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Gener som påverkar ovulationsfrekvens och kullstorlek hos får

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Abstract

Fertility traits typically have low heritability and selection based on phenotype is often inefficient and slow. It is more effective to select breeding animals according to their genotype to improve fertility traits like ovulation rate and litter size in sheep. Different mutations in genes of the transforming growth factor-β (TGFβ) superfamily have been shown to affect ovulation rate and litter size in sheep. These genes include BMPR-1B, BMP15, and GDF9. Mutations in the Lacaune gene (B4GALNT2) and Woodland gene (FecX2) have been shown to affect ovulation rate and litter size in sheep as well. The mutations have different effects and inheritance patterns. All mutations increase ovulation rate in heterozygous individuals, but some mutations cause infertility in homozygous individuals where ewes fail to develop ovaries and follicles properly. An increase in litter size may be critical for profitability in lamb production, but only focusing on increasing the number of lambs born is not optimal. The optimal litter size differs between production systems, breeds, climate and the availability of nutritious forage. The aim of this review is to study genes involved in ovulation rate and litter size in sheep. In conclusion, it is important to study fecundity genes to increase production efficiency, stabilizing optimal litter sizes and obtaining knowledge about infertility and genetic disorders associated to reproduction.

Sammanfattning

Fertilitetsegenskaper har ofta låga arvbarheter och selektion baserat på fenotyp är ofta ineffektiv och långsam. För att förbättra fertilitetsegenskaper som ovulationsmängd och kullstorlek hos får är det effektivast att selektera avelsdjur utefter deras genotyp. Olika mutationer i gener tillhörande transforming growth factor-β (TGFβ) superfamiljen har visat sig påverka ovulationsmängd och kullstorlek hos får. Dessa gener är BMPR-1B, BMP15 och GDF9. Även mutationer i den så kallade Lacaune genen (B4GALNT2) och Woodland genen (FecX2) har visat sig påverka ovulationsmängd och kullstorlek hos får. Mutationerna ger olika effekt och har olika nedärvningsmönster. Samtliga mutationer ökar ovulationsmängden hos heterozygota individer, men vissa mutationer orsakar infertilitet hos homozygota individer och hos dessa tackor utvecklas inte äggstockarna och folliklarna normalt. En ökad kullstorlek kan vara avgörande för lammproduktionens lönsamhet men att endast inrikta aveln på att öka antalet födda lamm är inte optimalt. Vilken kullstorlek som är optimal skiljer sig mellan produktionssystem, ras, klimat och tillgång på näringsrikt foder. Syftet med denna litteraturstudie är att undersöka vilka gener som påverkar ovulationsfrekvens och kullstorlek hos får. Sammanfattningsvis är det viktigt att studera fertilitetsgener för att öka effektiviteten i produktionen, kunna stabilisera optimala kullstorlekar samt att få kunskap om infertilitet och genetiska sjukdomar associerade till reproduktion.

Introduction

Fertility traits have a major impact on efficiency and profitability in lamb meat production. Swedish production is low and known to be non-profitable and ineffective due to small herds and a large amount of labor spent on each ewe. Developing fertility traits may therefore be of big interest for sheep producers (Kumm, 2008). Ovulation rate and litter size are important fertility traits in sheep and are of high economic value (Notter, 2008). Traits associated with fertility usually have low heritability and breeding improvements made on phenotypic selection based on observable data are often limited. Ovulation rate and litter size are also only expressed in one sex and is recordable only relatively late in the animal's life. This further obstructs the breeding progress and limits the inclusion of the traits into selection schemes

since selection of breeding candidates is complicated. It is important to study genes associated with fertility so that breeding can include genotypic information from animals. This will increase the genetic improvements in reproduction traits since it will be easier to collect data and information of the animal's traits. Focusing of development of fertility traits will have a long term effect on profitability in the sheep production (Pramod et al., 2013).

Follicle development includes a series of stages and takes place in the ovaries. Follicles contain oocytes, or egg cells, that are the haploid female reproductive gametes and are surrounded by epithelial cells. Development of follicles and the process of ovulation are influenced by endocrine factors. Hormones important in reproduction are follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both are gonadotrophins secreted by the anterior pituitary acting in the ovaries and stimulate ovulation (Sjaastad et al., 2003).

The domesticated sheep, *Ovis aries*, has 54 chromosomes containing genetic material in the form of deoxyribonucleic acid (DNA). A locus, or loci in plural, is a specific location for a gene on a chromosome. The corresponding loci in a chromosome pair may contain similar or slightly different segments of DNA, which in turn are called alleles (Simm, 1998). Reproduction is a complex process and fecundity traits such as ovulation rate and litter size can be genetically regulated by many genes with small effects, and sometimes also by single genes with major effects, called fecundity (Fec) genes (Drouilhet et al., 2009). Characteristics for the presence of a major gene affecting fertility in a population include high variation in ovulation rate and litter size combined with high repeatability. To detect major genes, high prolificacy ewes and rams are scanned and have their genome mapped.

By DNA testing rams and ewes for major genes and learning about inheritance patterns, sheep producers have been given the ability to increase ovulation rate and litter sizes and thereby productivity (Davis, 2005). It is important to note that breeding for an optimal and constant litter size should be more desirable than trying to increase the number of lambs born per ewe (SanCristobal-Gaudy et al., 2001). The optimal litter size may differ between production systems, climate, breeds and the availability of nutritious forage.

