Sustainable Solutions for Small Ruminants

Mapping a putative autosomal gene controlling ovulation rate and infertility in Cambridge sheep

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Teagasc

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SEVENTH FRAMEWORK PROGRAMME

Cambridge breed

- Composite breed developed from a set of 54 ewes with exceptional prolificacy selected in Britain in 1964.
- The foundation ewes had to have achieved a minimum prolificacy of three sets of triplets in consecutive lambings (primarily Clun Forest)
- Mated to Finnish Landrace rams
- Selected males from these mating retained for subsequent use
- Extreme variation in OR and occasional ovarian hypoplasia
- Mutations identified in *GDF9* and *BMP15* which affected OR[1]
- Further work suggested there was an additional autosomal recessive gene segregating that caused ovarian hypoplasia
- This gene may also be associated with canalisation of ovulation rate in heterozygous carriers.

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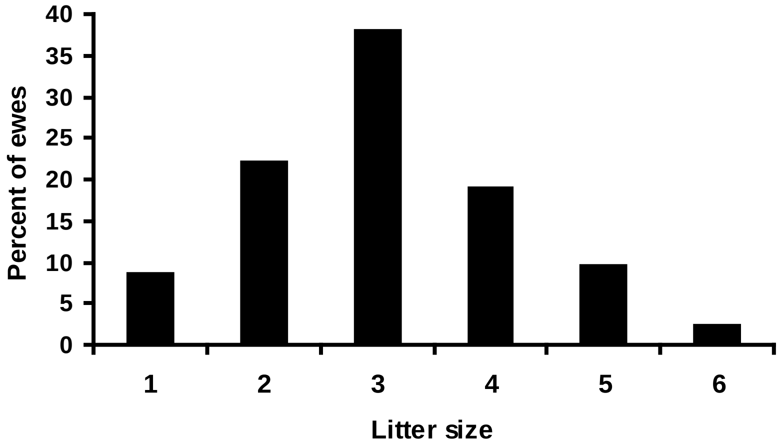
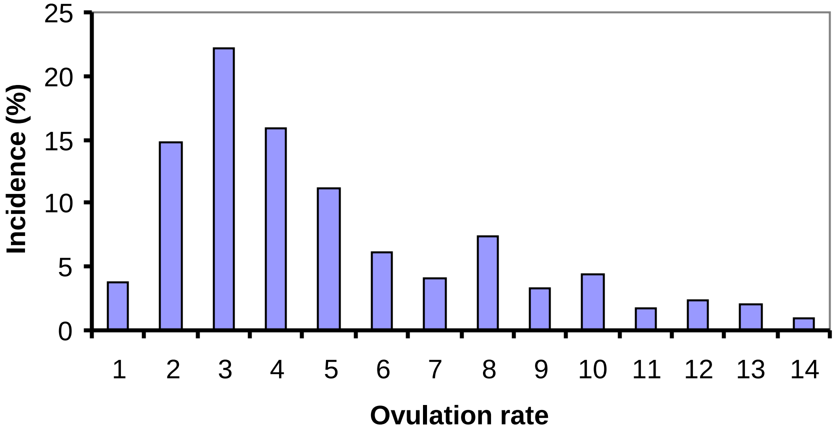
[1] Hanrahan et al., 2004

Performance Characteristics



- Mean Litter size = 3.1
- Max litter size = 8
- Litter size varies little with age



- Extreme variation in OR

Genotypic & Phenotypic characterisation of unexplained steriles

23 unexplained sterile ewes = p = 0.26
344

BMP15	***GDF9***	**No of Unexplained Sterile Ewes**
WT/WT	WT/WT	7
WT/WT	WT/$FecG^H$	4
WT/$FecX^G$	WT/WT	5
WT/$FecX^G$	WT/$FecG^H$	7

Genotype	**Phenotypic characterisation (follicles/cortex recorded)**
Unexplained Sterile	100%
GDF9 sterile	50%
BMP15 sterile	29%

