Hormone secretion patterns associated with increased ovulation rates or with ovarian dysfunction in Inverdale (FecX) ewes

BJ. McLEOD, S.E. KYLE, M.R. RAMSAY AND T.R. MANLEY

AgResearch, Invermay Agricultural Centre, Private Bag, Mosgiel, New Zealand.

ABSTRACT

Ewes that are heterozygous (I+) carriers of the Inverdale gene (FecX) have an increased ovulation rate, whereas ewes that are homozygous (II) for the gene are infertile and have 'streak' ovaries in which normal follicle development does not occur. A proportion of ewes with streak ovaries develop structures that have some characteristics of ovarian tumours. We have monitored patterns of secretion of gonadotrophin and ovarian hormones to (a) determine how the gene influences ovulation rate, (b) monitor the growth of ovarian structures and (c) assess the effectiveness of using hormone concentrations to identify the genotype of individual ewe lambs.

No differences in hormone secretion related to differences in ovulation rate were evident between I+ and non-carrier (++) Inverdale ewes (mean ovulation rates 3.1 ± 0.28 and 2.1 ± 0.18, respectively; n=10/group). During a synchronised oestrous cycle, neither mean LH (0.4 ± 0.01 ng/ml), FSH (2.1 ± 0.22 ng/ml) nor inhibin concentrations (7.5 ± 0.48 I.U./ml) differed significantly between these genotypes. In II ewes, mean plasma concentrations of gonadotrophins were consistently elevated (FSH 16.9 ± 1.8ng/ml; LH 4.5 ± 0.5 ng/ml; N=14) and were comparable to those observed in ovariectomised ewes. In contrast, inhibin was always undetectable (< 3.0 I.U./ml). These patterns of hormone secretion often change markedly when ovarian structures develop, with inhibin concentrations increasing acutely, which in turn causes FSH levels to fall.

To assess the potential of using these differences to identify II genotype, Inverdale ewe lambs were blood-sampled at two, five or seven months of age. Over 95% of II Inverdale ewe lambs had genotype assigned correctly (confirmed by laparoscopy) on the basis of plasma FSH concentrations alone.

Keywords: sheep; Inverdale gene; hormone patterns; FSH; LH; inhibin; ovulation rate; ovarian dysfunction.

INTRODUCTION

The Inverdale gene (FecX) is a major prolificacy gene, located on the X-chromosome of sheep, that exerts its effect by modifying ovarian function (Davis *et al.*, 1991a). In ewes that are heterozygous (I+) for the gene, follicle development is enhanced. The ovaries of these animals contain more large antral follicles than those of non-carriers of the gene, and this is reflected in a higher ovulation rate (Davis *et al.*, 1991a; Shackell *et al.*, 1993). This suggests that the gene directly influences follicle development, although the mechanism involved remains to be identified.

In contrast to the enhanced ovarian function observed in I+ ewes, homozygous (II) females are infertile and have non-functional 'streak' ovaries (Davis *et al.*, 1992). In these animals, normal resumption of follicle growth is disrupted, and antral follicles are completely absent from the ovaries (Braw-Tal *et al.*, 1993). As a consequence of the lack of antral follicles, and thus of ovarian steroid hormones, plasma concentrations of LH and FSH are elevated (comparable to those observed in ovariectomised ewes) and inhibin is undetectable in peripheral plasma (McLeod *et al.*, 1995a; Meikle *et al.*, 1992; McNatty *et al.*, 1995).

Routine laparoscopic examination has revealed that large structures periodically develop within the ovaries of a proportion of II ewes. It has been suggested that these may be ovarian tumours as there are some parallels with tumour development in women. For example, in women the peak incidence of ovarian cancer occurs after the menopause, a time when gonadotrophin concentrations are high, and the development of tumours is often associated with elevated levels of the ovarian hormone inhibin. These data provide circumstantial evidence that the structures that occur in II ewes may be ovarian tumours.

Successful use of the Inverdale gene in commercial flocks will depend on the availability of a reliable method to identify infertile II ewes to ensure that these animals are not inadvertantly introduced into breeding flocks. Thus, a crucial requirement is a reliable marker to segregate II ewes from I+ and ++ ewes.

In summary, hormone profiles from Inverdale ewes can provide valuable information regarding genotype and ovarian function in individuals. In this paper we have analysed patterns of secretion of FSH, LH and inhibin to determine whether these will increase our understanding of (a) how the Inverdale gene influences ovulation rate, (b) the factors that predispose to the development of putative ovarian tumours and (c) hormonal differences that can be used to identify the genotype of individual ewes.

