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Ovarian characteristics in Inverdale ewes heterozygous (I+) and homozygous (II) for the Inverdale gene (FecX)

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ABSTRACT

The Inverdale gene (FecX) affects ovarian development, during both foetal and adult life. There is evidence for affects on germ-cells, the follicle and the corpus luteum. In I+ ewes, follicles ovulate at a smaller diameter with fewer granulosa cells and the resulting corpora lutea are individually smaller compared to those in control (++) animals. This appears to be due to the granulosa cells in I+ follicles being more responsive to follicle-stimulating hormone (FSH) and acquiring receptors for luteinising hormone (LH) earlier than in cells from ++ ewes. These differences between I+ and ++ ewes in ovarian characteristics occur in the absence of any obvious differences in the peripheral plasma concentrations of FSH and LH or the ovarian secretion rates of inhibin, oestradiol and progesterone. In II animals, ovarian follicular growth is impaired at the primary stage of growth during foetal, neonatal and adult life. No normal follicles beyond this stage of growth have been observed. II animals also have castrate levels of FSH and LH and undetectable plasma levels of ovarian steroid or inhibin. In ~35% of II animals, abnormal surface-visible structures have been observed with a morphology consistent with that of a sertoli-cell or sex-chord stromal tumour. These structures, which secrete inhibin, appear to develop from clusters of oocyte-free follicles.

Keywords: sheep; Inverdale; FecX gene; ovary; follicle; germ cells; FSH; LH; inhibin; steroid; granulosa cells.

INTRODUCTION

The Inverdale gene (FecX) is a major gene located on the X-chromosome of sheep. In females carrying the FecX gene the level of ovarian activity is different relative to that in control (++) animals. For example, ewes with one copy of the gene (I+) have on average one more ovulation during each oestrous cycle than in ++ ewes, whereas ewes with two copies of the gene (II) have streak ovaries and are infertile (Davis *et al.*, 1991a, 1992). The evidence suggests that the X-linked mutation has direct effects on the ovarian development and function in foetal, neonatal and adult life, but the mechanism(s) of action remains obscure (Smith *et al.*, 1993).

The purpose of this paper is to review our current knowledge of the ovarian characteristics in I+ and II Inverdale ewes.

Ovarian characteristics in foetal life

Ovaries of Inverdale foetuses have been studied at 40, 90, 105, 120 and 135 days of gestation. To obtain these tissues the following matings were performed: carrier rams (I) were mated with ++ ewes to produce females which were all of the I+ genotype (authentic I+); non-carrier (+) rams were mated with ++ ewes to produce females of the ++ genotype, whilst I rams were mated with I+ ewes to produce a mixture of II and I+ foetuses - the putative II and I+ foetuses were unable to be distinguished by any overt characteristics.

No effects of the FecX gene in ++, authentic I+, putative I+ or II were noted with respect to the plasma concentrations of immunoreactive inhibin at days 40 and 90, or to the plasma concentrations of FSH at days 90, 105, 120 and 135 of gestation (data not shown).

The numbers of germ cells in the ovaries of ++, authentic I+, putative I+ and putative II foetuses are summarised in Table 1. The authentic I+ germ cell numbers at days 40 and 90 and 120, but not 105 and 135, were significantly different from those in ++ animals. The ovaries of putative I+/II foetuses at days 40 and 90, but not 120, were separated as two distinct populations based on numbers of germ cells. One population of foetuses had numbers of germ cells which were not different from that in the authentic I+ genotype so these animals were termed the putative I+ group. The remaining subset of foetuses had ovaries which contained a mean germ cell population significantly different from the putative I+ population but not different from the ++ genotype; this subset of animals was classified as the putative II genotype. At days 105, 120 and 135 one subset of foetal I+/II ovaries contained follicles growing beyond the primary follicle stage as did the authentic I+ ovaries; this subset was classified also as the putative I+ group. The remaining subset of ovaries had no follicles developing normally beyond the primary growth stage and/or had abnormal follicular structures: these were classified as putative II.

