Progress on major genes for high fecundity in ewes

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Abstract The existence of major genes affecting fecundity in sheep flocks throughout the world has been demonstrated. Three major genes whose mutations can increase ovulation rate have been discovered, and all related to the transforming growth factor β (TGF-β) superfamily. The mutant FecB of bone morphogenetic protein receptor 1B (BMPR1B) has an additive effect on ovulation rate. Six mutations (FecXα, FecXβ, FecXγ, FecXδ, FecXε, FecXζ) of bone morphogenetic protein 15 (BMP15) related with fertility have been identified that share the same mechanism. All the mutants can increase ovulation rate in heterozygotes and cause complete sterility in homozygotes. Homozygous ewes with two new mutations (FecXγr, FecXζr) of BMP15 had increased ovulation rate without causing sterility. There are five mutations in growth differentiation factor 9 (GDF9) associated with sheep prolificacy where FecGE and FecGF have additive an effect on ovulation rate and litter size. The newly identified β-1,4-N-acetylgalactosaminyltransferase 2 (B4GALNT2) gene of FecL is proposed as a new mechanism of ovulation rate regulation in sheep. Woodlands is an X-linked maternally imprinted gene which increases ovulation rate. In addition, several putative major genes need to be verified. This review is focused on the discovery of the major genes and putative major mutations affecting ovine ovulation rate and how these discoveries have provided new insights into control of ovarian function.

Keywords major gene, ovulation rate, sheep, reproduction

1 Introduction

Maintenance of high levels of ovulation rate and appropriate levels of fecundity are critical for sheep production. However, genetic improvement of traits associated with reproduction (such as ovulation rate, litter size and reproductive seasonality) in sheep is challenging because these traits have low heritability, are generally not expressed until puberty, and are normally recorded only in females [1]. Fortunately, in sheep, a large range in litter size has been observed between and within breeds. These sheep breeds provide opportunities for discovery of genes that underpin improved reproductive success. Genetic studies in sheep have indicated that the ovulation rate and litter size can be regulated by the action of single genes with major effects called fecundity (Fec) genes [2]. To date, three of these have been shown to belong to the transforming growth factor β (TGF-β) pathway and another newly discovered candidate beyond the TGF-β superfamily, has been identified as having mutations that lead to alterations of ovulation in sheep. In addition, multiple putative major genes affecting ovulation rate in sheep have been identified in several high prolificacy sheep lines. This review is focused on the discovery of the major genes and putative major mutations affecting ovine ovulation rate and how these discoveries have provided new insights into control of ovarian function.

2 Major genes with known mutations

2.1 BMPRIB and the mutation, FecB

In 1980, Piper and Bindon reported that the exceptional fecundity of the Booroola Merino may in part result from the action of a single major gene affecting ovulation rate according to their analysis of litter size records from Booroola Merino sheep [3]. In 1982, Davis et al. found that there was an autosomal mutation present in prolific Booroola sheep causing high ovulation of ewes [4]. The gene dosage effect of the mutation is additive for ovulation rate with an increase of 1.65 for each copy [5]. In 1989, the Sheep and Goat Genetic Nomenclature Committee assigned it FecB (Fecundity Booroola). By 2001, three groups found that the mutation is located in the BMPRIB gene [6–8]. This point mutation, which can significantly increase the ovulation rate of ewes [8,9], is at base 746 of the coding region (746 A → G) in the highly conserved intracellular kinase signaling domain of the BMPRIB
which causes a glutamine to arginine amino acid substitution [7].

BMPR1B, which belongs to the TGF-β receptor family, takes part in signal transduction of many factors, such as BMP15, BMP2, BMP4, BMP6, BMP7, growth differentiation factor 5 (GDF5) and Mullerian inhibiting substance/anti-Mullerian hormone (MIS/AMH) [10]. It mainly occurs in ovaries of sheep [11], but is also widely expressed in other tissues (ear, spinal cord, pituitary, bone, uterus, hypothalamus, kidney, skeletal muscle and fallopian tubes) [12] and is important in follicle development [13]. BMPR1B knockout mice were found to be infertile due to defects in the cumulus expansion and fertilization [14].

The FecB mutation is found in various sheep breeds, such as Booroola Merino sheep (Australia) [6–8], Garole sheep (India) [15,16], Javanese sheep (Indonesia) [15], Kendrapada sheep (India) [17], Bonpala sheep (India) [18], Kalekkoohi sheep (Iran) [19], Small Tail Han sheep (China) [20–28], Hu sheep (China) [20,23,28–31], Duolang (China) [32], Cele (China) [33], Wadi sheep (China) [34], Altay sheep (China) [35], and Bayanbulak sheep (China) [36].

Although the FecB gene has been widely used for sheep breeding improvement, it remains unclear how this mutation affects the receptor to cause changes in ovarian function. Although it has been reported that homozygous ewes have higher plasma FSH concentration, more granulosa cells and smaller diameter preovulatory follicles ewes have higher plasma FSH concentration, more granulosa cells and smaller diameter preovulatory follicles [57]. Further research found that FecXG, FecXH and FecXI have similar characteristics to FecX. This indicated that all four mutations may have the same action mechanism [41,58]. It was considered that granulosa cells in heterozygous ewes with mutations in BMP15 may develop an earlier responsiveness to LH for most of the follicles, increasing ovulation rate in sheep [59].

In general, heterozygous ewes with mutations in both BMP15 and GDF9 exhibited higher fertility than those having a mutation in only one of these genes. Liao et al. found that when mutant GDF9 was co-expressed with mutant BMP15, the secretion levels of both proteins were significantly lower than those of cells co-expressing wild-type GDF9 and mutant BMP15, suggesting a possible mechanism for the extreme fertility observed in the compound heterozygous mutant sheep [60].

Recently, two novel non-conservative mutations of BMP15 called FecXGr and FecXG, which lead to hyperproliferation, have been identified in the French Grivette and the Polish Olkuska breeds through genome-wide association studies. It is noteworthy that the homozygous ewes of these mutations also had an increased ovulation rate without becoming sterile [45]. Their findings suggest an additional role of the BMP15 protein in ovarian folliculogenesis.