Discovery Population

Population
23 unexplained sterile
17 carrier females
14 carrier males

Number of animals in Teagasc Cambridge population since 1994

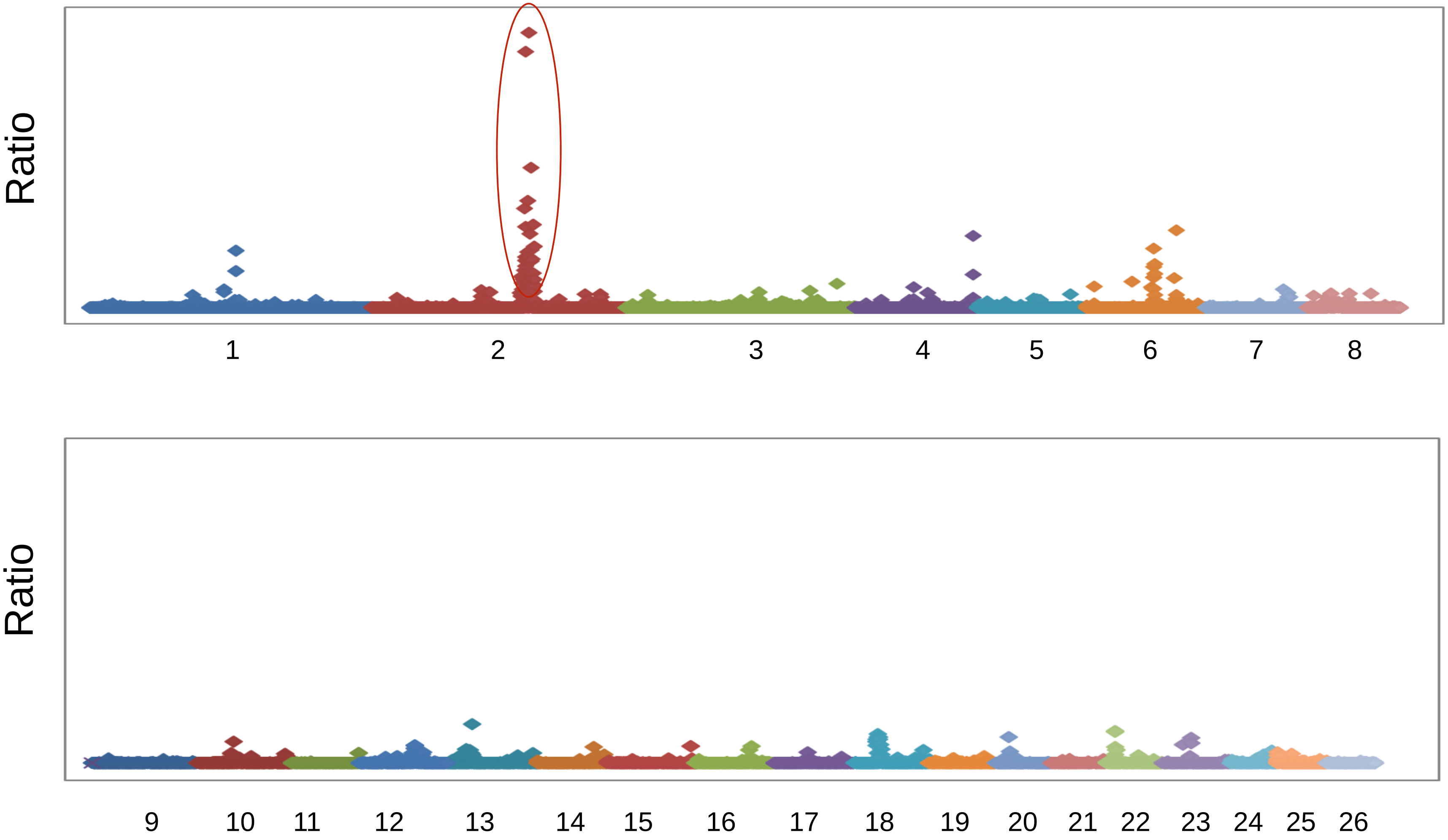
Number available for genotyping

50K genotypes
15 unexplained sterile
