USE OF GENE MARKERS IN THE NEW ZEALAND SHEEP INDUSTRY

K G Dodds

Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

SUMMARY

A number of gene marker tests have recently become available within the NZ sheep breeding industry. Gene marker tests are available for reproduction, meat yield, and health traits. The tests include those that are diagnostic for the causative mutation, single marker or haplotype based tests for use within specific populations and association based tests. Analysis systems have been developed to estimate genotypes at the causative gene using the marker genotypes of an individual. Genetic evaluation systems incorporating gene marker tests are in the early stages of development.

INTRODUCTION

The past 10-15 years has seen a considerable research effort, both in New Zealand (NZ) and overseas, directed towards finding genetic markers for sheep traits of economic importance. This effort has been aided by advances in genomic technology over this time, due primarily to the large international research effort in human genomics. Sheep genomics research has also benefited from cattle genomics research. The research effort for sheep has reached the point where a number of gene marker tests have recently become available to the NZ sheep breeding industry and are being used to assist selection decisions. This article presents the tests that are available (Table 1) and discusses issues related to their use.

GENE MARKER TESTS AVAILABLE TO THE NEW ZEALAND SHEEP INDUSTRY

Reproduction. Reproduction traits were the first traits in the NZ sheep industry to undergo investigation for marker tests. This was due to the discovery, without the aid of genetic markers, of segregating genes with large effects. The first these was the *Booroola* gene (Piper and Bindon 1982; Davis *et al*. 1982) discovered in a group of Merino sheep descending from members of the *Booroola* stud. One copy of the *Booroola* allele increases ovulation rate by about 1.5 and litter size by about 1.0, while two copies increases these by about 3.0 and 1.5 respectively (Davis, 2005). These large effects prompted a 'proof of concept' study investigating whether quantitative traits could be mapped and their underlying genetic basis possibly even discovered, using molecular genetic techniques. The gene was firstly mapped to sheep chromosome 6 (OAR 6) (Montgomery *et al*. 1993). Further refinement of the region involved allowed selection of carrier progeny of heterozygous parents using flanking markers to track the inheritance (Lord *et al.* 1998). Subsequently the causative mutation, which changes the 249th amino acid of *BMPR-1B* from a glutamine to an arginine, was found (Wilson *et al*. 2001) allowing genotype assisted selection. Genetic tests for Booroola are available from Genom*nz*^{TM} (Crawford *et al.* 2007). Due to practical difficulties in profitably managing the much higher litter sizes (Davis, 2005), the use of the *Booroola* gene in NZ has been predominantly for research purposes, and most commercial tests are for overseas clients.

Another reproduction gene, *Inverdale*, was identified by observing segregation in a line of Romney sheep (Davis *et al*. 1991). All known carriers are descended from a single carrier ewe which was screened into a research flock. Again the gene's effect was sufficiently large for its presence to

be discovered without the aid of genetic markers, but its pattern of inheritance indicated that it was on OAR X. The gene was also found to be overdominant, as although heterozygous ewes have increased ovulations rates (by 1.0) and litter sizes (by 0.6), homozygous ewes are infertile. Linkage mapping on the X chromosome established the approximate location (Galloway *et al*. 1998) allowing selection based on linked markers. Subsequently the causative mutation, which changes the 31st amino acid of *BMP15* from a valine to an aspartic acid, was found (Galloway *et al.* 2000) allowing genotype assisted selection. As a homozygous breeding flock cannot be established and sires cannot pass the allele to their sons, carrier rams are most efficiently generated by marker selection among sons of heterozygous ewes. Amer *et al*. (1998) compared a number of breeding strategies incorporating the *Inverdale* gene. Tests using either a linked marker or the causative mutation are available through Catapult Systems®. The less accurate linked marker test is available at lower cost, particularly if requested in conjunction with parentage testing and is used as a preliminary screening tool. The usefulness of the linked marker tests is because there are few generations back to the most recent common ancestor – the foundation ewe. The direct allele test is used when a more certain result is required, e.g. for sale rams. There have been over 10,000 samples tested for *Inverdale* using the direct gene test, up to January 2007 (Crawford *et al.* 2007), and about 1000 tested *Inverdale* rams are thought to have been used on commercial NZ farms in 2005 (see Dodds *et al.* 2007b). The marketing of *Inverdale* sheep in NZ has been licensed to Inverdale® New Zealand.