2.2 BMP15 and the mutations FecXg, FecXG, FecXI, FecXh, FecXa, FecXb and FecXo

BMP15, a member of the TGF-β superfamily located on the X chromosome, has a 1182 bp CDS mainly present in the ovaries [11]. To date, six mutations (FecX, FecXG, FecXI, FecXh, FecXb and FecXo) related with fertility have been identified in sheep, as shown in Table 1. These included three substituted mutations, two non-sense mutations, and one deletion mutation. All of these spontaneous mutations increased the ovulation rate in heterozygotes and caused complete sterility in homozygotes due to primary ovarian failure [40–44]. The effects of these mutations in various sheep are summarized in Table 1.

Biological activities of BMP15 on prolificacy in sheep have been reported. First, granulosa cell proliferation and mitosis can be stimulated by the expression of BMP15. Secondly, BMP15 stimulates the expression of granulosa cells kit-ligand that is required for the early development of the follicle. Thirdly, BMP15 can inhibit FSH receptor expression, as a result, the FSH-induced expression of steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage enzyme (P450scc), 3β-hydroxy-steroid dehydrogenase (3β-HSD), LH receptor, and inhibin/activin subunits (α, βA, and βB) were all inhibited by BMP15 [10]. Finally, BMP15 and GDF9 proteins showed synergistic action in the process of oocyte maturation, ovarian cumulus expansion and ovulation due to the similar structure of the heterodimer formed when they are co-expressed.

In the Inverdale ewes, compared with wild-type ewes, FecX heterozygotes have some distinctive characteristics such as more differentiated follicles in the ovary, fewer granulosa cell in these follicles, increased sensitivity of granulosa cells to LH in the early stage of follicle and smaller corpus luteum [57]. Further research found that FecXG, FecXH and FecXI have similar characteristics to FecX. This indicated that all four mutations may have the same action mechanism [41,58]. It was considered that granulosa cells in heterozygous ewes with mutations in BMP15 may develop an earlier responsiveness to LH for most of the follicles, increasing ovulation rate in sheep [59].

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2.3 GDF9 and the FecG mutants

Studies on mice showed that GDF9, a member of the TGF-β superfamily, is required for ovarian folliculogenesis [61]. GDF9, being an oocyte-specific expressed gene, is essential for normal follicular development from the early stage in sheep and has been mapped on ovine chromosome 5 [62,63]. After these findings were published, mutations in GDF9 were identified in some high-
fecundity sheep breeds around the world [64]. To date, the five mutations in GDF9, High Fertility (FecGH) [41], Thoka (FecGT) [46], Embrapa (FecGE) [47], G7 (FecGF) [49,50] and Vacaria (FecGV) [51], which introduce a non-conservative amino acid change, have been reported to be associated with the sheep prolificacy (Table 1). The peculiarity of the polymorphisms in GDF9 is that FecGE and FecGF have additive effects on ovulation rate and litter size, whereas FecGH, FecGT and FecGV cause increased ovulation rate and litter size in the heterozygotes and sterility in the homozygous carriers [51].

The mutation FecGH, which introduces serine to phenylalanine change at residue 395, was first identified and associated with sterility or increased ovulation rate in the Belclare and Cambridge sheep breeds. It was thought to disrupt the interaction with the type 1 TGF-β family [65]. One copy of FecGH was estimated to increase the ovulation rate by 1.4 in the Belclare and Cambridge breeds [2].

FecGT, which was characterized in Icelandic Thoka sheep, has a single base change (A1279C) resulting in a non-conservative amino acid change (S109R) in the C terminus of the mature GDF9 protein, predicted to disrupt binding to the type 2 receptor [46,65].

The mutant found in the Brazilian Santa Ines sheep is called FecGE. This mutation leads to a substitution of a phenylalanine with a cysteine in the mature peptide, which was predicted to be involved in dimer formation. The ewes of the FecGE allele homozygote carriers showed an increase in their ovulation rate (82%) and prolificacy (58%) and the heterozygous ewes had no change in ovulation rate compared to wild-types [47,48,65].

Recently, a point mutation (c.943C>T) of GDF9 was identified in Brazilian Ile de France ewes, resulting in an amino acid change (p.Arg315Cys) in the cleavage site of Table 1 Known mutations of major genes and the effects or their encoded proteins on ovulation rate of ewes heterozygous and homozygous for these mutations

| Gene | Line/breed | Allele | Coding residue | Chromosome | Change in ovulation rate 1 copy | Change in ovulation rate 2 copy | Change in litter size 1 copy | Change in litter size 2 copy | Reference |
|------|------------|--------|----------------|------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------|
| BMP1B | Hu, Small Tail Han, Booroola, Garole, Javanese | Booroola (FecB) | Q249R | 6 | +1.50 | +3.00 | +1.00 | +1.50 | [6–8] |
| | Romney, Inverdale | Inverdale (FecXc) | V299D | X | +1.00 | POF | +0.60 | POF | [40] |
| | Romney, Hanna | Hanna (FecXf) | Q291Ter | X | +1.00 | POF | +0.60 | POF | [40] |
| | Belclare | Belclare (FecXe) | S367I | X | +1.00 | POF | / | POF | [41] |
| | Belclare, Cambridge | Galway (FecXg) | Q239Ter | X | +0.70 | POF | / | POF | [41] |
| | Lacaune | Lacaune (FecXh) | C321Y | X | +1.50 | POF | / | POF | [42] |
| | Rasa Aragonesa | Rasa (FecXa) | 154-159delWVQKSP | X | / | POF | +1.30 | POF | [43,44] |
| | Grivette | FecXGr | T317I | X | 0.41 | 2.05 | / | / | [45] |
| | Olkuska | FecXO | N337H | X | / | / | 0.62 | 1.21 | [45] |
| | Belclare, Cambridge | High Fertility (FecXh) | S395F | 5 | +1.40 | POF | / | POF | [41] |
| | Icelandic Thoka | Thoka (FecXg) | S427R | 5 | +1.20 | POF | +0.70 | POF | [46] |
| | Embrapa | Embrapa (FecXe) | F345C | 5 | +0 | +0.82 | +0 | +0.58 | [47,48] |
| | Finnish Landrace, Belclare, Norwegian White Sheep | FecXe | V371M | 5 | +0.17 | / | / | / | [49,50] |
| | Ile-de-France sheep | FecXe | R315C | 5 | / | POF | / | POF | [51] |
| | Woodlands | Coopworth (FecXa) | / | X | +0.39 | ≥W+ | +0.25 | ≥W+ | [2,52] |
| | B4GALNT2 | Lacaune | FecL | / | 11 | +1.50 | +3.00 | 0.50 | / | [53,54] |
| | Otlkuska | FecW | / | / | / | +1.00 | / | +0.60 | / | [2,55] |
| | BELLE-ILE | Belle-Ile | / | / | / | / | / | / | [2] |
| | Unknown | Romeny | FecW | / | / | +0.80–1.00 | / | / | [56] |