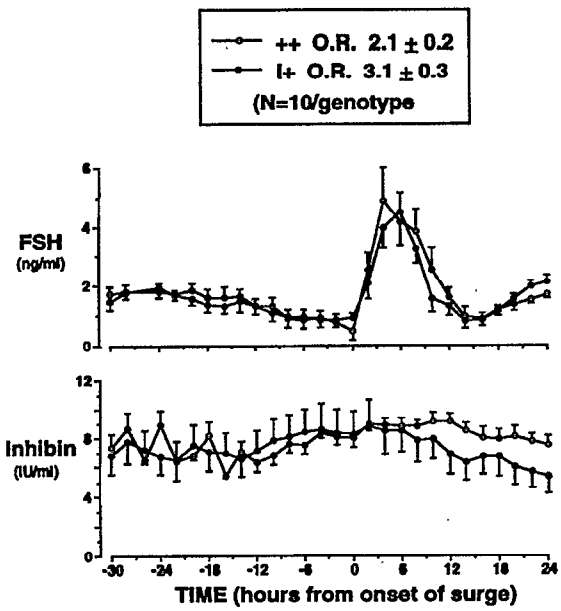
Increased ovulation rate

Oestrus was synchronised in Romney ewes that were either I+ or ++ (N=10/group), by administration of progesterone CIDR devices that were left *in situ* for 12 days. Blood samples (2 ml) were taken at 2 h intervals (via an indwelling jugular vein catheter), from 12 h before until 60 h after removal of the progesterone devices. All samples were assayed for plasma concentrations of FSH, LH and inhibin.

Ovulation rates were determined by laparoscopy, undertaken four days after the end of the blood sampling period.

Mean ovulation rates were significantly higher ($P < 0.01$) in I+ ewes (3.1 ± 0.3) than in ++ ewes (2.1 ± 0.2). Mean FSH, LH and inhibin concentrations did not differ significantly between genotypes at any stage of the sampling period. Profiles of mean FSH and inhibin concentrations are shown in Fig. 1.

FIGURE 1: Mean (± s.e.m.) plasma FSH (a) and inhibin (b) concentrations in heterozygous Inverdale and non-carrier ewes (N=10/group) over the follicular phase of the oestrous cycle. Blood samples (taken at 2 h intervals) have been standardised about the preovulatory FSH surge).

