Infertility in Ewes caused by
Prolonged Grazing on Oestrogenic Pastures:
Oestrus, Fertilization and Cervical Mucus

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Abstract

An experiment was conducted to investigate oestrus, fertilization and cervical mucus production in
two groups of ewes, one group being of reduced fertility due to prolonged grazing on oestrogenic
subterranean clover. Ewes within each of the oestrogenic (Yarloop subterranean clover) and control
(Mt Barker subterranean clover) treatments were divided into subgroups of ‘high’ and ‘low’ fertility
on the basis of total lambs born over the preceding 3 years.

The results confirmed previous findings concerning a failure of sperm transport and reduced
fertilization in affected ewes but the duration of oestrus (approx. 23 h) was normal. Ewes in the
Yarloop treatment, and in particular those in the low-fertility subgroup, were found to produce
significantly more cervical mucus than control ewes over the period −30 to +30 h relative to
time of onset of oestrus. This result is discussed in relation to the failure of sperm transport.

Introduction

Since the original work of Bennetts et al. (1946), it has been established that the major cause of permanent infertility in ewes affected with clover disease as the result of prolonged grazing on oestrogenic pastures is failure of fertilization due to impaired transport of spermatozoa through the genital tract (Turnbull et al. 1966; Lightfoot et al. 1967; Kaltenbach and Davies 1970). It is not known, however, why sperm transport is affected, although changes in the anatomy of the tract or in the nature of its secretions or both could be involved. In particular, cervical mucus seemed worthy of further study. Lightfoot et al. (1967) noted a lower proportion of motile sperm in mucus from clover-affected ewes and it is known (Gibbons and Mattner 1966; Mattner 1966) that the establishment and maintenance of the cervical sperm population in the ewe is dependent on the rheological properties of cervical mucus.

This paper describes part of a study concerning the physiological status of ewes that have reduced fertility due to clover disease. Data are presented on the incidence and duration of oestrus, fertilization and the production of cervical mucus.

Materials and Methods

The experiment was conducted at the ‘Avondale’ Agricultural Research Station, Beverley, W.A.,
during April–May 1970.

Experimental Animals

Two groups, each of 8-year-old Merino ewes, were studied. The ewes were the survivors of an
experiment that compared fertility of sheep grazing pastures sown initially to either the Yarloop or
Mt Barker cultivars of subterranean clover (*Trifolium subterraneum* L.). The following tabulation gives the lambing data from the original experiment over the years 1964–9 inclusive:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yarloop</td>
<td>64 (64)</td>
<td>86 (63)</td>
<td>90 (62)</td>
<td>80* (59)</td>
<td>78* (54)</td>
<td>50* (36)</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>77 (56)</td>
<td>89 (55)</td>
<td>89 (53)</td>
<td>96 (51)</td>
<td>96 (49)</td>
<td>76 (45)</td>
</tr>
</tbody>
</table>

* No. of ewes joined in parentheses.

The comparatively low fertility of the ewes grazing Yarloop clover was attributed to clover disease. Isoflavone analyses of the clovers were performed each year over the period 1966–9 inclusive. Typical results for formononetonin were 1.22% in Yarloop versus 0.06% in Mt Barker clover on 1 October 1968. A cervical mucus bioassay (Lindsay and Francis 1968) conducted at the same time showed the Yarloop pasture to produce responses equivalent to 10 μg stilboestrol/day whereas animals grazing Mt Barker clover did not differ from controls. When slaughtered in 1972, uteri from 10 of 15 Yarloop-treatment ewes showed cystic glandular hyperplasia of the endometrium compared with 1 of 18 Mt Barker ewes.

During the present investigation the ewes were run in pens under cover and fed a ration of cereal hay. The ewes had not eaten oestrogenic forage over the previous 6 months.

### Table 1. Ewe fertility in relation to clover treatment and fertility subgroup

<table>
<thead>
<tr>
<th>Clover cultivar</th>
<th>Fertility subgroup:</th>
<th>Both subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Yarloop</td>
<td>1.24 (17)</td>
<td>0.56 (16)</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>1.52 (17)</td>
<td>1.02 (17)</td>
</tr>
</tbody>
</table>

**Design of the Experiment**

The experiment was of factorial design (2 × 2) and compared previous grazing history (Yarloop versus Mt Barker) and previous ewe fertility (high versus low). For the later classification ewes from each clover treatment were ranked according to the mean number of lambs born over the three lambings, 1967–9 inclusive. Within each treatment the top 50% of ewes designated as most fertile were allocated to the ‘high-fertility’ subgroup and the remaining ewes to the ‘low-fertility’ subgroup (Table 1). Ewes from these four categories were then further subdivided at random into two groups. Ewes in group 1 were used to determine egg fertilization rates following artificial insemination while those in group 2 were used for the collection of cervical mucus and the determination of duration of oestrus.

