An investigation of genetic fecundity variants in Faroese sheep

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Ein rannsókn av arvaligum fruktbæri hjá føroyskum seyði

Eva Kjæld Hansen[1,5], Jens Ivan í Gerðinum[2], Dag Inge Våge[3], Svein-Ole Mikalsen[4,*]

Abstract

Several genes have variants that impact fecundity in mammals. We sequenced parts of some genes with internationally known high-fecundity variants in sheep from the northern Faroese islands. No known variant was found. Other variants were found, but none could be linked to twin/multiple births. This was further investigated using 362 ewes from four flocks and a genotyping array containing 51,646 ovine SNPs. We previously used these data to show that the genetic distance among the animals were clearly related to geography (as islands). When analysing genetic variations against fertility (measured as present and historical single or multiple births), only a single SNP showed statistical significance, and this was located outside any known coding region. When the four flocks were individually analysed, a few SNPs showed statistical significance in one flock, but not in other flocks. This strongly indicated that the hits were random and not functionally linked to high fertility. Thus, we were not able to find any gene or genome region that could be linked to high fertility or fecundity in Faroese sheep.

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[1] Faculty of Science and Technology, University of Faroe Islands, Nóatún 3, FO-100 Tórshavn, Faroe Islands. E-mail: rit@eva.fo

[2] Agricultural Agency (Búnaðarstovan), Frammi í Dali, FO-410, Kollafjørður, Faroe Islands. E-mail: jens_ivan@hotmail.com

[3] Faculty of Biosciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway. E-mail: daginge.vage@nmbu.no

[4] Faculty of Science and Technology, University of Faroe Islands, Nóatún 3, FO-100 Tórshavn, Faroe Islands. E-mail: sveinom@setur.fo. Tel: +298 292556.

[5] Present affiliation: BioFar, Mjólkagøta 7, FO-180 Kaldbak, Faroe Islands.

* Corresponding author.

Úrtak

Fleiri ílegur hava avbrigdir, ið hava ávirkan á fruktabærið hjá súgdjórum. Vit sekvenseraðu nakrar ílegur, sum í øðrum londum eru kendar fyri at hava avbrigdir, ið kunnu setast í samband við høgt fruktabæri, fyri at vita, um tær eisini vóru í seyði í norðara parti av Føroyum. Ongar av hesum fruktabærisavbrigdum vórðu funnar í føroyska seyðinum. Onnur avbrigdir vóru tó funnin, men eingi av teimum kundu knýtast at fleirlembum. Vit kannaðu eisini 362 ær úr fýra høgum í Føroyum, har vit nýttu eitt íleguúrval (genotyping array) við 51.646 seyðavbrigdum (Single Nucleotide Polymorphisms, SNPs). Vit hava áður brúkt hesar dátur til at vísa, at arvafrøðiliga fjarstøðan ímillum seyðin er týðiliga knýttur at, hvørjari oyggj seyðurin kemur úr. Tá arvaligi munurin varð samanborin við fruktabæri (núverandi og søguligar ein- og fleirlembur), var tað bert eitt avbrigdi, sum var hagfrøðiliga týðandi, men hetta avbrigdið var uttan fyri nakað kent proteinkotandi øki. Tá ærnar í hvørjum av teimum fýra høgunum vórðu samanbornar sínámillum, vóru tað nøkur fá avbrigdi, sum vóru hagfrøðiliga týðandi í einum haga, men sum ikki vóru tað í hinum høgunum. Hetta er ein sterk ábending um, at hesin munurin er tilvildarligur, og hevur ikki veruligt samband við fruktbærið hjá føroyska seyðinum. Sostatt funnu vit onga ílegu ella íleguøki, sum kann setast í samband høgt fruktabæri ella lambatal hjá føroyskum seyði.

Keywords: Fecundity, fertility, genetic variants, genotyping, QTL analysis, sheep.
Leitorð: Fruktbari, fruktabæri, arvalig avbrigdir, avbrigdiskanning, QTL kanning, seyður.

Introduction

The Faroe Islands are a small (1400 km^2) archipelago in the North Atlantic Ocean, approximately halfway between Scotland and Iceland. One etymologic interpretation of the name is originating from Old Norse "Færeyjar" – the sheep islands. This is a well-fitting interpretation, given that there are around 70 000 winter-fed sheep (Thorsteinson, 2020), while the number of inhabitants is presently around 55 000. Traditionally, it has been assumed that sheep were introduced to the islands with the Norse settlers, probably around 800-850 AD, but recent evidence could suggest that sheep were introduced at an earlier time, around 500 AD, possibly by Irish monks (Curtin et al., 2021). This fits well with the writings of Irish monk and geographer Dicuil from 825 AD (Debes, 1990; Dicuil, 825; Skårup, 2009), describing some island in the north filled with seabirds and sheep, but now being attacked by Norse pirates, so the people, described as “hermits from our Scottia” (“*heremitae ex nostra Scottia*”), had to flee (Dicuil, 825).

Around year 1600, a series of events is presumed to have essentially wiped out the existing Faroese sheep population, the most catastrophic called “svartafellið” (Svabo, 1789a, 1789b). Subsequently, sheep from Iceland were imported to the northernmost Faroese islands, and from Shetland and the

Orkneys to the southernmost Faroese islands (Svabo, 1789a, 1789b). However, Effersøe (1886, at p. 29) writes that the animals on the northern islands must be assumed to be imported from Norway. In addition, there were numerous instances of imports of a low number of animals from the UK, Greenland, Denmark, Norway, Iceland and Sweden (Jensen Beder, 2010; í Brekkunum, 2017). We recently confirmed that the northern Faroese animals indeed have Icelandic sheep as their closest genetic relatives, although the genetic distance was surprisingly large (Hansen et al., 2025).