The purpose of this review is to study genes involved in ovulation rate and litter size in sheep. Genes that are going to be examined include BMPR-1B (bone morphogenetic protein receptor-1B), BMP15 (bone morphogenetic protein 15) and GDF9 (growth differentiation factor 9) all of which have hyper prolificacy-associated mutations that affect the bone morphogenetic signaling system in the ovaries (Drouilhet et al., 2013). The effects of the B4GALNT2 (beta-1, 4-N-acetyl-galactosaminyl transferase 2 or Lacaune gene) and FecX2 (fecundity gene X2 or Woodland gene) are also going to be examined. All of these genes are in some way involved in ovulation rate and litter size in sheep, but have different effects and inheritance patterns. An overview of the mutations and their effect is presented in table 1 and mutation type and positions are presented in table 2 (see appendix). Some mutations in fecundity genes are also associated with infertility in ewes. Much research is currently being conducted in this field and new genes and mutations affecting fertility are being discovered constantly.

Table 1. Mutations in the fecundity genes BMPR-1B (bone morphogenetic protein receptor-1B), BMP15 (bone morphogenetic protein 15) and GDF9 (growth differentiation factor 9) B4GALNT2 (beta-1, 4-N-acetyl-galactosaminyl transferase 2, Lacaune gene) and FecX2 (fecundity gene X2, Woodland gene) and their effect on ovulation rate and litter size in sheep. Information of the mutations founder breed, name, allele symbol and chromosome location in sheep. The effect on ovulation rate and litter size is a comparison between heterozygous (A+) and homozygous (AA) to non-carriers of the mutations (++) rounded to one decimal place

Gene	Founder Breed	Name, allele symbol	Chro-mos-ome	Effect ovula-tion rate	Effect litter size	Reference
BMPR-1B	Booroola Merino, (Garole)	Booroola, $FecB^{B}$	6	B+: +1.3 BB: +3.6	B+: +0.7 BB: +0.8	[1]
BMP15	Romney	Inverdale, $FecX^{I}$	X	I+: +1.0 II: infertile	I+: +0.6 II: infertile	[2]
BMP15	Romney	Hanna, $FecX^{H}$	X	H+: +1.0 HH: infertile	H+: +0.6 HH: infer-tile	[2]
BMP15	Belclare	Belclare, $FecX^{B}$	X	B+: +1.0 BB: infertile	- BB: infer-tile	[2]
BMP15	Belclare and Cam-bridge	Galway, $FecX^{G}$	X	G+: +0.7 GG: infertile	- GG: infer-tile	[2]
BMP15	Lacaune	Lacaune, $FecX^{L}$	X	L+: +2.0 LL: infertile	- LL: infertile	[4]
BMP15	Rasa Ara-gonesa	Rasa Ara-gonesa $FecX^{R}$	X	- RR: infertile	R+: +1.3 RR: infer-tile	[5]
BMP15	Grivette	Grivette, $FecX^{Gr}$	X	- -	Gr+: +1.9 GrGr: +2.5	[3]
BMP15	Olkuska	Olkuska, $FecX^{O}$	X	O+: +2.0 OO: +3.3	- -	[3]
GDF9	Belclare and Cam-bridge	High Fer-tility, $FecG^{H}$	5	H+: +1.4 HH: infertile	- HH: infer-tile	[2]
GDF9	Icelandic	Thoka, $FecG^{T}$	5	I+: +1.2 II: infertile	I+: +0.7 II: infertile	[2]
GDF9	Santa Inês	Embrapa, $FecG^{E}$	5	- EE: +1.0	- EE: +0.7	[3]
GDF9	Norwegian white sheep (Finnsheep)	-	5	- -	N+: +0.2 NN: +0.5	[6]
B4GALNT 2	Lacaune	Lacaune, $FecL^{L}$	11	L+: +1.5 LL: +3.0	L+: +1.0 LL: +2.0	[7,8]
FecX2	Coopworth	Wood-land, $FecX2^{W}$	X	W+: +0.4 WW: ≥ +0.4	W+: +0.3 WW: ≥ +0.3	[2]

[1] Fogarty, 2009

[2] Davis, 2005
[3] Demars et al., 2013
[4] Bodin et al., 2007
[5] Monteagudo et al., 2009
[6] Våge et al., 2013
[7] Drouilhet et al., 2009
[8] Drouilhet et al., 2013

Ovulation and Litter size in sheep

Ovulation is a complex mechanism that differs among species and depends both on genetic and environmental factors. Mammals can be either mono- or poly-ovulatory based on how many oocytes that mature and are released during ovulation. Ruminants typically releases a single oocyte per ovulation compared to pigs and rodents which have high ovulation rates (Montgomery et al., 2001). The ovulation rate even differs between breeds. In sheep, it ranges from one egg per ovulation in Texel and Suffolk to ten eggs per ovulation in the prolific Booroola Merino breed (Hanrahan, 1984; Souza et al., 2001). Beside genetic background, the variation in ovulation rate between breeds can be influenced by age, season and nutrition.