11 carrier females
4 carrier males

Number available after editing

50K genotypes
12 unexplained sterile
9 carrier females
3 carrier males

Regions of Homozygosity



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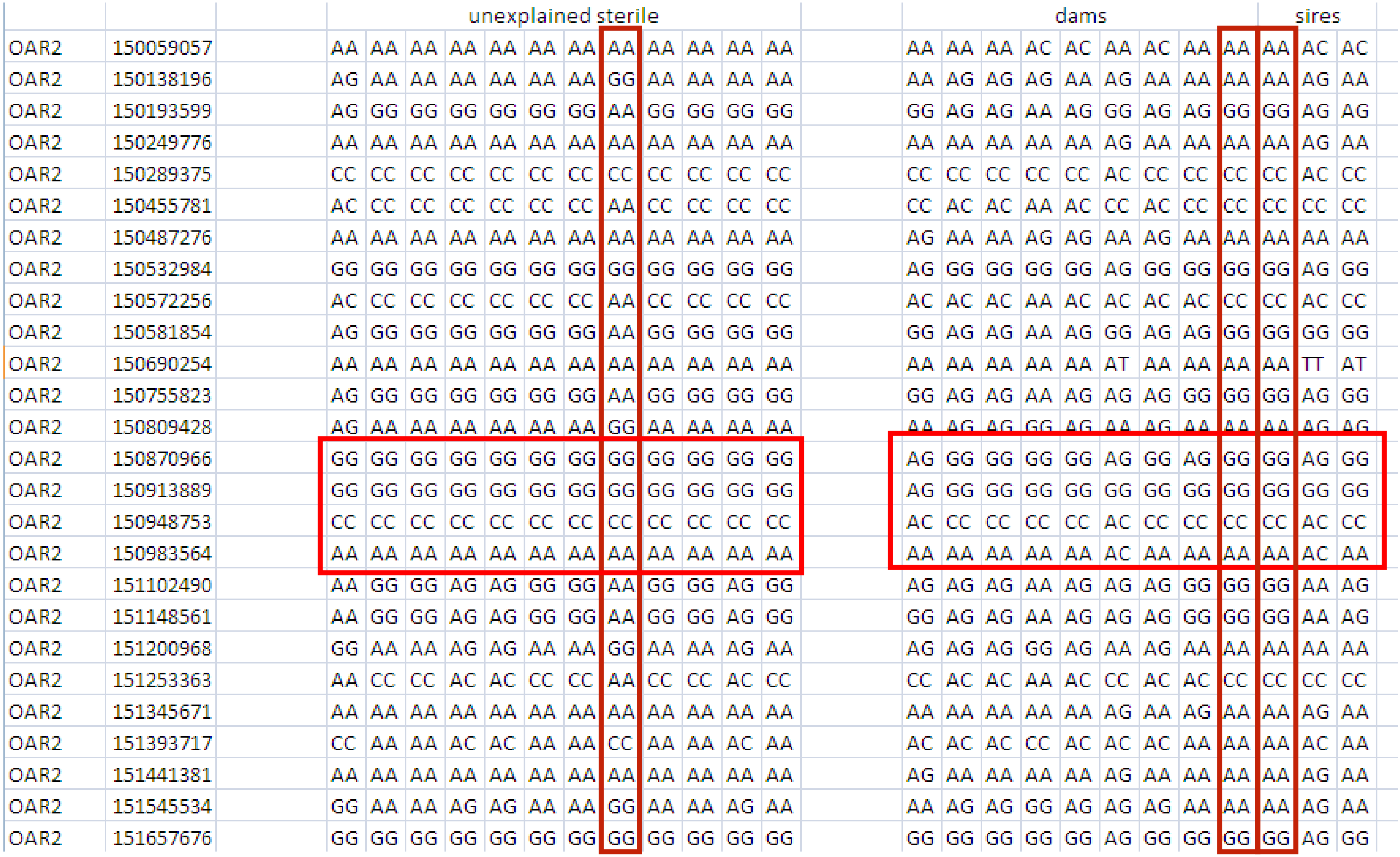
Haplotypes