The Faroese sheep belong to the North European short-tailed sheep, having a short, fluke-shaped and tapered tail (Dýrmundsson & Niznikowski, 2010; Wikipedia). The North European short-tailed breeds are genetically distinct from other North European breeds (e.g., Rochus et al., 2020).The longer tail of many modern breeds is a derived property (Lagler et al., 2022), so the short tail points to old origins (Deng et al., 2020). Present North European short-tail breeds are genetically the closest relatives to ancient sheep (4000 years old) from Åland and Gotland in the Baltic Sea (Larsson et al., 2024). The Faroese sheep are relatively small with long legs. Typical autumn slaughtering weight of the lambs is around 15 kg (around 32-35 kg total body weight). The animals have dual-layer curly wool with white, black, grey, brown, or red-brownish colours in a wide variety of patterns. Males generally have horns, while this is less common in ewes (Effersøe, 1886; Jensen Beder, 2010).

For the most part, the sheep are kept in different grazing fields during the entire year. With the high number of animals (approx. 45 winter-fed animals/km^2 when the total land area is considered), non-cultivated outfields are heavily grazed. This may affect the natural biodiversity (Austrheim & Eriksson, 2001), and may further cause more erosion (Arnalds & Barkarson, 2003; Centeri, 2022), leading to soil and peat loss, although the link between grazing and erosion may not always be evident (Rowntree et al., 2004), as the erosion may also be influenced by other human activities, directly or indirectly (Arnalds & Barkarson, 2003).

One way of counteracting a part of the ecological effects is to increase the average number of lambs per ewe. In this way, the number of winter-fed animals can decrease, but the meat yield can be kept at the same level as before. The total wool yield might decrease, but wool is not a major focus for Faroese sheep farming. Both genetic and non-genetic factors are involved in fecundity (the physiological potential for offspring, whereas fertility is the registered number of offspring) (Edwards & Juengel, 2017; Gebreselassie et al., 2019; Sadleir, 1968; Xu et al., 2018). However, there is no specific and universal genetic marker for high fecundity in sheep. Several investigations have pointed out variations in several genes that have considerable effect on the number of lambs per ewe (Abdoli et al., 2016; Gebreselassie et al., 2019; Tong et al., 2020; Xu et al., 2018). In particular, the *bone morphogenetic protein receptor type 1B* (*BMPR1B*; one known fecundity variant), *bone morphogenetic protein 15* (*BMP15*; nine known fecundity variants), *growth differentiation factor 9* (*GDF9*; seven known fecundity variants), *beta-1,4,-N-acetyl-galactosaminyl transferase 2* (*B4GALNT2*; one known fecundity variant) and *leptin receptor* (*LEPR*; one known fecundity variant) genes are known to affect fecundity according to the summary of Tong *et al.* (2020). Also other genes have been implicated in ovine reproductive traits (Ajafar et al., 2022). Thus, different sheep breeds may vary in which genes and which variants within the same gene that are linked to higher reproductive success (Gebreselassie et al., 2019; Tong et al., 2020; Xu et al., 2018).

Due to the potential mix of breeds in the Faroe Islands, a known genetic high fecundity variant in a specific imported breed does not necessarily apply to the local animals. We therefore analyzed DNA samples from four flocks of Faroese sheep from three of the northern Faroese islands (Streymoy, Eysturoy, Kalsoy) with fertility ranging from low (13.6% multiple births) to high (46.6% multiple births). We sequenced parts of some of the genes known to contain fecundity variants in 16 selected animals with known low or high fertility characteristics. Furthermore, we genotyped approximately 360 animals for 50,000 known single nucleotide polymorphisms (SNPs) to investigate if any genetic variant could be associated to high rate of multiples (defined as twins or higher) in Faroese animals.

Materials and Methods

Ethics

This project complies with all national guidelines and laws, including those concerning ethics, by being permitted by the Chief Veterinary Officer of the Faroe Islands. The blood samples were drawn by a certified veterinarian as required by the Chief Veterinary Officer. The authors declare that the practical execution of the project complies with all relevant and accepted international guidelines, including The International Council for Laboratory Animal Science Ethical Guidelines for Researchers (ICLAS). The informed consent was obtained from the animal owners, and they made their animals available for this project without any payment or compensation.

Animals and blood sampling

The animals are the same as previously were analyzed for the genetic relationship to other sheep breed on a global scale (Hansen et al., 2025). The ewes were taken from four flocks that had been bred by different criteria, from no focus on multiple births to it being a main focus. Please note that the large majority of multiple births is twin births, and triplets and higher are rare.

The characteristics of the animals are shown in Table 1. Blood samples from all animals were taken by a veterinarian. Subsequently the animals were scanned by ultrasound (Ovi-Scan 6 with axial scan sheep probe; BCF Technology, Bellshire, UK). Successful blood samples and scans were obtained from 486 animals during 7th to 18th February 2021. While in the field, the blood samples were kept at 0-4 °C using cooling blocks, and subsequently frozen at -70 °C.

Table 1. Characteristics of sheep flocks.

	Búnaðarstovan (Streymoy)			**Barmarnar (Streymoy)**	**Skála (Eysturoy)**	**Kalsoy**	
# of ewes	152			44	114	176	
	Group 1	Group 2	Group 3			Group 1	Group 2
Bred for twins	Yes	Yes	Yes	No	Yes	Yes	Yes
Flushing[A]	Yes	yes	yes	No	Yes	Yes	Yes
Winter housing[B]	Yes	Yes	No	Partially	Partially	Partially	Partially
Winter shearing[C]	No	No	No	No	No	Yes	Yes
Breeding started	27/1120	27/11/20	30/11/20	29/11/20	24/11/20	05/12/20	25/11/20
Ultrasound scan	12/02/21	12/02/21	12/02/21	15/02/21	18/02/21	07/02/21	07/02/21
Max gestational days at scan	77	77	74	78	86	64	74
Winter feed	Silage + Danish aggro (600 g) + Forage	Silage + fishmeal (150 g) + Forage feed	Forage booster (18%)	Hay + grass pellets	Silage + concentrated feed	Silage + concentrated feed + grass pellets + fishmeal + micromineral capsule	
Known multiple rate	Intermediate			Low	High	Very high	

A. Providing ewes with feed of high nutritional value for 3-4 weeks before and 2-3 weeks after mating period to increase body fat and weight. This can increase lamb yield by 10-20% (Shad et al., 2011).