Follicle development includes a series of stages classified by the number of granulosa cell layers surrounding the oocyte and depends on the presence of certain hormones (Montgomery et al., 2001). The development starts with the primordial phase (the primitive or non-growing phase) where the oocyte is surrounded by a single layer of epithelial cells. The follicle then develops into the primary and secondary phase where the epithelial cells proliferate into granulosa cells and theca cells separated by the basal lamina. The growth and differentiation of granulosa cells is stimulated by FSH and the proliferation of theca cells is influenced by LH. The theca cells secrete androgens that are converted into estrogen. Granulosa cells are essential for ovulation since they support the oocyte and secret the hormones estrogen and inhibin. The oocyte is also surrounded by a non-cellular material layer called the zona pellucida (Sjaastad et al., 2003). The oocyte starts to prepare for ovulation after being influenced by hormones (Montgomery et al., 2001). Estrogen stimulates preovulatory surge, or dramatic increase, of LH which induce maturation of the follicle which then is ready to ovulate. In mono-ovulatory species like sheep, only a few selected follicles mature and become dominant (Sjaastad et al., 2003). The stages in follicle development can also be divided into the preantral and antral stages which are gonadotrophin-responsive and gonadotropin-dependent respectively (Pramod et al., 2013).

Litter size differs between and within sheep breeds (Davis, 2005). It is a trait that depends on ovulation rate and is affected by the number of fertilized oocytes. The higher the ovulation rate, the more oocytes will be available for fertilization during the estrous and increase the possibility of bigger litters (Drouilhet et al., 2013). It has not been proven that there is a genetic difference between sheep breeds in the ability to support fertilized eggs in the uterus, although, an excess number of fetuses in mono-ovulatory species like sheep can result in major embryo loss (Hanrahan, 1980; Souza et al., 2001). Studies have shown that higher ovulation rates result in reduced embryo survival and bigger litter sizes lowers the birth weights of lambs (Fogarty, 2009). There is an evident genetic correlation between litter size, a discrete trait, and ovulation rate, a continuous trait, making indirect selection on ovulation rate more efficient for making genetic progress (Hanrahan, 1980). What litter size that is optimal differs between production systems and breeds. In intensive systems including dairy sheep or spring lamb production, the animals are stall-fed with nutritious forage and concentrate. Large litters with two lambs or more are often desirable. Semi-intensive systems often include tough

breeds with lower milk yield, managed in high land pastures in exposed locations. Nutritious forage may not be available and large litters are not desirable in those production systems (Liandrisa et al., 2012).

Genes in the transforming growth factor-ß superfamily

It has been observed that mutations in a closely linked group of genes significantly increase ovulation rate in sheep (Davis, 2005). Those genes are BMPR-1B, BMP15 and GDF9 which are all part of the ovary-derived transforming growth factor-β (TGFβ) superfamily. The genes code for proteins that are essential growth factors and receptors in follicular development in the ovaries. The genes have thus an important effect on ovulation rate and litter size (Pramod et al., 2013). Bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) stimulate proliferation of granulosa cells, modulate other growth factors and hormones and affect follicle growth and cell-survival signaling (Demars et al., 2013). These proteins probably exert their biological effect after binding to a type 1 receptor (BMPR-1A, BMPR-1B or TGFβR1) in the ovaries, which further combines with type 2 receptors (BMPR-2) (Mulsant et al., 2001; Feary et al., 2007). The mutations in the fecundity genes belonging to the TGFβ superfamily are all dominant meaning that one copy of a mutated allele is enough to express the trait in the phenotype (Davis, 2005). The GDF9 and the BMP15 mutations result in similar phenotypes of the ovaries, however, the inheritance patterns differ and the mutations have different effects on ovulation rate and litter size in sheep (Nicol et al., 2009). It has been suggested that the effect of some of the mutations in the BMP15 and GDF9 gene are examples of over-dominance, or heterozygous advantage. This is because individuals who are heterozygous with the mutations show an increase in ovulation rate and thus an increase in fitness, compared to homozygous individuals where most of the mutations in the BMP15 and GDF9 gene cause infertility (Gemmell & Slate, 2006).

BMPR-1B – the Booroola gene

BMPR-1B, or the Booroola gene, is located on ovine chromosome 6 and codes for bone morphogenetic protein 1B receptors in the ovaries (Davis, 2005). The BMP receptor is expressed by oocytes and granulosa cells from the primary stage to the late antral stage of follicle development and binds to BMP15. The detected mutation in the Booroola gene is a single nucleotide non-conservative substitution that has an additive effect on ovulation rate (Davis, 2005; Pramod et al., 2013). For the carriers of the mutation, follicles mature and ovulate at a smaller size and in greater numbers. This is due to an increase in concentration and higher responsiveness to FSH at an earlier stage compared to non-carriers of the mutation. The granulosa cells are also fewer, though they develop LH receptors earlier (Montgomery et al., 2001). The mutated receptor has been shown to be less responsive to BMPs, suggesting that the mutation decreases the signaling ability of the receptor (Feary et al., 2007). The mutation in the Booroola gene has a strong inhibitory effect on the ligands GDF5 and BMP4, which bind to BMPR-1B receptors. This action inhibits progesterone secretion from the granulosa cells which explains the increase of the FSH concentration. In homozygous ewes BMPR-1B is likely to be partially inactivated and the BMP pathway seems to be altered in the ovaries. The lower level of the oocyte-derived BMP15 combined with an earlier onset of responsiveness to LH in granulosa cells is likely to explain the high ovulation rates (Mulsant et al., 2001; Pramod et al., 2013).

