Sexual behaviour of ewes with clover disease treated repeatedly with oestradiol benzoate or testosterone propionate after ovariectomy

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Summary. Ovariectomized ewes, 14 with permanent clover disease infertility (affected ewes) and 14 controls, were injected daily with 40 µg oestradiol benzoate for 12 days, and run with 2 rams fitted with marking crayons. The control ewes were mated sooner ($P < 0.05$) but both groups became refractory at a similar rate. In a second experiment, 20 similar affected ewes and 19 controls were injected daily with 5 mg testosterone propionate for 31 days and observed daily for 50 min with rams. Affected ewes again were slower to show female behaviour ($P < 0.05$) but faster ($P < 0.05$) to show aggression against the rams and other ewes. Over the 31 days, the incidence of female sexual behaviour declined at a similar rate in affected and control ewes. When examined in individual pen tests with oestrous ewes on Day 28, affected ewes showed more male-like courting behaviour than did controls ($P < 0.05$). The changes in behaviour are too slight to account for the infertility but they do support the hypothesis that phyto-oestrogens can act on the ewe by some of the pathways of sexual differentiation, even after puberty.

Introduction

Ewes which have grazed oestrogenic clover pastures for several years can develop permanent infertility (Schinckel, 1948) as part of the syndrome called clover disease. Such ewes are infertile because of impaired transport of spermatozoa through the cervix (Lightfoot, Croker & Neil, 1967). This cervical dysfunction is associated with a low spinnbarkeit of the cervical mucus resulting from an abnormal responsiveness to endogenous oestrogen (Adams, 1979) and is accompanied by metaplasia of the cervix so that histologically it resembles the uterus (Adams, 1976). Oestrogen has not been reported to cause this type of histological change in adult females of other species, although analogous changes may be caused by oestrogen during the period of organogenesis (Forsberg, 1969).

Permanently affected ewes exhibit slightly less female sexual behaviour than do controls after a single dose of oestradiol benzoate (Adams, 1978). The present study was carried out to see whether the behavioural responses of affected ewes to repeated treatment with oestradiol benzoate or testosterone propionate were altered. Some of these results have been referred to previously (Adams, 1981).

Materials and Methods

Two studies were carried out on 8-year-old Merino ewes which had been ovariectomized for at least 1 year. Half of the ewes had grazed a highly oestrogenic pasture of Yarloop subterranean clover for 3 years and only 11% had lambed after the last year of exposure. The control ewes originated from

the same group, but had grazed non-oestrogenic pasture during this period and were of normal fertility (76% lambing). Subsequently all the ewes were run together on non-oestrogenic pasture for 4 years.

The effects of 40 µg oestradiol benzoate injected i.m. in oil were studied in 14 affected ewes and 14 controls which were injected daily for 12 days. The ewes were run with 2 vasectomized rams fitted with a harness and marking crayon. Each day the colour of the crayon was changed and the ewes marked were recorded.

The effects of daily i.m. injection for 31 days with 5 mg testosterone propionate in oil were studied in other ewes from the same flock (19 control and 20 affected ewes). The ewes were individually identified with a large number painted on the side, and were run in 2 large pens. Each day, 2 active rams were run in each pen for 50 min and the sheep were observed from seclusion. The number of times each ewe showed female sexual behaviour (standing to be mounted, soliciting the ram, looking over the shoulder at the ram and tail fanning; Banks, 1964) was recorded. The frequency was also recorded for bunting of rams and other ewes, and rubbing the side of the face or the horn buds on pen divisions or on other ewes in a manner similar to the rubbing and bush-threshing behaviour described by Grubb & Jewell (1973). The relative incidence of masculine sexual behaviour (ano-genital sniffing, Flehmen reaction, and courting by pawing or nudging; Banks, 1964) was determined by observing the ewes individually in a pen with 3 other oestrous ewes for 7 min on Days 14, 21 and 28 of treatment.

Data were analysed statistically by the *t* test or, if the variances were not homogeneous, by Wilcoxon's two-sample rank test.

Results

Oestradiol benzoate

All of the ewes displayed oestrus, but the onset of mating was earlier and less variable in the control than in the affected ewes (2.5 ± 0.3 compared with 4.0 ± 2.5 days, $P < 0.05$ Wilcoxon's test). The control and affected ewes continued to be marked by the ram for a similar time (5.1 ± 0.8 and 4.6 ± 0.8 days, respectively) and the mean for the last day on which matings occurred was not significantly different for the two groups (8.1 ± 0.7 and 8.8 ± 0.7 days).

Testosterone propionate

As shown in Table 1, the affected ewes treated with testosterone propionate took longer to stand and be mated by the ram ($P < 0.05$, Wilcoxon's test) and showed less soliciting behaviour in the first week of study (Table 1; $P < 0.05$). The number of ewes showing soliciting behaviour and the

Table 1. Female sexual behaviour in a group of 19 clover-affected and 20 control ovariectomized ewes during the first 7 days of daily treatment with 5 mg testosterone propionate

	No. of days to first observation		No. of times behaviour observed	
	Control	Affected	Control	Affected
Ewe accepted mounting by ram	1.73 ± 0.15 (16)	$3.14 \pm 0.51^*$ (15)	3.47 ± 0.56	2.65 ± 0.58
Ewe solicited ram	4.9 ± 1.1 (14)	7.3 ± 1.3 (11)	2.68 ± 0.78	$0.85 \pm 0.42^*$
Ewe looked over shoulder at ram	3.9 ± 1.1 (15)	5.3 ± 1.7 (15)	5.2 ± 1.2	4.1 ± 0.9
Tail fanning	5.0 ± 1.2 (12)	6.6 ± 2.3 (7)	1.2 ± 0.3	0.9 ± 0.5

Values are mean $\pm$ s.e.m. Figures in parentheses indicate the number of ewes showing this behaviour.

