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Clover disease: what do we know and what can we do

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Clover Disease
—What we know and what we can do

By T. Marshall, Adviser, Sheep and Wool Branch

The importance of subterranean clover in ley farming systems was recognised in Western Australia in the 1930's\textsuperscript{14}. Increases in soil fertility and stock carrying capacity of land sown to clover had a tremendous impact on agriculture and the clover ley system became widely accepted.

However, this was closely followed by reports of severe abnormalities in normal reproductive function among flocks grazing dominant subterranean clover pastures\textsuperscript{4-5}. The complex of these abnormalities became known as clover disease.

Fig. 1.—Per cent. lambs marked, five-year moving average for two rainfall zones in the agricultural areas of W.A. (Reference 20).

Although it is now over 30 years since the problem and its cause were recognised there is still no cure for ewes affected with clover disease.

A measure of the present effects of clover disease is seen in Figure 1, which compares ewe fertility in the 300 to 380 mm and 380 to 630 mm rainfall zones of the agricultural areas. Before 1940, sheep in the higher rainfall zone were more fertile than in the 300 to 380 mm zone but since then the relationship has changed. This change parallels the introduction of sub. clover into the higher rainfall zone and suggests that clover disease may be causing a reduction in fertility of about 8 per cent\textsuperscript{20}.
There are about 6,500,000 ewes in the 380 to 630 mm rainfall zone. This suggests an annual loss of about 520,000 lambs due to clover disease. Recent research has indicated that the reduction in ewe fertility due to clover disease may be as much as 15 to 20 per cent on individual properties in this zone.

The results of continued scientific investigations have constantly added to the knowledge of the disease and have allowed evasive and preventative measures to be evolved. The results of these investigations and the measures that can be taken to control the disease are discussed below.

WHAT IS CLOVER DISEASE?
The clover disease syndrome, which is caused by the ingestion of certain pasture plants containing relatively high concentrations of oestrogenic substances, manifests itself as

- reduced fertility of ewe flocks
- dystocia (difficult lambing)

Dystocia. The ewe is unable to complete the birth because of interruptions to the normal birth process. The lamb is dead in utero.

- prolapse of the reproductive tract
- high tail
- increased death rate among ewes
- lactation in virgin ewes and wethers
- enlarged bulbo-urethral glands (false bladder) in wethers
- urinary obstructions in wethers.

The fertility of rams grazing oestrogenic pastures is apparently not affected.

In W.A. the disease is confined to sheep, and there is no evidence to suggest that cattle are affected in this State.

Ewe fertility

Probably the most dramatic and certainly the most economically important effect of clover disease is the drastic reduction in ewe fertility in affected flocks. There are reports of ewe fertility falling to 10 to 20 per cent of normal levels following prolonged grazing of oestrogenic pastures.

Two types of infertility have been defined—temporary infertility and permanent infertility. Temporary infertility results from grazing oestrogenically potent pastures during the mating period and, as its name suggests, affected flocks usually recover normal fertility after being moved to safe pastures. Occurrence would be unusual in W.A. except on the extreme south coast. Permanent infertility results from prolonged periods of intake at any time during life and results in a progressive and permanent reduction in flock fertility. Permanent infertility, once it has occurred, is not reversed by removing the source of oestrogen.

Dystocia

Another factor causing low lambing performance in affected flocks is
dystocia, or difficult birth. In affected ewes the normal physiological functions of the reproductive tract are suppressed, preventing the normal birth process from continuing.

Dystocia results in the birth of either dead or very weak lambs due to the prolonged birth process, or a complete failure in delivery. Often the ewe manages to extrude only part of the lamb and requires assistance to complete the process; without help, the partially born lamb quickly dies.

If retained, the lamb either mummifies or decomposes, and increases the likelihood of uterine infections which could kill the ewe.

**Prolapse of the uterus**

Varying proportions of ewes in flocks grazing oestrogenic pastures have exhibited a prolapse (turning inside out) of the reproductive tract. The degree of eversion varies from partial, when only a small amount of the tract is turned inside out, to complete. Prolapse is most common in the spring on lush clover-dominant pastures.

