SYNCHRONIZATION OF OESTRUS IN SHEEP BY INTRAVAGINAL AND SUBCUTANEOUS APPLICATION OF PROGESTIN IMPREGNATED SPONGES

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I. INTRODUCTION

Shelton (1964) has described the testing of a number of progestins in the search for one which meets the specifications laid down by the author (Robinson 1960) as being necessary for the practical control of ovulation in farm animals. He found one compound, namely 17α-acetoxy-9α fluoro-1 1β-hydroxypregn-4-en 3, 20-dione (Searle, Chicago; Code No. SC-9880) which appeared to have all the characteristics of progesterone but which was 25 times more potent. Further, he obtained definite evidence that physiological quantities of some progestins may be absorbed through the vaginal wall, when topically applied.

This paper is a preliminary report of the development of a method of utilizing SC-9880 and vaginal application for the practical control of the time of ovulation and oestrus in the cyclic ewe. Two experiments have been conducted, one in collaboration with Dr. N. W. Moore at the McCaughey Memorial Institute, Jerilderie, N.S.W., in March 1964 and the other with Dr. S. Salamon on a private property at Blanket Flat, via Crookwell, N.S.W., in April.

II. METHODS

(a) Administration of progestin

Two progestins, progesterone and SC-9880, have been used. The principle has been to adsorb a quantity, known to be in excess of normal physiological amounts, on a polyurethane sponge with a string attached (Fig. 1). The sponge is then inserted into the vagina, using a duckbilled speculum, or into a subcutaneous pocket. It is removed after a predetermined interval. Previously both Shelton and the author (unpublished data) had tried a number of methods for inserting progestins into the vagina, using vehicles such as plastic tubes, linen tampons, and the like, but without success. Absorption of hormone was limited and serious infections occurred in some cases.

(b) Preparation of the sponge inserts

The sponges are prepared from polyurethane foam sheet, 2.5 cm in thickness and are cut out with a circular borer of 2.5 or 3.2 cm diameter. The larger sponges are the more satisfactory and a still larger size may be desirable for intravaginal

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Fig. 1.-Polyurethane sponges used for intravaginal or subcutaneous application. Two sizes and two colours (green and yellow) are illustrated. Fewer of the larger sponges were lost from the vagina, and fewer of the yellow than of the green. The latter was probably due to the firmer and finer texture of the yellow sponges, visible in the photograph. The top left hand sponge is impregnated with 800 mg progesterone (clearly visible) and the top right hand sponge with 50 mg SC-9880 (not visible). The lower sponges are not impregnated.
use to prevent loss. Of two different colours used (green and yellow), significantly more green sponges have been lost from the vagina than yellow. At removal the green sponges have been soft and friable whereas the yellow ones have been still firm. Hence the texture of the sponge is important. A 35 cm length of strong linen thread is passed through the sponge and doubled and tied. As the string often pulls through when removing sponges some modification is required.

The hormone is dissolved in ethanol to give the required amount in 5 or 10 ml. In the two tests conducted, 800 and 500 mg progesterone have been used and 50 and 30 mg SC-9880. The latter is readily dissolved in 5 ml ethanol but 10 ml is needed for the progesterone. The solution is pipetted on to the sponges, which are suspended by their linen threads, and the ethanol allowed to evaporate. Each sponge will readily absorb 5 ml. Preparation of the SC-9880 sponges presents no problems; the full quantity can be applied at one time and the fine crystals appear dispersed through the sponges after the ethanol has evaporated. The progesterone sponges are more difficult to prepare. The solution must be applied in two lots and after evaporation a mass of large crystals forms on the exterior of the sponge. These have been scraped off, re-dissolved and re-applied. The final product has the progesterone fairly well dispersed through the sponge.

(c) Insertion of the sponges

Immediately prior to insertion each sponge is liberally coated with “Savlon” ointment (Cetrimide B.P. 0.5 %, Hibitane 0.1% ; I.C.I.) . The vagina is dilated with a duckbill speculum and the sponge inserted into the anterior vagina with a perspex rod. Subcutaneous sponges are sutured into a pocket cut in the flank.

(d) Removal of the sponges

Removal still presents some problems. Some degenerative changes take place in the plastic material while in the vagina so that, although the sponges retain their integrity, the strings often pull out. They can be removed quite simply with a speculum and long forceps. In none of the 142 ewes treated has the vagina been seriously infected on removal of the sponges. Sponges have a characteristic but not offensive odour, and their removal is accompanied by a copious discharge of cloudy mucus. Subcutaneous sponges are expressed after cutting the sutures.

(e) Vaginal picture after removal

Vaginal smears have been taken from 50 ewes at the time of sponge removal. They have been stained and examined in the normal manner used in our laboratory (Robinson and Moore, 1956). In addition, a detailed bacteriological examination has been made of 18 sponges.

(f) Observations

The occurrence of oestrus during and immediately after treatment was recorded, together with data for returns of service up to 30 days later. Oestrus was detected by the use of rams carrying pigmented crayon on the brisket. In addition 18 ewes were examined by laparotomy, at Blanket Flat to check that blocking and release of ovulation had occurred.
III. RESULTS

(a) Vaginal picture after removal

Contrary to expectations, smears generally have not contained large numbers of leucocytes. They have consisted mainly of mucus, with a few desquamated cells, some cellular debris and a few leucocytes.