B. The possibility to seek indoors shelter (with roof and walls) in winter.

C. Relieving the wool of the pregnant animals during winter (Adalsteinsson, 1972; Vipond et al., 1987). Usually only done for animals that are housed or have access to housing during winter. Shearing increases the lamb birth weight by 10-15% due to higher food intake of the ewes (Vipond et al., 1987).

DNA purification

DNA was purified with the DNeasy Blood and Tissue kit (Qiagen, Venlo, The Netherlands) as described by the supplier, using 100 µl thawed blood. The purified DNA was eluted with 200 µl of the supplied elution buffer. Quality and quantity of the extracted DNA was measured spectrophotometrically (Pearl NanoPhotometer; Implen, Germany). DNA from 368 samples were deemed of sufficient quality and quantity for the subsequent work (Table 2), and all sequencing and genotyping results are from within this group of animals.

Table 2. Results from ultrasound scans and number of successful DNA purifications from the ewes.

		Búnaðarstovan (Streymoy)	**Barmarnar (Streymoy)**	**Skála (Eysturoy)**	**Kalsoy**
# of ewes		152	44	114	176
Ewes vs. number of foetuses	0	9	6	7	7
	1	104	32	69	87
	2	39	6	38	82
Multiple births		25.7 %	13.6 %	33.3%	46.6 %
# (%) successful DNA purifications[A]		114 (75 %)	39 (89 %)	86 (75%)	129 (73%)

A. Defined as >3 ng/µl with 260/280 nm ratio >1.7.

Genotyping

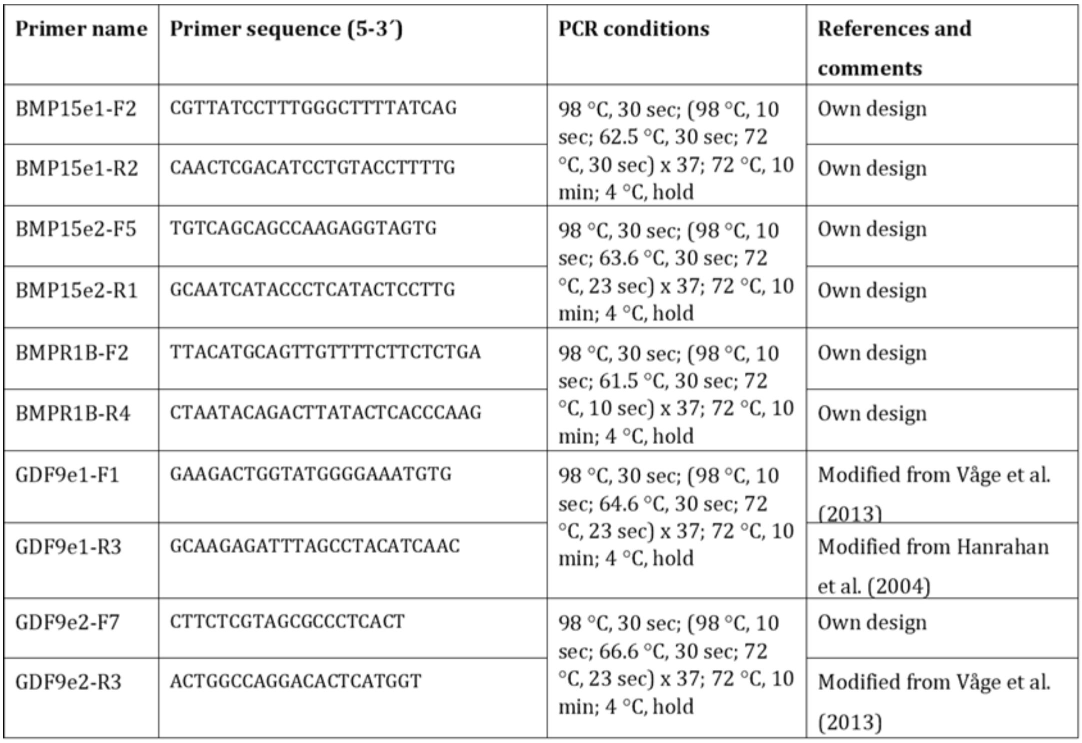
All ewes aged 3 years or older with adequate amounts of purified DNA (> 400 - 500 ng/sample) were included, and additionally four one-year old and 10 two-years old were included from the low fertility flock. Fifteen of the 16 samples that were used for DNA sequencing were included in the 368 samples sent for genotyping. The sixteenth sample was not included in genotyping as the remaining amount of DNA was too low. The DNA samples were dried down in 96 well plates. While still being liquid, the samples were exposed to a 30-minute period at 73 °C to inactivate any potential pathogens. Otherwise, the drying was done at 50 °C. Genotyping was done by Neogen (Ayr, Scotland) using the GGP Ovine 50K array.

The data obtained from the array were analysed using PLINK 1.9 (Purcell et al., 2007). Initially, the genotyping data were quality checked and filtered. The main criteria were "mind 0.1" (if the individual is missing > 10 % of the genotypes, the individual is excluded), "geno 0.1" (if an individual SNP is missing in > 10 % of the individuals, the SNP is excluded), and "hwe 1e-5" (Hardy-Weinberg equilibrium as described by Wigginton et al. (2005)). The association test was done by the Genome-wide Complex Trait Analysis (GCTA) software, using the --mlma-loco function (Yang et al., 2011). The genotyping data are available in the European Variation Archive under the accession number PRJEB81861 (https://www.ebi.ac.uk/ena/browser/view/PRJEB81861).