An additional line of NZ Romney sheep has also been found to display increased prolificacy that is inherited in the same fashion and has the same effect as the *Inverdale*. This was found to be another mutation in *BMP15* (Galloway *et al.* 2000) and is referred to as the *Hanna* allele. There were few known *Hanna* carriers, and commercialisation of the effect of *BMP15* variants within NZ has been via the *Inverdale* allele as this allows a simpler DNA testing system (one test instead of two or more). No other sources of these mutations or other *BMP15* variants with similar effects have been found in any breed in NZ, but several have been found overseas (Hanrahan *et al.* 2004, Bodin *et al.* 2006). There is evidence that other genes for prolificacy are also segregating in NZ sheep (Davis *et al*. 2001, 2006), but marker tests have not yet been developed for these.

Meat. There are two genes that influence meat production that are in use in the NZ sheep industry. The first of these is the *Carwell* gene, first identified in progeny and other descendants of two rams from the Carwell Poll Dorset flock in Australia. This gene has been mapped to OAR 18 (Nicoll *et al.* 1998), near *Callipyge* another gene that influences meat production, present in US Dorsets (Cockett *et al.* 1994). The *Carwell* allele increases *L. dorsi* weight by 8%, and cross-sectional area by 10%, or about 0.6 and 0.7 phenotypic standard deviations, respectively (Nicoll *et al.* 1998). It is currently thought that the gene is maternally imprinted, such that the effect is only present in progeny that have received the allele from their sire, although no supporting results have been formally published. Industry use is likely to be within terminal sire breeds, producing double copy rams, all of whose progeny (bred to slaughter) would express the effect. Since 1997 the Landcorp breeding programme has been using a set of markers that flank the gene to track its inheritance. More recently a test for the gene has become available from Catapult under the trademark LoinMAX®. This test uses a three marker haplotype where the outside markers are thought to flank the gene. All, or almost all, Poll Dorsets carrying the haplotype are also thought to carry the *Carwell* allele, while the haplotype is rare in other breeds (Campbell and McLaren 2007). There may be other haplotypes with the *Carwell* allele (e.g. if a recombination occurs within the three markers), but these are thought to be less common. Identification of such animals would help refine the *Carwell* position. The LoinMAX® test

appears to work in a wider set of animals than just the descendants of Carwell flock rams. This is likely due to relatively recent common ancestry of the NZ and Australian populations of the *Carwell* allele.

The myostatin (*GDF8*) gene, responsible for double muscling in cattle, was chosen as a candidate gene for influencing meat composition in sheep. A variant influencing carcass yield was found to be segregating in Texel sheep (Broad *et al.* 2000). The variant has its strongest effects on leg composition, with one copy increasing its muscle by 3% and decreasing fat by 10%, or about 0.5 phenotypic standard deviations each (Johnson *et al.* 2005). The mapped position of the variant did not exclude the myostatin region, so this gene remains as a candidate for the effect. The variant appears to have additive effects (two copies give twice the effect; Campbell and McLaren 2007). A two marker haplotype test for the variant is available from Catapult under the trademark MyoMAX®. The haplotype appears to be highly associated with the variant in NZ Texels (and descendants) while it is rare in other breeds (Campbell and McLaren 2007). The genetic bottleneck associated with the importation of Texels into NZ is likely to have resulted in the variant being present on a common background chromosomal region, helping an effective test to be established. A similar effect has also been reported in Texels in Belgium (Laville *et al*. 2004), and the causative mutation for this effect has been described by Clop *et al.* (2006).

Health. The gene marker that has been used the most worldwide is the prion protein (PrP) test for scrapie resistance (Dodds *et al.* 2007b). The test reports the amino acids encoded by codons 136, 154 and 171 on the prion gene, and different haplotypes have been shown to have differences in resistance to scrapie (Barillet *et al.* 2002). As scrapie has not been found in NZ, there is much less emphasis on this test in NZ than, for example, in Europe. Even so, PrP tests are available in NZ (e.g. Zhou *et al.* 2006) to monitor the risks that would be associated with the presence of the disease.