Note: POF, primary ovarian failure; /, no or unclear.

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the propeptide called FecG\(^i\) [49]. The SNP (c.1111G \(>\) A), responsible for a Val to Met substitution at position 371, which has previously been identified and not found to be associated with fertility in Belclare and Cambridge sheep, was shown to have strong association with litter size in Norwegian White Sheep [51].

### 2.4 FecL mutant

During the first 20 years of its operation the artificial insemination co-operative (OVI-TEST) implemented an on-farm selection scheme in France and the selection response for prolificacy in Lacaune sheep breed has been significant since 1975 [66]. In 1998, a major fecundity gene FecL, which genetically determined large variation in litter size, was initially described in the meat strain of the French Lacaune sheep breed [53]. In a previous study, FecL had been identified to be an autosomal major gene by a statistical approach and had been localized on sheep chromosome 11 (OAR11) by a full genome scan. Recently, fine mapping research reduced the interval between two SNP markers on OAR11 g.36910171T \(>\) C and g.37107627G \(>\) C. This interval was estimated at 197 kb based on ovine genome OARv3.1 and it was proposed that B4GALNT2, encoding the glycosylation enzyme β-1,4-N-acetyl-galactosaminyltransferase 2, was the best positional and expression candidate for FecL. Within this interval of localization, SNP g.36938224T \(>\) A and SNP g.37034573A \(>\) G have been found to be fully associated with the FecL\(^i\) mutation. These two SNPs, which were non-coding SNPs close or within the B4GALNT2 gene, caused ectopic expression in the ovarian follicles and overexpression of this protein in granulosa cells induced atypical glycosylation of follicular target proteins in granulosa cells [54,67]. Therefore, the fecundity gene FecL in sheep affected the ovulation rate in a different way compared to the TGF-B/BMP signaling genes. In the Lacaune meat breed population, the influence of FecL\(^i\) on inheritance of ovulation rate is additive as one copy increases ovulation rate (ova) by approximately 1.5 ova, and litter size by 0.5 lambs compared to the wild-type allele [53].

### 3 Inheritable major gene with unknown mutation (Woodlands)

Davis et al. studied the inheritance of ovulation rate which was recorded from a screened high prolificacy Coopworth sheep flock [52]. Breeding values for ovulation rate suggested that a major gene (Woodlands gene) for ovulation rate with a non-Mendelian inheritance pattern was segregating in this family line. Ovulation rate data indicated that it was expressed where females inherited a paternal allele but was silenced when they inherited a maternal allele. Also it was unlike either Inverdale or Hanna, thus this gene is on the X chromosome which is maternally imprinted. The locus FecX2 and the allele symbol FecX2\(^w\) have been assigned to this mutation. This was the first reported imprinted gene that can affect ovulation rate in sheep [2]. The increase in ovulation rate observed in the heterozygous sheep with the Woodlands mutation was 0.39. One copy of the Woodlands gene increases litter size by about 0.25 extra lambs per lambing ewe while the effect on litter size when homzygous has not been determined [2]. In 2002, Davis and Juengel et al. also identified another line of sheep (Metherell line) with the same inheritance pattern as the Woodlands gene [68]. At present, the mechanism by which the Woodlands gene is affecting ovulation rate is unknown [65]. The inheritance pattern of the Woodlands gene appears not to be that of the previously described mutations in BMP15 or GDF9. It is expected that further studies may reveal a new pathway, other than the TGF-β family, that can control ovulation rate in sheep.

### 4 Putative major genes need to be verified

#### 4.1 Olkuska

About 100 years ago, Olkuska sheep were bred by crossing long-wool Polish sheep with Friesian, Pomeranian, and Holstein sheep. In 1991, on the basis of ovulation rate records, Martyniuk and Radomska assigned Olkuska ewes a genotype and proposed that a major gene for prolificacy may exist in the Olkuska sheep, which is similar to the Booroola gene. They found that at least one record of ovulation rate of heterozygous ewes was ≥3, while it was ≥5 in homozygous ewes. They also estimated that one ewe may ovulate one extra egg per copy of the putative gene [2]. However, no significant relationships between the putative Olkuska genotype and blood protein polymorphisms were found by previous study. In 2002, from the result of DNA tests of a highly prolific Olkuska ewe, Davis et al. declared that there was no FecB mutant like BMPR1B in Booroola sheep and no FecX\(^i\) mutation like BMP15 in Inverdale sheep or in Olkuska sheep [15,65]. Progress in elucidating the inheritance of prolificacy in Olkuska sheep was slow. The putative major gene is still unknown. One of the reasons for this is that the Olkuska is an endangered breed with small population and flock sizes. This study reported that there were only 58 registered ewes in five flocks in 2000, and the endangered status of Olkuska was listed as “critical-maintained” [2]. Nevertheless, it would be potentially useful to identify the putative major gene in Olkuska.

