HORMONAL SYNCHRONIZATION OF OESTRUS IN ROMNEY EWES DURING THE BREEDING SEASON

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SUMMARY

The experimental flock comprised 24 balanced single-sire mating groups each of 27 mature Romney ewes. Representative samples of 7 to 9 ewes from each group were treated for 13 days with intravaginal sponges containing 60 mg 6α-methyl-17α-acetoxyprogesterone (MAP). Nine of the groups were mated with ram hoggets, the remaining 15 with two-tooth rams (18 to 20 months).

Among the older ram groups, 89% of the treated animals were marked over a 4-day period following sponge withdrawal, and 64% of these ewes lambed to matings at this time. Corresponding mean figures were 59% and 44% for the ram hoggets, which exhibited much greater variability between groups.

OESTRUS synchronization in sheep during the breeding season has possible applications in the promotion of a concentrated lambing, in the hormonal induction of superovulation, and in the simplification of flock management for large-scale artificial insemination programmes (Roberts, 1966).

Under free-grazing conditions, early lamb growth can be markedly affected by lambing date in relation to seasonal pasture production. Any technique which reduces environmental differences between animals, *e.g.*, the concentration of lambing, should increase the accuracy of assessment of genetic merit based on individual or progeny test performance. The object of the present trial was to study a method of synchronizing ovarian activity which might enable more precise comparisons of half-sib progeny groups in growth and development studies of export lamb production.

Lamond (1964) has given a detailed review of recent improvements in methods of synchronization that have been tried with sheep. The basic difficulty in the application of these methods has been the need for frequent administration of the hormones. Single injections of a progestagen alone have not given satisfactory synchronization. This

probably reflects variation between animals in endogenous progesterone levels and in the rate of absorption from the site of injection, with a consequent variation in the onset of oestrus and ovulation.

The intravaginal method of applying progestagens in polyurethane sponges (Robinson, 1964) offers a simple technique which is associated with relatively little handling of the treated animals. Robinson's trials demonstrated the effectiveness of the technique in cyclic Merino ewes, while the experiments of Roberts (1966) indicated the adequacy of a 13-day insertion period for mixed-age Merinos treated with 60 or 80 mg of MAP in early autumn.

The success of these experiments formed the background to the present study in Romney ewes during the breeding season.

EXPERIMENTAL

SHEEP

The ewes came from an experimental flock of bought-in 5½-year-old Romneys being run at the Ruakura Agricultural Research Centre (Hamilton) for progeny test comparisons of export lamb sires. Twenty-four rams which had given a satisfactory semen sample by electro-ejaculation within 3½ weeks of the commencement of mating were run with the ewes in single-sire mating groups. They were of six different breeds, 15 being 18 to 20 months of age, the remaining nine being Southdown ram hoggets aged 6 to 7 months at the beginning of mating.

TREATMENT AND MATING MANAGEMENT

Prior to mating, the ewes were randomized on a restricted liveweight basis into twenty-four groups of comparable mean liveweight, each comprising 27 or 28 ewes. From each of these groups, between seven and nine ewes were selected at random for treatment, again on a restricted liveweight basis. On March 11, 1965, these ewes each received a 2 in. long, by ¾ in. diameter, polyurethane sponge impregnated with 60 mg of 6α-methyl-17α-acetoxyprogesterone (MAP)*. The sponges were inserted into the anterior end of the vagina using a duck-billed speculum and plunger. They were removed on March 24 after a 13-day insertion period.

One ram was allocated at random to each group of ewes and the rams joined a total of 650 ewes on March 18, 7 days

* Repromap. Upjohn Pty. Ltd.