Genes within the ovine major histocompatibility complex (MHC), on OAR 20, are general candidates for disease resistance. Escayg *et al.* (1997) used this reasoning as a basis for investigating genetic differences in footrot resistance in a Corriedale flock, and found cosegregation of the footrot phenotype with markers in the class II region of the MHC in the progeny of one sire. Subsequent research has led to the "Footrot Gene Marker Test" becoming available from Lincoln University (Hickford *et al.* 2006). The test is based on polymorphism in the DQA2 and DQA2-like regions. Alleles are scored into 5 groups from the least susceptible ("1") to the most susceptible ("5"), and animals are given two scores. The alleles are assumed to act additively, with an estimated 10-fold difference in footrot susceptibility between extreme genotypes under sufficient challenge (Hickford *et al.* 2006).

An inherited semi-lethal disorder known as spider lamb syndrome (ovine hereditary chrondrodyplasia) has been found in NZ descendants of American Suffolks. This disorder is essentially an autosomal recessive condition, as most homozygous lambs have long limbs and other skeletal abnormalities, while individual heterozygotes are difficult to distinguish from non-carriers. The causative mutation, which changes the 700th amino acid of FGFR3 (on OAR 6) from a valine to a glutamate, has been found (Beever *et al.* 2006). The gene marker test is available from Massey University, and over 2,500 NZ Suffolks have been tested (Jolly *et al.* 2004).

An autosomal recessive condition known as microphthalmia causes blindness in Texel sheep (De Groot 1957). Microphthalmia has been mapped (Linscott *et al.* 2003), and a haplotype test is available from Catapult under the trademark i-Scan® and is mandatory for registration of Texel sheep in NZ. The test classes animals as "clear" (does not carry a microphthalmia associated haplotype),

"carrier" (does carry a microphthalmia associated haplotype) or "at risk" (genotype includes each of the alleles in a microphthalmia associated haplotype, but not necessarily on the same chromosome). For the latter class, the probability that the animal is a carrier provides more information. This probability is calculated using population estimates of haplotype frequencies to differentiate among the pairs of haplotypes the animal possibly carries (Excoffier and Slatkin 1995). Within NZ Texels, there is only one of these haplotypes known to be associated with microphthalmia, and this haplotype haplotype has not been observed in non-carriers of either the Texel or other breeds within NZ.

Forrest *et al.* (2003, 2006) have chosen β_3-adrenergic receptor (ADRB3) as a candidate for neonatal lamb cold survival, due to its role in the regulation of energy balance. Significant associations were found in Merinos, which has led to the provision of the ADRB3 cold-tolerance test by Lincoln University. Alleles are scored into 3 groups according to their associated risk of cold-related mortality – those with average or below average risk ("A"), those with a risk a little higher than average ("B"), and those with a markedly higher risk ("C") (Forrest and Hickford 2006). Individual allele effects differ by an estimated 2.5-fold and the allele with the highest risk corresponds to two amino acid changes (valine to alanine at position 52 and leucine to valine at position 322) in the ADRB3 protein (Forrest and Hickford 2006).

Table 1. Gene markers in the New Zealand sheep industry

Gene	Traits	Supplier[1]	Type of test[2]
Booroola	Reproduction	Genom*nz*	Gene
Inverdale	Reproduction	Catapult	Gene Linked Marker
Carwell	Meat	Catapult	Haplotype
Myostatin	Meat	Catapult	Gene Haplotype
Prion	Disease (scrapie)	Lincoln Catapult	Association
DQA2	Footrot	Lincoln	Association
FGFR3	Disease	Massey	Gene
Microphthalmia	Disease	Catapult	Haplotype
ADRB3	Survival	Lincoln	Association

[1] Genom*nz*: www.genomnz.co.nz; Catapult: www.catapultgenetics.com; Lincoln: www.lincoln.ac.nz; Massey: http://ivabs.massey.ac.nz/centres/EPAGSC/default.asp.

[2] Gene: Causative mutation is known and is detected by the test; Linked Marker: a single anonymous (not known to have any direct effects) marker close to the causative gene is used; Haplotype: a set of two or more anonymous markers flanking the likely location is used, with one or more haplotypes associated with the effect; Association: variants in a gene are associated with observed effects, and are either the causative variants or are very tightly linked (in linkage disequilibrium) with the causative position.