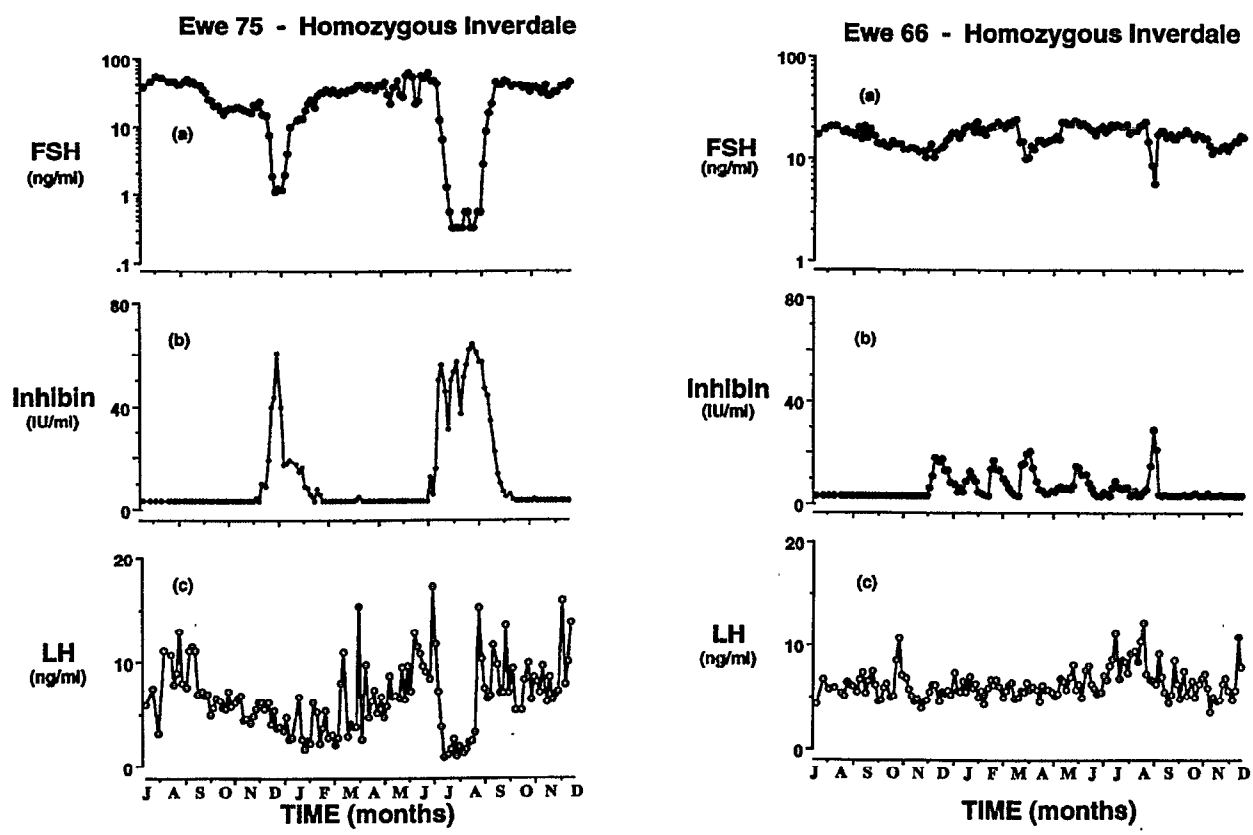


It has previously been shown that the increased ovulation rate in I+ ewes is associated with a higher number of antral follicles in these animals (Shackell *et al.*, 1993). It is feasible that this may reflect a greater degree of ovarian stimulation by gonadotrophic hormones. However, the present study failed to identify any differences in hormone concentrations between I+ and ++ ewes. It is possible that the higher numbers of antral follicles present, may be due to lower levels of ovarian hormones that contribute to negative feedback control, but the lack of any significant differences in patterns of inhibin secretion does not support this suggestion.

Ovarian tumour development

A total of 20 ewes that were putative II animals, classified as such on the basis of bilateral ovarian hypoplasia, were screened. The occurrence of abnormal ovarian structures was monitored by routine laparoscopic observation. Animals first underwent laparoscopy at approximately seven months-of-age. Thereafter, laparascopic observations were carried out at 6 to 12 week intervals. The ovaries of II ewes, which are about one third the size of normal ovaries, are typically flattened, streak-like organs on which follicles can not be observed macroscopically (McNatty *et al.*, 1995). The occurrence of any deviation from this 'normal' appearance of streak ovaries was recorded. From previous studies (McLeod *et al.*, 1995b),

FIGURE 2: Plasma FSH (a), inhibin (b) and LH (c) concentrations in two individual II Inverdale ewes in which ovarian structures were observed throughout the 18 month period of observation. Blood samples were collected twice weekly by jugular venepuncture.



ovarian structures can be described grossly as either (a) fluid-filled cysts that had translucent walls, (b) luteinised cysts that are similar in appearance to luteinised follicles but sometimes bear protruding knobs, or (c) as dense, white bodies that have a highly involuted surface. Blood-samples (5ml) were taken twice weekly by jugular venepuncture for a period of eighteen months. All blood samples were assayed for FSH, LH and inhibin content.

In 11 of the ewes, mean FSH and LH concentrations were elevated throughout the period of study (mean ± s.e.m. 18.6 ± 1.64 ng/ml and 5.8 ± 0.76 ng/ml for FSH and LH, respectively). In all these ewes inhibin was not detectable in any of the samples. In the remaining nine ewes, there were periods when FSH concentrations fell abruptly. Some, but not all of these, were associated with an elevation in plasma inhibin concentrations (see Fig 2). In five of these ewes, ovarian structures were recorded on at least one of the laparoscopic observations. There was no correlation between the type of ovarian structure and changes in hormone secretion patterns.

Segregation of Inverdale genotype

The ewe lambs that were monitored had been generated from single-sire mating groups in which known or potential Inverdale carrier rams were mated with known I+ Inverdale ewes. The total of 165 ewe lambs were the offspring of 14 sires (3 - 23 lambs/sire). Identification and pedigree of individual lambs was ensured by lambing ewes in single-sire groups, and eartagging all lambs at birth. Genotype was assessed on three separate occasions - when the ewe lambs were approximately two months of age (N=55), five months of age (N=112) and seven months of age (N=165). At each of these times ovaries were observed by laparoscopy, and blood samples (5 ml) for the determination of hormone concentrations were taken by jugular venepuncture. Laparoscopy and hormone analyses were undertaken by separate personnel and information on genotype of ewe lambs was not exchanged until completion of the study.