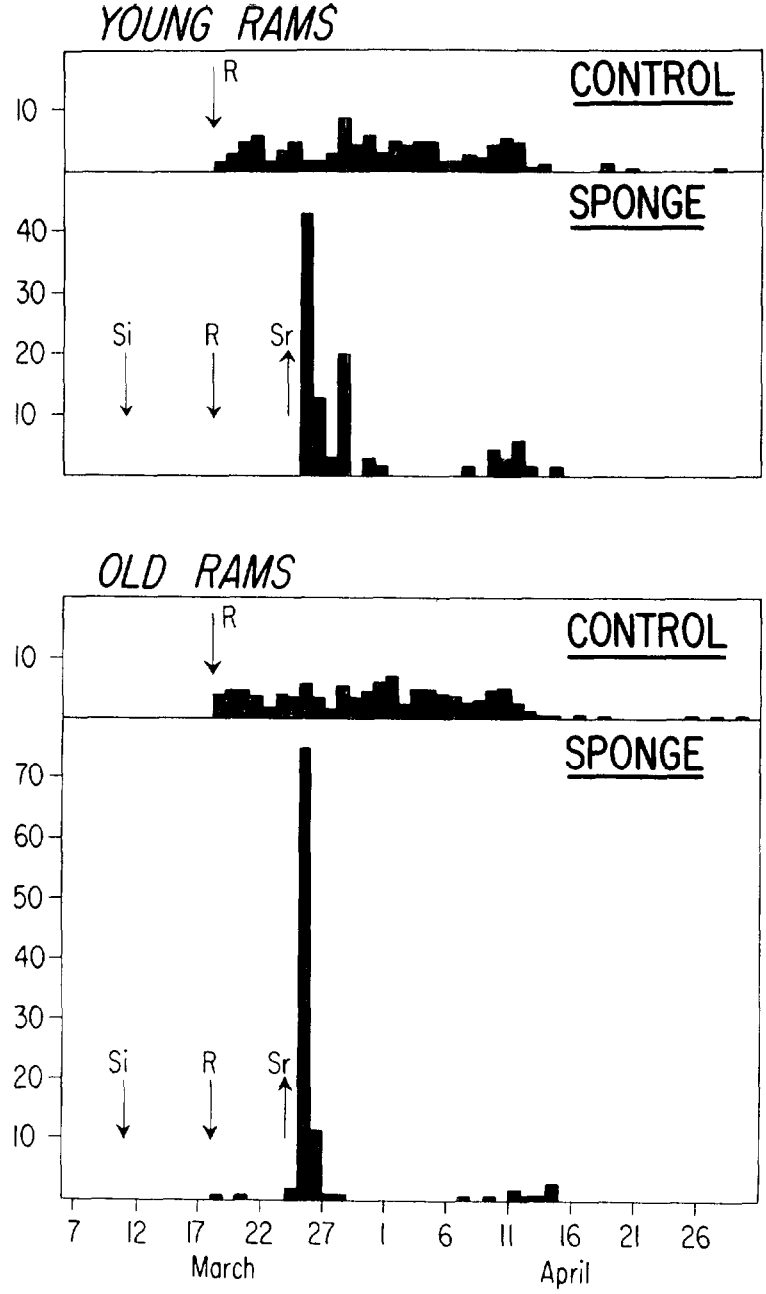

Fig. 1: Distribution of first marking — percentage of ewes marked per day (Si = sponges inserted; Sr = sponges removed; R = rams joined).

after the time of sponge insertion. Each remained with his group of ewes for a 9-week mating period, throughout which all rams were fitted with a crayon harness, the colour of the crayons being changed every fortnight. The ewes were individually identified, and those marked by the rams were recorded daily. Complete lambing records were obtained at parturition.

The mating groups were rotationally grazed over the experimental area with daily shifts to their next paddock. They were combined and managed as a single mob between the end of mating and the beginning of lambing.

Sponge Removal

During the treatment period, two of the 188 treated ewes lost their sponges and have been excluded from the analyses presented below.

On removal, two sponges were found to be slightly, and one other quite heavily, blood-stained, while a fourth seemed to be adhered to the wall of the vagina. These four ewes all subsequently conceived after exhibiting oestrous periods comparable with the remaining treated ewes. As found by Robinson (1964), the removal of the sponges was frequently accompanied by a discharge of cloudy mucus from the vagina.

RESULTS AND DISCUSSION

Date of First Marking

Figure 1 presents the daily distribution of first markings for the treated (sponge) and untreated (control) ewes, combined separately for the nine ram hogget (young rams) and the 15 two-tooth ram (old rams) mating groups. These histograms reflect the oestrous pattern of the animals only to the extent that the accidental or rape marking of ewes was not important, and to the extent that all ewes in oestrus were marked. It should be emphasized that each ewe had the opportunity of mating with only one ram, although the ewes were allocated to the various rams at random.