PCR

Several sets of primers targeting *BMPR1B*, *BMP15*, and *GDF9* were manually designed or taken from the sources given in Table 3, and synthesised by LGC BioSearch Technologies (Risskov, Denmark). PCR was done with Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Walham, Massachusetts) essentially as described by the supplier. PCR products were checked on 1.5 % Molecular Grade agarose gels (BioRad, Hercules, California) and visualised with GelRed (Biotium, Fremont, California). Different combinations of primers for each of the genes were tried out, and the primer combinations with the best results were selected for dideoxy sequencing. These primers and the PCR conditions are described in Table 3.

Table 3. Primers used in dideoxy sequencing and their PCR conditions.



Primer name	Primer sequence (5-3´)	PCR conditions	References and comments
BMP15e1-F2	CGTTATCCTTTGGGCTTTTATCAG	98 °C, 30 sec; (98 °C, 10 sec; 62.5 °C, 30 sec; 72 °C, 30 sec) x 37; 72 °C, 10 min; 4 °C, hold	Own design
BMP15e1-R2	CAACTCGACATCCTGTACCTTTTG		Own design
BMP15e2-F5	TGTCAGCAGCCAAGAGGTAGTG	98 °C, 30 sec; (98 °C, 10 sec; 63.6 °C, 30 sec; 72 °C, 23 sec) x 37; 72 °C, 10 min; 4 °C, hold	Own design
BMP15e2-R1	GCAATCATACCCTCATACTCCTTG		Own design
BMPR1B-F2	TTACATGCAGTTGTTTTCTTCTCTGA	98 °C, 30 sec; (98 °C, 10 sec; 61.5 °C, 30 sec; 72 °C, 10 sec) x 37; 72 °C, 10 min; 4 °C, hold	Own design
BMPR1B-R4	CTAATACAGACTTATACTCACCCAAG		Own design
GDF9e1-F1	GAAGACTGGTATGGGGAAATGTG	98 °C, 30 sec; (98 °C, 10 sec; 64.6 °C, 30 sec; 72 °C, 23 sec) x 37; 72 °C, 10 min; 4 °C, hold	Modified from Våge et al. (2013)
GDF9e1-R3	GCAAGAGATTTAGCCTACATCAAC		Modified from Hanrahan et al. (2004)
GDF9e2-F7	CTTCTCGTAGCGCCCTCACT	98 °C, 30 sec; (98 °C, 10 sec; 66.6 °C, 30 sec; 72 °C, 23 sec) x 37; 72 °C, 10 min; 4 °C, hold	Own design
GDF9e2-R3	ACTGGCCAGGACACTCATGGT		Modified from Våge et al. (2013)

Dideoxy sequencing of PCR products

To complement the genotyping, dideoxy sequencing was done on PCR products from 16 animals, 9 from the deemed high-fertility group (twin foetuses registered by scans or several multiple births known from documented history), and 7 from the deemed low-fertility group (history of only single births). The PCR products were purified with PureLink Purification kit (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing reaction was done with BigDye

Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific) both in forward and reverse direction for each PCR product, and the sequencing products were analysed on an ABI 3730xl Genetic Analyzer (Applied Biosystems). The electropherograms were handled and analysed using the MEGA7 software (Kumar et al., 2016). The variants of sequences with good-quality reads >200 nucleotides were deposited in GenBank with the accession numbers OR541123-OR541129.

Results and Discussion

As expected, the ultrasound scans showed different ratios of twin foetuses in the flocks (Table 2), ranging from 13.6 % to 46.6 %. However, one of the animals registered with twins during scanning in fact gave birth to triplets (see section "Sequencing" below). The animals showed an expected age distribution with regard to number of foetuses (Fig. 1), with nearly exclusively single foetuses in the very young (1 year; 64 animals) and very old (9 years; 2 animals).

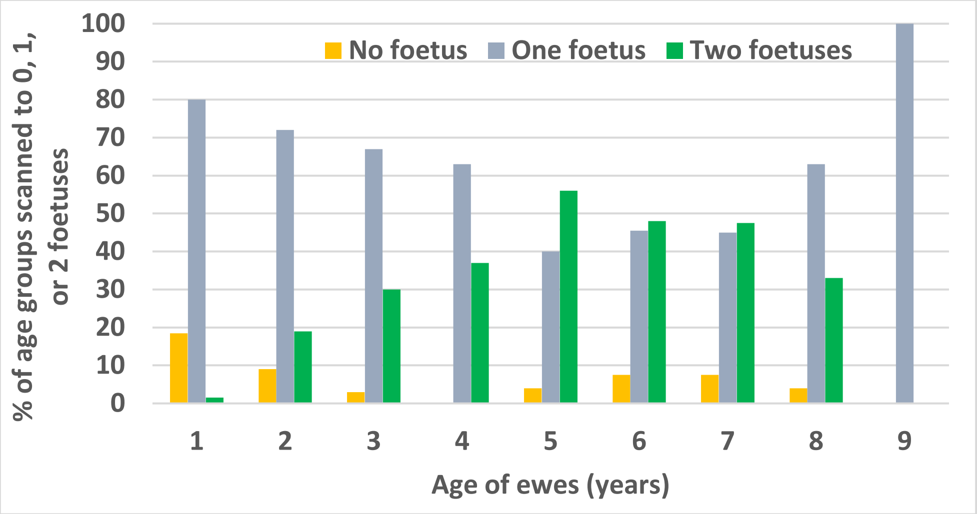


Fig. 1. Age of the ewes and proportion of the 486 ewes having: no, one, or two foetuses, when scanned.

Sequencing

The animals selected for sequencing are described in Table 4. Four of the nine selected animals in the high-fertility (H) group had twin births, one had triplets (but scanning indicated two foetuses), two animals had single lambs, and two animals had no lambs at the year of sampling (2021), but all were selected based on previous years with repeated twin births. For unknown reasons, two of the 16 animals consistently gave no readable sequences. These two animals belonged to the low-fertility (L) group. The sequencing results described below apply to the 14 remaining animals (9 H animals and 5 L animals).

Table 4. Fertility characteristics in high and low fertility groups selected for sequencing.

