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A genetic test to identify carriers of the ovine Inverdale fecundity gene

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ABSTRACT

The Inverdale high fecundity gene ($FecX^I$) is carried on the sheep X-chromosome. This provides a convenient means of producing prolific single copy carrier ewes because all daughters of a carrier ram will inherit the gene. A single copy of the gene increases litter size by about 0.6 lambs per ewe lambing. However, ewes homozygous for the gene are infertile. Production of elite rams that carry the gene requires the ability to distinguish between non-carrier and single copy carrier animals. Three DNA markers flanking the gene in a 10 cM (centiMorgan) region have been used to develop a genetic test that can identify single and double copy carriers in most cases. The test relies on detecting inheritance of the Inverdale region of the X-chromosome in offspring of known carriers. Validity of the genetic test has been confirmed by progeny testing of rams and laparoscopy of ewes. The test has recently been adapted to DNA obtained from a few plucked wool follicles.

Keyword: Inverdale gene; $FecX^I$; X-chromosome; genetic test; DNA markers; sheep (*Ovis aries*).

INTRODUCTION

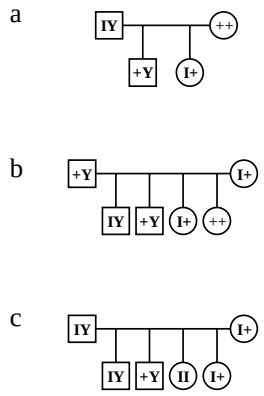
The Inverdale high fecundity gene ($FecX^I$) is a major gene for prolificacy in sheep which was first identified in descendants of a Romney ewe (A281) with consistently high litter sizes. Segregation studies showed that the gene is carried on the X-chromosome (Davis *et al*., 1991). A single copy of the gene in heterozygous (I+) ewes increases ovulation rate by about one extra egg, and litter size by about 0.6 lambs per ewe lambing. However, homozygous (II) ewes carrying two copies of the gene have small non-functional ovaries and are infertile (Davis *et al*., 1992).

Several important aspects of the Inverdale gene have been described in detail at a previous meeting of the NZSAP. These include discovery of the gene (Davis *et al*., 1995), segregation (Dodds *et al*., 1995), physiology (McNatty *et al*., 1995; McLeod *et al*., 1995), gene mapping (Galloway *et al*., 1995), commercial performance (Gray and Davis, 1995; Hanna, 1995) and suggestions for use in the industry (McEwan *et al*., 1995).

Inheritance of the X-chromosome differs from autosome pairs in that females have two X-chromosomes but males have an X-chromosome and a Y-chromosome. Males therefore pass the Y-chromosome to all sons and the X-chromosome to all daughters. This provides a convenient means of producing prolific single copy Inverdale carrier ewes because all daughters of an Inverdale carrier ram will inherit the gene (Fig. 1a).

However, production of elite rams which carry the Inverdale gene requires the ability to distinguish between non-carriers (++ females or +Y males) and single copy carriers (I+ females or IY males) (Fig.1b). To avoid producing infertile (II) ewes, matings should not be carried out between animals which both carry the gene (Fig.1c), but should this occur there needs to be a means of distinguishing between I+ carriers and infertile II ewe progeny.

FIGURE 1: Genotypes resulting from Inverdale matings. Males are represented by squares and females by circles. Symbols are ++ non-carrier female; I+ single copy (heterozygous) female; II double copy (homozygous) female; +Y non-carrier male; IY carrier male.



Infertile II ewes can be identified by laparoscopy at 6 months due to the obvious difference in the structure of their unformed 'streak' ovaries (Davis *et al*., 1992). They can also be identified later in life as dry (unmarked) ewes at tupping. Carrier IY rams can be progeny tested by mating them to single copy I+ ewes and detecting infertile female offspring with 'streak' ovaries by laparoscopy. Infertile II ewes can also be identified on the basis of differences in reproductive hormones. Increased plasma FSH and LH levels in II ewe lambs as young as two months was able to distinguish between infertile (II) and carrier (I+) females in 96 percent of cases (McLeod *et al*., 1997), but this test is unable to identify carrier males or distinguish between ++ and I+ females.