Two treated ewes were marked by a ram during the last 6 days of the treatment period. However, neither conceived at this time, and their returns to the ram 22 and 24 days later were in line with the majority of the treated animals. One ewe conceived at this second mating, while the other held to a service 17 days later. Thus, the suppression of

oestrus was good, during the period of treatment after the rams were joined. However, the results of Southcott *et al.* (1962), who used the oral route of administering MAP to ewes during the breeding season, indicate that failure of suppression is more likely to occur in ewes due to exhibit oestrus within 4 days of the start of treatment, than in those at a more advanced stage of their oestrous cycle.

Whether ovarian activity was sufficiently suppressed in the treated ewes of this trial must be inferred from the degree of synchronization achieved. Studies with progesterone have indicated that suppression of oestrus is not necessarily a reliable criterion for the effectiveness of ovarian suppression in relation to subsequent synchronization and fertility (Lamond, 1964). Lamond considers that the occurrence of oestrus in 80 to 90% of the ewes within a period of 36 to 48 hours is indicative of satisfactory suppression.

For the two-tooth rams, 86% of the treated ewes were marked within a 48-hour period during the second and third days after sponge removal, indicating a satisfactory level of synchronization. The degree of synchronization in the ram hogget mating groups is on average less satisfactory. This is indicated by a greater proportion of animals being marked 15 to 22 days after sponge removal and by a broader peak of marking activity within 8 days of sponge removal. These features are summarized in Table 1 which shows the proportion of first markings recorded for the treated ewes during the first and second four-day periods, and for all ewes during the 8-day period after the cessation of treatment.

TABLE 1: PROPORTION OF FIRST MARKINGS IN THE 8-DAY PERIOD FOLLOWING SPONGE REMOVAL

Treatment	*Sponge*			*Control*
	1–4 days	*5–8 days*	*1–8 days*	*1–8 days*
Young rams	41/70 58.6%	17/70 24.3%	58/70 82.9%	56/168 33.3%
Old rams	103/116 88.8%	1/116 0.9%	104/116 89.7%	93/289 32.2%
Difference (Young vs. old rams)	**	**	n.s.	n.s.

**: $p<.01$; ns: $p>.05$.

The figures of 59% and 89% for the proportion of first markings recorded for the treated ewes within 4 days of sponge removal in the young and old ram mating groups, respectively, are both lower than Roberts' (1966) figure of 96% of ewes mated within 4 days of sponge removal for Merinos treated with 60 mg MAP for 13 days. However, Robinson (1964) found that 82% of young ewes treated with a different progestagen, and 54% of similar ewes treated with 500 mg progesterone, exhibited oestrus within 4 days of a 17-day insertion period.

For both age groups of rams, the proportion of treated ewes marked in the 8 days following sponge removal was significantly ($p<.01$) greater than for the untreated controls over the same period. However, as indicated in Table 1, a significantly lower proportion of the treated ewes were marked by the ram hoggets than by the two-tooth rams in the first 4-day period, and a significantly higher proportion in the second 4-day period. For the entire 8-day period, the overall differences between the two age groups of rams were not significantly different for either the treated or the control ewes. A noteworthy feature of the results was the large variation among individual ram hoggets in the proportion of treated ewes marked in both the first and second 4-day periods. Analysis revealed significant ($p<.01$) heterogeneity in these proportions, whereas much less variation was observed among the older rams, and in the proportion of untreated ewes marked over the 1–8 day period by both the young and old rams.

In this trial, a total of 83% and 90% of the first markings to treated ewes by the young and old rams, respectively, were recorded within 8 days of sponge removal. The majority of the remaining first markings were recorded after an interval corresponding to one ovarian cycle (*i.e.*, 15 to 22 days after treatment), although this interval tended to be shorter for the treated ewes mating young rams (15.1 days) than for those mating old rams (17.5 days).