#### 4.2 Belle-Ile

Belle-Ile sheep in France have high average ovulation rate (2.54) and high average litter size (2.23). The variation in
4.3 NZ Longwool

Davis et al. found the evidence of segregating major genes in commercial flocks of Perendale, Romney and Border Leicester × Romney sheep in New Zealand. In these flocks the Booroola BMP1B mutation was not found by DNA testing. Although it was found that one flock of Border Leicester × Romney had the Inverdale BMP15 mutation (FecX), this does not explain all the highly prolific sheep in these flocks. From the pedigree information, the imprinted Woodlands FecX2 prolificacy gene is not present in any of these flocks [55]. In 2006, Davis et al. measured the ovulation rates in 547 Romney ewes with high prolificacy. They suggested that a segregating major gene (FecW) was increasing prolificacy of this flock. One copy of the putative gene increased ovulation rate by 0.8–1.0 eggs per ovulating ewe, which showed autosomal inheritance [56]. Further research on the mode of inheritance of these genes and the genetic basis of the prolificacy in these flocks is needed.

5 Gene interaction effects between mutants

Ewes carrying mutations in more than one major gene have also been identified and the combination of mutants has been found to produce synergistic effects, with higher ovulation rates than in animals with a single mutation.

Interactions between different mutations in the BMP15 gene can lead to sterility. The crossbred females with FecX and FecX mutations had stripy ovaes and were sterile [58]. Hanrhan et al. reported that the crossbred Beclare females with FecX and FecX mutations also had stripy and sterile ovaries [41].

The crossbred females with FecX and FecB mutations exhibited higher ovulation rate than wild-types or those with an individual mutation [2,27]. The combined effect of FecX and FecB appeared to be multiplicative, suggesting that there is a functional interaction between BMP15 and BMP1B [69,70]. Interactions among these genes, and other mutations yet to be identified, have also been demonstrated. Ewes that carry mutations in both the BMP1B and BMP15 genes, as well as the Woodlands allele, have very high ovulation rates, and the effects of the genes appear synergistic [55,71]. Analysis of another putative gene, the Davisdale, segregated in the AgResearch Fertility flock, also indicates an additive effect with the Inverdale mutation [72].

One copy of the BMP15 mutation together with one copy of the GDF9 mutation had an additive effect on ovulation rate [2,41,73,74]. Active immunisation of ewes with BMP15 and/or GDF9 peptides affected ovarian follicular development and ovulation rate [75]. Moreover, the analysis of GDF9 and BMP15 in oocyte lysate suggested that GDF9 and BMP15 may be secreted as a complex mix of forms by follicular fluid and from cell lines expressing GDF9 or BMP15 [60,75–78]. Lacaune ewes carrying mutations in both FcL and FecX loci had a greater ovulation rate than those with either mutation alone [42,66,79].

6 Genetic mutations and their association with high prolificacy in sheep native to China

China has a long history of sheep breeding and there are 42 native sheep breeds reared in China [80]. Most of these have only single births, while some of them have high prolificacy, for example, Small Tail Han (STH) sheep and Hu sheep [81].

It has been shown that the FecB mutation, found in Booroola Merino ewes, occurs in many Chinese sheep breeds. The procedure to identify the ovine fecundity gene FecB has now been established in China [82]. Chinese farmers gain considerable benefit from improving flock prolificacy or breeding prolific strains through cross-breeding. FecB can be used to breed prolific sheep, for example, the Chinese Merino prolific strain and STH super high prolificacy strain. Since the 1990s, Hu sheep germplasm was introduced into Chinese Merino (Xinjiang type) though reciprocal crossing and phenotype selection [83]. Now, STH sheep have been widely introduced into several sheep breeds to breed prolific strains or to improve the fecundity of different breeds for mutton or wool production in China.

Our group also found that STH sheep carry the same FecX mutation of the BMP15 gene as do Belclare and Cambridge ewes [84–87]. Ewes with the heterozygous mutant had 0.55 (P < 0.01) more lambs than wild-type ewes. After comprehensive analysis, the STH ewes carrying both mutations in BMP1B and BMP15 genes had greater litter size than those with either mutation alone [27].
In addition, one novel single nucleotide mutation (G729T) in exon 2 of the GDF9 gene in the CD genotype that resulted in an amino acid change (Gln243His) was identified in STH sheep. The ewes with heterozygous mutant CD genotype had 0.77 ($P < 0.01$) more lambs than those with wild-type CC genotype [86,88].

7 Conclusions and future directions

Implementation of effective programs of reproductive management involving synchronization of genetic potentials for reproductive ability with the production environment could maximize profitability of sheep production [1]. The use of selected prolific ewes with genetic mutation affecting ovulation rate has proven to be an exceptional tool to detect major genes. The incorporation of a major gene for prolificacy into flocks by using marker-assisted selection (MAS) could substantially enhance selection pressure on other traits leading to genetic improvement. As shown above, the TGF-β superfamily now represents a key system in the control of ovulation rate and ovarian folliculogenesis in sheep. Also B4GALNT2 being the FecL fecundity gene opens new lines of investigation regarding ovarian glycosylation. However, there is still considerable knowledge required to be gained regarding the regulation and function of these identified major genes.

This includes, but is not limited to, a better understanding of the mechanism of BMPR1B in ovulation. As stated above, neither physiology nor protein interaction can illustrate how FecB mutation affects the receptors to cause changes in ovulation. Researchers did not observe similar effects when FecB was introduced into the mouse BMPR1B gene, suggesting this mutation has a species-specific function [89]. Thus a more appropriate animal model is needed to perform functional analysis. In addition, due to extreme difficulties in obtaining the crystal structure, the effect of the Booroola mutation on the kinase activity of ovine BMPR1B has not been verified [65]. Currently it is likely that more information on BMPR1B and related pathways can be obtained by the help of high-seq technologies with elaborate design.

GDF9 and BMP15 may be secreted as a complex mix of forms to regulate ovarian follicular development [76,78]. Unfortunately, the biological activity of the complex formed from GDF9 and BMP15 and the interaction with receptors are all unclear. The close interactions between GDF9 and BMP15, therefore, are likely to have a critical biological impact that should be taken into account in future studies [90].

Several approaches and new methods can be used to study the high prolificacy trait in sheep native to China. Most previous studies carried out by researchers regarding to Chinese native sheep breeds were by genetic association analysis at the single gene level. There was no genome wide association study (GWAS) performed based on pedigree records. Thus GWAS conducted in sheep breeds with different prolificacy is needed. Also, knockout sheep models will be vital for future understanding of how oocyte-derived growth factors regulate ovarian functions.