DISCUSSION

Contributing factors. A number of gene tests are now available to the NZ sheep industry. Several factors have contributed to their discovery and commercial development. Techniques for assaying genetic variation have become less expensive, due to the large effort being directed at understanding human genetic variation, and marker development is aided by the increasing amount of sequence from sheep and cattle deposited in public databases. Increasing knowledge of genetic effects in sheep and other species has also helped formulate useful hypotheses about other possible genetic factors. The footrot and cold tolerance tests both arose from candidate gene studies. The *Booroola* mutation was discovered using knowledge of the *Inverdale* mutation. The myostatin test was discovered using knowledge of myostatin effects in cattle. The *Carwell* gene was mapped quickly as it happened to be close to another gene affecting muscle growth in sheep.

Another reason enabling commercial development of these tests has been Catapult's strategy of providing these tests in conjunction with parentage testing. The lowering cost of parentage testing, and the availability of genetic evaluation using fractional pedigree assignments (Dodds *et al.* 2005) has resulted in the uptake of this technology, with over 250,000 sheep having being genotyped for parentage in NZ to date (Crawford *et al.* 2007).

Types of test. The simplest type of gene marker test is one that directly assays the causative mutation. Such tests are available for *Booroola* and *Inverdale*. These tests can be applied in any population to determine the genotype of an individual. It is possible that allele effects could vary between populations, for example if other genes interact with them. Using the gene test in different populations will help unravel any possible population differences.

A linked marker or haplotype test relies on having one or more markers close to the causative gene, and usually with one specific allele that has been determined to be associated with the gene allele of interest. This type of test will work well when the gene allele traces back to a relatively recent single founder within the population where the test is to be applied. This is the case for *Inverdale* where the linked marker screens for likely carriers among the descendants of a founder ewe. The (more costly) direct test for the mutation can be applied to likely carriers if it is important to know their status definitively. The LoinMAX® test was originally derived in descendants of two related Poll Dorset rams, but appears to be associated with the *Carwell* mutation across the Poll Dorset breed in NZ. The MyoMAX® and i-SCAN® tests also appear to be highly associated with their respective mutations within the NZ Texel population, and this will in part be due to the relatively recent and narrow importation that has established the breed here. With all these tests there is the possibility that the same mutation is present in their respective or other populations and could be associated with the same or different marker alleles. Therefore, although the linked marker and haplotype tests in use are highly specific (a positive test result will likely also be a gene allele carrier), their sensitivity (probability that a true carrier is detected) is generally unknown. In addition, the tests need to be validated before they can be used in other populations.

One issue with haplotype tests is that an animal's haplotype needs to be derived from its genotypes at the loci that comprise the test. The haplotype tests that are currently in use in the NZ sheep industry are implemented by using test results on the individual alone (i.e. no information on relative is used to help estimate the haplotype). This means a test result can be returned without needing to verify pedigree or ensure that parent samples are obtained. It relies on population-based haplotype estimation procedures (Excoffier and Slatkin 1995). Marker alleles are classed into those associated with the mutant allele ('1') and all others ('2'), so that haplotype estimation procedures for

biallelic markers can be used. This protects against needing to estimate the frequency of all the marker haplotype combinations possible. Reference sets of animals, based on breed and the particular gene test, are used to help stabilise the estimation of different haplotype frequencies in the populations. A diagnostic test is used to check that the set of animals submitted for the haplotype test come from a similar population to the reference set.

Association tests provide marker alleles associated with varying degrees of trait values. This could be due to a number of different causative alleles, or to a biallelic gene variant, with differing allele frequencies at the different marker alleles. An association test is generally seen as a method of increasing the frequency of a favourable allele, rather than discriminating between causative genotypes. An association test will be effective within the population(s) where it was discovered, but would need to be re-validated for use in other populations, e.g. the favourable allele in one breed may not be the favourable allele in another breed.

