Ovulation rate and oocyte numbers in ewes after prolonged exposure to oestrogenic pasture

N. R. Adams, C. M. Oldham* and R. A. Heydon†

*Division of Animal Health, CSIRO, Wembley, and *Institute of Agriculture, University of Western Australia, Nedlands, Western Australia 6009, Australia*

Summary. Ewes which had been exposed to oestrogenic clover for 3 years showed an elevated ovulation rate but no difference in numbers of primordial follicles compared with normal ewes.

Introduction

Ewes exposed to oestrogenic pasture for prolonged periods suffer a reduction in fertility which persists for several years after their removal to non-oestrogenic pasture (Schinckel, 1948). This infertility results mainly from impaired transport of spermatozoa through the cervix (Lightfoot, Croker & Neil, 1967) and abnormal cervical mucus has been observed in affected ewes (Smith, 1971). The ovarian function of affected ewes has not been studied in any detail.

While ewes are grazing oestrogenic pasture, large numbers of developing follicles accumulate in their ovaries (Adams 1977). If this occurs as a result of increased initiation of follicular development, the population of primordial follicles may become depleted. It has also been suggested that, even some months after removal from the oestrogenic pasture, the ovulation rate may be elevated in clover-affected ewes, compared with that in controls (1.57 versus 1.50 corpora lutea/ewe: Lightfoot *et al.*, 1967; 1.89 versus 1.73: Fels & Neil, 1968; 1.67 versus 1.50: Lightfoot *et al.*, 1974). However, in each study, group sizes were too small for the differences to be statistically significant. Land (1970) and Trounson, Chamley, Kennedy & Tassell (1974) have shown that, in groups of ewes which differ in their genetic potential for multiple ovulations, there is an inverse correlation between the numbers of primordial follicles in the ovary, and the ovulation rate.

The present study was carried out in ewes which had previously grazed oestrogenic clover to determine whether the apparent elevation in the ovulation rate was real, and if so, whether this was associated with reduced numbers of primordial follicles in the ovary.

Materials and Methods

The Merino ewes originated from a single flock, but 100 of them had grazed oestrogenic Yarloop subterranean clover for 3 years and at the last lambing only 11% lambed. The other 80 ewes had grazed non-oestrogenic pasture during this period and 76% lambed. The ewes were run together on non-oestrogenic pasture for 6 months before the start of the present study.

The number of corpora lutea in the ovaries was determined by laparoscopy in December, March and July, i.e. early, middle, and late in the normal Merino breeding season in Western Australia. After the last laparoscopy, the ovaries were removed from 14 control and 11 affected ewes taken at random, and the number of primordial follicles present in the right ovary was counted as described by Trounson *et al.* (1974).

† Present address: Department of Agriculture, South Perth, Western Australia.

Table 1. Number of phyto-oestrogen-affected and control ewes with 0, 1 or 2 corpora lutea at 3 stages of the breeding season

	14 December		26 March		8 July	
	Affected	Control	Affected	Control	Affected	Control
No. of CL						
0	17	17	8	5	13	7
1	61	55	52	57	61	52
2	7	1	39*	16	19	16
Total no. of ewes†	85	73	99	78	93	75
Ovulation rate for ewes ovulating	1.10	1.02	1.43	1.22	1.24	1.24
Body weight (kg)	50.6 ± 0.5	50.4 ± 0.6	48.9 ± 0.4	49.0 ± 0.5	39.4 ± 0.3	37.1 ± 0.4

* $\chi^2_1 = 7.97$, $P < 0.01$.

† The total number of ewes recorded varies between times because the genitalia of some ewes could not be readily visualized at laparoscopy.

Results

The frequency of the primordial follicles was not normally distributed, being skewed to the right. The mean number of primordial follicles per ovary was 25 180 (range 5607–53 523) in the control ewes and 24 217 (range 8595–43 704) in the affected ewes.

Despite the similar numbers of primordial follicles, the ovulation rate was increased in the phyto-oestrogen affected ewes, due to an increase in the frequency of twin ovulations in the ovulating ewes (Table 1). The difference was statistically significant at the peak of the breeding season ($P < 0.01$) and was not associated with differences in body weight or nutrition (Table 1). The proportion of ewes with no visible corpus luteum in their ovaries was similar for both groups at all stages of the breeding season.

Discussion

The failure to observe any depletion of primordial follicles in affected ewes indicates that the increased number of primary and secondary follicles in ovaries of ewes ingesting phyto-oestrogens (Adams, 1977) results from an altered pattern of follicular development, rather than from an increase in the rate of initiation of growth.

When the ovaries could not be visualized readily, no record was taken for that ewe. This happened more frequently in phyto-oestrogen-affected ewes, in which abnormalities such as hydrops uteri and uterine adhesions were more common. Since these ewes were presumably most affected with 'clover disease', it is not felt that this omission accounted for differences observed between groups.

The increased ovulation rate in affected ewes may be due to a change in hypothalamic control of the ovary. Studies on the weight of ovaries before and after unilateral ovariectomy have suggested that the function of the hypothalamic-ovarian axis is altered in ewes with permanent phyto-oestrogenic infertility (Adams, 1976). The hypothalamus of affected ewes is less sensitive to oestrogen (Findlay *et al.*, 1973), and may permit increased numbers of ovarian follicles to develop to meet its increased threshold for oestrogen. Evidence that an increased ovulation rate is accompanied by greater oestrogen production has been provided by Land, Thompson & Baird (1972) and Wheeler, Baird, Land & Scarramuzzi (1977). A slight elevation in levels of endogenous oestrogen may explain some apparently conflicting data on ewes with permanent phyto-oestrogenic infertility. Although oestrogen is less efficient at releasing LH from

the pituitary of affected ewes (Findlay *et al.*, 1973), ovulation, which is dependent on a preceding LH peak, proceeds normally (Lightfoot *et al.*, 1967, 1974). Furthermore, although the behavioural sensitivity to exogenous oestrogen is decreased in affected ewes (Adams, 1978), the duration of oestrous behaviour at natural oestrus is not diminished (Smith, 1971; Lightfoot *et al.*, 1974). This circumstantial evidence, together with the elevation in ovulation rate, suggests that endogenous oestrogen production may be slightly increased in ewes with permanent phyto-oestrogenic infertility.

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