Booroola was the first major fecundity gene detected and the mutation was identified by segregational studies on litter size and ovulation rate in sheep in Australia in the 1980s (Davis et

al., 1982; Piper & Bindon, 1982). The genotype was used with the purpose to increase fertility in other Merino flocks and breeds in the same way (Fogarty, 2009). It is likely that the mutation was introduced in the Booroola Merino strain from importations of Garole (also known as Bengal) sheep from India in the 1790s. Studies have shown that the Garole sheep is probably the original source of the mutation in the Booroola gene. It has apparently survived for 40-50 generations in the Booroola Merino breed since the interbreeding with the Garole sheep, without any specific selection for prolificacy or litter size until the mid 1940s. The gene has also been found in other Asian breeds. The mutation is present in the Small Tailed Han and Hu sheep in China, the Kendrapada sheep in India and the Javanese thin-tailed sheep in Indonesia but seems to be absent in European breeds (Davis et al., 2002; Davis et al., 2006). A DNA test with high accuracy has been developed for the mutation in the Booroola gene and is used in intensive production systems mainly in Asian countries (Davis, 2005; Walkden-Brown et al., 2008).

Lamb survival and birth weight have been reported to be lower among Booroola Merino cross sheep that carry the mutation compared to local breeds without the mutation (Davis et al., 1991; Gootwine et al., 1993). There have also been reports that lambs from ewes that carry the Booroola mutation have a lower growth rate compared to lambs from non-carrier ewes, and in contrast there are studies reporting that the mutation has no effect on growth rate (Davis et al., 1991; Meyer et al., 1994). It has also been shown that lambs from ewes carrying the mutation need a significantly higher intake of energy and protein per kilogram of average daily gain (Visscher et al., 2000). The negative effects seem to be more noticeable in homozygous ewes managed in extensive production systems and those can be viewed as inferior to heterozygous ewes due to lower lamb survival at an almost equivalent litter size (Walkden-Brown et al., 2008). The small size at maturation and low growth rate is seen in the Garole sheep, and it may be the case that those traits are only correlated with increased fecundity (Fogarty, 2009).

BMP15 gene

The BMP15 gene (also known as FecX or GDF9B) codes for the bone morphogenetic protein 15 which is an ovary-derived growth factor that is essential for follicular development in sheep (Hanrahan et al., 2004). The protein is expressed in the primary stage of follicular development and onwards and acts within the ovaries (Nicol et al., 2009). The action of BMP15 is regulated by the binding protein follistatin, which can bind in BMP15 and inhibit its effect on the ooocyte. BMP15 inhibits FSH receptor expression in the ovaries, thus follistatin is important to maintain the granulosa cells responsiveness to FSH (Pramod et al., 2013). The mutations in the BMP15 gene increase ovulation rate in heterozygous individuals. Heterozygous ewes show multiple ovulations, earlier maturation of granulosa cells and reduced follicle size. The phenotype shows more follicles in the antral stage compared to non-carriers but has fewer granulosa cells that also develop receptors for LH earlier. On the other hand, most of the mutations in this gene block follicular development in homozygous individuals (Montgomery et al., 2001). In six out of eight known mutations in the BMP15 gene, homozygous individuals are found to be infertile with small, undeveloped ovaries. The phenotype is described as ovarian hypoplasia which is failure of ovarian follicles to grow beyond the primary stage of development which further inactivates them (Hanrahan et al., 2004). Two copies of the mutation impair the production of the biologically active mature form of BMP15 which is essential for normal follicular development (Bodin et al., 2007). Failure of the BMP15 signaling results in failure of the granulosa cells to divide and support the oocytes which contributes to infertility (Montgomery et al., 2001).

The BMP15 gene is located on the X-chromosome and to recent date eight different mutations have been discovered in different sheep breeds and populations. The mutations in the BMP15 gene are Inverdale, Hanna, Belclare, Galway, Lacaune, Rasa Aragonesa, Grivette and Olkuska. BMP15 is a pre-proprotein and consists of three regions; a signal peptide, a large proregion and a mature peptide. Two of the mutations (Rasa Aragonesa and Galway) are located to the proregion, and the other six are located at the mature peptide. The BMP15 mutations differ slightly in type and effect. The Rasa Aragonesa mutation is an amino acid deletion while the Galway and Hanna mutations are premature stop codons. The other mutations are likely to be amino acid substitutions that alter the shape of the proteins thereby changing their function (Demars et al., 2013).

Inverdale and Hanna mutations

The Inverdale ($FecX^{I}$) and Hanna ($FecX^{H}$) mutations were the first two mutations in the BMP15 gene to be discovered. Both mutations were found in New Zealand in the 1990s, in highly prolific populations of Romney sheep where one ewe had produced 33 lambs in 11 lambings. Although both mutations give a similar phenotype and are the result of a single nucleotide substitution, Inverdale affects the mature coding sequence while Hanna results in a premature stop codon. Ewes that are heterozygous with the Inverdale or Hanna mutation show an increase in litter size, while homozygous ewes have small, undeveloped ovaries which results in infertility (Davis, 2005). Montgomery et al. (2001) suggested that the Inverdale and the Hanna mutation were located on the same locus and showed this by crossing sheep homozygous with one of the mutation respectively, thus creating the phenotype $FecX^{I}/FecX^{H}$. The researchers confirmed their hypothesis as the resulting ewes were infertile with undeveloped ovaries, phenotypically indistinguishable from ewes heterozygous with the Inverdale mutation.