	High fertility (H)	Low fertility (L)
# ewes	9[A]	7
# lambs	13[A]	4
Average # lambs	1.44	0.57
Multiple births this year	5[A]	0
% Multiple births this year	55.6 %	0 %
% ewes with multiple births this year or previous years	100 %	0 %

A. One of the ewes had twin foetuses as determined by scanning, but did actually give birth to three lambs. Two of the ewes yielded no lamb this year, but had a history of multiple births.

BMP15 exon 1

The obtained variants of sequences are deposited in GenBank with accession numbers OR541125.1 and OR541126.1. The readable part of the sequence started 30 nucleotides before the initiation codon, continued through the whole exon 1 (322 or 325 nucleotides, see below), and approximately 380 nucleotides into intron 1-2. Among the Faroese sheep, there was only one polymorphism detected in the sequenced area. This was an in-frame triplet indel, CTT (Fig. 2), giving one or two Leu in amino acid positions 10 and 11 of the protein. We detected both variants of homozygotes and the heterozygote (Fig. 2). The allele distribution with regard to fertility characterisation is shown in Table 5, and it does not give any indication that any of these variants contribute to the fertility characteristics. This indel variant is known from before, with some breeds known to have a single CTT [Rambouillet (Ensembl ARS-UI_Ramb_v2.0/GCA_016772045.1), Balochi (GenBank JN655672), Rahmany (GenBank KT238844)], and other breeds have double CTT [Texel (Ensembl Oar_v3.1/GCA_000298735.1), Lohi (GenBank JN655671), small-tailed Han (GenBank AY885263), Pelibuey (GenBank KT853038), Ahshari (GenBank KT013295), Inverdale (GenBank NM_001114767)]. Otherwise, the Faroese sequences were fully identical to the Ensembl Rambouillet and Texel reference sequences in the area covered here, including the intron sequence.

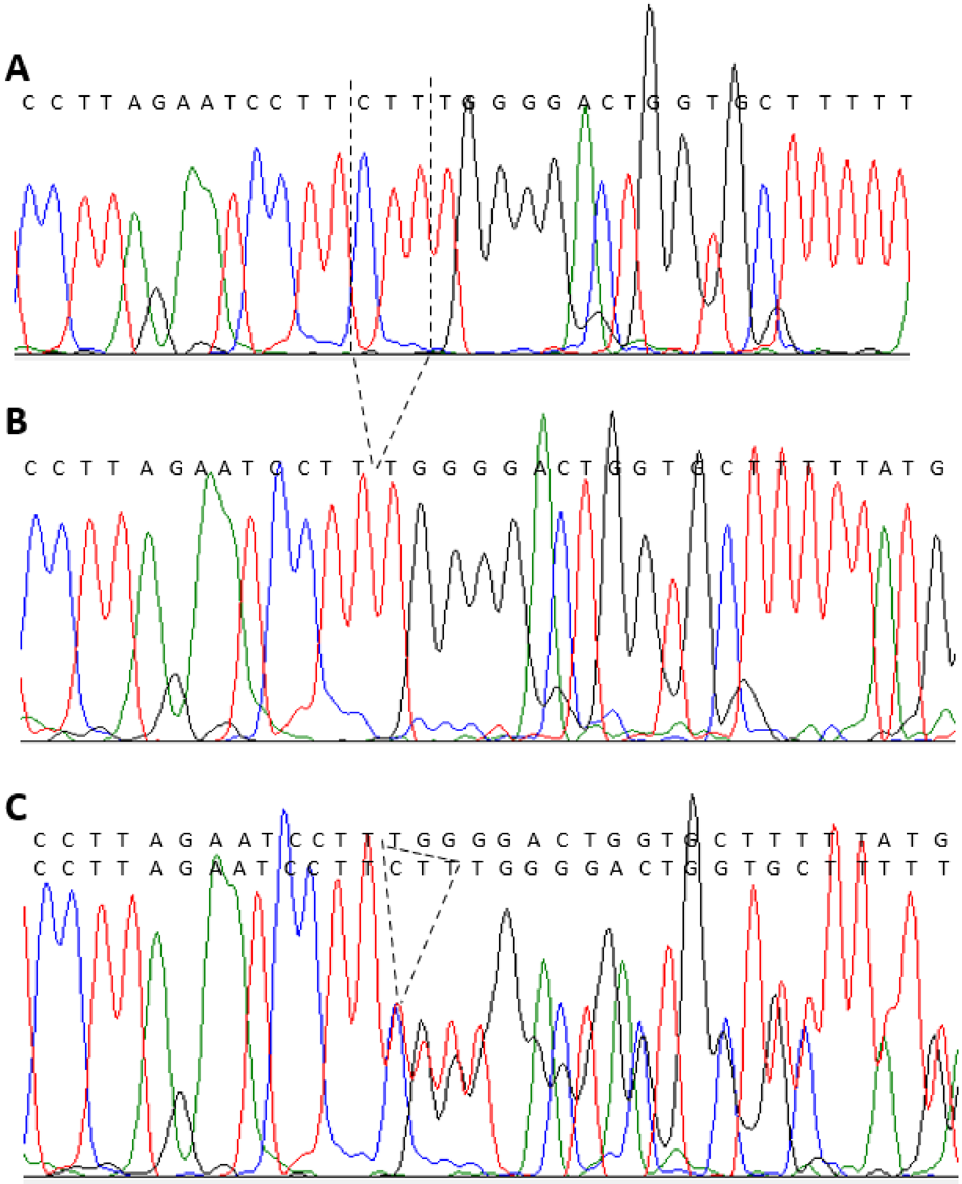


Fig. 2. Indel of in-frame triplet CTT in *BMP15* exon 1. **A.** Homozygous animal with doublet CTT triplet (GenBank OR541126.1). **B.** Homozygous animal with singlet CTT triplet (GenBank OR541125.1). The dotted lines between A and B indicate the indel. **C.** Heterozygous animal, where the lower sequence indicates the double CTT triplet allele, and the upper sequence indicates the singlet CTT triplet allele. Colours in the electropherograms: red, T; black, G; Green A; blue, C. The shown electropherograms are from forward sequencing, and the same results were obtained from reverse sequencing.