As part of the search for the gene responsible for the Inverdale trait we have constructed a genetic linkage map of the sheep X-chromosome (Galloway *et al*., 1996) and localised the Inverdale gene to a 10 cM (centiMorgan)

region of the chromosome. Although the exact location of the gene has not been determined, the proximity of flanking DNA microsatellite markers have allowed us to develop a genetic test for carriers of the Inverdale gene. The accuracy of the test has been evaluated by progeny testing and laparoscopy of offspring.

MATERIALS AND METHODS

DNA sampling

DNA was purified from the white blood cells present in 5 to 10 ml of whole blood from each animal as previously described (Montgomery and Sise, 1990). DNA was also obtained from the follicles of 15-20 fibres of plucked wool for some animals (M. Tate, AgResearch (Genom*nz*), pers. comm.).

Animals

The animals tested in this study were from AgResearch Inverdale breeding flocks located at the Invermay Agricultural Centre and Woodlands Research Station, and from the commercial flocks of Mr Arnold Gray, Orawia, Southland. All Inverdale carrier animals were descendants of the original Inverdale ewe (A281) from which the Inverdale gene was first detected.

DNA markers

Microsatellite (dinucleotide repeat) markers which amplified DNA from sheep were developed within the AgResearch Molecular Biology Unit as previously described (Galloway *et al*., 1996) or were from the cattle and sheep mapping literature. New markers were mapped onto the sheep X-chromosome as previously described (Galloway *et al*., 1996).

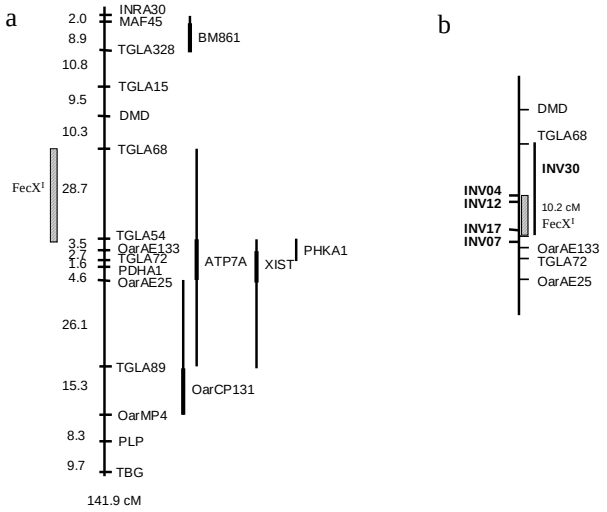
Progeny testing of rams

Carrier status of rams was assessed in one of two ways. Early in the study rams were assigned on the basis of ovulation rates of their daughters. With the discovery of 'streak' infertile ovaries in II ewes a faster method for progeny testing of rams was employed by mating each ram to seven to ten I+ ewes and carrying out laparoscopic examination of the ovaries of their daughters at 6 months. Infertile II offspring with 'streak' ovaries confirm the sire as a carrier. The aim was to produce five daughters per ram, as the probability of an IY ram having no daughters with streak ovaries in a sample of five daughters is only 0.031 (Davis *et al*., 1994).

RESULTS AND DISCUSSION

The Inverdale gene was initially located within a large (30 cM) gap in the genetic linkage map of the sheep X-chromosome (Fig. 2a). Five new DNA markers (INV04, INV07, INV12, INV17, INV30) have now been mapped into this region and the closest of these flank the gene by about 10 cM (Fig. 2b). Nine of the nearby markers have been assessed for suitability as genetic markers to follow the inheritance of the Inverdale gene. DNA markers mark positions along the X-chromosome at which it is possible to detect differences between a region of an X-chromosome

FIGURE 2: Partial sheep X-chromosome genetic linkage map showing (a) the extent of the Inverdale ($FecX^I$) region defined by the original map and (b) flanking markers which currently define the gene region.



which is inherited from the father and one which is inherited from the mother. Homologous recombination between the two X-chromosomes during meiosis in the mother results in inheritance of an X-chromosome which contains a mixture of the mother's two X-chromosomes. The markers allow identification of which X-chromosome regions have been passed on from which chromosome. Flanking markers on either side of the Inverdale gene can show whether the offspring has received the entire Inverdale region (and hence the Inverdale gene) or not.