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References

1. Notter D R. Genetic aspects of reproduction in sheep. Reproduction in Domestic Animals, 2008, 43(Suppl 2): 122–128
2. Davis G H. Major genes affecting ovulation rate in sheep. Genetics Selection Evolution, 2005, 37(Suppl 1): S11–S23
3. Piper L R, Bindon B M. The Booroola Merino and the performance of medium non-Booroola crosses at Armidale. In: Piper LR, Bindon BM, Nethery RD, eds. The Booroola Merino. Melbourne, Australia: CSIRO, 1982: 9–20
4. Davis G H, Montgomery G W, Allison A J, Kelly R W, Bray A R. Segregation of a major gene influencing fecundity in progeny of Booroola sheep. New Zealand Journal of Agricultural Research, 1982, 25(4): 525–529
5. Piper L R, Bindon B M, Davis G H. Single gene inheritance of the high litter size of the Booroola Merino. In: Land RB, Robinson DW, eds. Genetics of Reproduction in Sheep. London: Butterworths, 1985: 115–125
6. Mulsant P, Lecerf F, Schibler L, Monget P, Lanneluc I, Pisselet C, Riquet J, Monniaux D, Callebaut I, Criibi E, Thimonier J, Teyssier J, Bodin L, Cognie Y, Chitour N, Elsen J M. Mutation in the bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceedings of the National Academy of Sciences of the United States of America, 2001, 98(9): 5104–5109
7. Wilson T, Wu X Y, Juengel J L, Ross I K, Lumsden J M, Lord E A, Dodds K G, Walling G A, McEwan J C, O’Connell A R, McNatty K P, Montgomery G W. Highly prolific Booroola sheep have a mutation in the intracellulare kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biology of Reproduction, 2001, 64(4): 1225–1235
8. Souza C J H, MacDougall C, Campbell B K, McNeilly A S, Baird D T. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. Journal of Endocrinology, 2001, 169(2): R1–R6
9. Fabre S, Pierre A, Pisselet C, Mulsant P, Lecerf F, Pohl J, Monget P, Monniaux D. The Booroola mutation in sheep is associated with an alteration of the bone morphogenetic protein receptor-IB function-
10. Shimasaki S, Moore R K, Otsuka F, Erickson G F. The bone morphogenetic protein system in mammalian reproduction. *Endocrine Reviews*, 2004, 25(1): 72–101

11. Feary E S, Juengel J L, Smith P, French M C, O’Connell A R, Lawrence S B, Galloway S M, Davis G H, McNatty K P. Patterns of expression of messenger RNAs encoding GDF9, BMP15, TGFBR1, BMPR1B, and BMPR2 during folliculogenesis and characterization of ovarian follicular populations in ewes carrying the Woodlands FecX2W mutation. *Biological Reproduction*, 2007, 77 (6): 990–998

12. Yang H, Liu S R, Zhong F G, Yang Y L, Zhang Y S. Different expression of BMPR-IB in tissues of sheep. *Chinese Journal of Animal Science*, 2009, 45(11): 6–8 (in Chinese)

13. Silva J R, van den Hurk R, van Tol H T, Roelen B A, Figueiredo J R. Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. *Molecular Reproduction and Development*, 2005, 70(1): 11–19

14. Yi S E, LaPolt P S, Yoon B S, Chen Y Y, Lu J K, Lyons K M. The type I BMP receptor BMPR1B is essential for female reproductive function. *Proceedings of the National Academy of Sciences of the United States of America*, 2001, 98(14): 7994–7999

15. Davis G H, Galloway S M, Ross I K, Gregan S M, Ward J, Nimbkar C, Gray G D, Nimbkar C, Ghalsasi P M, Nimbkar A, Batabyal S, Pan S, Brahma B, Tiesnamurti B, Martyniuk E, Eythorsdottir E, Mulsant P, Lecerf F, Arora J S, Pan S, Samanta A K, Datta T K, Goswami S L. Polymorphism of fecundity genes (*BMPR-IB* and *BMP-15*) mutations in Chinese sheep using high resolution melting analysis. *Chinese Journal of Animal Science*, 2011, 8(6): 295–301

16. Jia C L, Li N, Zhao X B, Zhu X P, Jia Z H. Association of single nucleotide polymorphisms in exon 6 region of BMPR-IB gene with litter size traits in sheep. *Asian-Australasian Journal of Animal Sciences*, 2005, 18(10): 1375–1378

17. Yue Y J, Yang B H, Liang X, Liu J B, Jiao S, Guo J, Sun X P, Niu C E, Feng R L. Simultaneous identification of FecB and FecX<sup>G</sup> mutations in Chinese sheep using high resolution melting analysis. *Journal of Applied Animal Research*, 2011, 39(2): 164–168

18. Yan Y D, Chu M X, Zeng Y Q, Fang L, Ye S C, Wang L M, Guo Q K, Han D Q, Zhang Z X, Wang X J, Zhang X Z. Study on bone morphogenetic protein receptor IB as a candidate gene for prolificacy in Small Tailed Han sheep and Hu sheep. *Journal of Agricultural Biotechnology*, 2005, 13(1): 66–71 (in Chinese)

19. Yin Z H, Jiang Y L, Fan X Z, Wang Y, Tang H, Yue Y S. Polymorphism, effect and linkage analysis of BMPR-IB gene in Small Tailed Han sheep. *Acta Veterinaria et Zootechnica Sinica*, 2006, 37(5): 510–513 (in Chinese)

20. Fan Q C, Ye L L, Liu N, Cai Z F, Wang X Y, Qu X X, Yuan P X. Research of the relevance between BMPR-IB genotypes and litter size in Small Tailed Han sheep. *Anhui Agricultural Sciences*, 2011, 39(20): 12216–12218 (in Chinese)

21. Liu S F, Jiang Y L, Du L X. Studies of BMPR-IB and BMP15 as candidate genes for fecundity in Little Tailed Han sheep. *Acta Genetica Sinica*, 2003, 30(8): 755–760 (in Chinese)