Using these methods to segregate the putative II and I+ genotypes, there were no stages of foetal development where the total number of germ cells in the putative II differed from that in the ++ genotype. In contrast, the authentic and putative I+ ovaries had different total numbers of germ cells at days 40 and 90 but not at 105, 120 and 135. These differences in numbers of germ cells related to the numbers of oogonia at day 40, and oogonia and oocytes, but not primordial follicles, at day 90. In addition to genotype effects on the timing of oogonia and oocyte growth, differences were also noted in the mean diameters of oocytes but not oogonia. For example, at day 90 the geometric mean (and 95% confidence limits) diameters of isolated oocytes in the ++, I+, putative I+ and putative II genotypes were 20.5 (19.6, 21.3), 17.9 (17.2, 18.5), 18.0 (17.3, 18.7) and 19.8 (18.3, 21.5) μm respectively

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TABLE 1: Effect of genotype on numbers of germ cells ($x10^{-3}$) in ++, I+, putative I+ and putative II Inverdale foetuses with respect to day of gestation. Values are geometric means (and 95% confidence limits).

Genotype	Day of gestation				
	40	90	105	120	135
++	47^{a} (38,58) [15]	151^{a} (107,212) [14]	191^{a} (131,278) [12]	136^{a} (95,197) [14]	161^{a} (125,207) [15]
I+	24^{b} (19,31) [11]	306^{b} (212,442) [11]	219^{a} (153,313) [11]	220^{b} (150,321) [8]	135^{a} (48,374) [6]
Putative I+	27^{b} (19,37) [4]	268^{b} (190,378) [9]	229^{a} (183,287) [5]	$150^{a,b}$ (109,207) [8]	126^{a} (66,238) [8]
Putative II	42^{a} (31,58) [5]	102^{a} (70,150) [6]	150^{a} (84,271) [6]	$144^{a,b}$ (101,205) [9]	133^{a} (74,240) [8]

The ovarian data are for the left ovary. For each column, values with different alphabetical superscripts are significantly different. a v b = $p<0.05$ (Duncan's multiple range test).
Numbers in [ ] are number of foetuses.

(authentic and putative I+ both $p<0.05$ compared to ++ and putative II genotypes). Likewise, the mean diameters of the oocytes in primordial follicles of the authentic and putative I+ genotypes were significantly smaller than in the other two genotypes.

Therefore, to summarise, it seems reasonable to suggest that the migration of oogonia into the ovary in early gestation (e.g. day 40) and the timing of onset of atresia in mid-gestation (e.g. day 90) are both retarded in I+ relative to that in the ++ and II genotypes. Moreover, as the oogonia in the I+ genotype enter meiosis and become enclosed by follicle cells, the oocytes do not reach the diameters achieved in the ++ and II genotypes. Whether the diameters of the oocytes are smaller in adult ewes is not yet known.

At days 105, 120 and 135 no normal growing secondary follicles were noted in the putative II foetuses. Indeed most of the ovaries from putative II foetuses contained 'oocyte-free' follicles and/or abnormal follicular-like structures; 'oocyte-free' follicles have only rarely been observed in controls or in authentic I+ foetuses. Thus the results suggest that a double dose of the X-linked mutation blocks the normal transformation of a primordial follicle into a primary or secondary structure.