Robinson (1960) has interpreted a 17-day delay in the onset of first post-treatment marking as evidence of inadequate ovarian suppression in these animals. He suggests that ewes first marked at this time may have ovulated without oestrus within 8 days of the cessation of treatment. If this is true, it could offer a clue to explaining the mating group differences observed for the treated ewes of the present experiment, since it would seem reasonable to expect an absence of oestrus in some of the ewes to be associated with an altered intensity or length of oestrus in

other treated ewes. However, differences in the mating behaviour of young ram hoggets would also need to be invoked to fully account for the present results. Reduced marking activity on the part of some of the younger rams may indeed be the sole explanation. Nevertheless, it is possible that ram hoggets may have on average differed from the older rams through their influence on the response of the ewes to the treatment. Perhaps relevant in this respect is the finding in some instances by Edgar and Bilkey (1963) of apparently differential responses by ewes, in terms of the onset of the breeding season, to the presence of entire in comparison with vasectomized rams.

Whatever the explanation, it is apparent that the technique has resulted in a sub-optimal synchronization of marking activity for ewes mated to some of the younger rams. Five of the nine ram hogget mating groups had less than 70% ewes marked for the first time within 4 days of treatment, while only one of the two-tooth ram groups fell in this category.

Return Intervals to Second Marking

The mean intervals between first and second marking for ewes not lambing to first marking, together with the corresponding standard deviations are shown in Table 2.

Table 2: Interval Between First and Second Marking (Days)

Treatment	*Sponge*		*Control*	
	Mean	*Stand. Devn.*	*Mean*	*Stand. Devn.*
Young rams	15.5	1.74	16.8	1.32
Old rams	16.7	1.40	17.2	1.15

Analysis revealed significant ($p<.05$) heterogeneity of the variances among the four categories of Table 2. The high variability among treated ewes mating young rams stemmed largely from the shorter but more variable return intervals of ewes first marked in the 5 to 8 day period after treatment (mean 14.0 days), as compared with ewes first marked in the 1 to 4 day interval (mean 16.4 days). The corresponding standard deviations (1.66 and 1.01) differed significantly ($p<.05$).

The shorter return interval for the treated ewes first marked 5 to 8 days after sponge removal suggests that these ewes may have ovulated 2 to 3 days earlier than the time

suggested by their observed markings. If true, this would mean that they were comparable with the majority of the treated animals in terms of their ovulatory activity.