Table 5. Distribution of single and double CTT (indel) in *BMP15* exon 1 in Faroese sheep. Readable sequences were obtained from 14 animals, 9 in the high fertility group and 5 in the low fertility group.

	Homozygote single CTT	**Homozygote double CTT**	**Heterozygote**
High fertility group (H)	3	1	5
Low fertility group (L)	0	0	5

BMP15 exon 2

The obtained sequence is deposited in GenBank with accession numbers OR541127.1. *BMP15e2* contains a number of known fecundity variants. We sequenced an area corresponding to positions 643 to 1136 in the reference entry NM_001114767.2. The area contains at least seven known fecundity variants. The covered area was identical to the reference sequence NM_001114767.2 and the ovine genome sequences for Texel and Rambouillet breeds in Ensembl. The forward and reverse sequencing confirmed each other (data not shown). Thus, no known fecundity variants were indicated in this area.

BMPR1B exon 8

The obtained variants of sequences are deposited in GenBank with accession numbers OR541123.1 and OR541124.1. This gene contains one of the best known fecundity variants in sheep, the Booroola variant c.746A>G, which is located early in exon 8. We designed primers that surrounded exon 8 and were able to sequence the whole exon (except for that the downstream primer included the five last nucleotides of the exon). Both sheep genomes in Ensembl (Texel and Rambouillet) and the GenBank reference sequence NM_001009431 contained the wildtype variant at the Booroola position 746, and so did all Faroese sheep (Fig. 3), confirmed by both forward and reverse sequencing.

However, the sequencing revealed SNPs at positions 750 and 753 in Faroese animals. For position 750, we found both homozygous A/A and G/G animals, and we also found heterozygous animals 750A/G. All three reference sequences (Rambouillet, Texel and NM_001009431) showed G in this position. A similar situation was found for position 753. Both homozygous (753A/A or 753G/G) and heterozygous (753A/G) animals were found in our sequences (Fig. 3). The Rambouillet genome and NM_001009431 have 753A, while the Texel genome has 753G.

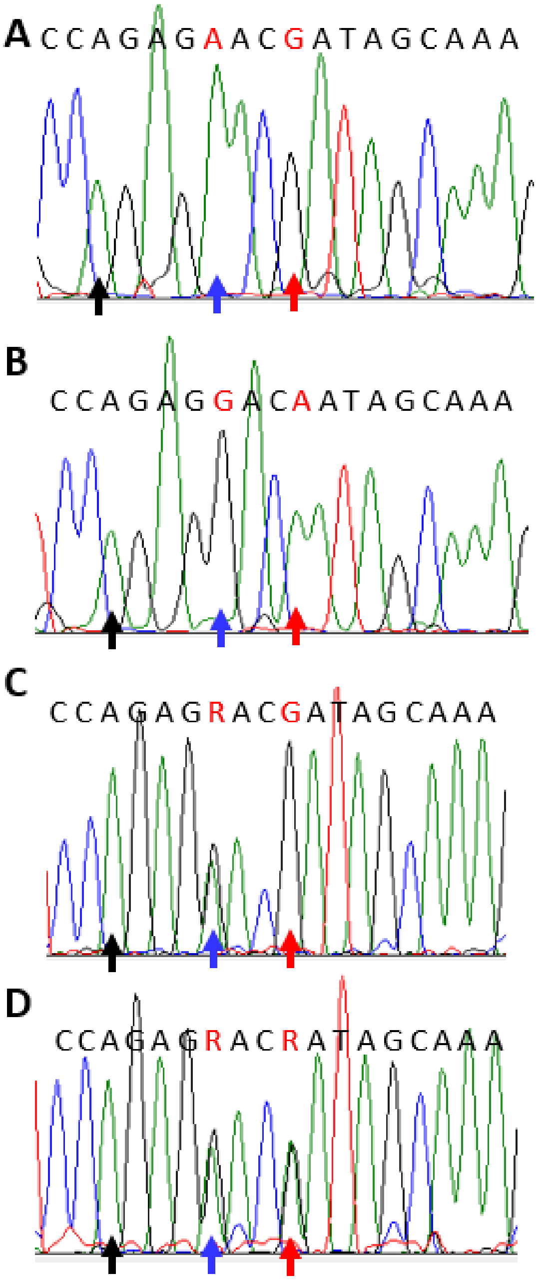


Fig. 3. Heterozygosity in positions 750 (blue arrow) and 753 (red arrow) in the *BMPR1B* gene (exon 8), close to the Booroola position 746 (black arrow; see text). All animals were found to have A (wild type) in position 746 (black arrow). **A.** Wildtype homozygous sequence with 750A and 753G (GenBank OR541123.1). **B.** "Inverse wildtype" with double homozygote with 750G and 753A (GenBank OR541124.1). **C.** Single heterozygote (750R)/single homozygote (753G). **D.** Double heterozygote with 750R (=A+G) and 753R.

Interestingly, we found both 750G+753A (2 animals, 1L + 1H; Fig. 3A), 750A+753G (4 animals, 1L + 3H; Fig. 3B), 750R+753G (6 animals, 2L + 4H; Fig. 3C), and 750R+753R (2 animals, 1L + 1H; Fig. 3D), where R signifies heterozygosity A+G. Although the low number of animals in each of the sequence groups precludes any reasonable statistics, the distribution suggests that among these particular sequence combinations, none can be linked to high or low fertility. The 750R+753G sequence implies that there ought to be some animals with the sequence 750G+753G, but we did not find any such animals in our limited group.