			unexplained sterile													dams									sires		
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OAR2	150138196		AG	AA	AA	AA	AA	AA	AA	GG	AA	AA	AA	AA		AA	AG	AG	AG	AA	AG	AA	AA	AA	AA	AG	AA
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OAR2	150249776		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA
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OAR2	150487276		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AG	AA	AA	AG	AG	AA	AG	AA	AA	AA	AA	AA
OAR2	150532984		GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG		AG	GG	GG	GG	GG	AG	GG	GG	GG	GG	AG	GG
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OAR2	150690254		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AA	AA	AA	AA	AA	AT	AA	AA	AA	AA	TT	AT
OAR2	150755823		AG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG		GG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AG	GG
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OAR2	150870966		GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG		AG	GG	GG	GG	GG	AG	GG	AG	GG	GG	AG	GG
OAR2	150913889		GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG		AG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
OAR2	150948753		CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC		AC	CC	CC	CC	CC	AC	CC	CC	CC	CC	AC	CC
OAR2	150983564		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AA	AA	AA	AA	AA	AC	AA	AA	AA	AA	AC	AA
OAR2	151102490		AA	GG	GG	AG	AG	GG	GG	AA	GG	GG	AG	GG		AG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AA	AG
OAR2	151148561		AA	GG	GG	AG	AG	GG	GG	AA	GG	GG	AG	GG		GG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AA	AG
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OAR2	151253363		AA	CC	CC	AC	AC	CC	CC	AA	CC	CC	AC	CC		CC	AC	AC	AA	AC	CC	AC	AC	CC	CC	CC	CC
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OAR2	151545534		GG	AA	AA	AG	AG	AA	AA	GG	AA	AA	AG	AA		AA	AG	AG	GG	AG	AG	AG	AA	AA	AA	AG	AA
OAR2	151657676		GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG		GG	GG	GG	GG	GG	AG	GG	GG	GG	GG	AG	GG

Flock Haplotypes

- Can we generate unexplained sterile animals by mating animals heterozygous for the haplotype of interest on OAR 2