22. Chu M X, Liu Z H, Jiao C L, He Y Q, Fang L, Ye S C, Chen G H, Wang J Y. Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (*Ovis aries*). *Journal of Animal Science*, 2007, 85(3): 598–603

23. Chu M, Jia L, Zhang Y, Jin M, Chen H, Fang L, Di R, Cao G, Feng T, Tang Q, Ma Y, Li K. Polymorphisms of coding region of BMPR-IB gene and their relationship with litter size in sheep. *Molecular Biology Reports*, 2011, 38(6): 4071–4076

24. Guan F, Liu S, Shi G, Ai J, Mao D, Yang L. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. *Acta Genetica Sinica*, 2006, 33(2): 117–124

25. Wang Q G, Zhong F G, Li H, Wang X H, Liu S R, Chen X J, Gan S Q. Detection of major gene on litter size in sheep. *Hereditas*, 2005, 27(1): 80–84 (in Chinese)

26. Wang G L, Mao X, Davis G H, Zhao Z S, Zhang L J, Zeng Y Q. DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (*FecB*) mutation. *Chinese Journal of Animal Science*, 2014, 5(1): 111–115

27. Yin Z H, Jiang Y L, Fan X Z, Wang Y, Tang H, Yue Y S. Polymorphism, effect and linkage analysis of BMPR-IB gene in Small Tailed Han sheep. *Acta Veterinaria et Zootechnica Sinica*, 2006, 37(5): 510–513 (in Chinese)

28. Chu M X, Liu Z H, Jiao C L, He Y Q, Fang L, Ye S C, Chen G H, Wang J Y. Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (*Ovis aries*). *Journal of Animal Science*, 2007, 85(3): 598–603

29. Chu M, Jia L, Zhang Y, Jin M, Chen H, Fang L, Di R, Cao G, Feng T, Tang Q, Ma Y, Li K. Polymorphisms of coding region of BMPR-IB gene and their relationship with litter size in sheep. *Molecular Biology Reports*, 2011, 38(6): 4071–4076

30. Guan F, Liu S, Shi G, Ai J, Mao D, Yang L. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. *Acta Genetica Sinica*, 2006, 33(2): 117–124

31. Wang Q G, Zhong F G, Li H, Wang X H, Liu S R, Chen X J, Gan S Q. Detection of major gene on litter size in sheep. *Hereditas*, 2005, 27(1): 80–84 (in Chinese)

32. Wang G L, Mao X, Davis G H, Zhao Z S, Zhang L J, Zeng Y Q. DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (*FecB*) mutation. *Journal of Nanjing Agricultural University*, 2003, 26(1): 104–106 (in Chinese)

33. Shi H C, Gao Z Y, Niu Z G, Bai J, Feng L J, Li H B, Jia B, Zhang Y. Detection of FecB mutation and its relationship with litter size in Xinjiang Duolang sheep(*Ovis aries*). *Journal of Agricultural Biotechnology*, 2011, 19(2): 330–334 (in Chinese)

34. Shi H C, Niu Z G, Bai J, Feng L J, Yang Q Y, Liu M J, Jia B. The research of BMPRIB gene mutations effect on litter size and genetic law in Cele black sheep. *Chinese Journal of Animal Science*, 2012, 48(3): 14–17 (in Chinese)

35. Li D, Sun W, Ni R, Zhang X N, Zhang Y L, Chu M X, Zhang Y F, Shen G Z, Chen L, Wu W Z, Zhou H, Han F, Xu H S. Genetic diversity of FecB gene and association analysis of its litter size. *Journal of Animal Science and Veterinary Medicine*, 2012, 31(2): 1–5 (in Chinese)

36. Tian X E, Sun H X, Wang Y J. Genetic polymorphism of BMPR-IB gene and effect on litter size in three sheep breeds. *Journal of Northwest A&F University*, 2009, 37(11):31–36 (in Chinese)
36. Zuo B, Qian H, Wang Z, Wang X, Nisa N, Bayier A, Ying S, Hu X, Gong C, Guo Z, Wang F. A study on BMPR-IB genes of Bayanbuluk sheep. *Asian-Australasian Journal of Animal Sciences*, 2013, 26(1): 36–42.

37. McNatty K P, Henderson K M. Gonadotrophins, fecundity genes and ovarian follicular function. *Journal of Steroid Biochemistry and Molecular Biology*, 1987, 27(1–3): 365–373.

38. Heath D A, Caldani M, McNatty K P. Relationships between the number of immunostaining gonadotropes and the plasma concentrations of gonadotrophins in ewes with and without the FecB<sup>B</sup> gene. *Journal of Reproduction and Fertility*, 1996, 106(1): 73–78.

39. Montgomery G W, Galloway S M, Davis G H, McNatty K P. Genes controlling ovulation rate in sheep. *Reproduction*, 2001, 121(6): 843–852.

40. Galloway S M, McNatty K P, Cambridge L M, Laitinen M P, Juengel J L, Jokiranta T S, McLaren R J, Liu K, Dodds K G, Montgomery G W, Beattie A E, Davis G H, Ritvos O. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics*, 2000, 25(3): 279–283.

41. McNatty J P, O’Connell A R, Everett-Hincks J M, Davis G H, Powell R, Galloway S M. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of Reproduction*, 2004, 70(4): 900–909.

42. Bodin L, Di Pasquale E, Fabre S, Bontoux M, Monget P, Persani L, Mulans P. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology*, 2007, 148(1): 393–400.

43. Martinez-Royo A, Jurado J J, Smulders J P, Marti J I, Alabart J L, Roche A, Fantova E, Bodin L, Mulans P, Serrano M, Folch J, Calvo J H. A deletion in the bone morphogenetic protein 15 gene causes sterility and increased prolificacy in Rasa Aragonesa sheep. *Animal Genetics*, 2008, 39(3): 294–297.

44. Montecagudo L V, Ponz R, Tejedor M T, Lavina A, Sierra I. A 17 bp deletion in the Bone Morphogenetic Protein 15 (BMP15) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. *Animal Reproduction Science*, 2009, 110(1–2): 139–146.