GDF9 exon 1

The obtained variants of sequences are deposited in GenBank with accession numbers OR541128.1 and OR541129.1. The sequenced amplicon covered the whole exon 1 (397 nt), and approx. 180 nt into intron 1-2. In this area, the fecundity variant FecG^{I}, c.260G>A, is known. Texel and Rambouillet genomes are identical in the covered area, and their exon 1 was also identical to NM_001142888, including the wildtype 260G. All sequenced Faroese animals showed wildtype sequence for this position, and across the whole exon 1 (not shown). However, we found a variant in the sequenced part of intron 1-2 (Fig. 4), which was a 4 nt insertion (TACT) relative to both the Rambouillet and Texel reference genomes in Ensembl (Rambouillet_v1.0: 5:56546981_46546980insTACT; Texel oar_v3.1: 5:4182970_4182969insTACT; note that the numbering and sequence is given according to the *GDF9* gene direction) linked with a substitution 15 nt downstream (corresponding to Rambouillet v1.0 5:46546966C>G and Texel oar_v3.1 5:41842956C>G). Eleven animals were homozygous for the insertion and substitution, one animal was homozygous wildtype (without insertion and substitution; in the low fertility group), and two animals were heterozygous (one low fertility, one high fertility) (Fig. 4), and thereby the variant cannot be linked to high or low fertility. This variant has been observed earlier (GenBank AF078545) in an unspecified sheep breed (Bodensteiner et al., 1999) and in many named sheep breeds, including another Texel genome assembly (GenBank JAJTAA010000241 and JAJSZZ010001299). Thus, this is a common gene variant that might coexist with wildtype also in other sheep breeds.

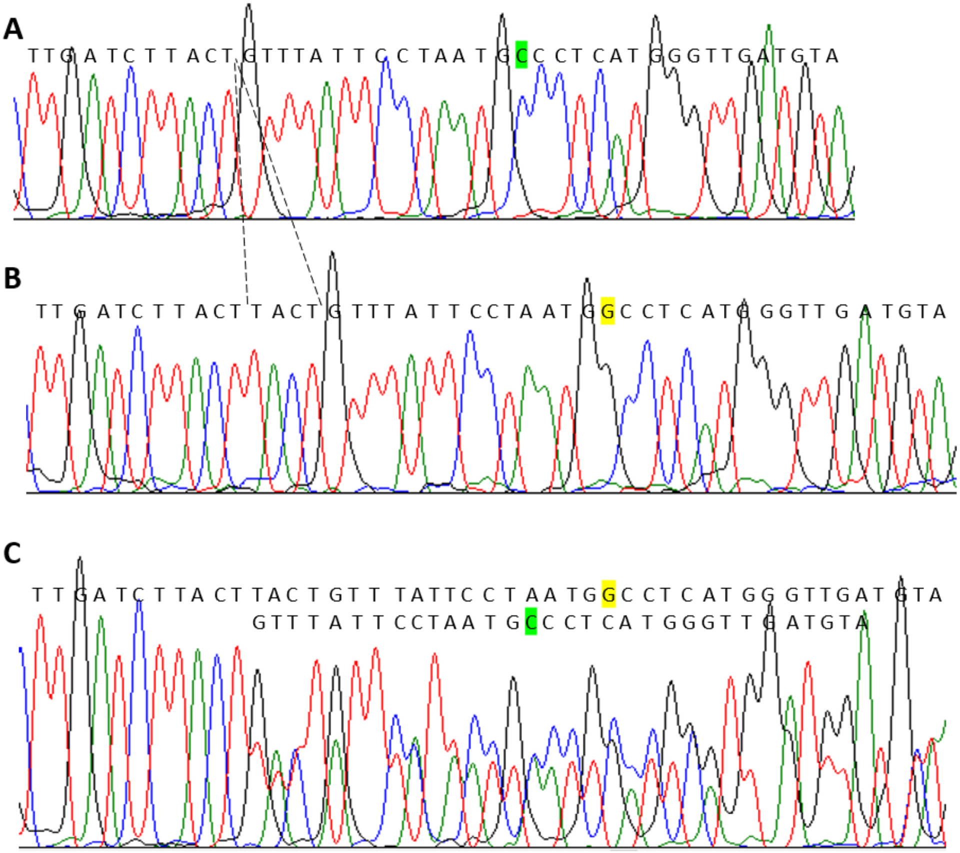


Fig. 4. Variants in intron 1-2 of the *GDF9* gene. **A.** Wildtype sequence found as homozygote in a single animal (GenBank OR541128.1). **B.** Homozygous variant found in the majority of animals (GenBank OR541129.1). The four-nucleotide insertion relative to the wildtype is indicated by the dotted lines, and the substitution is indicated by the nucleotide marked in yellow. The corresponding nucleotide in the wildtype sequence (A) is marked in green. **C.** Heterozygous animal with the doublet variant indicated as the upper sequence, and the wildtype sequence as the lower line.

GDF9 exon 2

In exon 2 of the *GDF9* gene, there are at least 6 known fecundity variants in the area stretching from position 943 (FecG^V c.943C>T) to 1279 (FecG^T c.1279A>C) (summed up by Tong et al., 2020). However, only a four (3H + 1L) animals gave reasonable sequence quality over longer stretches, and we were able to cover five of the six variant positions. All four animals showed wildtype sequence at these positions.

Genotyping

Quality control removed three animals from the dataset due to missing genotype data. No variants were removed by Hardy-Weinberg exact test or by minor allele threshold, but 1,721 variants were removed due to missing genotype data. This left 49,925 variants and 359 animals that passed filters and quality controls. Two animals were removed from the fertility analyses (because of missing fertility data), but were included in the initial relatedness analyses (Hansen et al., 2025), where the multidimensional scaling (MDS) analysis indicated that the samples clustered according to geography (Hansen et al., 2025). The two flocks that were on the same island (Streymoy) grouped together and separated clearly from each of the two other flocks that were from different islands (Eysturoy and Kalsoy). The geographical clustering of Faroese sheep decreases the power of the subsequent association analyses between fertility and genetic variants. When all four flocks (357 animals) were included, a single variant in chromosome 3 (OAR3_95120667.1) was at significance ($-\log_{10}(p) > 6$) (Fig. 5). This variant is located in a non-coding region, approximately 170,000 bp away from the closest known protein-coding gene (ENSOARG00000010279; probably originating from an integrated retrovirus). There were a few variants that were close to significance ($5 < -\log_{10}(p) < 6$). None of these variants were among the known fecundity variants.