- Designed a SNP assay for 34 SNP across 10 MB in region of interest on chr 2

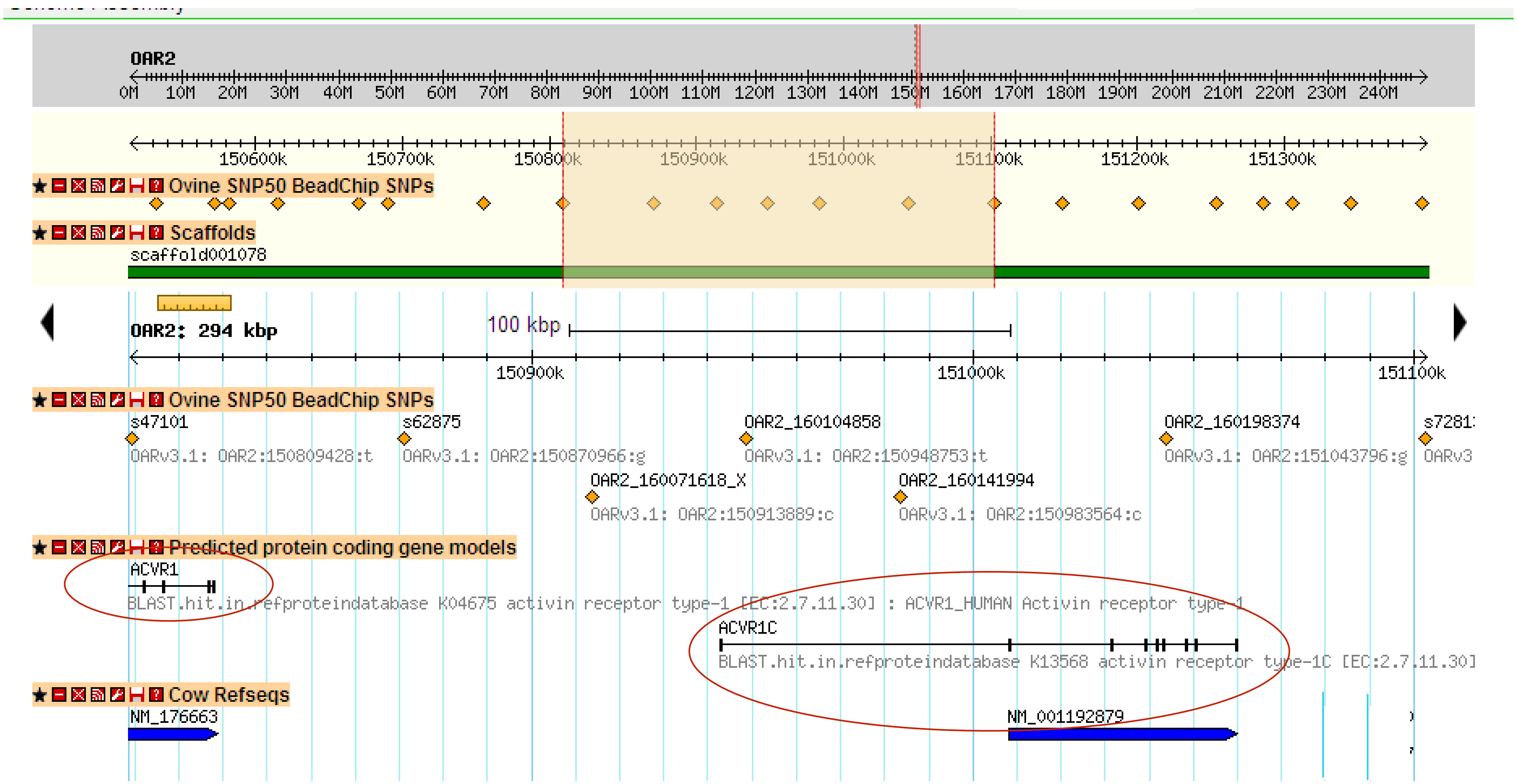
- Genotyped flock (n = 142) for region of interest

- 23 SNP remaining after quality control

- Assigned haplotypes with FastPHASE

- Haplotype of interest on chr 2 is a common haplotype in the population

Sheep Genome Browser



OARv3.1: OAR2:150809428:t

OARv3.1: OAR2:150870966:g

OARv3.1: OAR2:150948753:t

OARv3.1: OAR2:151043796:g

OARv3

OARv3.1: OAR2:150913889:c

OARv3.1: OAR2:150983564:c

BLAST.hit.in.refproteindatabase K04675 activin receptor type-1 [EC:2.7.11.30] : ACVR1_HUMAN Activin receptor type-1

BLAST.hit.in.refproteindatabase K13568 activin receptor type-1C [EC:2.7.11.30]

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ACVR1 & ACVR1C sequencing

- *ACVR1* & *ACVR1C* encode type I TGF- β superfamily member ligand receptors

- TGF- β superfamily ligands bind to a TGF- type II receptor. The type II receptor is a serine/threonine receptor kinase, which recruits and catalyses the phosphorylation of the Type I receptor which subsequently transduces the signal via SMADs