Although the proportion of affected ewes is usually small, uterine prolapse is economically important under extensive grazing conditions because treatment is difficult and affected animals usually die or must be destroyed.

**High Tail**

Associated with uterine prolapse is the condition known as high tail. Both result from relaxation of the ligaments in the pelvic region. High tail is characterised by an apparent elevation of the base of the tail. It is not necessarily associated with loss of fertility but can be taken as an indication that ewes have been exposed to oestrogenic clover.

**Ewe deaths**

A common effect of clover disease is a large increase in ewe deaths. It has been shown that ewe death rate in clover disease-affected flocks can be as much as twice that among similar ewes grazing non-oestrogenic pastures. While the occurrence of dystocia and prolapse can influence the death rate post mortem examinations have shown that many ewes also die from a variety of other causes.

**Lactation**

Udder development and lactation among non-lambing ewes and wethers is a common sign of current intake of oestrogenic pastures. Lactation has also been seen occasionally in rams. While the condition has no economic importance and no known harmful effects, it can serve as a useful guide to the potency of the pasture being grazed at the time.

**Enlarged bulbo urethral glands**

The bulbo urethral glands which are present on the upper surface of the urethra of all male sheep may become enlarged, cystic and finally break down to form a large fluid-filled sac in wethers which have been grazing oestrogenic pastures. This sac is evident as a swelling just below the anus. Badly affected animals lose condition and may die. If mildly affected animals are moved to "safe" pastures they usually recover.

**Urinary obstructions in wethers**

Sediments may form in the urethral passage of wethers grazing highly oestrogenic pastures. The sediments may be solid or of a pasty consistency. In severe cases the blockages can stop the passage of urine, rupture the bladder and kill the wether.

**WHAT WE KNOW ABOUT CLOVER DISEASE**

**Strain variation in potency**

Following the initial investigations it was concluded that clover disease was due to the presence of oestrogenic substances in the subterranean clover pastures being grazed by affected flocks. Three oestrogenic compounds (isoflavones)—formononetin, genistein and biochanin A—were isolated in significant quantities from green clover. The potency in dry clover material was found to be greatly reduced and in most cases is negligible.

It was at first thought that all strains were potent and would cause the disease. However, field observations and investigations showed that there were differences in potency among varieties. It was noted that isoflavone levels were relatively constant for any one variety under normal conditions.

Further research showed that formononetin was the major causative agent in clover disease. Genistein and biochanin A are rapidly metabolised by the rumen to non-oestrogenic compounds and have no net oestrogenic effect in sheep.

The metabolic products of formononetin differ from those of genistein and biochanin A. Up to 70 per cent of the ingested formononetin has been recovered from...
Enlarged bulbo-urethral gland in a wether. It can be seen as a large swelling just below the tail.

Table I—Oestrogenic potencies for sheep of some common leguminous pasture species

| LOW OESTROGENIC POTENCY |  |
|-------------------------|  |
| (usually less than 0.3 per cent of formononetin) |  |

Trifolium subterraneum (subterranean clover)
strain (in order of maturity)

- Northam A
- Uniwager
- Blackwood
- Dalíak
- Seaton Park
- Woogenellup
- Clare
- Bacchus Marsh
- Mt. Barker
- Redwest

T. pratense (red clover)

- Geraldton
- Dwellanup
- Dinninup
- Howard
- Yarloop
- Tallarook
- All varieties except Redwest

T. hirtum (rose clovers)
T. cherleri (cupped clovers)
T. repens (white clovers)
T. fragiferum (strawberry clovers)
T. campestris
T. tomentosum
T. arvense
T. glomeratum
Vicia sativa (vetches)
Ornithopus species (serradella)
Lupinus species (lupins)

Semen quality was not affected in a flock of rams which were lactating as a result of grazing oestrogenic pastures. It seems unlikely sheep as equol and a further 5 per cent as O-desmethyl angolensin, both of which are oestrogenic in sheep. Thus the oestrogenicity of formononetin is largely due to the equol formed from formononetin in the rumen.17

This knowledge plus the development of methods for rapidly measuring the isoflavone levels of plants17 has now made it possible to categorise varieties on the basis of formononetin content into those which are likely to cause clover disease and those which are not. Reductions in ewe fertility have been recorded in flocks grazing pastures containing about 0.8 per cent or more formononetin, while those containing 0.3 per cent or less are usually considered safe.