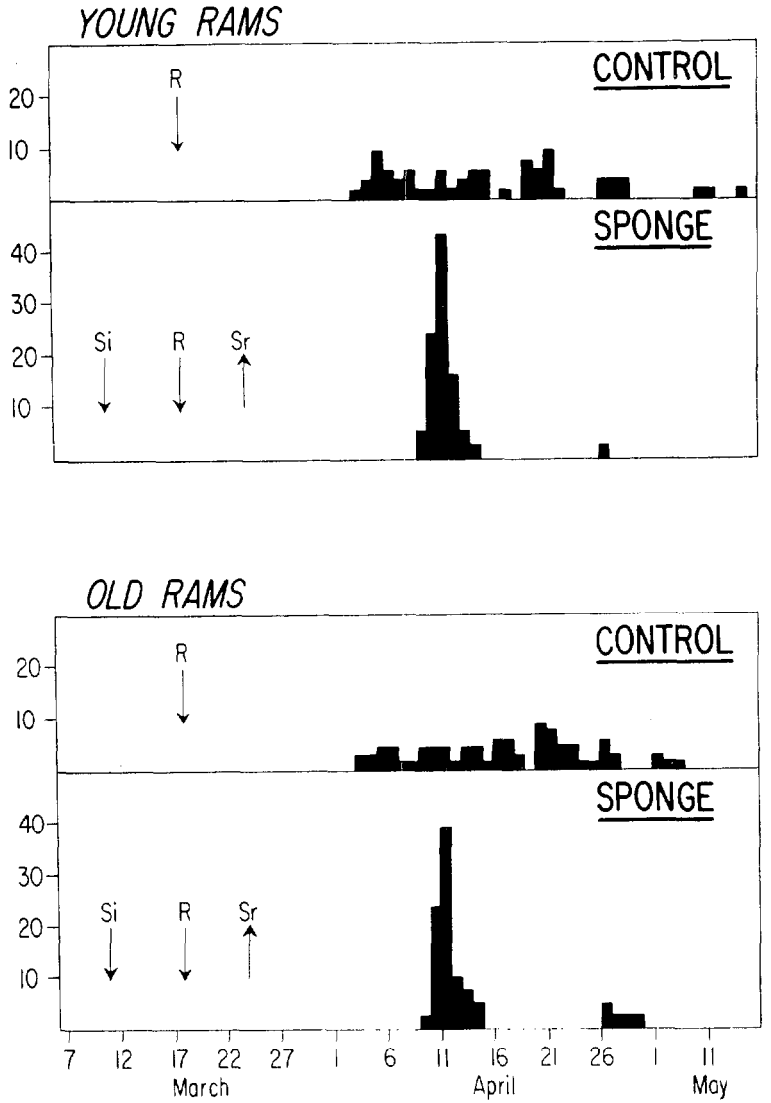

Fig. 2: Distribution of second marking — percentage of ewes marked per day (Si = sponges inserted; Sr = sponges removed; R = rams joined).

Date of Second Marking

The distribution of second post-treatment marking shown in Fig. 2 is similar for both young and old ram mating groups with 97% and 87%, respectively, of the treated ewes marked a second time being mated over a 6-day period from April 10 to 15, and with a peak of second markings on April 12. The remaining second markings occurred approximately 16 days later.

The peak of second marking on April 12 occurred after an interval of 17 days from the day on which the peak of first marking was recorded. It thus corresponds with the first markings recorded 15 to 22 days after sponge removal.

Ewes Lambing to First Marking

The number of ewes lambing to first marking as a percentage of all ewes marked is shown in Table 3. Ewes not returning to service, but failing to lamb as a result of death or presumed abortion, have been excluded from this table and subsequent analyses.

Among the older ram mating groups, the proportion of treated ewes lambing to first marking was less, but not significantly so, than for untreated ewes. On the other hand, the overall lambing rate to first marking of the treated ewes mated to ram hoggets was significantly ($p<.01$) less than for their untreated counterparts. The same conclusions apply in respect of the lambing rate to first markings recorded in the period 1 to 8 days after sponge removal.

As indicated in Table 3, the lambing rate to first marking was on average significantly lower for the treated ewes running with the ram hoggets than for those with the older rams. The lambing rate for the treated ewes mating the younger rams was particularly poor for those marked over the 5 to 8 day period after sponge removal. This is in line with the disturbed oestrus-ovulation relationships suggested by the return intervals of these animals. For the untreated ewes, the lambing rates to first marking were somewhat higher for the older ram groups than for the ram hogget groups but in this case the differences were not significant.

Variation amongst individual rams was again strikingly manifest in the case of treated ewes mated to the ram hoggets. Within this category, the proportion of ewes lambing to first marking ranged from zero to 100%. Although significant differences between rams were also established for the untreated ewes, the variance between mating

TABLE 3: PROPORTION OF EWES LAMBING TO FIRST MARKING

Treatment	*Sponge*				*Control*			
	1–4 Days	*5–8 Days*	*1–8 Days*	*Total*	*1–4 Days*	*5–8 Days*	*1–8 Days*	*Total*
Young rams	17/39 43.6%	3/17 17.6%	20/56 35.7%	30/67 44.8%	12/19 63.2%	25/35 71.4%	37/54 68.5%	106/162 65.4%
Old rams	65/101 64.4%	1/1 100.0%	66/102 64.7%	71/114 62.3%	26/40 65.0%	39/51 76.5%	65/91 71.4%	200/274 73.0%
Difference (Young vs. old rams)	*	n.s.	**	*	n.s.	n.s.	n.s.	n.s.

*: $p<.05$; **: $p<.01$; n.s.: $p>.05$.

groups in the proportion of ewes lambing to first marking was significantly greater ($p<.05$) for treated ewes mated to the younger rams than for the other three categories.

In the case of the control ewes, the lower lambing rates to first service during the four days following removal of sponges from the treated ewes may be due to heavy