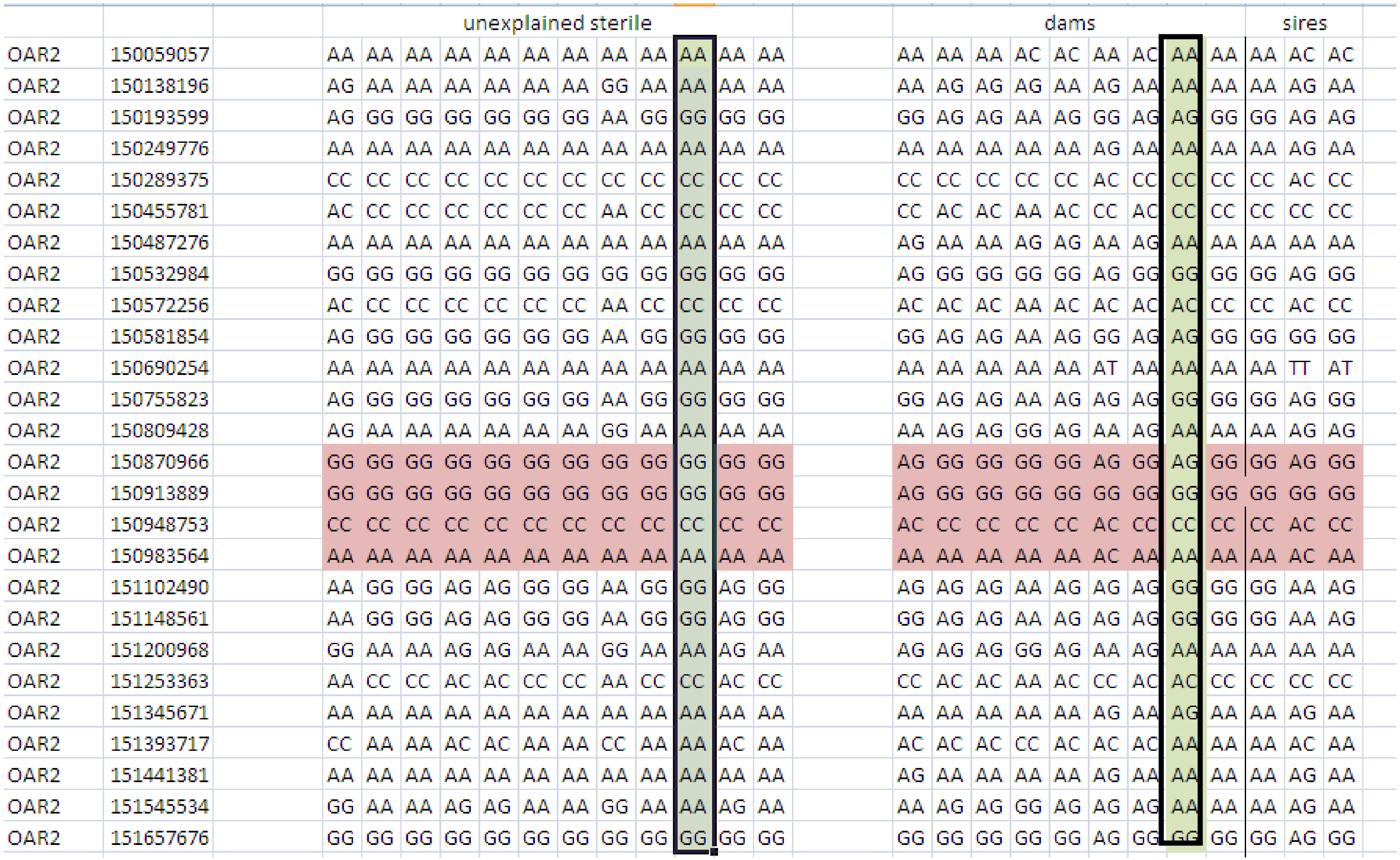
- *ACVR1* – sequenced CDS

-No polymorphims found that explain the phenotype

- *ACVR1C* – sequenced CDS

- 5′ end of *ACVR1C* failed to amplify
- *ACVR1C*-positive BAC sequenced
- No polymorphisms found that explain the phenotype