Classification of some common pasture species into high and low oestrogen categories is given in Table 1.

Mechanism of clover infertility

Ewe fertility could be impaired at varying stages in the reproductive cycle. These possible breakdown points will be discussed in sequence.

Sperm production

Although reductions in sperm motility have been induced in rams injected with synthetic oestrogens, there have been no reports of reduced ram fertility following the grazing of oestrogenic pastures. Semen quality was not affected in a flock of rams which were lactating as a result of grazing oestrogenic pastures.30
that ram fertility would be affected under field conditions.

**Oestrus and ovulation**

Oestrus (or heat period) is that time when the ewe will accept the ram. Ovulation (egg release) normally occurs 20 to 30 hours after the onset of oestrus.

There have been numerous reports of abnormal oestrus patterns among ewes during periods of intake of potent pastures. These effects are generally grouped under the heading of temporary infertility. The occurrence of behavioural oestrus without ovulation (ewes allow the ram to mount but ovulation does not occur), delayed first oestrus, anoestrous (failure of oestrus to occur), reduced ovulation and reduced conception rate among ewes currently grazing oestrogenically potent pastures have been reported. However, oestrus and ovulation appear to return to normal after removal to "safe" pastures.

Although some variability in the incidence and duration of oestrus has been reported in ewes affected with permanent infertility, it is considered that there is no relationship between oestrus cycle abnormalities and flock fertility.

**Sperm transport and egg fertilisation**

Reduced egg fertilisation due to impaired sperm transport through the reproductive tract is considered to be the major reason for reduced fertility among ewes affected with clover disease.

Fertilisation takes place in the fallopian tubes. To reach this site sperm have to travel from the vagina through the cervix and uterus and up the tubes. Research has shown that sperm numbers in the cervix and in particular the number finally reaching the fallopian tubes are greatly reduced in ewes affected with clover disease (see Table 2). The lower number of sperm present results in a lesser proportion of eggs being fertilised (see Table 3).

Recent research has shown that the cervical mucus produced by

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**Table 2**—Effect of clover disease on sperm transport. Numbers of sperm in cervix, uterus and fallopian tubes 24 hours after service.

<table>
<thead>
<tr>
<th>Division of tract</th>
<th>Clover disease affected ewes</th>
<th>Control ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>85,000</td>
<td>2,529,000</td>
</tr>
<tr>
<td>Uterus</td>
<td>20,000</td>
<td>291,000</td>
</tr>
<tr>
<td>Fallopian tube (Average of the two tubes)</td>
<td>350</td>
<td>17,160</td>
</tr>
</tbody>
</table>

Reference 22

**Table 3**—Effect of oestrogenic clover on fertilisation rate. Fertilisation rates among ewes grazing oestrogenic clover and non-clover pastures.

<table>
<thead>
<tr>
<th>Type of pasture</th>
<th>Age of ewes</th>
<th>Eggs fertilised as a per cent of eggs shed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clover</td>
<td>Young</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>69</td>
</tr>
<tr>
<td>Non-clover</td>
<td>Young</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>91</td>
</tr>
</tbody>
</table>

References 28, 37

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**Table 4**—Effect of oestrogenic clover on embryo mortality. Embryo mortality among ewes grazing oestrogenic clover and non-clover pastures.