Marker assisted selection. Marker tests in NZ sheep have generally been used through independent culling strategies – high index (not including gene test results) animals are chosen from among those with the desired gene test results. In some cases NZ industry consultants provide more sophisticated procedures incorporating gene test and estimated breeding values (EBVs) into a single index. In some cases gene test results are incorporated into the genetic evaluation as a factor in the evaluation, providing polygenic (i.e. other than tested gene) EBVs as well as gene test EBVs. There are a number of issues with these analyses – use of pedigree information to give better haplotype estimates, how to incorporate animals that have not been marker tested, whether to fix or estimate gene test effects and how to combine polygenic and gene test EBVs into an index. The choice of analysis method will be influenced by the type of test being used (causative mutation, haplotype or association), the population to which the test might be applied (could it be wider than the population where it was validated?) and the mode of action of the gene (dominance, imprinting, epistasis). At this time only Inverdale is ready for routine analysis by Sheep Improvement Limited in the national evaluation system. The Inverdale effect is estimated with each analysis of number of lambs born, and the effect is reported back along with the polygenic EBV.

Future developments. There are ongoing research programmes within NZ to identify additional gene marker tests for use in the sheep industry. These include traits for which gene tests are already available (reproduction, meat yield) as well as a number of other traits such as meat quality or resistance to specific diseases. The development of a single nucleotide polymorphism (SNP) chip for sheep (http://www.sheephapmap.org/) is likely to move discovery from mainly linkage-based to mainly association-based methods. Dodds *et al.* (2007a) discuss the prospects for using genome wide selection, based on SNP chip results, in the NZ sheep industry.

CONCLUSIONS

There are currently nine gene marker tests available within the NZ sheep breeding industry. Most of these tests are being increasingly adopted by the industry, often in association with marker based parentage. The way in which the marker tests are being used depends on the specific test, but sophisticated genetic evaluation systems incorporating gene marker tests are in the early stages of development.

ACKNOWLEDGMENTS

I wish to thank my colleagues – John McEwan, Benoît Auvray, Allan Crawford, George Davis, Rayna Anderson, Jude Sise and Theresa Wilson for informative discussions.

REFERENCES

Amer, P.R., McEwan, J.C., Dodds, K.G. and Davis, G.H. (1998) *Proc. N.Z. Soc. Anim. Prod.* **58**:157.

Barillet, F., Andreoletti, O., Palhière, I., Aguerre, X., Arranz, J.M., Minery, S., Soulas, C., Belloc, J.P., Briois, M., Frégeat, G., Teinturier, P., Amigues, Y., Astruc, J.M., Boscher, M.-Y. and Schelcher, F. (2002) *Proc. 7th World Cong. Genet. Appl. Livest. Prod.* Communication 13-20.

Beever, J.E., Smit, M.A., Meyers, S.N., Hadfield, T.S., Bottema, C., Albretsen, J. and Cockett, N.E. (2006) *Anim. Genet.* **37**:66.

Bodin, L., Lecerf, F., Bessière, M. and Mulsant, P. (2006) *Proc. 8th World Cong. Genet. Appl. Livest. Prod.* Communication 4-04.

Broad, T.E., Glass, B.C., Greer, G.J., Robertson, T.M., Bain, W.E., Lord, E.A. and McEwan, J.C. (2000) *Proc. N.Z. Soc. Anim. Prod.* **60**:110.

Campbell, A.W. and McLaren, R.J. (2007) *Proc. N.Z. Soc. Anim. Prod.* **67**:160.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibe, B., Bouix, J., Caiment, F., Elsen, J., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C. and Georges, M. (2006) *Nature Genet.* **38**:813.

Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D. and Georges, M. (1994) *Proc. Nat. Acad. Sci. U.S.A.* **91**:3019.

Crawford, A.M., Anderson, R.M. and McEwan, K.M. (2007) *Proc. N.Z. Soc. Anim. Prod.* **67**:168.

Davis, G.H. (2005) *Genet. Sel. Evol.* **37**:S11.

Davis, G.H., Dodds, K.G., Wheeler, R. and Jay, N.P. (2001) *Biol. Reprod.* **64**:216.

Davis, G.H., Farquhar, P.A., O'Connell, A.R., Everett-Hincks, J.M., Wishart, P.J., Galloway, S.M. and Dodds, K.G. (2006) *Anim. Repro. Sci.* **92**:65.

Davis, G.H., McEwan, J.C., Fennessy, P.F., Dodds, K.G. and Farquhar, P.A. (1991) *Biol. Reprod.* **44**:620.

Davis, G.H., Montgomery, G.W., Allison, A.J., Kelly, R.W. and Bray, A.R. (1982) *N.Z. J. Agric. Res.* **25**:525.

