

## BREEDING IMPLICATIONS OF THE STREAK OVARY CONDITION IN HOMOZYGOUS ( $FecX^1/FecX^1$ ) INVERDALE SHEEP

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### SUMMARY

Ewes that are homozygous carriers ( $FecX^1/FecX^1$ ) of the Inverdale prolificacy gene have small non-functional 'streak ovaries', and are infertile. This streak ovary condition can be utilised in a simplified progeny test enabling the Inverdale genotype of a ram to be established by the time it is 14 months of age. The presence or absence of streak ovaries is also a useful test where screening for new major genes is being undertaken. To avoid producing ewes with streak ovaries in a commercial breeding programme, breeders should not mate carrier rams ( $FecX^{1/Y}$ ) with carrier ewes ( $FecX^1/FecX^+$ ). The Inverdale gene has the potential to increase productivity in the sheep industry, although the identification of a reliable genetic marker is essential if matings producing ewes with streak ovaries are to be prevented. Because the streak ovary condition accurately differentiates  $FecX^1/FecX^1$  and  $FecX^1/FecX^+$  genotypes, it should facilitate the early identification and validation of genetic markers for the Inverdale gene. If structures observed on some streak ovaries are confirmed to be ovarian tumours, flocks of  $FecX^1/FecX^1$  ewes may be established specifically for medical research.

### INTRODUCTION

The Inverdale prolificacy gene ( $FecX^1$ ) located on the X-chromosome, was discovered in Romney sheep (Davis *et al.*, 1991).  $FecX^1/FecX^+$  ewes have ovulation rates about 1.0 higher than non-carriers ( $FecX^+/FecX^+$ ). However, it has been shown that  $FecX^1/FecX^1$  ewes have small non-functional streak ovaries and are infertile (Davis *et al.*, 1992). In adults, these ovaries are about one-eighth the size of normal ovaries and are pink coloured, flattened, streak-like organs showing no signs of follicular activity. They contain no normal developing ovarian follicles beyond the primary stage of development (Braw-Tal *et al.*, 1993).

In the first report of streak ovaries in Inverdale sheep, Davis *et al.* (1992) recorded an incidence of 48% in 59 daughters of four  $FecX^{1/Y}$  rams mated with  $FecX^1/FecX^+$  ewes. We now have data from 160 daughters of 12  $FecX^{1/Y}$  rams mated with  $FecX^1/FecX^+$  ewes, and the incidence of streak ovaries is 46.2%, which is not significantly different from the expected 50%. Gross examination of the ovaries of stillborn lambs has failed to distinguish between streak and normal ovaries, but we have found that laparoscopic examination of lambs aged only two months can be used to identify streak ovaries. At this age the size of streak and normal ovaries was similar, but the characteristic pink colour and complete absence of follicles were both evident.

### PROGENY TESTING - INVERDALE RAMS

The streak ovary syndrome can be identified in young lambs, which enables a progeny test of putative Inverdale carrier rams to be completed in less than one year. In 1992, nine Inverdale ram lambs aged seven months, that were the offspring of  $FecX^1/FecX^+$  ewes, were progeny tested. Half of these rams were expected to be  $FecX^{1/Y}$  and half non-carriers ( $FecX^{+/Y}$ ). Each ram lamb was joined with 7-8  $FecX^1/FecX^+$  ewes, and the female progeny were identified at birth. If a ram was  $FecX^{1/Y}$ , on average half of the daughters would have streak ovaries, whereas none of the daughters of a  $FecX^{+/Y}$  ram would have streak ovaries. The aim was to produce five daughters per ram, as the probability of a  $FecX^{1/Y}$  ram having no daughters with streak ovaries in a sample of five daughters is only 0.031 (i.e.  $(1/2)^5$ ). The number of daughters available for laparoscopy at five months of age ranged from 3-5 per sire. Four of the progeny groups had 1-3 daughters with streak ovaries, indicating that the sire was  $FecX^{1/Y}$ . These four rams were thus known to be  $FecX^{1/Y}$  before they were joined in 1993 at 19 months of age, which is the normal age for first using rams.

The same progeny testing procedure has been carried out in a commercial flock. This breeder also had the female progeny laparoscoped as lambs, but an alternative option for commercial breeders would be to send five daughters per sire to a slaughter-house as lambs and examine each reproductive tract at the time the carcass is eviscerated. The breeder would receive full commercial value for the carcass and would avoid the relatively high cost of laparoscopy.