Like the Boorola mutation, a cheap DNA test with high accuracy for the Inverdale mutation has been developed that does not require information from the parent animals. It is available on the commercial market and is popular in the New Zealand sheep industry where rams are tested to breed heterozygous progeny (Davis, 2005).

Belclare and Galway mutations

A highly prolific population of Belclare sheep in Ireland that demonstrated an inconsistent inheritance pattern of fertility traits was examined for major fecundity genes. There was also a high presence of infertile ewes in the population, suggesting that they carried more than one major gene. Studies have shown that Belclare sheep carry three mutations in fecundity genes, including two different mutations of the BMP15 gene, the Belclare ($FecX^{B}$) and the Galway ($FecX^{G}$) mutation (the third mutation being the high fertility mutation in GDF9). The Galway mutation was also found in a population of Cambridge sheep that had been established from high prolificacy ewes from the United Kingdom's national sheep flock. Ewes that are heterozygous for one of those mutations in the BMP15 gene show an increase in fertility (Davis, 2005). On the other hand, ewes that are heterozygous for both the Galway and the Belclare mutations are infertile. Also homozygous ewes are infertile (Hanrahan et al., 2004). The homozygous phenotype includes abnormalities of the reproductive tract with inactive or undeveloped ovaries (Davis, 2005). Homozygous ewes of the Cambridge and Belclare breeds show the same infertility symptoms as the Inverdale mutation, but closer histological examination of the ovaries indicate some significant differences in the oocytes. Unlike the Inverdale

mutation the phenotypes of the Belclare and Galway mutations show a thickening of the zona pellucida and disorganized layers of granulosa cells (Hanrahan et al., 2004).

The Chinese Han sheep has the same BMP15 mutation as the Belclare and Cambridge sheep in the $FecX^G$ allele. The Han breed is apparently unrelated to the Belclare and Cambridge breeds. Examining the genetic distances between breeds would reveal if the mutation has originated separately twice or if some interbreeding has occurred. The last-mentioned scenario is unlikely because of the geographical distance between the breeds (Chu et al., 2007).

$FecX^L$ mutation

The BMP15 mutation found in Lacaune sheep is located at the $FecX^L$ allele and is a missense non-conservative substitution resulting in an amino acid change. In heterozygous ewes, the mutation causes a large increase in ovulation rate but results in infertility in homozygous ewes. The action of the Lacaune mutation in homozygous individuals is an impairment of the maturation process of BMP in the ovaries. This causes premature blockage of the primary stage of folliculogenesis and the follicle fails to develop further than to the primordial phase (Bodin et al., 2007). Some follicles show a thickening of zona pelllucida and disorganized granulosa cell layers, similar to the Belclare and Galway mutations (Hanrahan et al., 2004; Bodin et al., 2007). The Lacaune mutation likely increases ovulation rate more than the other mutations in the BMP15 gene because of an additional effect of the Lacaune breed's phenotypic background of high fertility and the presence of other fecundity genes (Bodin et al., 2007).

Rasa Aragonesa mutation

The Rasa Aragonesa breed is the most common sheep in the river basin of Ebro in Spain (Monteagudo et al., 2009). The breed is an entrefino type that is polled with short wool and mainly used for meat production. Selection schemes for improving fertility traits in this breed have been going on since the 1990s including usage of elite sire semen from rams with high breeding values for litter size (Martinez-Royo et al., 2008). The mutation found in the BMP15 gene was the sixth of its kind and the first one discovered in Spanish sheep breeds. The allele is called $FecX^R$ and the mutation causes a deletion of 17 base pairs, corresponding to 6 amino acids, which leads to a premature stop codon in the bone morphogenetic protein. This results in a loss of functionality in the protein. Heterozygous ewes show an increase in prolificacy and litter size and homozygous ewes are expected to show primary ovarian failure (Monteagudo et al., 2009). It was concluded that in homozygous ewes the ovaries did not show any obvious follicular structure but was not similar to the undeveloped streaked ovaries observed in the phenotype of the Inverdale or the Lacaune mutations. More research needs to be done on this specific mutation to identify the effect on ovulation rate and the phenotype of homozygous individuals (Martinez-Royo et al., 2008).

Grivette and Olkuska mutations

Recent studies have shown that the highly prolific French Grivette sheep and the Polish Olkuska sheep carry major fecundity genes. Each breed has a different mutation in the BMP15 gene in the $FecX^{Gr}$ and $FecX^O$ allele respectively. In contrast to all the other known mutations in the BMP15 gene, sheep homozygous for the Grivette and Olkuska mutations are hyper-prolific and not infertile (Demars et al., 2013).

GDF9 gene

The GDF9 gene, also called FecG, is located on chromosome 5 and codes for oocyte-derived growth differentiation factor 9 and is essential for normal folliculogenesis. The growth factor is present in oocytes from the primary stage of follicular development until ovulation (Hanrahan et al., 2004). Mutations in the GDF9 gene show similar expression as the BMP15 mutation in the ovaries, however, it increases the ovulation rate in animals even more (Pramod et al., 2013). The mutation in the GDF9 gene increases ovulation rate in heterozygous individuals but in two out of four mutations follicle development is disrupted in homozygous individuals resulting in infertility (Nicol et al., 2009). The ovarian failure is due to a block in follicular growth at an early stage of development. The homozygous phenotype of the ovaries differs from the BMP15 mutations, since the ovarian follicles continue to develop to the antral stages with the majority of the follicles showing abnormal granulosa cells and oocyte morphology (Pramod et al., 2013).