45. Demars J, Fabre S, Sarry J, Rossetti R, Gilbert H, Persani L, Tosser-Klopp G, Mulans P, Nowak Z, Drobiw K, Martyniuk E, Bodin L. Genome-wide association studies identify two novel BMP15 mutations responsible for an atypical hyperprolificacy phenotype in sheep. *PLOS Genetics*, 2013, 9(4): e1003482.

46. Nicol L, Bishop S C, Ponnong, Rendiben C, Holm L E, Rhind S M, McNeill A S. Homozygosity for a single base-pair mutation in the oocyte-specific GDF9 gene results in sterility in Thoka sheep. *Reproduction*, 2009, 138(6): 921–933.

47. Silva B D, Castro E A, Souza C J, Paiva S R, Sartorii R, Franco M M, Azevedo H C, Silva T A, Vicera A M, Neves J P, Melo E O. A new polymorphism in the Growth and Differentiation Factor 9 (GDF9) gene is associated with increased ovulation rate and prolificacy in homozygous sheep. *Animal Genetics*, 2011, 42(1): 89–92.

48. Melo E O, Silva B, Castro E A, Silva T, Paiva S R, Sartorii R, Franco M M, Souza C J, Neves J P. A novel mutation in the growth and differentiation factor 9 (GDF9) gene is associated, in homozygosis, with increased ovulation rate in Santa Ines sheep. *Biology of Reproduction*, 2008, 78(141): 371.

49. Vage D I, Hudsal M, Kent M P, Klemetsdal G, Boman I A. A missense mutation in growth differentiation factor 9 (GDF9) is strongly associated with litter size in sheep. *BMC Genetics*, 2013, 14(1): 1.

50. Mullen M P, Hanrahan J P. Direct evidence on the contribution of a missense mutation in GDF9 to variation in ovulation rate of Finnsheep. *PLoS ONE*, 2014, 9(4): e95251.

51. Souza C J, McNeill A S, Benavides M V, Melo E O, Moraes J C. Mutation in the protease cleavage site of GDF9 increases ovulation rate and litter size in heterozygous ewes and causes infertility in homozygous ewes. *Animal Genetics*, 2014, 45(5): 732–739.

52. Davis G H, Dodds K G, Wheeler R, Jay N P. Evidence that an imprint on the X chromosome increases ovulation rate in sheep. *Biology of Reproduction*, 2001, 64(1): 216–221.

53. Martin P, Raoul J, Bodin L. Effects of the FecL major gene in the Lacaune meat sheep population. *Genetics Selection Evolution*, 2014, 46(1): 48.

54. Drouilhet L, Mansanet C, Sarry J, Tabet K, Bardou P, Poloszyn F, Lluch J, Harichaux G, Vugue C, Monniaux D, Bodin L, Mulans P, Fabre S. The highly prolific phenotype of Lacaune sheep is associated with an ectopic expression of the B4GALNT2 gene within the ovary. *PLOS Genetics*, 2013, 9(9): e1003809.

55. Davis G H. Fecundity genes in sheep. *Animal Reproduction Science*, 2004, 82–83: 247–253.

56. Davis G H, Farquhar P A, O’Connell A R, Everett-Hincks J M, Wishart P J, Galloway S M, Dodds K G. A paternal autosomal gene increasing ovulation rate in Romney sheep. *Animal Reproduction Science*, 2006, 92(1–2): 65–73.

57. Shackell G, Hudson N, Heath D, Lun S, Shaw L, Condell L, Blay L, McNatty K. Plasma gonadotropin concentrations and ovarian characteristics in Inverdale ewes that are heterozygous for a major gene (Fec<sup>L</sup>) on the X chromosome that influences ovulation rate. *Biology of Reproduction*, 1993, 48(5): 1150–1156.

58. Davis G, Bruce G, Dodds K. Ovulation rate and litter size of prolific Inverdale (Fec<sup>K</sup>) and Hanna (Fec<sup>I</sup>) sheep. *Proceeding of Association for the Advancement of Animal Breeding and Genetics*, 2001, 14: 175–178.

59. McNatty K P, Heath D A, Hudson N L, Lun S, Juengel J L, Moore L G. Gonadotrophin-responsiveness of granulosa cells from bone morphogenetic protein 15 heterozygous mutant sheep. *Reproduction*, 2009, 138(3): 545–551.

60. Liao W X, Moore R K, Shimasaki S. Functional and molecular characterization of naturally occurring mutations in the oocyte-secreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. *Journal of Biological Chemistry*, 2004, 279(17): 17391–17396.

61. Dong J, Albertini D F, Nishimori K, Kumar T R, Lu N, Matzuk M M. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature*, 1996, 383(6600): 531–535.

62. Sadighi M, Bodensteiner J K, Beattie A E, Galloway S M. Genetic mapping of ovine growth differentiation factor 9 (GDF9) to sheep chromosome 5. *Animal Genetics*, 2002, 33(3): 244–245.

63. Bodensteiner K J, Clay C M, Moeller C L, Sawyer H R. Molecular cloning of the ovine growth/differentiation factor-9 gene and
expression of growth/differentiation factor-9 in ovine and bovine ovaries. *Biology of Reproduction*, 1999, 60(2): 381–386

64. Eghbalsaeidi S, Ghaedi K, Shahmoradi S, Pirestani A, Amini H, Saiedi T, Nicol L, McNeillly A. Presence of SNPs in GDF9 mRNA of Iranian Afshari Sheep. *International Journal of Fertility & Sterility*, 2012, 5(4): 225–230

65. Juengel J L, Davis G H, McNatty K P, Using sheep lines with mutations in single genes to better understand ovarian function. *Reproduction*, 2013, 146(4): R111–R123

66. Bodin L, SanCristobal M, Lecerf F, Mulsant P, Bibe B, Lajous D, Belloc J P, Eychenne F, Amigues Y, Olsen J M. Segregation of a major gene influencing ovulation in progeny of Lacaune meat sheep. *Genetics Selection Evolution*, 2002, 34(4): 447–464

