Altered response of cervical and vaginal epithelia to oestradiol benzoate in ewes after prolonged exposure to oestrogenic pasture

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Summary. Ovariectomized ewes, 10 with clover disease infertility and 10 controls, were injected daily for 3 days with 25 µg oestradiol benzoate. At the end of this period, the Spinnbarkeit of cervical mucus and the keratinization of the vaginal epithelium were less in the infertile ewes. It is suggested that the infertility in clover-affected ewes is due to an inability of target organs to give a continued response to the 'priming' action of oestrogen.

Introduction

Ewes which have grazed oestrogenic subterranean clover pastures for several years may develop "clover disease", which is marked primarily by a persistent decrease in fertility (Schinckel, 1948). The infertility is caused mainly by impaired transport of spermatozoa through the cervix (Lightfoot, Croker & Neil, 1967), due to production of abnormal mucus (Smith, 1971) which has a low Spinnbarkeit (Adams, 1976). Although abnormal mucus is produced by entire ewes, after ovariectomy the mucus produced by the cervix of affected ewes in response to a dose of oestradiol benzoate is indistinguishable from that produced by controls (Smith, 1971; Adams, 1977a). It was therefore concluded that the abnormal mucus was produced at oestrus because of an increased stimulation of the cervix by endogenous ovarian hormones (Smith, 1971). However, Smith (1975) showed that plasma progesterone levels are unchanged in affected ewes, and studies on oestrous behaviour in affected ewes suggest that endogenous oestrogen levels are not greatly altered (Smith, 1971; Lightfoot *et al.*, 1974; Adams, 1978). Because there is no obvious difference in ovarian hormones, the present work was carried out to re-examine the response of the cervix of affected ewes to oestrogen.

Materials and Methods

Twenty (20) 5-year-old strong-wool Merino ewes were studied. These originated from the same flock, but 10 had grazed an almost pure sward of the highly oestrogenic Yarloop cultivar of subterranean clover for 3 years and were infertile; none had lambed to the last mating. The other 10 ewes had grazed non-oestrogenic pasture for this period and were of normal fertility. All ewes had run together on non-oestrogenic pasture for 12 months before the study commenced.

At the start of the study, the ewes were run with a ram fitted with a mating harness and crayon, and with an apron to prevent intromission of the penis. The ewes were examined daily, and when they came on heat the mucus in the cervico-vaginal area was aspirated into a pipette. The weight of mucus, the Spinnbarkeit, the proportion of dry matter, and the arborization score were determined as described previously (Adams, 1976).

The ewes were ovariectomized within 1 week of coming on heat. Commencing 3 weeks after ovariectomy, the ewes were given daily i.m. injections of 10 mg progesterone for 11 days to simulate a normal oestrous cycle, and left for 48 h after the last progesterone injection. The ewes were then injected i.m. with 25 µg oestradiol benzoate in oil daily for 3 days. Samples of mucus were collected 24 h after each injection with oestrogen and examined as before. At the same time, a smear was taken from the anterior vagina with a cotton swab, and stained by the method of Ayoub and Shklar (1963) which stains keratinized cells red, cells with prekeratin yellow, and non-keratinized cells blue. A logarithmic transformation was applied to the data on mucus weight because of wide variations in amount. Characteristics of mucus obtained after ovariectomy were analysed statistically by analysis of variance, and other data by Student's *t* test.

Results

As shown in Table 1, similar amounts of mucus were collected from the infertile and control ewes at the normal oestrus, but mucus from the infertile ewes had a low Spinnbarkeit. After ovariectomy, no mucus was available for collection until the ewes were treated with oestrogen. After a single dose of oestradiol benzoate, the Spinnbarkeit of mucus from control ewes was reduced compared with that at oestrus, but it increased markedly during the 3 days of oestrogen treatment (*P* < 0.001). In contrast, the Spinnbarkeit of mucus from affected ewes failed to increase to the same extent (Table 1). The amount of mucus collected was similar in both groups on the first 2 days, but declined slightly in affected ewes on the 3rd day (Table 1). At no time was there any difference between affected and control ewes in the proportion of dry matter, or the degree of arborization in the mucus.