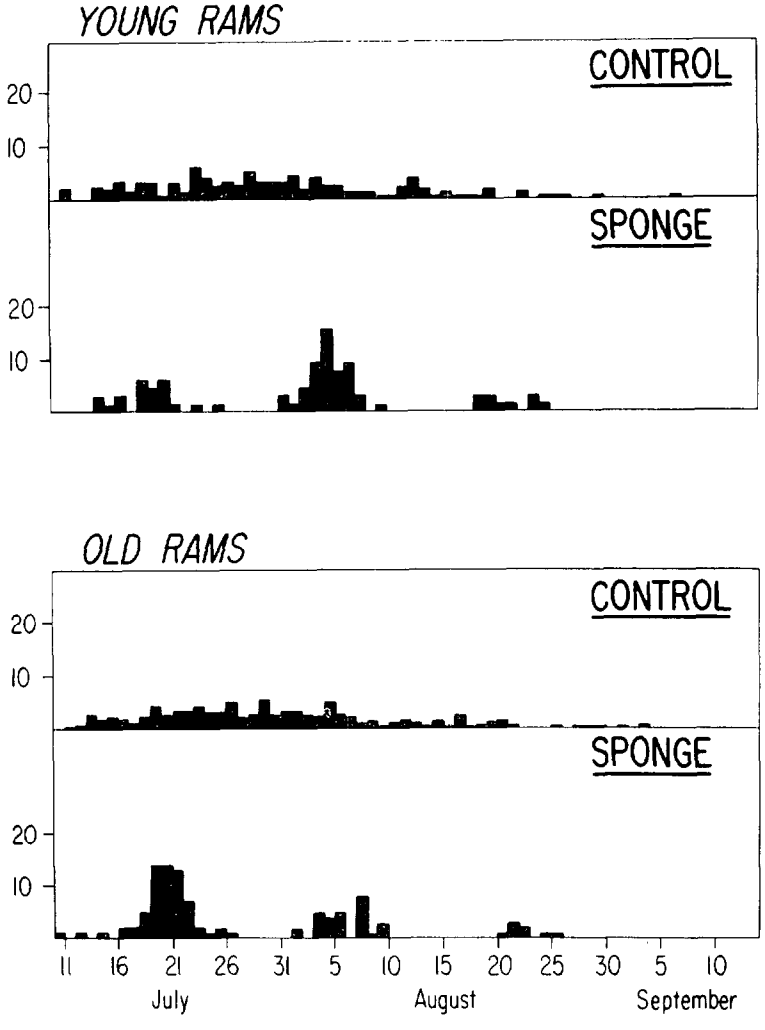

Fig. 3: Distribution of ewes lambing — percentage of ewes lambing per day.

demands made on the rams at this time. The reduction was greater for the older ram groups, in which on one day (day 2) an average of seven ewes were marked by each ram, than for the young ram groups where the highest daily marking average was four ewes per ram. It may be noted that, over this four-day period, the same proportion (65%) of treated as of untreated ewes held to service by the older rams.

Thus, with respect to the success of the treatment technique in producing a concentrated lambing period, differences in the effective fertility of treated and control ewes have tended to aggravate the poor synchronization of marking activity achieved in some of the ram hogget groups, and to nullify the effectiveness of a satisfactory synchronization in other young ram groups. Only two of the nine ram hogget mating groups had more than 50% of the treated ewes lambing to matings within four days of sponge removal, whereas a significantly greater ($p<.05$) proportion — 11 out of 15 — of the two-tooth ram groups fell in this category. No completely satisfactory explanation can be put forward to account for this difference, although it seems likely that disturbed oestrus-ovulation relationships and their interaction with mating behaviour, semen, or sexual response differences are involved. Lamond (1964) has suggested that the presence of the male and/or coitus may act to overcome a physiological state inhibiting fertility which is brought about by hormonal methods of synchronization, and rams may well differ in this connection.

The net result of the effects of both marking patterns and fertility differences were lambing distributions that were quite different for the treated ewes in the young and old ram groups. These are presented in Fig. 3 as the number of ewes lambing on each day as a percentage of the total number of ewes lambing in the four age of ram-treatment groups. The peak of lambing for the treated ewes mating young rams occurred after an interval corresponding to one ovarian cycle later than the peak for the treated animals in the older ram groups.

Ewes Lambing to Second Marking

In contrast to the lambing rate recorded at first post-treatment marking, there appeared to be a higher proportion of treated ewes lambing to their second marking in comparison with the control ewes. There were, however, no significant differences between the means recorded in Table 4.

TABLE 4: PROPORTION OF EWES LAMBING TO SECOND MARKING

Treatment	*Sponge*	*Control*
Young rams	27/37 73.0%	35/53 66.0%
Old rams	27/41 65.9%	40/68 58.8%
Difference (Young vs. old rams)	n.s.	n.s.

n.s.: $p > .05$.