Initial tests used more distant markers (TGLA68, OarAE25 etc) but these were less accurate in predicting the presence of the Inverdale gene, as the likelihood of recombination between markers increases the further apart they lie on the chromosome. As new markers were mapped into the region the distant markers have been replaced. Suitability of markers was also dictated by their ability to easily amplify sheep DNA under standard polymerase chain reaction (PCR) conditions (Galloway *et al*., 1996), by the number of different variants (informative alleles) the markers are able to detect in the sheep population, and by the ease of interpreting the data and assigning clear and unambiguous alleles. Several potential markers were superceded for these reasons and the present test relies on following the inheritance of three markers (INV12, INV17 and INV07).

Thirty-one rams predicted by the genetic marker test to be IY carriers were progeny tested to confirm this assignment (Table 1). Twenty-nine rams (94%) were clearly confirmed as Inverdale carriers. The remaining two rams have not been confirmed as carriers as they did not produce infertile daughters. One had insufficient daughters (two) to provide a definitive result and is still likely to be a carrier ram. The second produced no infertile daughters out of eight daughters and while this does not rule it out as a carrier, the likelihood of it being a carrier is only 0.004. (Davis *et al*., 1994).

To date the genetic test has been carried out for 215

TABLE 1: Genotype prediction of Inverdale carrier rams. Flanking DNA markers were used to identify rams carrying the Inverdale region. The rams were also assigned a carrier status on the basis of progeny testing. Progeny tests were based on the ovulation rates of daughters (Ov. rate) or on the presence of infertile daughters of I+ mothers (Infert. daughters).

Number of rams	DNA marker prediction	Method of progeny test	Assigned carrier status on basis of progeny test
12	INV	Ov. rate	IY
17	INV	Infert. daughters	IY
29			
2	INV	Infert. daughters	? [a]

[a] one ram produced no infertile daughters out of two daughters and the other ram produced no infertile daughters out of eight daughters

rams (to distinguish IY from +Y) and 507 ewes (to distinguish II, I+ and ++) in commercial and research flocks. Most tests have been based on DNA extracted from blood samples of adult animals or lambs at about 6 months, but animals can be sampled at any age. The test has also been used to distinguish between the II or I+ status of fetal tissue samples (K. McNatty, pers. comm.). A recent adaptation has been to use DNA extracted from the follicles of 10 to 15 wool fibres, enabling farmers to collect samples themselves. Work is also currently underway to develop the test from a drop of blood on blotting paper.

The current DNA marker test has a number of limitations in its present form. Prediction of Inverdale carrier status relies on the inheritance of the entire region flanked by the three markers. The test cannot be relied upon to predict the Inverdale status of animals in which recombination has occured between these markers. Recombination could be expected to affect 10% of samples over the genetic distance of 10 cM. Until the Inverdale gene itself is identified, the DNA test relies on prior knowledge of the carrier status of the parents or grandparents in a pedigree. However, as all Inverdale animals currently tested have descended from one animal, the pattern of alleles for close markers around the Inverdale gene are relatively constant in all offspring. It remains to be seen how frequently this Inverdale pattern of alleles occurs more widely in non-Inverdale flocks into which the gene is being introduced but tests to date on Inverdale x East Friesian and Inverdale x Texel crosses have still been able to distinguish the Inverdale gene region.

In summary, the Inverdale genetic test is able to distinguish all Inverdale genotypes in both sexes, can be carried out at (or before) birth and only requires a small amount of DNA. The test is currently being added to the genetic tests available from AgResearch which include the Booroola test (Lord *et al*., 1998), sheep parentage tests and product tracing. Closer DNA markers are being developed as part of the continuing effort to determine the exact location and identity of the Inverdale gene. Any new markers which further narrow the Inverdale region and which are suitable for a diagnostic test will replace the current markers in order to improve the accuracy of the test.