* Value significantly different from control, $P < 0.05$.

number which stood to be mounted by the ram declined over the 5-week treatment period (Text-fig. 1). The affected and control ewes did not differ from one another in the rate of decline in responses with continued treatment.

Text-fig. 1. Total numbers out of 19 control or 20 affected ewes which (a) accepted mounting, (b) solicited, and (c) looked over their shoulder, in each week, during daily 50-min observation periods.

Some ewes challenged and head-bunted the rams, and sometimes other ewes as well. Such aggressive behaviour was observed earlier ($P < 0.05$) in the affected (mean onset 15.7 ± 1.6 days) than in the control (21.8 ± 2.1 days) ewes, and did not appear to be related to female sexual behaviour. Ewes also rubbed the poll or the side of the face on pen divisions or on other ewes; this behaviour was also exhibited sooner by affected ewes than by controls (6.0 ± 1.0 compared with 10.8 ± 1.4 days, $P < 0.05$).

When ewes were tested for male sexual behaviour, the total number of ewes showing courting behaviour increased after the first test (Table 2; 12 on Day 14 compared with 21; $\chi^2_1 = 4.25$, $P < 0.05$). In each test, affected ewes showed more courting behaviour than did controls and, by Day 28, this difference was statistically significant (Wilcoxon's test, $P < 0.05$; Table 2). There was no significant difference between groups or tests in Flehmen behaviour or in ano-genital sniffing.

Only 2 ewes (both controls) were observed to mount other ewes throughout the study.

Table 2. Number of times that 19 control and 20 clover-affected ovariectomized ewes injected daily with 5 mg testosterone propionate showed male behaviour during pen tests

	Day 14		Day 21		Day 28	
	Control	Affected	Control	Affected	Control	Affected
Courting	8.8 ± 5.1 (6)	11.7 ± 4.3 (6)	7.7 ± 4.1 (9)	10.8 ± 3.0 (12)	6.4 ± 4.5 (7)	$14.0 \pm 2.8^*$ (11)
Flehmen	2.5 ± 0.8 (6)	1.6 ± 0.4 (5)	2.3 ± 1.3 (4)	2.9 ± 1.1 (8)	1.2 ± 0.2 (6)	1.4 ± 0.2 (5)
Ano-genital sniffing	3.5 ± 0.5 (13)	4.1 ± 0.9 (11)	3.7 ± 0.8 (14)	3.6 ± 0.6 (16)	3.6 ± 0.7 (14)	4.5 ± 1.6 (12)

Values are mean $\pm$ s.e.m. for the no. (in parentheses) of ewes showing the behaviour.

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

Discussion

The behaviour of the ewes in the present study was consistent with that observed previously in ewes treated with oestradiol benzoate (Adams, 1978) or testosterone propionate (Signoret, 1975). Adams (1978) showed that mating and soliciting behaviours in oestradiol-treated ewes were more dose-

dependent than was tail fanning or looking over the shoulder, and in the present study the former behavioural features were also the most sensitive indicators of a difference between affected and control ewes treated with testosterone propionate. In the previous study on ovariectomized ewes (Adams, 1978), delayed onset or reduced incidence of female sexual behaviour was observed in clover-affected ewes given a single injection of oestradiol benzoate after treatment with progesterone for 10 days. The similar delay in onset of female mating behaviour in affected ewes given oestradiol benzoate alone in the present study indicates that the impairment results from an altered responsiveness to the oestradiol, and not from a reduced effectiveness of progesterone priming.

It is unlikely that the relatively minor changes in sexual behaviour in affected ewes have any direct bearing on the infertility. However, the nature of the changes does provide an insight into other changes which have been observed in permanently affected ewes. The uterine-like histological metaplasia of the cervix in affected ewes is best interpreted as an oestrogen-dependent differentiation (Lightfoot & Adams, 1979). In addition, ovariectomized affected ewes have increased protein and glycoprotein synthesis in the uterus and cervix and increased epithelial cell keratinization in the vagina, in the absence of any hormonal stimulation (Tang & Adams, 1981). Similar changes have been reported in female rodents treated neonatally (i.e. at the time of sexual differentiation) with oestrogen or testosterone (Kohrman & Greenberg, 1968; Takasugi & Kamishima, 1973). Such animals also have decreased female sexual behaviour and increased male behaviour (Phoenix, Goy, Gerall & Young, 1959; Gorski, 1973; Whalen & Etgen, 1978) and increased aggressive behaviour (Bronson & Desjardins, 1970) when stimulated with hormonal steroids during adult life. The parallel between the results of the present study and the organizational effects of neonatal oestrogen or testosterone on sexual behaviour in rodents is obvious. A similar parallel between the organizational effects of steroids and changes in permanently infertile ewes is the inability of ovariectomized affected ewes to release a surge of LH in response to oestradiol (Findlay *et al.*, 1973).

The ewes in the present study and those of Adams (1976) and Tang & Adams (1981) had never been exposed to phyto-oestrogens until after puberty. Thus, the permanent effects of phyto-oestrogens on the ewe mimic at least some of the changes caused by hormonal steroids during differentiation, even though the phyto-oestrogens are administered outside the normal period of organogenesis. It is not known whether steroidal oestrogens given to the adult ewe over a long period can also induce permanent differentiation.

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