Because an Inverdale ram may be designated  $FecX^{I/Y}$  on the basis of just one daughter with streak ovaries, it is critical that pedigrees are accurately assigned at birth. Each sire group should be lambled in a separate paddock to avoid any cross fostering between progeny of different rams.

### PROGENY TESTING - PUTATIVE MAJOR GENES

The streak ovary condition can be useful in the quest for new major genes affecting prolificacy. At present the  $FecX^I/FecX^I$  Inverdales are the only genotype in sheep known to have streak ovaries. Ewes that are homozygous for the Booroola prolificacy gene ( $Fec^B/Fec^B$ ) have functional ovaries (Davis *et al.*, 1982) and ewes with one copy of both the Booroola and Inverdale genes also have functional ovaries (Davis *et al.*, 1992). As the streak ovary condition appears to be a unique characteristic of homozygous Inverdale ewes, this can be used to test whether a new putative major gene is in fact the Inverdale gene.

Daughters of a ram that carries a putative major gene for prolificacy can be mated with a  $FecX^{I/Y}$  ram (or alternatively, the ram in question can be mated with  $FecX^I/FecX^+$  ewes) and the female offspring examined for the presence of streak ovaries. The absence of streak ovaries would indicate that the putative major gene is not the Inverdale gene.

The Inverdale line of sheep was identified within an extensive project involving highly prolific sheep screened from throughout New Zealand (Kelly *et al.*, 1983) and can be traced back 28 years to a flock in Canterbury in the central South Island of New Zealand, but no earlier records are available. Soon after the Inverdale discovery was publicised we were approached by a Romney breeder from the central North Island of New Zealand who had some highly prolific ewes. Records from 19 daughters of a ram (70-84) indicated a mean litter size at their first lambing (aged two years) of 2.37, which was 0.75 higher than their contemporaries sired by another Romney ram. Some of the dams of these 19 prolific ewes had very low litter sizes (e.g. five lambs born in four lambings). There had been high barrenness (27%) in the two-year-old daughters of ram 70-84, and five of the seven barren daughters came from ewes that had at least one record of triplets. The sire of ram 70-84 had progeny with 'normal' litter sizes, but his dam had produced three sets of triplets in six lambings. Collectively, these data were strong evidence of a major gene for prolificacy in this flock.

In April 1993 five daughters of ram 70-84 that had a lifetime mean litter size of 2.75 were mated with a progeny-tested  $FecX^{I/Y}$  Inverdale ram to test for the Inverdale gene. These matings resulted in only two surviving female lambs, but both were found to have streak ovaries. This result shows that sheep in this flock have either the Inverdale gene or a gene allelomorphous with the Inverdale gene. This flock can be traced back 29 years and no South Island sheep were introduced during that time. Rams from this flock have been sold for breeding.

Romney ewes tracing back to another South Island ewe obtained in the prolific sheep project of Kelly *et al.* (1983), were also mated to a  $FecX^{I/Y}$  ram in 1993. When the progeny were laparoscoped as lambs, six of the twelve daughters were found to have streak ovaries. This line will also be further investigated.

In flocks with unexpectedly high barrenness in maiden ewes the streak ovary condition can be used to diagnose whether the Inverdale gene is present. Because the Romney is the major sheep breed in New Zealand and the Inverdale gene has now been identified within flocks in both the North and South Islands, it may be quite widely distributed through the national flock. Farm consultants and veterinarians need to become acquainted with the

streak ovary condition because it may be the cause of unexplained barrenness in flocks where the breeder, not realising that the Inverdale gene is present, has mated rams carrying the gene with carrier ewes.

### GENETIC MARKERS

An accurate genetic marker is the key to the utilisation of major genes in industry. In the case of the Inverdale gene, significant increases in prolificacy can be achieved from  $FecX^1/FecX^+$  ewes (Davis *et al.*, 1993), but matings between  $FecX^{1/Y}$  rams and  $FecX^1/FecX^+$  ewes should be avoided because half of the daughters will be infertile due to streak ovaries. A genetic marker would allow the identification of genotype early in life and form the basis of selection by ram breeders. Flock replacements would be retained from ewes identified as  $FecX^1/FecX^+$ ; and rams identified as  $FecX^{1/Y}$  would be sold for breeding purposes. In a structured industry these  $FecX^{1/Y}$  rams would be mated with non-carrier ewes to produce  $FecX^1/FecX^+$  daughters which would comprise a prolific flock that was subsequently mated to terminal sires for specialist meat production.