The tendency for a satisfactory lambing rate to be obtained from treated ewes at their second rather than their first post-treatment oestrus is characteristic of a number of studies on methods of hormonal synchronization, and favours the practice of delaying mating until after the first post-treatment oestrus has occurred. On an applied basis, this would have been achieved in the present trial by delaying the joining of the rams until the ninth day following sponge withdrawal, providing, of course, that the earlier introduction of the rams was not important in determining the distribution of first post-treatment oestrus for these animals.

CONCLUSIONS

Robinson (1960) listed as one of the major problems in the field of controlled sheep reproduction the need for a technique of synchronization of ovarian activity that was simple to apply but which would be accompanied by a level of fertility comparable to that obtainable naturally. Intravaginal sponges would seem to offer great possibilities in this direction, although the present trial indicates that the level of fertility may not be adequate in certain circumstances associated with the use of ram hoggets in single-sire mating groups. Just what these circumstances are in physiological terms cannot be ascertained from this investigation, for the experimental design and mating programme adopted were not sufficiently critical to allow even a crude investigation of the underlying mechanisms. As Lamond (1964) has stressed, this limitation is a feature of a large number of investigations upon which the present knowledge of the mechanics of synchronization is based, and there is obviously a real need for experiments aimed specifically at understanding the physiology of ovarian suppression and release by progestagens.

It is at least known that the subject is one of considerable

complexity, depending upon such factors as type of progestagen, dose, frequency and method of administration, duration of treatment, breed, location, season, nutrition and social factors, time of day and lactation. In view of this array, it has been suggested that a single dosing system is unlikely to give optimum results for all animals treated. However, it is only when there exists a better understanding of the important physiological pathways which govern the animals' responses that results will be able to be specified with a satisfactory degree of assurance.

With respect to the aim of obtaining a concentrated lambing distribution, any treatment technique applicable to the ewes will be limited by the proportion of ewes that will conceive and lamb to a single oestrous period, at least to the extent that this is governed by the male. As a result, some type of overmating technique becomes necessary to allow the exclusion from the flock of those ewes failing to conceive over a short mating period. In the present trial, an average of 36% of the treated ewes mated to two-tooth rams failed to lamb to matings within 4 days of sponge removal. However, the degree of overmating necessary will vary with the fertility of the rams used, and may need to be more pronounced in some mating groups than in others. The efficiency of the method is further reduced by between animal variation in gestation length. This is indicated by a 13-day spread in lambing date for the treated ewes lambing to matings by old rams in the first 4 days following treatment, although 75% of these ewes lambed over a 4-day period.

A further limitation to synchronized lambing could be the ability of the ram or rams to successfully cover a large number of ewes over a 3 to 4-day period. This aspect is likely to be more important the greater the number of ewes to be mated by any one ram.

ACKNOWLEDGEMENTS

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REFERENCES

Edgar, D. G.; Bilkey, D. A., 1963: *Proc. N.Z. Soc. Anim. Prod., 23*: 79.

Lamond, D. R., 1964: *Anim. Breeding Abstr., 32*: 269.

Roberts, E. M., 1966: *Proc. Aust. Soc. Anim. Prod., 6* (In press).
Robinson, T. J., 1960: *Proc. N.Z. Soc. Anim. Prod., 20*: 42.
——— 1964: *Proc. Aust. Soc. Anim. Prod., 5*: 47.
Southcott, W. H.; Braden, A. W. H.; Moule, G. R., 1962: *Aust. J. agric. Res., 13*: 901.

DISCUSSION

PROFESSOR M. C. LANCASTER: *What was the previous history of the ewes? How prolonged was the lambing period?*

J. N. CLARKE: The previous reproductive performance of the ewes, all 5½ years of age, is not knkown.

Lambing was spread over about 8 weeks for the untreated animals and about 7 weeks for the treated animals.

DR D. G. EDGAR: *The ram hoggets appear to have marked some ewes later than expected and perhaps even after the ewes were in oestrus. This seems to be supported by the shorter period to second oestrus, and by the low conception rate at first marking of the late marked ewes in the ram hogget groups.*

MR CLARKE: This would appear to be the case, and it would be interesting to know whether these late marked ewes were really in oestrus at this stage.