Plasma concentrations of FSH, LH and inhibin are shown for individual lambs sampled at five months of age are shown in Figure 3. These animals have been arranged in order of ascending FSH concentrations with the animal with the lowest plasma FSH level shown at the extreme left and that with the highest concentration at the extreme right (Fig 3a). Plasma LH (Fig 3b) and inhibin (Fig 3c) concentrations recorded in these animals are aligned to their FSH value. It is apparent that the greater variation in hormone concentrations was in plasma FSH levels. Cluster analysis techniques were employed to separate ewe lambs into two groups with high or low FSH levels. The accuracy of this method of segregating infertile II ewe lambs from fertile I+ or ++ ewe lambs is summarised in Table 1.

FIGURE 3: Hormone concentrations in 112 individual lambs sampled at five months of age. FSH (a) concentrations are arranged in ascending order with the animal with the lowest plasma FSH level shown at the extreme left and that with the highest concentration at the extreme right. Plasma LH (b) and inhibin (c) concentrations recorded in these animals are aligned to their FSH value.

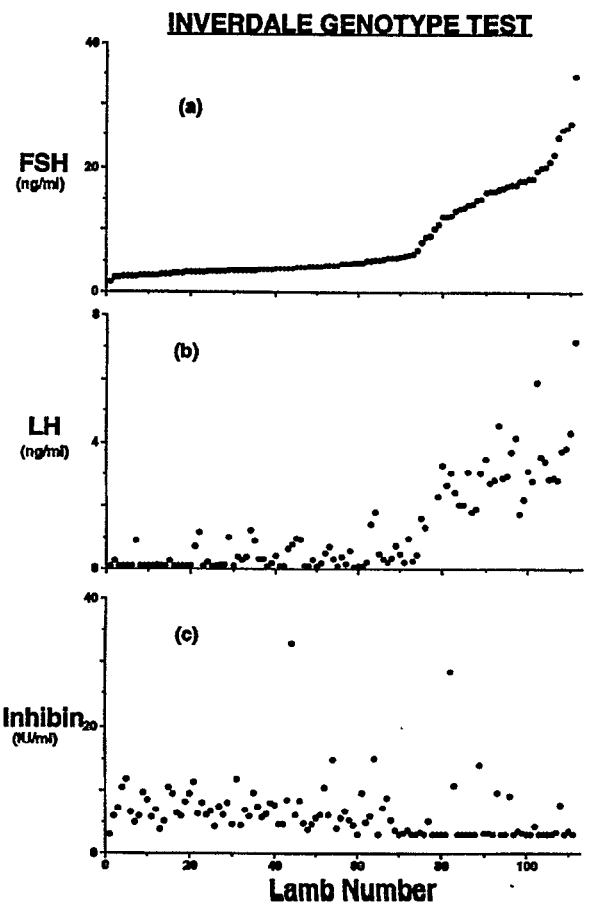


TABLE 1: Accuracy of attributing Inverdale genotype on the basis of plasma FSH concentrations. Figures in parenthesis show number of animals in which assigned genotype was verified as correct by laparoscopy at seven months of age.

Age (months)	Genotype	GENOTYPE ASSIGNED FSH		Lap	
2	I+	22	(21)	23	(21)
(N=48)	II	26	(25)	25	(24)
5	I+/++	73	(72)	63	(63)
(N=109)	II	36	(35)	46	(35)
7	I+/++	109	(106)	106	
(N=168)	II	59	(57)	57	

Summary

Hormone profiles from Inverdale ewes can provide valuable information regarding genotype and ovarian function in individuals. The wide differences in gonadotrophin (especially FSH) concentrations between infertile II and fertile I+ or ++ Inverdale ewes offer a reliable means of identifying these animals at a very early age. Patterns of hormone secretion appear to be markedly disrupted with the development of ovarian structures in II ewes. However, with the limited data available to date, it is not possible to relate changes in hormone concentrations to the type or stage of development of these structures. No significant differences in gonadotrophin or inhibin secretion have been observed that are related to the increase in ovulation rate that occurs in I+ Inverdale ewes.