<table>
<thead>
<tr>
<th>Type of pasture</th>
<th>Age of ewes</th>
<th>Per cent. embryo mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clover</td>
<td>Young</td>
<td>1971</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>1972</td>
</tr>
<tr>
<td>Non-clover</td>
<td>Young</td>
<td>1971</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>1972</td>
</tr>
</tbody>
</table>

References 28, 37
ewes affected with clover disease differs in its physical structure and volume to that of normal mucus. It is thought that these changes in the physical characteristics of the mucus may be the immediate cause of reduced sperm transport in affected ewes.

**Development of the fertilised egg**

Following fertilisation the egg begins to divide into two cells, then four, eight and so on. At the same time it is travelling down the Fallopian tube and reaches the uterus about three days after ovulation. The timing of fertilisation in relation to the position of the egg in the tract is important if a successful pregnancy is to take place.

As sperm transport is severely retarded in ewes affected with clover disease it is reasonable to expect that in some cases sperm will not come into contact with the egg until later than normal. This could result in abnormal patterns of cell division or the egg reaching the uterus at other than the optimum time.

![Graph](image-url)

**Fig. 3.** The effect of grazing oestrogenic pasture during the weaner year on ewe fertility in subsequent years of grazing the same oestrogenic pasture (Reference 26).

There are some reports of abnormal patterns of cell division and losses of eggs after fertilisation (embryo mortality) in ewes affected with clover disease. However, work with large numbers of ewes in W.A. suggests that there is no clear and consistent effect of clover disease on embryo mortality in the field (Table 4).

**Effect of length of grazing history**

As a general rule the longer the history of grazing oestrogenic pastures then the greater will be the effect on fertility of the ewe flock. This relationship of course varies with pasture variety, seasonal effects, pasture composition, management practices and a number of other factors.

Various experiments have shown a general decline in fertility with increasing length of time the ewes have grazed potent pastures.

Figure 2 details the changes in fertility of two similar groups of ewes, one of which continually grazed non-oestrogenic pastures and...
the other which grazed oestrogenic subterranean clover pastures.

When ewe weaners are grazed on potent pastures their fertility can be expected to be affected more quickly than when they spend their weaner year on non-oestrogenic pastures (Figure 3).

Effect of botanical composition of pastures

The dilution of potent pastures by non-oestrogenic species reduces the severity of the effects on ewe fertility. An experiment comparing the effects of pastures containing varying proportions of oestrogenic clovers showed clearly that as the proportion of clover in the pasture increased ewe fertility became more and more affected (Figure 4).

Field observations suggest that sheep given a choice prefer certain strains of clover. Recent research suggests that strains high in total isoflavones (e.g. Yarloop and Dinninup) may be less acceptable to sheep. However, in the field situation, especially at moderate to high stocking rates, it is unlikely that sheep would be able to select out the non-oestrogenic component of the pasture completely. They could therefore be expected to eat a certain amount of oestrogenic material. This suggests that pastures containing even as little as 20 per cent of oestrogenic clover may not be completely safe.

Environmental effects on pasture potency

The effects of a number of environmental factors are known to increase the formononetin content of subterranean clover. These include waterlogging, low temperature, defoliation, and deficiencies of phosphate, sulphur, nitrogen, and a number of trace elements.

Of these the most likely effect to occur in the field is that of elevated formononetin levels due to phosphate deficiency. The data presented in Figure 5 show the responses of ewes (cervical mucus bio-assay) to pastures of the same clover variety topdressed at varying rates in their third year. The available evidence suggests that pastures should receive adequate phosphate to sustain healthy plant growth to reduce the risk of clover disease but applying rates of super above those required for normal plant growth will not lower the formononetin levels. Care should be exercised where sheep are grazing clover pastures in areas which normally experience very cold temperatures or are subject to water-logging, both of which can increase formononetin levels.

Immunity of some ewes to clover disease

In practically all flocks which become affected with clover disease some ewes maintain normal fertility while others in the flock become infertile. This suggests that some ewes are capable of resisting the effects of pasture oestrogens. The mechanisms of this apparent resistance are unknown but it is possible that the resistance is genetically controlled.

South Australian workers suggested that there may be an association between blood haemoglobin type and susceptibility or resistance to clover disease. However, observations in W.A. with much larger numbers of ewes have failed to show any relationship between haemoglobin type and susceptibility or resistance to the disease.