Research into genetic markers for prolificacy at the AgResearch Molecular Biology Unit in the Biochemistry Department at the University of Otago is focused on the Booroola and Inverdale genes, and requires the accurate identification of genotypes. The search for a genetic marker relies on studying families in which a major gene is known to be segregating. Booroola ewes have been assigned genotypes on the basis of ovulation rate records, but it is known that these classifications are prone to error (Davis *et al.*, 1982). Furthermore, ovulation rate records can only be collected on ewes after puberty. In Inverdale ewes segregation occurs where  $FecX^{1/Y}$  rams are mated with  $FecX^1/FecX^+$  ewes, as half the daughters are  $FecX^1/FecX^+$  and half  $FecX^1/FecX^1$ . Because  $FecX^1/FecX^+$  ewes have normal ovaries and  $FecX^1/FecX^1$  ewes have streak ovaries, it is possible to accurately assign genotypes. This assignment can be carried out at an early age (two months) because the lambs do not need to have attained puberty.

At present the streak ovary condition can be used to assign genotypes within progeny test programmes where candidate sires are mated with ewes known to be  $FecX^1/FecX^+$ . However, in future this is likely to be achieved by genetic markers, and because streak ovaries can be used to precisely determine genotypes it should help facilitate the early identification of effective genetic markers.

### APPLICATIONS IN THE SHEEP INDUSTRY

An increase in litter size of 0.58 has been achieved from  $FecX^1/FecX^+$  ewes in an industry flock (Davis *et al.*, 1993), showing that the Inverdale gene has the potential to significantly increase the productivity of sheep flocks. The gene would fit particularly well in a structured industry where, for example, surplus hill country ewes are mated to a  $FecX^{1/Y}$  ram and the prolific daughters sold to specialist meat-producing lowland farmers who mate them to terminal sire breeds, and all progeny are sold for slaughter. This system requires a continuing supply of  $FecX^{1/Y}$  rams.

The breeder of  $FecX^{1/Y}$  rams should avoid matings that produce the infertile  $FecX^1/FecX^1$  ewes, and would therefore need a flock of  $FecX^1/FecX^+$  ewes which would be mated with  $FecX^{1/Y}$  rams. Replacement ewes for this flock would be selected from the 50% of ewe lambs that were  $FecX^1/FecX^+$ , while the 50%  $FecX^1/FecX^+$  ewe lambs would be culled. The  $FecX^{1/Y}$  rams for sale would be selected from the 50% of ram lambs that were  $FecX^{1/Y}$ . Replacement rams could be selected from the 50% of  $FecX^{1/Y}$  rams or they could be bought-in non-carrier rams. Although flock replacements would only be available from 50% of the progeny, the fact that  $FecX^1/FecX^+$  ewes have greatly increased prolificacy will ensure that high selection pressure can still be applied to other traits.

In such a system the hill country farmer can specialise in producing Inverdale carrier ewes for sale and wool production, the Inverdale ram breeder's flock has high prolificacy which allows him to concentrate on other traits such as parasite resistance and wool, and the terminal sire breeder can specialise in growth rate and carcass quality. All of these attributes, as well as hybrid vigour, combine in the highly productive intensive lowland

flock. The system avoids matings that result in progeny with streak ovaries. However, it is dependent on the identification of the genotype of progeny in the Inverdale ram breeder's flock, and thus the availability of a reliable genetic marker test is crucial.

#### OVARIAN TUMOUR RESEARCH

Serial laparoscopy of FecX<sup>1</sup>/FecX<sup>1</sup> ewes has shown the spontaneous appearance of luteal- or granulosa cell-like structures and other unusual formations on the streak ovaries of 8 out of 27 ewes (Braw-Tal *et al.*, 1993; McLeod *et al.*, 1993). These structures have first appeared at 2-34 months of age and their morphology is consistent with that observed for ovarian tumours. If these structures are confirmed as a type of ovarian tumour, Inverdale ewes with streak ovaries have the potential to become an important model for the study of human ovarian cancer. At the Invermay Agricultural Centre a flock of more than 100 FecX<sup>1</sup>/FecX<sup>1</sup> ewes is being established for the study of these structures and possible future medical research.

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