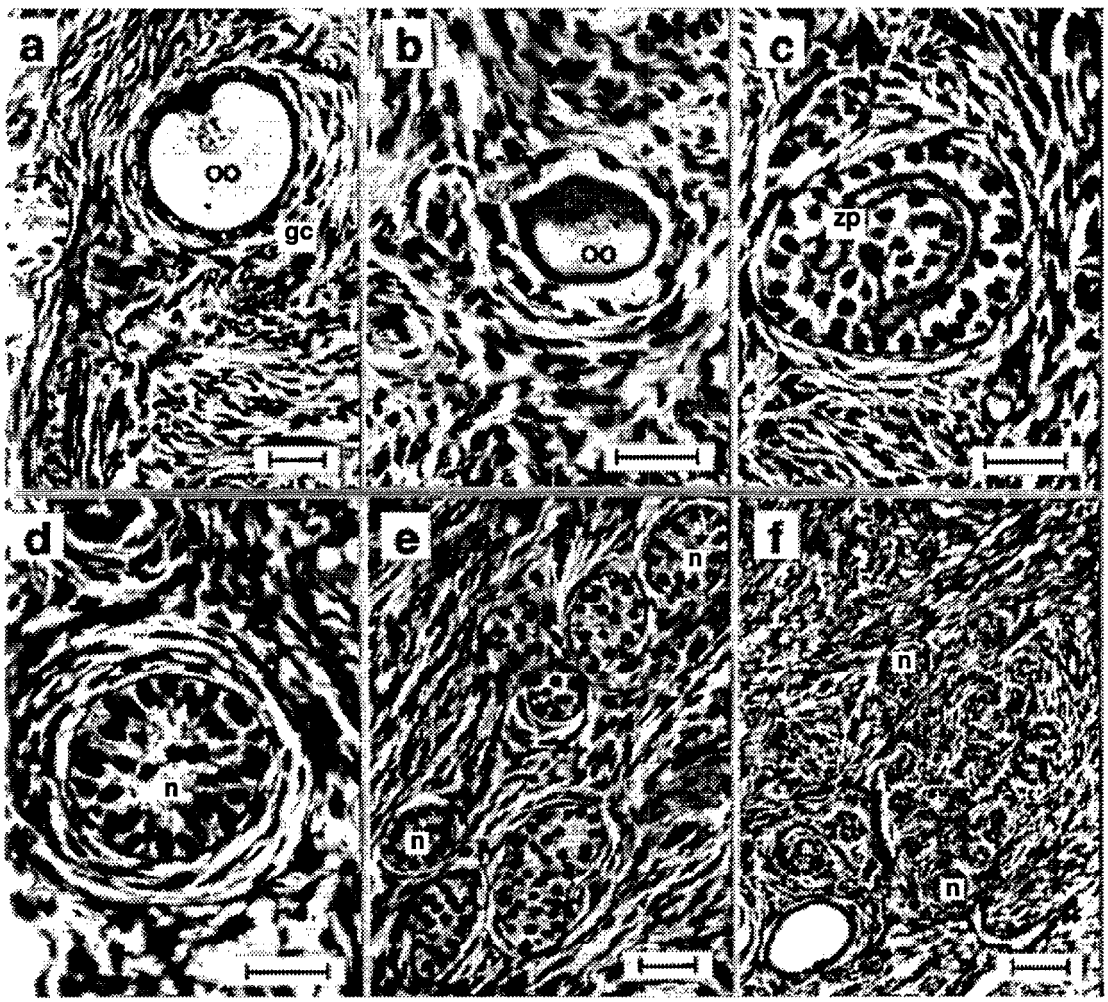
Ovarian characteristics in adult life

Heterozygous ewes. In adult I+ animals, the plasma concentrations of FSH and LH and the ovarian venous secretion rates of inhibin, oestradiol and progesterone were not different from those in ++ animals, notwithstanding the difference in ovulation rate (Shackell *et al.*, 1992). Whilst the mean weights of the ovaries in the two genotypes were not different, there were significant differences in follicular and luteal characteristics. For example, the total number of antral follicles (≥1 mm diameter) was greater in the I+ genotype (mean ± s.e.m. I+ = 30±2 follicles, n=11; ++ = 23±2 follicles, n=14; $p<0.05$): this difference in follicle numbers was due to more small (i.e. 1-2.5 mm diam.) follicles. In addition, the mean numbers of granulosa cells in small (i.e. 1-2.5 mm) and large (i.e. >4.5 mm) diameter non-atretic follicles were significantly lower (both $p<0.01$) in I+ compared to that in ++ ovaries. In the I+ genotype the individual corpora lutea (CL) were found to be significantly lighter than in the ++ genotype (i.e. 0.33±0.03 g vs 0.59±0.05 g; $p<0.01$). It appears that the preovulatory follicles in I+ ewes mature at a smaller diameter, perhaps with one less doubling of the granulosa cell population than is the case for ++ ewes. This earlier maturation of follicles in I+ ewes is likely to be due to a greater sensitivity to FSH with respect to cyclic 3',5'-adenosine monophosphate (cAMP) synthesis and an earlier acquisition of receptors for luteinising hormone in granulosa cells (Shackell *et al.*, 1993).

The finding in I+ ewes of significantly more 1-2.5 mm diameter follicles with fewer granulosa cells relative to that in ++ ewes suggests a greater accumulation of follicular fluid relative to cell mass. One possibility is that granulosa cells in small follicles of I+ ewes produce a different composition of glycosaminoglycans, which are known to effect fluid accumulation (see Chang *et al.*, 1978). This production of glycosaminoglycans may or may not be under the control of some trophic factor such as FSH. From studies *in vitro*, the higher sensitivity of granulosa cells in >2.5-4.5 mm diam. follicles with respect to FSH-induced cAMP synthesis and the presence of LH receptors in granulosa cells of I+ but not ++ ewes is consistent with the notion that granulosa cells in the I+ genotype are functionally different. Whether this difference in granulosa cell responsiveness is a consequence of earlier alterations in glycosaminoglycan (extracellular matrix) synthesis or other factors remains to be determined.