67. Drouillet L, Taragnat C, Fontaine J, Duittoz A, Mulsant P, Bodin L, Fabre S. Endocrine characterization of the reproductive axis in highly prolific Lacaune sheep homozygous for the *FecL* mutation. *Biology of Reproduction*, 2010, 82(5): 815–824

68. Davis G, Dodds K, Wheeler R, Bruce G. Further evidence of non-mendelian inheritance at the *FecX2* locus in a prolific sheep flock. In: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production. Montpellier: Institut National de la Recherche Agronomique (INRA), 2002: 0–4

69. Davis G H, Dodds K G, Bruce G D. Combined effect of the Inverdale and Booroola prolactin genes on ovulation rate in sheep. In: Proceedings of the Thirteenth Conference Association for the Advancement of Animal Breeding and Genetics. 1999, 13: 74–77

70. McNatty K P, Galloway S M, Wilson T, Smith P, Hudson N L, O’Connell A, Bibby A H, Heath D A, Davis G H, Hanrahan J P, Juengel J L. Physiological effects of major genes affecting ovulation rate in sheep. *Genetics Selection Evolution*, 2005, 37(Suppl 1): S25–S38

71. Davis G H, Galloway S M, O’Connell A, Farquhar P A, McNatty K P, Juengel J L. Hyper-prolific ewes carrying copies of three major genes: a model for studying genes controlling ovulation rate. *Biology of Reproduction*, 2008, 78: 110

72. Juengel J L, O’Connell A R, French M C, Proctor L E, Wheeler R, Farquhar P A, Dodds K G, Galloway S M, Johnstone P D, Davis G H. Identification of a line of sheep carrying a putative autosomal gene increasing ovulation rate in sheep that does not appear to interact with mutations in the transforming growth factor beta superfamily. *Biology of Reproduction*, 2011, 85(1): 113–120

73. Barzegari A, Atashpaz S, Ghabili K, Nemati Z, Rustaei M, Azarbaiajani R. Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Mughali and Ghezel sheep in Iran. *Reproduction in Domestic Animals*, 2010, 45(4): 666–669

74. Gemmell N J, Slate J. Heterozygote advantage for fecundity. *PLoS ONE*, 2006, 1(1): e125

75. McNatty K P, Lawrence S, Groome N P, Meerashab M F, Hudson N L, Whiting L, Heath D A, Juengel J L. Meat and Livestock Association Plenary Lecture 2005. Oocyte signalling molecules and their effects on reproduction in ruminants. *Reproduction, Fertility and Development*, 2006, 18(4): 403–412

76. Edwards S J, Reader K L, Lun S, Western A, Lawrence S, McNatty K P, Juengel J L. The cooperative effect of growth and differentiation factor-9 and bone morphogenetic protein (BMP)-15 on granulosa cell function is modulated primarily through BMP receptor II. *Endocrinology*, 2008, 149(3): 1026–1030

77. McIntosh C J, Lun S, Lawrence S, Western A H, McNatty K P, Juengel J L. The proportion of mouse BMP15 regulates the cooperative interactions of BMP15 and GDF9. *Biology of Reproduction*, 2008, 79(5): 889–896

78. Lin J Y, Pitman-Crawford J L, Bibby A H, Hudson N L, McIntosh C J, Juengel J L, McNatty K P. Effects of species differences on oocyte regulation of granulosa cell function. *Reproduction*, 2012, 144(5): 557–567

79. Drouillet L, Lecerf F, Bodin L, Fabre S, Mulsant P. Fine mapping of the *FecL* locus influencing prolificacy in Lacaune sheep. *Animal Genetics*, 2009, 40(6): 804–812

80. China National Commission of Animal Genetic Resources. *Animal Genetic Resources in China: Sheep and Goats*. Beijing: China Agriculture Press, 2011 (in Chinese)

81. Wang J Q, Cao W G. Progress in exploring genes for high fertility in ewes. *Hereditas*, 2011, 33(9): 953–961 (in Chinese)

82. Chu M, Di R, Ye S, Fang L, Liu Z, Peng Z, Zhang G, Jia L, Sun J, Feng T. Establishment of molecular detection methods for high prolificacy major gene *FecB* in sheep and its application. *Journal of Agricultural Biotechnology*, 2009, 17(1): 52–58 (in Chinese)

83. Hua G H, Yang L G. A review of research progress of *FecB* gene in Chinese breeds of sheep. *Animal Reproduction Science*, 2009, 116 (1–2): 1–9

84. Chu M, Sang L, Wang J, Fang L, Ye S. Study on BMP15 and GDF9 as candidate genes for prolificacy of Small Tail Han sheep. *Acta Genetica Sinica*, 2005, 32(1): 38–45 (in Chinese)

85. Chu M, Chen R, Chen G, Fang L, Ye S. Study on bone morphogenetic protein 15 as a candidate gene for prolificacy of Small Tail Han sheep and Hu sheep. *Journal of Anhui Agricultural University*, 2005, 32: 278–282 (in Chinese)

86. Chu M, Sun J, Chen H, Fang L. Detection of the *FecR* mutation of BMP15 gene in sheep. *Chinese Agricultural Science Bulletin*, 2007, 23(10): 85–88 (in Chinese)

87. Di R, Feng T, Chu M, Zhang Y, Fang L. Detection of the *FecR* mutation of BMP15 gene in sheep and goats. *Chinese Journal of Animal and Veterinary Sciences*, 2009, 40(9): 1303–1307 (in Chinese)

88. Chu M X, Yang J, Feng T, Cao G L, Fang L, Di R, Huang D W, Tang Q Q, Ma Y H, Li K, Li N. *GDF9* as a candidate gene for prolificacy of Small Tail Han sheep. *Molecular Biology Reports*, 2011, 38(8): 5199–5204

89. Ho C C, Bernard D J. Bone morphogenetic protein 2 signals via BMPRIA to regulate murine follicle-stimulating hormone beta subunit transcription. *Biology of Reproduction*, 2009, 81(1): 133–141

90. Persani L, Rossetti R, Di Pasquale E, Cacciator E, Fabre S. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. *Human Reproduction Update*, 2014, 20(6): 869–883