**Observations on Oestrus**

Oestrus was synchronized by vaginal insertion (14 days) of pessaries impregnated with progestagen (Robinson 1965).

Vasectomized rams (fitted with aprons to prevent intromission) were used to detect both the first and second synchronized oestrus. For the first oestrus, ewes marked by the rams were recorded at intervals of 12 h. Commencing 16 days after pessary withdrawal, tests to detect the second synchronized oestrus were conducted at intervals of 4 h. To determine duration of oestrus in group 2, the 4-hourly tests were continued until ewes had failed to allow a ram to mount at two successive tests.
**Mucus Collection**

Mucus was collected by intravaginal cotton wool swabs (Smith 1971). Collection commenced 16 days after removal of intravaginal pessaries and continued at intervals of 6 h until 30 h after the onset of oestrus.

**Ovulation and Fertilization**

Ewes in group 1 were artificially inseminated 8–12 h after the onset of the second synchronized oestrus with 0·1 ml (3–4 × 10⁸ sperm) of fresh pooled semen collected by artificial vagina from three Merino rams. Seventy-two hours after onset of oestrus these ewes were subjected to laparotomy under general anaesthesia, and their ovaries examined. Eggs recovered by flushing the appropriate fallopian tube(s) with saline were examined for cell cleavage and number of sperm on the zona pellucida.

**Statistical Evaluation**

Total mucus weight was analysed by fitting the model \( y = m + ax + bz + cxz \) by the least squares method of Kemphorne (1957), where \( y \) is mucus weight, \( x = 1 \) for Yarloop and \(-1\) for Mt Barker, and \( z = 1 \) for high fertility and \(-1\) for low fertility.

Data for duration of oestrus and ovulation rate were analysed by analysis of variance and those for egg recovery, fertilization and sperm number by \( \chi^2 \) tests.

**Table 2. Pattern of onset of oestrus after synchronization in relation to clover treatment**

<table>
<thead>
<tr>
<th>Clover cultivar</th>
<th>No. of ewes</th>
<th>Days after pessary withdrawal:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1·0</td>
<td>1·5</td>
</tr>
<tr>
<td>Yarloop</td>
<td>33</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>34</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

**Results**

**Oestrus**

The distributions of ewes classified according to day of onset of oestrus are shown in Table 2. At the first oestrus after synchronization Yarloop-treatment ewes tended to be slower in exhibiting oestrus than Mt Barker ewes \((X^2_3 = 6·81; P < 0·1)\). There was no difference between treatments at the second oestrus, however, nor between fertility subgroups at either oestrus.

Means for duration of oestrus [time (h) ± standard error] were as follows:

<table>
<thead>
<tr>
<th>Clover cultivar</th>
<th>Fertility subgroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Yarloop</td>
<td>21·3 ± 3·0</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>26·0 ± 2·8</td>
</tr>
</tbody>
</table>

There were no significant effects of either treatment or subgroup, the overall mean for the 35 ewes being 22·8 ± 2·0 h.

**Mucus Weight**

There was a significant \((P < 0·01)\) effect of clover cultivar on total mucus weight over the period −30 to +30 h at about the time of onset of oestrus. Yarloop-treatment ewes yielded more mucus than those in the Mt Barker treatment. Some doubt is cast, however, on the biological significance of this main effect as the inter-
action between clover treatment and fertility subgroups approached conventional levels of statistical significance (0.05 < P < 0.1). An examination of the data presented in Table 3 and Fig. 1 reveals that, of the four subgroups under study, only the Yarloop-low-fertility subgroup was markedly different. Ewes in this subgroup yielded approximately 1.5 times the weight of mucus produced by ewes in the other three subgroups.

<table>
<thead>
<tr>
<th>Clover cultivar</th>
<th>Fertility subgroup:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yarloop</td>
<td>High: 24.2</td>
<td>5</td>
<td>35.5</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>Low: 23.8</td>
<td>5</td>
<td>30.5</td>
<td>27.0</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>High: 23.6</td>
<td>5</td>
<td>24.7</td>
<td>24.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5</td>
<td>30.5</td>
<td>27.0</td>
</tr>
</tbody>
</table>

**Table 3. Effect of clover treatment and fertility subgroup on total cervical mucus collected from 30 h before to 30 h after the onset of oestrus**

Values are mean mucus weights (g) per ewe

**Fig. 1.** Effects of clover treatment and fertility subgroup on cervical mucus production relative to time of onset of oestrus.