Further work is required in this area to determine the reasons for this apparent resistance in some ewes and to investigate ways of using the resistance factor, if it is proven, to advantage.

WHAT CAN BE DONE?

Despite the large volume of research into clover disease and the improving knowledge of the causes of the reduced fertility there is still no known cure of permanent infertility. There are, however, a number of husbandry practices which can be employed to reduce the effects of clover disease.

Avoid potent strains

The most obvious way of avoiding clover disease is to prevent sheep having access to potent pastures. When establishing new pastures this can be achieved by sowing suitable strains which have a low formononetin content.

Such strains are now available for most environments. Where highly potent pastures are already established it is not practicable to avoid grazing them completely so measures designed to dilute their effects have to be devised.

Introduce low potency strains

The development of low potency strains provides the opportunity to replace existing potent strains. Before a new strain can be introduced
to a pasture the soil seed bank of the existing strain must be reduced to a level that will allow the new strain to become established. This could be achieved by heavy spring grazing before cropping, spring fallow or spraying, and continuous cropping. These practices will reduce the seed set of the existing strain and will effectively use up the soil seed bank.

The new strain must be able to compete successfully with the existing strain or it will be quickly dominated and the pasture will revert to its original condition. A number of the low oestrogen strains now available are able to compete successfully with the potent strains of similar maturity already sown into pastures.

Suitable replacement strains and replacement methods vary with the environment concerned. Advice should be sought as to the best strains and methods to use in any particular environment.

Fertilize pasture adequately
As it has been shown that superphosphate-deficient pastures have higher formononetin levels than usual, fertiliser applications should be sufficient for normal healthy pasture growth. Adequate superphosphate will also hasten the invasion of the pastures by other herbs and grasses which will dilute the oestrogenic effect of the pasture.

Manipulation of stocking rates and fertiliser applications may also be used as tools to increase the proportion of non-oestrogenic species in a pasture. Management practices should be aimed at encouraging the non-oestrogenic component in the pasture and reducing the proportion of the potent strain.

Avoid mating on potent pastures
Mating on oestrogenically potent pastures may result in the occurrence of temporary infertility described above.

While it is not common for green clovers to be on offer to ewes during normal mating times (November to March) in the Mediterranean type climate, it is possible that in seasons where there is substantial early rain, particularly along the south coast of W.A., appreciable amounts of potent clovers could be eaten. In these situations ewes should be shifted if possible to non-oestrogenic pastures to avoid possible temporary infertility.

**Treatment of breeding stock**
Breeding stock are the most important animals on the farm; young breeders are more important than old breeders as they have most of their reproductive life ahead of them. On properties where oestrogenic pastures are established the sheep should be allocated in such a way that the most important animals are grazed on the least oestrogenic pasture.

The classes of sheep in decreasing order of importance on a farm which has areas sown to oestrogenic pasture and which carries out a normal breeding programme are ewe weaners, young ewes, old ewes, young wethers and old wethers.

The importance of strategic allocation of grazing among the various classes of sheep is demonstrated in Table 5. In the table two farm model situations, one with no oestrogenic pastures and the other which has five grazing units each of 0, 25, 50, 75 or 100 per cent oestrogenic pasture, are examined.

In these models it is assumed that each farm runs Merinos and breeds its own replacements, that ewes are mated for the first time after the fourth lambing, that a permanent and progressive depression of fertility occurs after successive seasons of grazing oestrogenic pastures and that the magnitude of these depressions is 0, 5, 10, 15 or 20 per cent for each year of grazing pastures containing 0, 25, 50, 75 or 100 per cent oestrogenic species respectively. For simplicity death rates have been ignored in these examples.

In the model dealing with the oestrogenic farm three grazing strategies are examined:

- the ewes are grazed with no consideration to ewe age or pasture composition but are moved from paddock to paddock at frequent intervals (random grazing),
- the youngest ewes graze the most clover dominant pastures (disaster grazing),
- the youngest ewes graze the least clover dominant pastures (strategic grazing).

