al.*, 2000). Sheep inheriting the QTL allele on the same haplotype as the Booroola allele in the founding sires are, on average, 1.4 kg lighter at weaning. Therefore, gene-specific differences observed in fetal size may result from effects of a QTL linked to the *FecB* locus. Consequently, direct effects of the mutation or mutations affecting ovulation rate may be restricted to the developing ovary, delaying development at several stages, similar to effects of the *FecX* locus.

Although the different genes may act first at different stages of follicular development, increased ovulation rate is associated with reduced size of mature follicles and fewer granulosa cells. Thus, it appears that a common mechanism may be operating. BMP15 has been identified as a key gene

influencing ovulation rate. Characterization of the genes and mutations in other breeds and strains will show whether they form part of the transforming growth factor β (TGF β) signalling pathway or are part of other intersecting pathways that provide the control of ovulation rate and distinct species variation in litter size. Understanding some of the key molecular events may also help us to understand the mechanisms for effects of age, nutrition and season on ovulation rate.

Conclusions

In summary, genes influencing ovulation rate in sheep act principally in the ovary, resulting in delays in ovarian development during fetal life and different patterns of follicular development in adults. There is evidence that there are multiple loci in different sheep populations and further segregation and genetic marker studies should be carried out to characterize the nature of genetic variation in the Cambridge, Belclare and other breeds. These studies will help to clarify the number of loci and the relationships (if any) to *FecB* and *FecX* loci. There are some similarities in the gene effects on follicular development leading to increased ovulation rate across the different genotypes, possibly acting through the same pathway as the BMP15 mutations in heterozygous *FecX* carriers. The discovery of the first mutations to alter ovulation rate directly provides a key to the pathways in the ovary that control follicular development and ovulation rate. Genes affecting ovulation rate in the other breeds may provide clues to additional players in the BMP15 pathway, or uncover alternative mechanisms. There remains much to learn about follicular development and the control of litter size from genetic models in sheep.

Note added in proof

Since writing this review, it has been shown that sheep carrying the *FecB* locus have a mutation (Q249R) in the intracellular kinase domain of bone morphogenetic protein 1B receptor (BMPR1B; Wilson *et al.*, 2001). This is the second mutation in a gene from the TGF β signalling pathway influencing ovulation rate in sheep. BMPR1B is expressed in the oocyte and granulosa cells in the ovary, and in other tissues including the pituitary gland.

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