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**Ovulation, Fertilization and Spermatozoa on the Zona Pellucida**

Data for these parameters are presented in Table 4. The mean numbers of ovulations per ewe and percentages of eggs recovered to eggs shed were similar for both clover treatments and fertility subgroups. Overall estimates were 1.58 and 86% respectively.

A lower proportion of ewes in the Yarloop treatment (54%) yielded fertilized eggs when compared with results for the Mt Barker treatment (88%). The major component of this effect was the low fertilization rate for the Yarloop-low-fertility subgroup ewes (33%).

The distribution of eggs according to whether or not spermatozoa were attached to the zona pellucida was similar to those for fertilization, although the effects were more marked. Significantly fewer eggs from the Yarloop than from the Mt Barker
treatment yielded sperm \( P < 0.05 \). Within the Yarloop treatment none of the eggs recovered from the low-fertility subgroup had attached sperm, compared with 73% for the high-fertility subgroup \( P < 0.01 \).

### Table 4. Ovulation, egg recovery, fertilization and sperm on the zona pellucida in relation to clover treatments and fertility subgroups

<table>
<thead>
<tr>
<th>Clover cultivar</th>
<th>Fertility subgroup</th>
<th>No. of ewes:</th>
<th>No. of eggs:</th>
<th>Shed</th>
<th>Recovered</th>
<th>Fertilized</th>
<th>With sperm^A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Examined</td>
<td>Yielding eggs</td>
<td>With fertilized eggs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yarloop</td>
<td>High</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>14</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>11</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Mt Barker</td>
<td>High</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>13</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

^A On zona pellucida.

### Discussion

Results from the present study, showing that ewes affected with clover disease had a lower fertilization rate and fewer sperm on the zona pellucida of recovered eggs, are in agreement with previous reports (Turnbull et al. 1966; Lightfoot et al. 1967; Kaltenbach and Davies 1970) concerning the nature of the permanent infertility in ewes following prolonged grazing on oestrogenic pastures. New evidence was presented to show that the duration of oestrus in affected ewes is comparable with that in control animals.

It has been established that the rheological properties and macromolecular structure of mucus are of functional importance with regard to the establishment and maintenance of a cervical population of spermatozoa in the ewe (Gibbons and Mattner 1966). The finding that cervical mucus production was disturbed in clover-affected ewes may therefore be of significance and could prove to be the immediate cause of reduced sperm transport discussed above.

Since the volume of cervical mucus produced in the ovariectomized ewe is a linear function of exogenous oestrogen (Lindsay and Francis 1968; Smith 1971), the present results may be explained if the ewes in the Yarloop treatment had a higher level of oestrogen production, or alternatively if their cervical sensitivity was increased. The former suggestion is not supported by the finding that oestrus duration, which is also related to dose of oestrogen (Fletcher and Lindsay 1971; Scaramuzzi et al. 1971), was similar in both affected and control ewes. Moreover, with regard to cervical sensitivity, Smith (1971) reported that the cervical mucus response to injected oestrogen in ovariectomized ewes was similar when comparing sheep with previous histories of high versus low fertility. Further research is obviously needed to clarify the situation.

An important feature of the results was the demonstration that some ewes appear to be resistant to the deleterious effect of prolonged grazing on oestrogenic pastures. Despite identical histories of grazing and management, ewes in the Yarloop–high-fertility subgroup not only produced more lambs than the low-fertility subgroup ewes in the years previous to this study, but they were found to be comparable to control ewes with regard to number of sperm on the zona pellucida, egg fertilization rates and cervical mucus production.
A suggestion (Obst et al. 1971) that ‘resistance’ to clover disease is associated with blood haemoglobin type was not supported by a subsequent study (Wroth et al. 1973) involving much larger numbers of ewes. It is possible that the resistant (Yarloop–high-fertility subgroup) ewes studied here were representative of a class that are capable of grazing selectively to achieve a diet containing less phyto-oestrogen. One of several alternative suggestions is that endogenous progesterone, as the result of more frequent pregnancies, provides protection against phyto-oestrogens. The question is important as clear definition of the physiological basis by which some ewes are resistant to clover disease could offer promise towards finding a practical solution to the problem.

Acknowledgments

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