Whole Genome Sequencing



		unexplained sterile												dams									sires		
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OAR2	150138196	AG	AA	AA	AA	AA	AA	AA	GG	AA	AA	AA	AA	AA	AG	AG	AG	AA	AG	AA	AA	AA	AA	AG	AA
OAR2	150193599	AG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG	GG	AG	AG	AA	AG	GG	AG	AG	GG	GG	AG	AG
OAR2	150249776	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA
OAR2	150289375	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	AC	CC	CC	CC	CC	AC	CC
OAR2	150455781	AC	CC	CC	CC	CC	CC	CC	AA	CC	CC	CC	CC	CC	AC	AC	AA	AC	CC	AC	CC	CC	CC	CC	CC
OAR2	150487276	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AG	AG	AA	AG	AA	AA	AA	AA	AA
OAR2	150532984	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	AG	GG	GG	GG	GG	AG	GG	GG	GG	GG	AG	GG
OAR2	150572256	AC	CC	CC	CC	CC	CC	CC	AA	CC	CC	CC	CC	AC	AC	AC	AA	AC	AC	AC	AC	CC	CC	AC	CC
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OAR2	150948753	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	AC	CC	CC	CC	CC	AC	CC	CC	CC	CC	AC	CC
OAR2	150983564	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AC	AA	AA	AA	AA	AC	AA
OAR2	151102490	AA	GG	GG	AG	AG	GG	GG	AA	GG	GG	AG	GG	AG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AA	AG
OAR2	151148561	AA	GG	GG	AG	AG	GG	GG	AA	GG	GG	AG	GG	GG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AA	AG
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OAR2	151253363	AA	CC	CC	AC	AC	CC	CC	AA	CC	CC	AC	CC	CC	AC	AC	AA	AC	CC	AC	AC	CC	CC	CC	CC
OAR2	151345671	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AG	AA	AA	AG	AA
OAR2	151393717	CC	AA	AA	AC	AC	AA	AA	CC	AA	AA	AC	AA	AC	AC	AC	CC	AC	AC	AC	AA	AA	AA	AC	AA
OAR2	151441381	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA
OAR2	151545534	GG	AA	AA	AG	AG	AA	AA	GG	AA	AA	AG	AA	AA	AG	AG	GG	AG	AG	AG	AA	AA	AA	AG	AA
OAR2	151657676	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	AG	GG	GG	GG	GG	AG	GG