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REFERENCES

Davis, G.H.; McEwan, J.C; Fennessy, P.F.,; Dodds, K.G.; Farquhar, P.A. 1991: Evidence for the presence of a major gene influencing ovulation rate on the X-chromosome of sheep. *Biology of Reproduction* **44:** 620-624.

Davis, G.H.; McEwan, J.C.; Fennessy, P.F.; Dodds, K.G.; McNatty, K.P.; O, W-S. 1992: Infertility due to bilateral ovarian hypoplasia in sheep homozygous ($FecX^{I}$ $FecX^{I}$) for the Inverdale prolificacy gene located on the X-chromosome. *Biology of Reproduc*tion **46:** 636-640.

Davis, G.H.; Bruce, G.D.; Reid, P.J. 1994: Breeding implications of the streak ovary condition in homozygous ($FecX^{I}$/$FecX^{I}$) Inverdale sheep. *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production* **19:** 249-252.

Davis, G.H.; McEwan, J.C.; Fennessy, P.F.; Dodds, K.G. 1995: Discovery of the Inverdale gene (FecX). *Proceedings of the New Zealand Society of Animal Production* **55:** 289-290

Dodds, K.G.; Davis, G.H.; McEwan, J.C.; Fennessy, P.F. 1995: Elucidation of the genetic nature of the Inverdale gene (FecX): Segregation with a twist. *Proceedings of the New Zealand Society of Animal Production* **55:** 291-293

Galloway, S.M.; Hanrahan, V.; Potts, M.D.; Hill, D.F. 1995: Genetic markers in Inverdale (FecX) sheep. *Proceedings of the New Zealand Society of Animal Production* **55:** 307-309.

Galloway, S.M.; Hanrahan, V.; Dodds, K.G.; Potts, M.D.; Crawford, A.M.; Hill, D.F. 1996: A linkage map of the ovine X chromosome. *Genome Research* **6:** 667-677.

Gray, A.J.; Davis, G.H. 1995: Commercial performance of sheep carrying the Inverdale gene. *Proceedings of the New Zealand Society of Animal Production* **55:** 294-295.

Hanna, M.M. 1995: Living with the Inverdale gene (FecX) in a Romney flock. *Proceedings of the New Zealand Society of Animal Production* **55:** 296-297.

Lord, E.A.; Davis, G.H.; Dodds, K.G.; Henry, H.M.; Lumdsen, J.M.; Montgomery, G.W. 1998: Identification of Booroola carriers using microsatellite markers. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production* **27:** 19-22.

McEwan, J.C.; Davis, G.H.; Fennessy, P.F.; Dodds, K.G. 1995: Industry use of the Inverdale gene (FecX). *Proceedings of the New Zealand Society of Animal Production* **55:** 298-300.

McLeod, B.J.; Kyle, S.E.; Ramsay, M.R.; Manley, T.R. 1995: Hormone secretion patterns associated with increased ovulation rates or with ovarian dysfunction in Inverdale (FecX) ewes. *Proceedings of the New Zealand Society of Animal Production* **55:** 304-306.

McLeod, B.J.; Fenton, L.F.; Davis, G.H.; Bruce, G.D.; Manley, T.R.; Johnstone, P.D. 1997: Identifying infertile homozygous Inverdale ($FecX^{I}$) ewe lambs on the basis of genotype differences in reproductive hormone concentrations. *Animal Reproduction Science* **47:** 291-302.

McNatty, K.P.; Smith, P.; Hudson, N.L.; Lun, S.; Heath, D.; Shackell, G.; Corrigan, K. 1995: Ovarian characteristics in Inverdale ewes heterozygous (I+) and homozygous (II) for the Inverdale gene (FecX). *Proceedings of the New Zealand Society of Animal Production* **55:** 301-303.

Montgomery, G.W.; Sise, J.A. 1990: Extraction of DNA from sheep white blood cells. *New Zealand Journal of Agricultural Research* **33:** 437-441.