Four mutations in the GDF9 gene affecting ovulation rate have been discovered to date. It was first discovered in Belclare and Cambridge sheep ($FecG^H$ or the high fertility mutation) which have the mutation together with mutations in the BMP15 gene (Davis, 2005). Studies have concluded that the long known Thoka gene found in the prolific Icelandic sheep is also a mutation of the GDF9 gene (Nicol et al., 2009). A mutation has also been found in the Brazilian Santa Inês sheep, called the Embrapa mutation. The Embrapa mutation differs from the previous mutations in the GDF9 gene because homozygous ewes are not sterile but show an increase in ovulation rate (Silva et al., 2011). This is also true for the fourth mutation in GDF9. Våge et al. (2013) showed that a polymorphism in the GDF9 that had previously been identified but not considered to have an effect on ovulation rate, is present in Norwegian white sheep and gives an increase in ovulation rate.

Thoka mutation

Icelandic sheep have long been known for large litter sizes, and it was concluded early that this breed was under influence of fecundity genes (Jonmundsson & Adalsteinsson, 1985). A mutation was discovered in the progeny from a high prolificacy ewe called Thoka and recent studies have shown that the Thoka mutation is located in the GDF9 gene (Nicol et al., 2009). The Thoka mutation, $FecG^T$, results in an amino acid substitution that increases ovulation rate in heterozygous individuals and causes infertility in homozygous ewes (Pramod et al., 2013). The ovaries and the follicles are abnormal and undeveloped and sheep homozygous with the mutation are infertile and have no records of litter size. The reason homozygous ewes are infertile may be because of the inhibition of theca cells forming a layer around the follicles which further inhibits development. The level of inhibin A and estradiol are lower in homozygous ewes resulting in low levels of progesterone which can inhibit gestation (Nicol et al., 2009).

Embrapa mutation

A mutation in the GDF9 gene was detected in the Brazilian Santa Inês sheep which is a breed well-adapted to tropical conditions and is known for frequent births of twins. The mutation is a point mutation resulting in an amino acid substitution in the mature peptide and is called the Embrapa mutation, located at the $FecG^E$ allele (Silva et al., 2011). This was the first mutation in the GDF9 gene discovered that did not cause infertility in homozygous individuals (Demars et al., 2013). Ewes homozygous for the mutation show an increase in ovulation rate by 82 % compared to non-carrier ewes. The effect of the Embrapa mutation does not show any other change in phenotype than an increase in ovulation rate and a higher frequency of twin

births. An additive effect of the mutation is suggested, though no significant difference in ovulation rate between heterozygous ewes and non-carrier ewes has been detected. More research must be conducted on this specific mutation to be certain (Silva et al., 2011).

GDF9 mutation in Norwegian white sheep

A polymorphism of the GDF9 gene already detected in Cambridge and Belclare sheep, at that time not considered to affect ovulation rate, was recently also detected in the Norwegian white sheep (Våge et al. 2013). In this breed, the polymorphism GDF9 results in an increase in ovulation rate. The Norwegian white sheep is a synthetic breed that is a crossing between local and foreign prolific breeds. The mutation was detected after scanning rams with both extreme high and low breeding values based on their daughter's number of progeny. The mutation is expected to have originated from interbreeding with the prolific Finnish landrace that occurred in the 1970s with the aim to increase fertility among Norwegian breeds. The mutation is located at the bioactive part of the GDF9 protein and at the mature region of the gene and is an amino acid substitution. Since homozygous ewes are fertile this mutation could have a great importance for commercial use to increase productivity among sheep producers. Våge et al. (2013) concluded that further studies on this polymorphism must be conducted including a larger amount of ewes with records of reproductive performance. By genotyping the ewes the effect on ovulation rate can be estimated (Våge et al., 2013).

Lacaune gene – B4GALNT2

The French Lacaune breed is known for its high prolificacy and big litter sizes. Litters with up to four lambs are common and so are high ovulation rates with an average of 5.8 eggs per ovulation which is likely due to several mutations in fecundity genes (Bodin et al., 1998; Davis, 2005). To recent date, two major genes affecting ovulation rate have been detected in this breed. One is the X-linked BMP15 gene (the mutation in the $FecX^{L}$ allele) and one autosomal gene called the Lacaune gene located at chromosome 11 at the FecL locus. The mutation in the Lacaune gene has an high additive effect on ovulation rate. The FecL locus contains two genes, IGF2BP1 (insulin-like growth factor 2 mRNA binding protein 1) and B4GALNT2 (beta-1, 4-N-acetyl-galactosaminyl transferase 2). Studies have shown that the B4GALNT2 gene is most likely responsible for the high fecundity in Lacaune sheep. That theory is supported by the fact that B4GALNT2 transferase activity is localized to the granulosa cells which are important in follicular development. The B4GALNT2 gene encodes a glycosylation enzyme and is not related to the BMP family. The Lacaune breed shows an over expression of the B4GALNT2 gene leading to atypical glycosylation of the hormone inhibin in the ovaries, which regulates ovarian functions by inhibiting the secretion of FSH from the anterior pituitary gland (Sjaastad et al., 2003; Drouilhet et al., 2013). Homozygous individuals show an increase in the number of follicles in the antral stage and an increase in plasma estradiol concentrations and the frequency of LH. Those mechanisms distinguish the Lacaune mutation from the mutations in the BMPR-1B, BMP15 and GDF9 genes. The exact position of the mutation on the FecL locus has not yet been identified (Drouilhet et al., 2013).