A large number of bacteria were present in all sponges. In the first experiment (Jerilderie), with one exception, the flora was non specific and no pathogens were detected. In the second experiment (Blanket Flat) *Pseudomonas aeruginosa* was isolated from all 12 sponges. Five showed polymorphs and two appeared to represent severe infections. Two or three days later, at insemination, the vagina of all sheep except one appeared clinically normal. These sheep had recently been shorn and dipped. The dip was the possible source of the *Pseudomonas* infection. Hence incorporation of an antibiotic and a bacteriostat into the sponges may be desirable. However, fertility did not appear to be impaired.

(b) Control of oestrus and ovulation

The results of the Jerilderie experiment are summarised in Table 1. Each progestin administered in sponges, alone or in combination, and inserted subcutaneously or intravaginally, was effective in blocking oestrus and presumably ovulation. The intravaginal route was highly effective. Thirty ewes retained the sponges and, of these, 29 did not exhibit oestrus until 2 or 3 days after removal of sponges. Apparent fertility to natural service was high as indicated by the 73 % of ewes which did not return to service.

<table>
<thead>
<tr>
<th>Site of Sponge</th>
<th>Number of Ewes</th>
<th>Sponges</th>
<th>Blocked Oestrus</th>
<th>Released Oestrus</th>
<th>Non-returns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous §</td>
<td>36</td>
<td>36</td>
<td>31</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Intravaginal §</td>
<td>36</td>
<td>30</td>
<td>29</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>66</td>
<td>60</td>
<td>55</td>
<td>40</td>
</tr>
</tbody>
</table>

‡2-3 days after removal

§Number of ewes not detected in oestrus up to 30 days after natural service

§Data are pooled for 3 types of impregnation, namely with:

(a) 800 mg progesterone
(b) 50 mg SC-9880 (17α-acetoxy-9αfluoro-11βhydroxypreg-4-en-3, 20 dione)
(c) 800 mg progesterone + 50 mg SC-9880

 Twelve of each were inserted both subcutaneously and intravaginally and the response to each type was indistinguishable.
The intravaginal use of polyurethane sponges impregnated with progesterone or SC-9880 for the control of oestrus in the ewe

Summary of Results-Blanket Flat

<table>
<thead>
<tr>
<th>Type of Progestin</th>
<th>Sponges Total</th>
<th>Blocked 2-4 days</th>
<th>Released 5-6 days</th>
<th>Oestrus &gt;6 days</th>
<th>Oestrus Total</th>
<th>Inseminated</th>
<th>Non-returns†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone‡</td>
<td>54</td>
<td>46</td>
<td>25</td>
<td>5</td>
<td>24</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>SC-9880¶</td>
<td>52</td>
<td>51</td>
<td>42</td>
<td>9</td>
<td>0</td>
<td>51</td>
<td>42</td>
</tr>
<tr>
<td>Totals</td>
<td>106</td>
<td>97</td>
<td>97</td>
<td>14</td>
<td>24</td>
<td>105</td>
<td>67</td>
</tr>
</tbody>
</table>

†Number of ewes not detected in oestrus up to 30 days after artificial insemination.
‡Progesterone — 500 mg.
¶SC-9880 — 30 mg.
§Plus 1 anoestrus ewe
||Many “silent heats” during progesterone treatment

The results of the Blanket Flat experiment are summarised in Table 2. The sheep were poorly grown four- and six-tooth ewes (mean liveweight 32.5 kg) which, as judged by the ovaries and reproductive tracts of the 18 animals examined by laparotomy, had never borne a lamb. The doses of progesterone and SC-9880 were lower than those used at Jerilderie (Tables 1 and 2). Progesterone was less effective than SC-9880. Oestrus was blocked in all 51 four- and six-tooth ewes which retained their intravaginal SC-9880 sponges, and ovulation was blocked in each of the 18 ewes examined by laparotomy. Only 1 ewe lost her sponge. The release of oestrus in these ewes was less precise than in the older more mature animals at Jerilderie (Fig. 2).

These ewes were artificially inseminated using 0.15 ml undiluted semen containing not less than 150 $\times 10^6$ live sperm. Of 67 inseminated, 54 (81%) had not returned to the ram 30 days after insemination.

IV. CONCLUSIONS

The use of intravaginal sponges impregnated with a progestin, and particularly with a suitable 9α-fluoro compound such as SC-9880, offers a simple effective method of controlling ovulation and oestrus in the cyclic sheep, and possibly in other farm animals. Oestrus and ovulation are completely blocked and are effectively released in mature animals 2 or 3 days after removal. In immature animals a wider time interval may be expected. Limited data suggest that normal fertility following natural service or artificial insemination may be expected. Subcutaneous sponges are also effective but not practical.
V. ACKNOWLEDGMENTS

The author wishes to thank Mr. R. V. S. Bain for making the bacteriological examination, Mr. H. Sinclair for locating and preparing suitable types of foam sponge and Dr. S. Salamon for artificially inseminating ewes.

This work was supported by the Australian Sheep and Wool Research Committee, aided by the facilities of the McCaughey Memorial Institute and of Mr. H. Zouch, Blanket Flat, N.S.W.

VI. REFERENCES