Homozygous ewes. Adult II animals have plasma concentrations of gonadotrophins similar to those in castrate ewes and low or undetectable concentrations of inhibin or ovarian steroid. In animals at 18 months of age, the ovarian volumes as geometric means (and 95% confidence limits) in II (n=6) and ++ (n=6) genotypes were 84 (43, 162) and 365 (219, 607) mm^3 respectively. Despite this difference in volume, the germ cell numbers (geometric means and 95% confidence limits) in the two genotypes were not different [i.e. II = 38715 (25331, 59172), ++ = 55825 (45681, 68222)]. None of the ovaries of 16 II ewes was found to have normal follicular growth beyond the primordial stage (Braw-Tal *et al.*, 1993). In II ewes, as some oocytes enlarged beyond 40 μm, there was no concomitant increase in granulosa cell numbers as normally occurs (Fig. 1a). A common feature of II ovaries was the presence of follicular structures which contained degenerating oocytes (Fig. 1b, c) or follicles completely devoid of oocytes (i.e. 'nodules'). Many 'nodules' were present in large clusters (Fig. 1d). 'Nodules' were rarely

FIGURE 1: Sections through ovaries of adult homozygous Inverdale ewes showing abnormal follicular structures (a,b), 'nodule' formation (c,d), and the clustering of nodules to form a putative tumour (e,f). Fig. 1a shows an abnormally large oocyte (oo) with a flattened layer of granulosa cells (gc). Fig. 1b shows a degenerating oocyte (oo). Fig. 1c shows a follicular-like structure with the remnant of the zona pellucida (zp) whilst Fig. 1d shows an oocyte-free follicular structure or 'nodule' (n). Fig. 1e and 1f show clusters of nodules (n), with those shown in Fig. 1f appearing as a large solid ('tumour-like') structure often visible on the ovarian surface. Scale bars are all 25 μm except for f where the scale bar is 50 μm.



observed in control ovaries. Often structures thought to have arisen from the clusters of 'nodules' were present (Fig. 1e) and these were sometimes visible on the ovarian surface. The surface visible structures have been variously described at laparoscopy as (a) translucent fluid-filled cysts; (b) opaque cysts with the appearance of luteinised follicles or as (c) dense white bodies with a highly involuted surface. The structures as shown in Fig. 1e have a morphology consistent with sertoli cell or sex cord-stromal cell tumours and functionally these structures secrete immunoreactive inhibin.

In four cases where blood samples were taken during and after removal of the ovary containing a putative 'tumour', the mean ± s.e.m. concentrations of inhibin (I.U./ml) were 16±1 (before) and 4±1 (after) and of FSH (ng/ml) were 10±1 and 17±1 respectively. Surgical removal of the structures therefore resulted in a significant ($p<0.01$) fall in inhibin, and a significant ($p<0.01$) increase in FSH concentrations. Since there were no concomitant changes in follistatin or steroid (McNatty *et al.*, 1994) after removal of the putative tumours it is likely that the immunoreactive inhibin from these structures was biologically active in suppressing FSH. In a separate study of 21 II ewes which were blood sampled twice weekly and laparoscoped at three monthly intervals for 15 months, surface visible structures were observed in six animals and all of these animals had elevated inhibin concentrations (i.e. maximum concentrations 8-48 I.U./ml). In four of six animals, the elevated inhibin concentrations were associated with a fall in FSH concentrations from ≥12 ng/ml to undetectable concentrations (i.e. <0.5 ng/ml).

Thus, in Inverdale ewes a double dose of the X-linked mutation blocks normal ovarian follicular growth at the primordial/primary stage of development in foetal, neonatal and adult life. As follicles begin to grow, the oocyte dies, and 'nodules' form. In many instances the nodules cluster and appear to develop into tumour-like structures which actively secrete inhibin.

In summary, the FecX gene affects ovarian development and function in both foetal and adult life. In heterozygous carriers, germ-cell development, follicular growth and cell composition and gonadotrophin responsiveness are all affected. In homozygous carriers, follicular growth is impaired and the development of ovarian tumour-like structures are not uncommon.