Woodland gene – FecX2

The mutation in the Woodland gene, or FecX2, was discovered in 1999 in a screened prolific flock of Coopworth sheep that had a history of interbreeding with Border Leicester and Romney sheep. The gene is located on the X-chromosome, though, the identity of FecX2 and the mechanism by which it affects ovulation rate is unknown to date (Feary et al., 2007). Studies have concluded that the Woodland gene is not BMPR-1B or BMP15 (Hanrahan et al., 2004).

The mutation may affect ovulation rate by changing the expression patterns of BMP15, BMPR-1B and TGFβR1 in the ovaries. The phenotype of the mutation is ovaries showing an increase in number of follicles smaller than 1 millimeter in diameter in the antral stage. Oocytes are also smaller, however, when oocyte diameter in proportion to follicle diameter was examined, the oocytes where bigger compared to non-carriers of the mutation. This can also be seen in ewes carrying the Booroola ($FecB^B$) and Inverdale ($FecX^I$) mutation. The follicles also show an earlier formation of an antrum with fewer layers of granulosa cells which may be the effect of enhanced signaling by GDF9 due to higher concentrations of TGFβR1 mRNA in the oocyte (Feary et al., 2007). Since the gene is located on the X chromosome ewes can inherit it from either carrier parent while rams can only inherit it from their dam. The mutation in the Woodland gene is that it is maternally imprinted which means that it is only expressed in ewes when inherited from their sire. The effect of the mutation is silenced if the ewe inherits it from the dam and will not give an increase in ovulation rate (Davis et al., 2001; Davis, 2005). Also, the inheritance pattern does not follow simple Mendelian segregation. The mutation is only expressed in ewes that have inherited the gene from their sire that were progeny of a dam with the mutation silenced (Montgomery et al., 2001). Furthermore, if the grand dam expressed the mutation, her son's daughters will have the mutation silenced (Davis, 2005).

Discussion and Conclusion

The study of fecundity genes is of great importance in the farming industry (Nicol et al., 2009). The identification and use of major fecundity genes in sheep production will enable an increase in reproductive traits and thus an increase in genetic improvement. Additionally, an increase in ovulation rate and litter size will result in higher productivity of the ewe, and lamb production will become more efficient as a result since more lambs is produced per ewe. In most sheep production systems the main objective is to maximize farm profit which in lamb meat production includes maximizing the number of lambs butchered and the weight and composition of the carcass. Thus, increasing litter size should be secondary to the primary goal of production. The use of the mutations in the farming industry requires carefully constructed breeding programs (Notter, 2008). Direct marker tests with high accuracy are developed for the Booroola and Inverdale mutations. Genetic screening of the Inverdale mutations in sheep is a method that is frequently utilized to increase productivity among producers, primary in Australia and New Zealand (Davis et al., 2002; Pramod et al., 2013). Crossbreeding and introgression of the Booroola mutation in sheep populations in India, China and Israel has been successful and interest in using the mutation has seen a major shift to Asia. The genotypes have been most successful in intensive production systems where FecB carriers perform better and where the lambs are reared artificially. There is no economic benefit in production systems with poor nutrition and extensive grazing systems without housing are not suitable for introgression of the Booroola mutation. The introduction of the Booroola mutation has not been successful in Australia, USA and France. Despite being present in Australia for over two centuries, the Booroola mutation is unpopular and has a low impact on sheep production due largely to excessive litter sizes and increased lamb mortality. The problem is more noticeable in homozygous ewes that have higher ovulation rate and lamb mortality compared to heterozygous individuals, despite similar litter sizes (Walkden-Brown et al., 2008). The reason to why commercial sheep production outside Asia has not utilized the mutation in the Booroola gene in their herds is likely because of studies reporting lower lamb survival, birth weights and lamb growth. The low birth weights can be a consequence of bigger litter sizes and not solely an effect of the Booroola mutation (Fogarty, 2009).

Selecting for high ovulation rates and maximizing litter sizes may not be acceptable for the sheep producer. Extreme litter sizes with five lambs or more has occurred in the Romanov and the Finnsheep breeds. Big litters are not acceptable for lamb and ewe viability (SanCristobal-Gaudy et al., 2001). There is also a natural restriction of how many lambs that can suckle since ewes only have two teats and milk production culminates at a certain level, although, the optimal level of fecundity is often below the maximum attainable level of fecundity in most production systems. What is defined as the optimal litter size differs among production systems. In most pastoral environments, twins are considered to be ideal and triplets are undesirable (Notter, 2008). In intensive lowland systems the optimal litter size may be two, three or even four lambs, because feeding is under control and the ewe has access to enough nutritious forage and concentrate to provide the lambs with milk (Simm, 1998). The Greek Chios sheep have high milk yield and is often kept in intensive dairy production systems with good forage and nutrition. In that case, large litters with twins or triplets are desirable (Liandrisa et al., 2012). In contrast, the extensive systems in the harsh highlands where ewes do not have enough forage and nutrients available, an average litter size of two or less lambs may be more reasonable (Simm, 1998). In the Lacaune sheep managed in semi-intensive systems, the economic optimal litter size is two lambs per litter since twins have showed to be the most profitable. Selection should not be based on maximizing litter size but on breeding for a consistent litter size where the number of lambs born is most profitable for the production system (SanCristobal-Gaudy et al., 2001).

