
THE MECHANISM OF TEMPORARY INFERTILITY IN EWES GRAZING OESTROGENIC SUBTERRANEAN CLOVER PRIOR TO AND DURING JOINING

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Summary

Ewes in which oestrus had been synchronised were artificially inseminated (at the second synchronised oestrus) 17 to 21 days after placement on plots containing either oestrogenic (Trifolium subterraneum L. cv. Dinninup) or non oestrogenic pasture. A comparison of the reproductive performance indicated that grazing oestrogenic pasture prior to and during joining reduced the proportion of ewes in oestrus, the ovulation rate and the fertilization rate. Evidence presented suggested adverse effects on the rate of egg transport and the number of sperm reaching the site of fertilization. Effects on corpus luteum weights, and embryo mortality are also reported.

I. INTRODUCTION

There are several reports of infertility in ewes joined on pastures containing phyto-oestrogens. It has been demonstrated that such infertility is of a temporary nature, (Engle, Bell & Davis 1957; Ch'ang 1961), normal fertility returning some 5 weeks after the ingestion of oestrogenic forage ceased (Morley, Axelsen & Bennett 1966). This is distinctly different from the infertility known as clover disease described by Bennett, Underwood & Shier (1946), which is of a permanent and progressive nature, and in which ewes are normally joined on dry non oestrogenic pasture.

This study was conducted to examine in more detail the nature of the infertility occurring in ewes grazing green subterranean clover around joining.

II. MATERIALS & METHODS

The experiment was conducted at the Badgingarra Agricultural Research Station, approximately 224 km north of Perth in June-July, 1970. Pastures at the time were green and well grown following germination in May.

Oestrus was synchronised in a flock of 183 five to eight year old Merino ewes by vaginal insertion of pessaries containing progesterone for a period of 14 days (Robinson 1964). One day after pessary removal the flock was randomized into two groups and these placed on plots containing either dominant (> 90%) Trifolium subterraneum L. cv. Dinninup (92 ewes: Oestrogenic Treatment) or a mixture of West Australian Serradella (Ornithopus compressus), grasses and capeweed (91 ewes: Control Treatment). For the seven months prior to randomization the ewes had either grazed dry (non oestrogenic) pasture or been fed in pens with cereal hay.

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Eleven vasectomised rams wearing Sire Sine harnesses and crayons were used to detect the second oestrus after pessary removal. Oestrous ewes were drafted twice daily at 0800 and 1630 hours for insemination. Ejaculates were collected by artificial vagina from 2 rams, pooled and diluted 1:1 with glucose-yolk-citrate diluent. Each ewe was inseminated once two to three hours after drafting (2 to 18 hrs after oestrus onset) with 0.1 ml of diluted semen (approximately 150 x 10^6 motile sperm). The ewes were returned to their treatment plots within 12 hrs of insemination.

Pastures on each plot were sampled (6 samples per plot) for isoflavone assay (Francis & Millington 1965) on July 6 (4-8 days post insemination).

A proportion of the ewes inseminated each day were allocated at random for laparotomy (54-79 hrs post oestrus onset). Eggs recovered by flushing the Fallopian-tubes with saline were examined for cell cleavage, polar bodies and number of sperm on the zona pelucida. The remaining ewes were slaughtered 21-25 days post insemination, their reproductive tracts were examined and the number and weight of corpora lutea and the number of embryos present were recorded. Statistical analyses were performed by Chi Square.

III. RESULTS

(a) Pasture Isoflavone Levels

Mean levels of formononetin, genistein and biochanin A for Dinninup from the Oestrogenic treatment plot on July 6 (4-8 days post insemination) as a percentage of dry petiole weight (± standard error) were 0.91 (± 0.14), 1.20 (± 0.17) and 1.57 (± 0.08) respectively. No isoflavones were detected in samples of Serradella taken from the Control plot.

(b) Incidence of Oestrus

The distribution of ewes according to time of onset of oestrus at the second oestrus after synchronisation was similar in both groups. In the control group, however, more ewes were detected in oestrus over the 5 day period (72/91, 79% versus 61/92, 66%; 0.05 < p < 0.1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of ewes</th>
<th>LAPAROTOMY</th>
<th>Slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of ewes with</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 C.L.*</td>
<td>2 C.L.</td>
</tr>
<tr>
<td>Oestrogenic</td>
<td>61</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>71**</td>
<td>16</td>
<td>13</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 4.62, \ p < 0.05 \]

**Corpus Luteum  ** One ewe died between insemination and slaughter
(c) Ovulation Rate

Ovulation rates for the Oestrogenic versus Control treatments were 1.15 versus 1.45 at laparotomy and 1.12 versus 1.55 at slaughter, respectively (Table 1). Pooling all data the overall rates were 1.13 for the Oestrogenic treatment, and 1.51 for the Controls, this difference being highly significant (p < 0.001).

