Genes controlling ovulation rate in sheep

Grant W. Montgomery1, Susan M. Galloway2, George H. Davis3 and Kenneth P. McNatty4

1Genetic Epidemiology Laboratory, Queensland Institute of Medical Research and Joint Genetics Program, The University of Queensland, Brisbane, Queensland, Australia; 2AgResearch Molecular Biology Unit, Department of Biochemistry, University of Otago, Dunedin, New Zealand; 3Invermay Agricultural Centre, Mosgiel, New Zealand; and 4AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand

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.

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, FecXI) 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

Email: grantM@qimr.edu.au

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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 (Johnsdottir and Adalsteinsson, 1985), Javanese (Bradford et al., 1986), Olksclas (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+B 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...
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) 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...
from 11 lamblings 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 (FecXI). 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 (FecXH), 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 FecXI with FecXH animals produces FecXI/FecXH infertile females with streak ovaries indistinguishable from FecXI/FecXI 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 FecXI in females during fetal development.** No effects of FecXI 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.

Germin 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 FecXI 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 follicle 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

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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 McM53, OarJL1A, OarHH55, BM143, and OarJMP36.
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 XIST (inactive X-specific transcript). Sheep and cattle microsatellite markers are ILSTS0I7, McMM551, OarAE25, OarAE133, OarMP1, TGLA54 and TGLA68.

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 ATPA7A (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 E1α), PHKA1 (phosphorylase kinase α-1), TIMP1 (tissue inhibitor of metalloproteinase 1) and XIST (inactive X-specific transcript). Sheep and cattle microsatellite markers are ILSTS0I7, McM551, OarAE25, OarAE133, OarMP1, TGLA54 and TGLA68.

**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 ATPA7A (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 E1α), PHKA1 (phosphorylase kinase α-1), TIMP1 (tissue inhibitor of metalloproteinase 1) and XIST (inactive X-specific transcript). Sheep and cattle microsatellite markers are ILSTS0I7, McM551, OarAE25, OarAE133, OarMP1, TGLA54 and TGLA68.

**FecX locus**

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**I+ phenotype**
- Multiple ovulation
- More antral follicles than wild-type
- Follicles mature at a smaller size
- Have fewer granulosa cells
- Granulosa cells acquire LH receptors earlier

**II phenotype**
- Streak ovaries
- Follicles fail to grow normally beyond primary stage (i.e. Type 2)

**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 ATPA7A (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 E1α), PHKA1 (phosphorylase kinase α-1), TIMP1 (tissue inhibitor of metalloproteinase 1) and XIST (inactive X-specific transcript). Sheep and cattle microsatellite markers are ILSTS0I7, McM551, OarAE25, OarAE133, OarMP1, TGLA54 and TGLA68.
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 FecX 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 Lleyn breed and, since infertile ewes have been reported in the Lleyn 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/BMP15 knockout acts early in the growth phase (type 2 follicles; Fig. 1). Failure of the BMP15 signal from the early developing oocyte in II 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/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 (+) carriers of the FecX 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 inhibit 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 (TGFβ) signalling pathway or are part of other interacting 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 TGFβ 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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