De Groot, T. (1957) *Landbouwkundig Tijdschrift* **69**:819.

Dodds, K.G., Amer, P.R., Spelman, R.J., Archer, J.A. and Auvray, B. (2007a) *Proc. N.Z. Soc. Anim. Prod.* **67**:162.

Dodds, K.G., McEwan, J.C. and Davis, G.H. (2007b) *Small Rum. Res.***70**:32.

Dodds, K.G., Tate, M.L. and Sise, J.A. (2005) *J. Anim. Sci.* **83**:2271.

Escayg, A.P., Hickford, J.G.H. and Bullock, D.W. (1997) *Res. Vet. Sci.* **63**:283.

Excoffier, L. and Slatkin, M. (1995) *Mol. Biol. Evol.* **12**:921.

Forrest, R.H. and Hickford, J.G.H. (2006) *Proc. Soc. Sheep and Beef Cattle Vet. N. Z. Vet. Assoc.* **36**:141.

Forrest, R.H., Hickford, J.G.H., Hogan, A. and Frampton, C. (2003) *Anim. Genet.* **34**:19.

Forrest, R.H., Hickford, J.G.H., Wynyard, J., Merrick, N., Hogan, A. and Frampton, C. (2006) *Anim. Genet.* **37**:465.

Galloway, S.M., Cambridge, L.M., Dodds, K.G., Tate, M.L., Davis, G.H. and Montgomery, G.W. (1998) *Anim. Genet.* **29S1**:6.

Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P.E., Juengel, J.L., Jokiranta, T.S., McLaren, R.J., Luiro, K., Dodds, K.G., Montgomery, G.W., Beattie, A.E., Davis, G.H. and Ritvos, O. (2000) *Nature Genet.* **25**:279.

Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R. and Galloway, S.M. (2004) *Biol. Reprod.* **70**:900.

Hickford, J.G.H., Zhou, H., Fang, Q. and Forrest, R.H. (2006) *Proc. 14th Int. Symp. and 6th Conf. Lameness in Ruminants*:170.

Johnson, P.L., McEwan, J.C., Dodds, K.G., Purchas, R.W. and Blair, H.T. (2005) *J. Anim. Sci.* **83**:1988.

Jolly, R.D., Blair, H.T. and Johnstone, A.C. (2004) *N. Z. Vet. J.* **52**:52.

Laville, E., Bouix, J., Sayd, T., Bibe, B., Elsen, J.M., Larzul, C., Eychenne, F., Marcq, F. and Georges, M. (2004) *J. Anim. Sci.* **82**:3128.

Linscott, E.M., Stuart, L., McEwan, J.C., Greer, G.J., McLean, N., Dodds, K.G., Crawford, A.M., Nijman, I.J., Moll, L., Vellema, P., Houwers, D.J. and Lenstra, J.A. (2003) *XIX Int. Cong. Genet*:135.

Lord, E.A., Davis, G.H., Dodds, K.G., Henry, H.M., Lumsden, J.M. and Montgomery, G.W. (1998) *Proc. 6th World Cong. Genet. Appl. Livest. Prod.* **27**:19.

Montgomery, G.W., Crawford, A.M., Penty, J.M., Dodds, K.G., Ede, A.J., Henry, H.M., Pierson, C.A., Lord, E.A., Galloway, S.M., Schmack, A.E., Sise, J.A., Swarbrick, P.A., Hanrahan, V., Buchanan, F.C. and Hill, D.F. (1993) *Nature Genet.* **4**:410.

Nicoll, G.B., Burkin, H.R., Broad, T.E., Jopson, N.B., Greer, G.J., Bain, W.E., Wright, C.S., Dodds, K.G., Fennessy, P.F. and McEwan, J.C. (1998) *Proc. 6th World Cong. Genet. Appl. Livest. Prod.* **26**:529.

Piper, L.R. and Bindon, B.M. (1982) *The Booroola Merino*:9.

Wilson, T., Wu, X.-Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Connell, A.R., McNatty, K.P. and Montgomery, G.W. (2001) *Biol. Reprod.* **64**:1225.

Zhou, H., Fang, Q. and Hickford, J.G.H. (2006) *Proc. Soc. Sheep and Beef Cattle Vet. N. Z. Vet. Assoc.* **36**:157.