(d) Weight of Corpora Lutea

Corpora lutea from non-pregnant animals showing only one ovulation were heavier for the Control than the Oestrogenic treatment (0.63 g ± 0.06 versus 0.50 g ± 0.02). Corpora lutea were classified as either "mid" (approx. 4-10 days post ovulation) or "late" (approx. 11-17 days post ovulation) cycle. All 19 non-pregnant Control ewes showed corpora lutea classified as "mid" cycle, whereas 3 of the 23 Oestrogenic treatment ewes were classified as "late" cycle. Assuming a 17 day cycle all non-pregnant ewes should have been between days four to eight of the oestrus cycle.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of ewes</th>
<th>No. of eggs</th>
<th>Yielding eggs</th>
<th>Yielding fertilized eggs</th>
<th>Shed</th>
<th>Recovered</th>
<th>Fertilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrogenic</td>
<td>27</td>
<td>17</td>
<td>9</td>
<td>31</td>
<td>20</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>26</td>
<td>18</td>
<td>42</td>
<td>36</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

(e) Egg Recovery, Fertilization and Sperm on the Zona Pelucida

The proportion of ewes from which eggs were recovered to ewes examined (Table 2) was significantly lower in the Oestrogenic compared with the Control treatment (63% v 90%; p < 0.05). The proportion of eggs fertilized to eggs recovered was also lower in the Oestrogenic treatment (45% versus 75%; p = 0.05). The difference between treatments in fertilization rate was associated with fewer sperm on the zona pelucida of eggs recovered from the Oestrogenic treatment ewes. Six (33%) of 20 eggs from the Oestrogenic treatment ewes had more than 1 sperm on the zona pelucida versus 23 (64%) of 36 from the Controls (p < 0.05).

(f) Pregnancy, 21-25 days Post Insemination

Fifty five percent of the Control ewes had one or more embryos at slaughter compared with 32% of the Oestrogenic treatment ewes (Table 3). Because of the higher ovulation rate in the Controls the superiority of this treatment is increased when expressed in terms of the percentage of embryos recovered to corpora lutea present (41% versus 71%).
TABLE 3
Embryo recovery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of ewes</th>
<th>No. of ewes with 1 C.L. yielding</th>
<th>No. of ewes with 2 C.L. yielding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 embryo</td>
<td>1 embryo</td>
</tr>
<tr>
<td>Oestrogenic</td>
<td>34</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>42</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

(g) Embryo Mortality

Estimates of embryo mortality were calculated from the difference between fertilization rate (laparotomy group) and pregnancy rate (slaughter group) as shown below.

\[
\text{% Embryo mortality} = \frac{100 (F-P)}{F}
\]

where

\[
F = \frac{\text{No. ewes yielding fertilized eggs} \times 100}{\text{No. eggs yielding eggs}}
\]

Egg basis

\[
P = \frac{\text{No. ewes with embryos} \times 100}{\text{No. ewes slaughtered}}
\]

IV. DISCUSSION

Using the cervical mucus bioassay Lindsay and Francis (1968) found that Dinninup subterranean clover with isoflavone levels similar to those reported here produced responses almost equivalent to 40 \( \mu g \) stilboestrol. Morley, Bennett and Axelsen (1963) found that daily injections of 32 \( \mu g \) stilboestrol around mating drastically reduced fertility in Merino ewes. It seems reasonable therefore to ascribe the deleterious effects on fertility that were observed here to the phyto-oestrogens present in Dinninup particularly as the use of artificial insemination precluded any confounding effects due to ram fertility.

A reduction in the incidence of oestrus, as suggested by the results of the present experiment, has been noted previously (Engle, Bell & Davis 1957; Coop & Clark 1960) for ewes grazing oestrogenic pastures. Similar effects have resulted from daily injections of stilboestrol (Morley, Bennett & Axelsen 1963).
which initially stimulated but subsequently inhibited oestrous behaviour.

A most striking feature of the results reported here was the reduction in ovulation rate seen in ewes grazing the oestrogenic pasture. Although reductions in ovulation rate or twinning percentage have been a feature of previous studies using stilboestrol (Morley, Bennett & Axelsen 1963) and some studies with lucerne (Coop & Clark 1960) and red clover (Ch'ang 1961; Morley, Axelsen & Bennett 1964, 1966) the effect has not always occurred on oestrogenic pastures (Engle, Bell & Davis 1957; Clark 1965; Holst & Braden 1972). Possibly the depression in ovulation rate depends on the oestrogenicity of the pasture and/or the time of observation relative to commencement of grazing. Obst & Seamark (1970) reported that peripheral plasma progesterone levels fell two to three days earlier in ewes grazing Yarloop subterranean clover and observations on corpus luteum weights presented here support their suggestion that phyto-oestrogens may affect luteal function. It seems likely that both the reduced incidence of oestrus and lowered ovulation rates may be related in this respect.

The finding that egg fertilization rate was significantly depressed in ewes grazing oestrogenic subterranean clover is supported by previous results for both ladino clover (Sanger & Bell 1961) and pelleted red clover (Holst & Braden 1972). It seems likely that fertilization was affected via reduced sperm transport as few of the eggs recovered from the oestrogenic treatment in the present experiment had sperm on the Zona Pelucida. Differences in the rate of ovum transport may also be involved, however, as both the present results and those of Holst & Braden (1972) suggest that phyto-oestrogens may hasten the passage of the egg through the Fallopian tube.

Whether or not oestrogenic grazing induces additional embryonic mortality was not satisfactorily determined from the present study. Certainly the findings indicate that total losses of pregnancy on a ewe basis were higher in the oestrogenic treatment. The results serve to emphasise the problems inherent in detecting differences in embryonic loss between treatments when large differences in ovulation rate also occur.

V. ACKNOWLEDGMENTS

The authors wish to thank Mr. R. Randall, Manager, Badgingarra Research Station and Mr. R.B. Guthrie for assistance during the conduct of the trial, Dr. C.M. Francis for isoflavone analyses and the Australian Wool Board for financial support.

VI. REFERENCES