Inverdale as a model

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ABSTRACT

The Inverdale gene (FecX) is extraordinary. Located on the X-chromosome, it increases ovulation rate in the heterozygous state, but in the homozygote the ewe has undeveloped or "streak" gonads and is sterile. The phenotype is almost certainly due to the action of the gene in early embryonic/foetal development.

The Inverdale may be a valuable model for investigation of similar phenotypes in other species, especially the human female. Identification of the gene itself will enable substantial progress in understanding mechanisms of ovarian and follicle development. However, even without knowledge of the specific gene function, there are the areas where the phenotype itself may be of real value. Two such areas are the "tumour-like" structures in the homozygote as a model for ovarian tumours and the "steroid-free" environment of the homozygote as a model for investigating the regulation of seasonality in sheep.

Keywords: sheep; Inverdale gene; streak ovaries; prolificacy.

INTRODUCTION

The Inverdale gene provides fascinating opportunities both in research and in its practical application on farms. Other papers in this symposium contain essential background. I will discuss the value of Inverdale as a model and the various possibilities and insights which it offers. In this respect, a model is a representation of something else and to be a useful model, Inverdale must offer advantages over more direct research on the phenomenon of interest. That is, the model must be more accessible than the real thing, and it must offer potential for both greater understanding and for insights into the "real thing" and its underlying causes. Before considering Inverdale as a model, there is one critical question which must be addressed: how important is it to know the actual underlying cause of the Inverdale mutation with its high ovulation rate in the heterozygous state and streak gonads in the homozygous state? The appropriate answer depends on the specific question being asked.

The Inverdale gene

No effects of the gene have so far been described for the male but the effect of the Inverdale gene in the female carrier is paradoxical. The heterozygote female exhibits a marked increase in ovulation rate while the homozygote has an undeveloped or "streak" ovary. The Inverdale is a classical example of "overdominance", that genetic phenomenon where one copy of a gene is advantageous but where two copies of a gene exert a disadvantageous effect on the "phenotype" of the carrier. In effect the Inverdale is a "lethal" gene. In the last two years knowledge has grown considerably. Comparisons of heterozygotes and non-carrier ewes have shown that developmental differences are evident in the foetus at around day 40 (McNatty *et al.*, 1995). In the homozygote, early oocyte and follicle development appear to be normal but the primary follicle development, which starts at around day 90 in the sheep foetus, is seriously perturbed. Thus the effect of the gene is evident in early foetal development. As a developmental defect, does the gene affect the oocyte itself, the follicle (especially the granulosa cells) or even the communication between the oocyte and its follicle?

Since Inverdale is a gene on the X-chromosome, it follows that carrier females can have one or two copies while carrier males have only one copy. This is itself a paradox in that despite the X-chromosome carrying a number of important "house-keeping" or regulatory genes, the success of males indicates that one copy of the X-chromosome is clearly sufficient. In the female, one copy of a gene on the X-chromosome is also often sufficient as many of the genes on one of the X-chromosomes do not function due to the process of X-inactivation, a special form of gene regulation, in which much of one of the X-chromosomes is turned off in early embryogenesis of the mammalian female (Disteche, 1995). Thus for most genes on the X-chromosome the female has only one effective copy, but in this respect the Inverdale is clearly different because, at least during the critical developmental stage where the Inverdale gene is expressed, both copies of the gene must be active.

Inverdale - phenotype or genotype

In considering the Inverdale gene as a model or research tool both the phenotype and the genotype are interesting. With the phenotype it is actually what we see which is of interest, whereas with the genotype it is the genes themselves which are of interest. There are two areas where such a model may be valuable in studying aspects relevant to animal production and to reproductive problems in humans and in other species.

Phenotypic models

The increased ovulation rate of the heterozygote carrier offers potential for insights into the regulation of oocyte and

follicle development. However, the complexity of folliculogenesis itself means that such investigations are unlikely to be particularly rewarding in the absence of gene identification. A possible exception may be crosses between the Inverdale and the Booroola, another major gene for ovulation rate, which may prove particularly interesting as there is evidence that the two genes are additive in terms of their effect on ovulation rate (G.H. Davis, pers. comm.).