The effects on ewe fertility of the three different grazing strategies on the oestrogenic farm clearly illustrate the importance of grazing the youngest ewes on the least potent pastures.

If potent pastures occupy a large proportion of the farm then consideration should be given to sowing special purpose crops (e.g. oats or barley) for winter grazing. This would effectively reduce the intake of potent pasture and could also operate in conjunction with the replacement of the potent pasture with a low oestrogen strain.

Where replacement breeders are bought in and not bred on the property care should be taken to ensure that they have not been grazing oestrogenic pastures before purchase. Regular purchase of

<table>
<thead>
<tr>
<th>Ewe age</th>
<th>Per cent ewes lambing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-oestrogenic farm</td>
</tr>
<tr>
<td></td>
<td>Theoretical inherent fertility</td>
</tr>
<tr>
<td>1-5</td>
<td>70</td>
</tr>
<tr>
<td>2-5</td>
<td>85</td>
</tr>
<tr>
<td>3-5</td>
<td>95</td>
</tr>
<tr>
<td>4-5</td>
<td>95</td>
</tr>
<tr>
<td>Average</td>
<td>86</td>
</tr>
</tbody>
</table>

Reference 21
suitable replacement ewes can be a most effective procedure on properties where clover disease is severe.

**General flock husbandry**

Normal recommended husbandry practices for breeding ewes should be followed. Ewes should be in the best possible condition at mating and should be adequately fed in late pregnancy and during lactation. At least 2 per cent of mature rams should be used at mating. If maiden rams are being used, then more than 2 per cent should be joined with the flock.

Because of the large proportion of ewes which fail to conceive to first service a joining period of at least eight weeks should be used to ensure that ewes have the opportunity to return to the ram. However, if mating is extended too long, management problems may arise because of the extended lambing period.

It was often thought in the past that culling ewes which failed to lamb in two consecutive years would remove clover disease affected ewes from the flock. However, recent examination of data from a series of experiments (see Table 6) has shown that this practice would not lead to any major improvement in flock fertility. The data suggest that ewes should be grouped in flocks according to their history of grazing potent pastures. Those flocks with the longest history of grazing oestrogenic pasture will be most affected by clover disease and when their fertility falls to a critical level the whole flock should be sold and replaced with young breeders.

On properties where clover disease is likely to be a problem, management practices should be aimed at reducing the intake of potent clovers. The effects of clover disease are progressive in their nature and any reduction in intake of potent clover which can be achieved will result in a decrease in the potential severity of the problem.

**Acknowledgments**

Grateful acknowledgment is made for the help and constructive criticism afforded by many colleagues within the Department of Agriculture and other organisations during the preparation of this paper.

**References**


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**Table 6—The effect of culling "dry" ewes on flock fertility.**

<table>
<thead>
<tr>
<th>Sub. clover</th>
<th>Age of ewes at lambing (years)</th>
<th>5(i)</th>
<th>5(ii)</th>
<th>5(iii)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Yarloop</td>
<td>82*60†</td>
<td>79/53</td>
<td>49/45</td>
<td>46/39</td>
</tr>
<tr>
<td>Dinninup</td>
<td>78/79</td>
<td>72/71</td>
<td>53/64</td>
<td>35/63</td>
</tr>
<tr>
<td>Dinninup</td>
<td>87/73</td>
<td>55/69</td>
<td>47/64</td>
<td>18/56</td>
</tr>
<tr>
<td>Dwalganup</td>
<td>89/37</td>
<td>56/34</td>
<td>30/33</td>
<td>6/31</td>
</tr>
<tr>
<td>Overall</td>
<td>84/249</td>
<td>64/227</td>
<td>47/206</td>
<td>28/189</td>
</tr>
</tbody>
</table>

(i) Original unselected flock.
(ii) Culling ewes that were dry at both 2nd and 3rd lambings.
(iii) Culling ewes dry at 3rd lambing.
* Number of ewes joined.