Whole Genome Sequencing

- Genomes sequenced to approximately 12 X depth

- Sequence aligned and compared to Texel reference sequence

- 1779 SNP detected in ROI that were

 - homozygous for alternative allele in sterile case
 - heterozygous in carrier

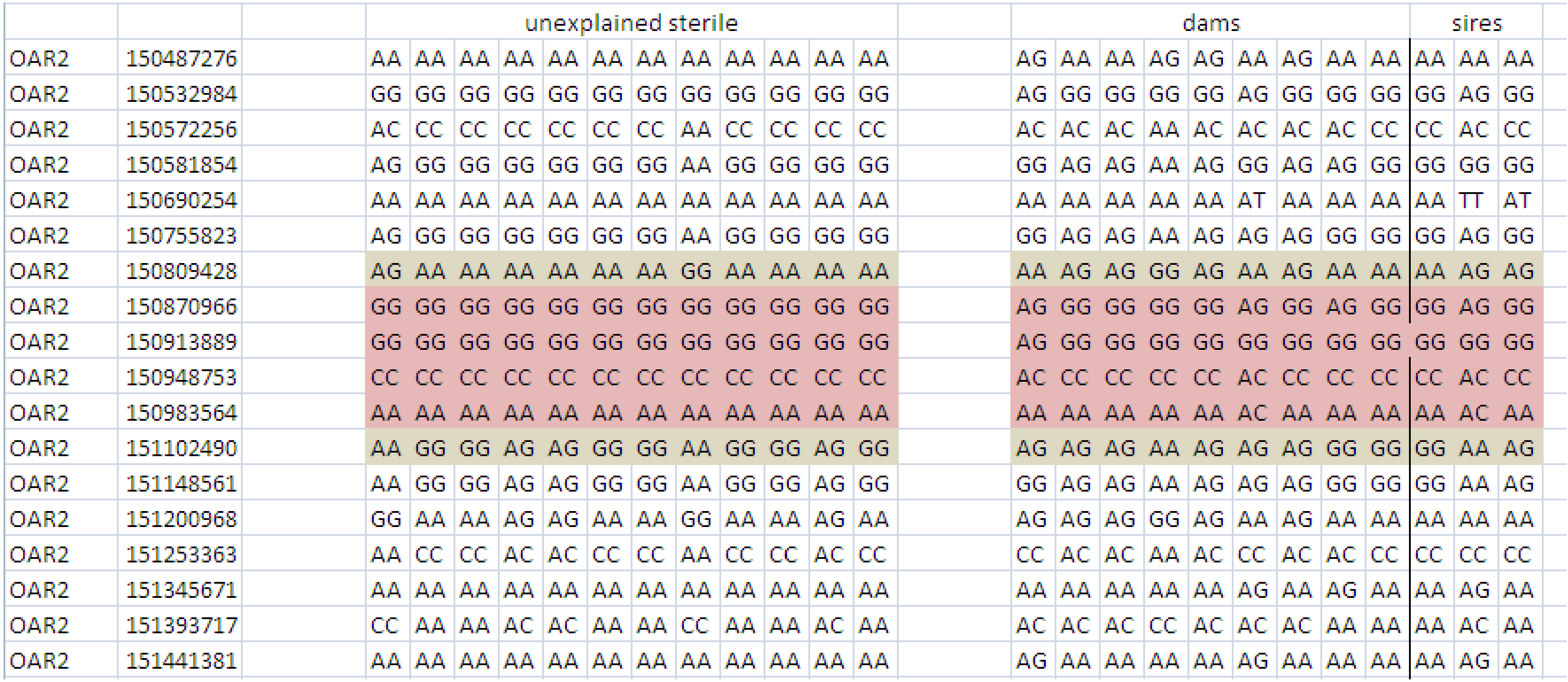
- Filtered SNP using

 - 3 sequenced Laucaune animals
 - 75 genome data set (ISGC)

- 49 SNP in large ROI
- 2 SNP in small ROI

SEVENTH FRAMEWORK PROGRAMME

Genotyping 6 SNP in additional animals



			unexplained sterile													dams									sires		
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OAR2	150581854		AG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG		GG	AG	AG	AA	AG	GG	AG	AG	GG	GG	GG	GG
OAR2	150690254		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AA	AA	AA	AA	AA	AT	AA	AA	AA	AA	TT	AT
OAR2	150755823		AG	GG	GG	GG	GG	GG	GG	AA	GG	GG	GG	GG		GG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AG	GG
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OAR2	150913889		GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG		AG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
OAR2	150948753		CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC		AC	CC	CC	CC	CC	AC	CC	CC	CC	CC	AC	CC
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OAR2	151148561		AA	GG	GG	AG	AG	GG	GG	AA	GG	GG	AG	GG		GG	AG	AG	AA	AG	AG	AG	GG	GG	GG	AA	AG
OAR2	151200968		GG	AA	AA	AG	AG	AA	AA	GG	AA	AA	AG	AA		AG	AG	AG	GG	AG	AA	AG	AA	AA	AA	AA	AA
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OAR2	151441381		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA		AG	AA	AA	AA	AA	AG	AA	AA	AA	AA	AG	AA

Genotyping 6 SNP surrounding region of interest in

- 4 unexplained sterile
- 9 carrier

Future Work

- Genotype additional sterile animal born 2013 and her parents
- Genotype 19 new SNP in ROI in all available cases/controls
- Complete genotyping of 6 SNP in additional sterile and carrier animals
- Sequence genome of 3 additional Cambridge animals
- Call SNP in region of interest
- If necessary fill gaps in regions of interest

SEVENTH FRAMEWORK
PROGRAMME

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- Pavel Senin (INRA)
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- International Sheep Genomics Consortium

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