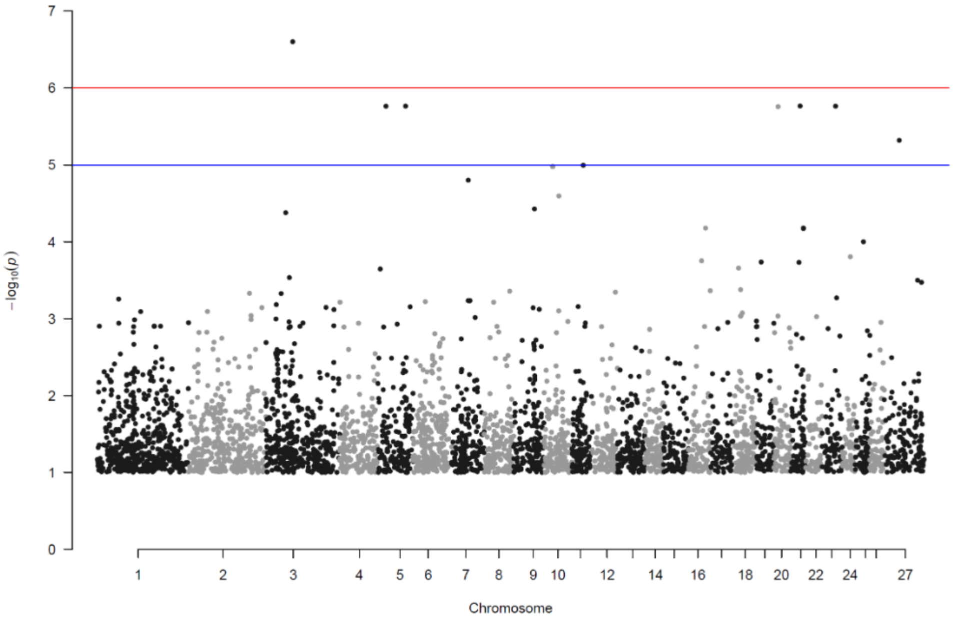


Fig. 5. Manhattan plot for genetic variants in 359 Faroese ewes scanned for number of foetuses. The red line indicates statistical significance at $-\log_{10}(p) > 6$, while the blue line indicates near significance at $-\log_{10}(p) > 5$.

We then analysed each of the flocks separately (data not shown). No cluster of fertility-related variants were indicated by any of the analyses. A total of 53 variants had statistical significance ($-\log_{10}(p) > 6$) in one or another of the flocks, and another 100 variants showed near significance ($5 < -\log_{10}(p) < 6$). Importantly, none of these variants showed significance in two or more flocks, including the specific variant mentioned above (it was highly significant only in the animals from Eysturoy). This strongly suggested that the significance was randomly obtained.

As noted above, the genetic stratification of the animals into island-dependent groups (which in this work also coincides with flocks, except that the Streymoy animals consist of two flocks) decreased the power of the association analyses. Additionally, we have used a medium-density genotyping array (50 K). Thus, we cannot fully exclude the possibility that some undetected major genetic fecundity variants ("major" understood as one or a few variants that have high explanatory power) exist in the examined Faroese sheep, although it is more likely that external factors like shelter and feed (see Table 1), potentially combined with several minor variants, can explain the differences in rates of multiple births in the present animals. Such minor variants could of course be located in genes such as those mentioned in the Introduction but could also be in genes concerned with metabolism and energy efficiency, nutrient transport over the placenta, or many other types of genes. It is well known that many animal (or human) properties with high genetic inheritance is associated with numerous minor genetic variants, where each variant only explains a tiny bit of the genetic inheritance of the property, and additionally, external/environmental factors have significant contributions to the property. For example, one study found 29 genetic variants associated with human hypertension and cardiovascular disease risk (International Consortium for Blood Pressure Genome-Wide Association et al., 2011), and more extremely, >1,000 genetic variants are associating with human obesity (reviewed in Loos & Yeo, 2022), and 12,111 SNPs clustered within 7,209 non-overlapping genomic segments are associated with human height (Yengo et al., 2022).

It is recognized that the fertility is inversely correlated to inbreeding (Charlesworth & Willis, 2009). Hypothetically, the differences in multiple birth frequencies in the flocks of Faroese sheep, could therefore be explained by differences in the degree of inbreeding among the flocks. The average individual inbreeding coefficient (F) for the four investigated flocks varies between 0.12 and 0.15 (Hansen et al., 2025), where the flock with the highest multiple birth frequency has the second highest F. The Faroese inbreeding coefficients are at level with or lower than those of Icelandic sheep and Norwegian Spael, and minimally higher than Finnsheep and Rambouillet (Hansen et al., 2025). Less formally, this is also shown by the numerous variants we found in our sequencing of limited areas of known fecundity-related genes (Figs. 2-4). It is therefore unlikely that inbreeding depression can explain the differences in multiple birth rates among the flocks.

It should be noted that the present genotyped Faroese animals are only representative for sheep in the northern part of the Faroe Islands, as (i) the animals in the southern part of the Faroe Islands are assumed to have a distinct origin (Svabo, 1789a, 1789b), and ii) there is limited transport of animals between the islands due to actions against scrapie (Logir.fo, 1990).

Conclusions

This study was not able to point out any genetic variants that were associated with increased fecundity in Faroese sheep. This does not fully exclude the possibility that there in fact could be such major genetic variants, but they must be different from any of the nearly 50,000 variants investigated here and not showing any particular genetic linkage to the present variants. It appears more likely that several minor variants (that in the present work did not reach significance) could interact with external factors like feed and shelter to explain the differences in fecundity between the flocks.

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