Understanding the regulation of seasonality is an important aspect of reproductive research. In this respect Karsch and his co-workers (Karsch *et al.*, 1984) have proposed that the onset of the breeding season in the ewe is due to a switch in sensitivity of the hypothalamus to steroid feedback. Thus the homozygous Inverdale ewe is a particularly useful model for investigating seasonal changes in hormonal patterns since it has developed in a steroid-free environment. Preliminary data indicate that the pronounced seasonal changes in LH patterns characteristic of the onset of the breeding season are also evident in homozygous Inverdale ewes (McLeod *et al.*, 1995b). Thus, while steroid feedback may be involved, there appear to be other factors which influence the seasonal hormonal patterns and the onset of the breeding season.

Turner's syndrome is a well-described phenotype in women that is generally, but not exclusively characterised by X-chromosome monosomy (i.e. XO; Migeon *et al.*, 1993). While most XO females are spontaneously aborted, some survive foetal life. The XO human female has an abnormal phenotype including short stature and ovarian failure (usually streak gonads), whereas XO mice have a phenotype which is virtually normal (Disteche, 1995). This difference may well be due to differences in the inactivation status of genes on the X-chromosome. Although the phenotypic similarity of the homozygous Inverdale and the human XO female in respect of streak ovaries is interesting, real progress in understanding Turner's syndrome and the Inverdale gene are unlikely to be made without knowledge of the genes which are involved. Similarly, investigations of Inverdale as a possible model for other human genetic conditions with the streak ovary phenotype (e.g. premature ovarian failure - Powell *et al.*, 1994) are unlikely to result in substantive progress without knowledge of the gene itself.

Around one-third of homozygous Inverdale ewes develop tumour-like structures on the ovary. The "tumours" which develop and regress appear to secrete inhibin (Braw-Tal *et al.*, 1993, McNatty *et al.*, 1994) and have been described as "Sertoli cell-like" or stromal tumours (McNatty, et al., 1995). The possibility that these structures may be tumours is now the subject of intense investigation and could well become the area where the Inverdale phenotype has the most to offer as a model.

Genotypic models

As indicated previously, identification of the Inverdale gene would lead to considerable progress especially in relation to expression of the gene and the role of X-chromosome inactivation. It is possible that the gene is one which normally escapes inactivation, or the developmental defect occurs at a stage of development when both copies of the gene are active, or that the gene affects the oocyte itself. The Inverdale gene may also be important in sex determination in that genes that escape X-inactivation in humans, but have no Y-chromosome homologue, may be important in female-specific and dosage-sensitive functions involved in processes such as ovarian development which are compromised in Turner's syndrome (Disteche, 1995). There is evidence for such dosage-sensitive loci which may have a role in ovarian development and/or which function as a link between ovary and testis formation in that in humans the presence of two active copies of a particular Xp locus results in male to female sex reversal (Bardoni *et al.*, 1994).

Identification of the Inverdale mutation would also open the possibility of investigations of the effect of the gene in the male carrier of the Inverdale. The site of expression of the defect itself is of great interest. The two prime candidates are the oocyte and the granulosa cell. However, the somatic cells have a major role in the regulation of oocyte growth and development and the two are independent (Buccione *et al.*, 1990).

Linkage analysis is being used to search for the location of the Inverdale gene (Galloway *et al.*, 1995) on the X-chromosome. Other possible approaches include the development of cDNA libraries from populations of germ cells and somatic cells from foetal gonads and investigation of the differences in gene expression (Urven *et al.*, 1993) to identify potential candidates. Identification of the gene, the search for homologues in other species and identification of the gene product are all major tasks. Thereafter the incorporation of the gene into a mouse would probably offer the greatest possibilities for the use of Inverdale as a model.