There was no significant difference between affected and control ovariectomized ewes in the proportion of keratinized cells in vaginal smears after progesterone priming (Day 0; Table 2). After oestrogen treatment, the proportion of fully keratinized cells in the vaginal smears

Table 1. The weight (geometric mean, with confidence limits) and Spinnbarkeit (mean ± s.e.m.) of cervical mucus from 10 clover-affected and 10 control ewes before and after ovariectomy

	At oestrus	After ovariectomy and daily treatment with 25 µg oestradiol benzoate		
		Day 1	Day 2	Day 3
Mucus weight (mg)				
Control ewes	95 (65–135)	291 (226–375)	318 (234–430)	178 (127–249)
Affected ewes	54 (32–91)	143 (83–245)	246 (175–345)	50 (29–87)
Spinnbarkeit (cm)				
Control ewes	9 ± 0.7	6.3 ± 1.5	14 ± 1.5	19.3 ± 1.2
Affected ewes	2.3 ± 1	2.7 ± 0.9	8.3 ± 1.9	5 ± 1.9

Table 2. Changes in vaginal smears (mean ± s.e.m. % of cells) of ovariectomized clover-affected (CA) and control (C) ewes (10/group) injected daily with 25 µg oestradiol benzoate

Cell type	Day 0		Day 1		Day 2		Day 3	
	C	CA	C	CA	C	CA	C	CA
Keratin	31 ± 4	36 ± 6	22 ± 4	33 ± 6	55 ± 4	45 ± 6	68 ± 3	55 ± 5*
Prekeratin	49 ± 3	36 ± 6	42 ± 5	33 ± 2	26 ± 3	26 ± 3	23 ± 3	26 ± 3
No keratin	20 ± 4	28 ± 6	36 ± 7	34 ± 7	19 ± 4	29 ± 5	9 ± 2	19 ± 3*

* Significantly different from control values, *P* < 0.05.

increased, and the proportion of non-keratinized cells and cells containing pre-keratin decreased (Table 2). This change was more obvious in control ewes and by Day 3 it was significantly more advanced than in the affected ewes ($P < 0.05$; t test).

Discussion

Both the cervix and the vagina of ewes with clover disease failed to respond normally to oestrogenic stimulation. This was due mainly to a failure of the responses to increase normally over the 3-day period; responses to oestrogen were not greatly different from those of controls in the first 24 h of treatment. Even in normal ewes, repeated treatment with oestrogen is known to inhibit the continuation of the oestrogenic response, both as cervical mucus production (Adams, 1977b; Kelly & Lindsay, 1978) or as a behavioural response (Scaramuzzi, Lindsay & Shelton, 1972). In the ewes with clover disease, it may be that this 'refractory' condition began earlier than usual, and prevented the continued expression of the normal response.

The lower Spinnbarkeit of mucus in the clover-affected ewes at oestrus agrees with previous results (Adams, 1976). The present study indicates why previous work involving only a single dose of oestrogen (Smith, 1971; Adams, 1977a) failed to detect a difference in response between clover-affected and control ewes. Firstly, responses to oestrogen are not greatly different in affected and control ewes for the first 24 h. Secondly, the Spinnbarkeit of mucus produced by normal ovariectomized ewes 24 h after treatment with oestrogen was lower than that at oestrus (Table 1), so that the difference normally observed between affected and control ewes at oestrus was less apparent 24 h after oestrogenic stimulation. The Spinnbarkeit of mucus from normal ewes increased during repeated treatment with oestradiol benzoate, although the amount of mucus produced did not vary significantly with the duration of oestrogen treatment. It is suggested that the clover-affected ewe is less able to give a sustained response to oestrogen, and so endogenous oestrogen is unable to 'prime' the cervix sufficiently to produce mucus with a normal Spinnbarkeit at oestrus.

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