The study of genes associated with fertility is important and has many applications both in the animal and human health sector, thus sheep can provide a genetic model for the study of fecundity genes and ovulation rate (Montgomery et al., 2001). BMPs are essential for female fertility and knowledge of its function allows direct manipulation of ovulation rate and litter size in farm animals and can also provide useful information in treating infertile individuals. The study of fecundity genes is also important to gain knowledge about genetic disorders associated with reproduction (Pramod et al., 2013).

Mutations in fecundity genes have different effects on ovulation rate and litter size. Some mutations have additive effects and others result in infertility in homozygous individuals. The forth mutation in GDF9 found in Norwegian white sheep by Våge et al. (2013) had already been detected in Cambridge and Belclare sheep by Hanrahan et al. (2004) but at that time was not considered to be associated with fertility, or have limited effect on the trait. The expression in the phenotype may depend on other fixed alleles or multiple mutations interacting which could be the reason to why it has an effect in some breeds and not in others. There are with absolute certainty more genes involved in fertility which are unknown to date, and much research is currently being conducted on the topic. Highly prolific breeds are scanned for fecundity genes and it is possible that both already known and new mutations will be discovered. Fecundity genes can be expected to be found in prolific breeds with high variations in litter size and ovulation rate. Inconsistent inheritance patterns and high repeatability can also be a sign indicating the presence of fecundity genes in sheep. This may include Swedish Finewool, Finnsheep, East Friesian dairy, Romanov, Blueface Leicester and Barbados Blackbelly breeds (Davis et al., 2006).

In conclusion, there are fecundity genes with major effect on ovulation rate and litter size in different sheep breeds. The most mentionable are BMPR-1B, BMP15 and GDF9, all belonging to the TGFβ superfamily. The Lacaune gene and the Woodland gene are also of importance. The knowledge of genes that are involved in ovulation rate and litter size and the effects they have provides useful information for breeding and selection on those traits. Since

fertility traits usually have low heritability and selection on phenotype can be ineffective, the study of fecundity genes, inheritance patterns and genotyping of individuals will make it easier to select breeding candidates, shorten the generation interval and speed up genetic improvement. The study of fecundity genes is important for increasing ovulation rate and litter size in sheep with the purpose to make lamb production more efficient. The litter size should be adapted to the production system and stabilized to an optimal size since too big or too small litters can be critical for profitability. The knowledge of fecundity genes is also important to understand the process of fertility and infertility in mammals and thereby be able to treat genetic disorders associated to reproduction.

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Appendix

Table 2. BMPR-1B (bone morphogenetic protein receptor 1B), BMP15 (bone morphogenetic protein 15) and GDF9 (growth differentiation factor 9), B4GALNT2 (beta-1, 4-N-acetyl-galactosaminyl transferase 2, Lacaune gene) and FecX2 (fecundity gene X2, Woodland gene) mutations, amino acid position, mature peptide residue and coding base pair position and effect

Gene	Allele symbol	Mutation type	Effect	Amino acid position	Mature peptide residue	Coding base pair position	Reference
BMPR-1B	FecB^{B}	missense single nucleotide non-conservative substitution	Glutamine → Arginine	249	-	746	[1;2]
BMP15	FecX^{I}	missense single nucleotide non-conservative substitution	Valine → Aspartic acid	299	31	896	[4; 10]
BMP15	FecX^{H}	premature stop codon	premature stop codon	291	23	871	[4; 10]
BMP15	FecX^{B}	missense single nucleotide non-conservative substitution	Serine →Isoleucine	367	99	1100	[3]
BMP15	FecX^{G}	premature stop codon	premature stop codon	239	-	718	[3]
BMP15	FecX^{L}	missense single nucleotide non-conservative substitution	Cysteine →Tyrosine	321	53	962	[4; 10]
BMP15	FecX^{R}	nucleotide deletion	premature stop codon	154	-	525-541	[6; 7; 10]
BMP15	FecX^{Gr}	missense single nucleotide non-conservative substitution	Threonine → Isoleucine	317	-	950	[11]
BMP15	FecX^{O}	missense single nucleotide non-conservative substitution	Asparagine → Histidine	337	-	1009	[11]
GDF9	FecG^{H}	missense single nucleotide substitution	Serine → Phenylalanine	395	77	1184	[3]
GDF9	FecG^{T}	missense single nucleotide non-conservative substitution	Serine → Arginine	427	109	1279	[8]
GDF9	FecG^{E}	missense single nucleotide substitution	Phenylalanine → Cysteine	-	345	-	[9]
GDF9	FecG	missense single nucleotide substitution	Serine → Phenylalanine	371	-	-	[10]
B4GALNT2	FecL^{L}	missense single nucleotide non-conservative substitution	-	-	-	-	[5; 12]
FecX2	FecX2^{W}	-	-	-	-		-

[1] Mulsant 2001
[2] Souza 2001
[3] Hanrahan et al., 2004

[4] Bodin et al., 2007
[5] Drouilhet et al., 2009
[6] Martinez-Royo et al., 2008
[7] Monteagudo et al., 2009
[8] Nicol et al., 2009
[9] Silva et al., 2011
[10] Våge et al., 2013
[11] Demars et al., 2013
[12] Drouilhet et al., 2013