# Fertility in Ewes Following Controlled Breeding Techniques

By D. R. LAMOND*

## SUMMARY

Groups of Merino ewes were given daily intramuscular injections of 10 mg of progesterone for 12-14 days. In one experiment conducted during the breeding season, the ewes received pregnant mares' serum gonadotrophin (PMS) on the day of the final progesterone injection and human chorionic gonadotrophin (HCG) 48 hours later, then were inseminated with undiluted semen on the day of the HCG injection. There was a highly significant difference in fertility between two farms. Dosage levels of PMS did not influence results but a significant interaction was observed between farms and dosage of HCG.

In a second experiment carried out during the period June to December, which includes the anoestrus period, ewes received PMS on the day of the final progesterone injection and some were placed with rams, while others were given HCG 48 hours later and artificially inseminated. Fertility during June and December was significantly greater than during the intervening months. In addition while fertility following natural service was normal, that following HCG and artificial insemination was extremely low.

## INTRODUCTION

Braden, Lamond and Radford (1960) developed a technique for synchronization of ovulation in groups of ewes which is a modification of the progesterone-pregnant mares' serum (PMS) method used by Robinson (1956). Before the technique can be recommended for field use, the variability in response due to breeds, time of year and environmental factors such as flock management and nutrition should be investigated.

This report describes experiments designed to give quantitative information on some of the above factors.

## MATERIALS AND METHODS

Experiment 1.

Forty Merino ewes were selected at random on each of two New England farms. Each ewe received a daily intramuscular injection of 10 mg. progesterone in oil (donated by Boots Pure Drug Company) for 14 days. On the day of the final progesterone injection (Day O) ewes were allotted at random to four groups of ten animals each. Groups 1 and 2 were injected subcutaneously with 200 IU of purified PMS (Gestyl — Organon Labs. Ltd.) and groups 3 and 4 with 600 IU of PMS. Approximately 48 hours later the ewes in groups 1 and 3 received 300 IU and those in groups 2 and 4 received 900 IU of human chorionic gonadotrophin, HCG (Pregnyl — Organon Labs. Ltd.), intramuscularly. All ewes were artificially inseminated on the day of the HCG injection.

The design of this experiment was therefore a $2 \times 2^2$ factorial, the three factors being:

(a) farms;

(b) dose of PMS;

(c) dose of HCG.

* Faculty of Rural Science, University of New England, Armidale, N.S.W.

**Experiment 2.**

Commencing in June, 1959, and continuing at approximately five week intervals until December, 1959, groups of ten Merino ewes were given a daily injection of 10 mg. progesterone in oil for 12-14 days. On the day of the final progesterone injection 500 IU of PMS were injected. On Day 2 (2 days after the final progesterone injection ) five sheep were placed with rams and the remainder injected with 500 IU of HCG intravenously and then artificially inseminated.

The factors varied in this experiment were thus time of year, of which there were six treatments, and method of insemination, of which there were two. The essential differences in the methods of insemination were that the ewes run with the rams were naturally inseminated at oestrus and ovulated spontaneously whereas the others were ovulated at a predetermined time and inseminated approximately 20 hours before the expected time of ovulation.

**Examination for pregnancy.**

Pregnancy was determined in the ewes in Experiment 1 from mammary gland palpation and confirmed by lambing results.

Fertility in Experiment 2 was determined from pregnancy examination by exploratory laparotomy at 6-8 weeks and confirmed by lambing results. An incision about 1 in. long is made 1 in. to the side of the midline and about 1 in. anterior to the mammary gland. One finger is inserted and the small uterus of non-pregnant ewes is easily identified. If it is thought. the ewe is pregnant, the incision is enlarged to enable two fingers to be inserted into the abdomen and the contour and contents of the uterus palpated. In later pregnancy cotyledons are readily palpated. A single mattress suture closes the wound.

## RESULTS

**Experiment 1.**

The experiment commenced on farm A on 2/4/59. During the period of the experiment the ewes were on pasture and were run into yards for injections. On Day 2 (17/4/59) HCG was given at 10 a.m. All ewes were inseminated between 11 a.m. and 1 p.m. and again between 3 p.m. and 4 p.m. Conditions in the shed were not ideal and some delay was experienced in inseminating the first few sheep in the morning. Rather than inseminate these ewes again, it was decided to carry out a second series of insemination in all ewes. Ewes were not inseminated twice with semen from the same ram. Semen was collected from three rams (two Cheviot and one Merino) using an electro-ejaculator and semen quality determined by colour, volume, density and motility. In so far as could be judged all collections were of satisfactory quality. 0.1-0.2 ml. of undiluted semen were placed in and around the vaginal opening of the cervix. Semen was used within 30 minutes of collection. The ewes were kept free from rams for five weeks after insemination, and were examined for pregnancy two weeks prior to the expected date of lambing. The date of birth of each lamb was recorded.

It was not possible to commence progesterone injections on farm B until 12/5/59. These ewes were brought in from pasture two weeks prior to the commencement of the experiment and they remained in the yards until after insemination. The ration was a standard maintenance diet of lucerne chaff, grain and supplements, normally used on C.S.I.R.O. field Stations for similar ewes. On Day 2 (25/5/59) the ewes were given HCG in the morning and inseminated once only in mid-afternoon. Semen of good quality was obtained from four Merino rams and semen from pairs of rams was mixed prior to insemination. Mixing is reported to have no effect on fertility (Dun 1959). Rams were not run with the ewes at any time from insemination to lambing, which took place in yards.

The number of ewes which conceived and lambed is shown in Table I. Only one ewe had twins as indicated in the Table. These data were converted to angles and an analysis of variance, a summary of which is appended to the Table, was carried out. There was a highly significant difference in fertility between the two farms. In addition there was the possibility of an interaction between the dosage level of HCG and farms.

TABLE I.

**Lambing Results in Experiment 1.**

10 ewes per group; all ewes received daily I/M injection of 10 mg progesterone in oil for 14 days; PMS given Day 0; HCG and insemination Day 2.

| FMS (IU) : | 200 | | 600 | |
|---|---|---|---|---|
| HCG (IU) : | 300 | 900 | 300 | 900 |
| Group : | 1 | 2 | 3 | 4 |
| Farm | No. of Ewes Lambing | | | |
| A | 3 | 4 | 4 (5)* | 5 |
| B | 3 | 0 | 2 | 1 |

* One ewe had twins.

**Analysis of Variance**

| Source of Variation | DF | Mean Square |
|---|---|---|
| Between Farms | 1 | 768** |
| PMS | 1 | 70 |
| HCG | 1 | 110 |
| Interaction Farms x HCG | 1 | 354 * |
| Remainder | 3 | 52 |
| Error Variance | 60 | 82.1 |

* $0.01 < P < 0.05$. ** $0.001 < P < 0.01$.

The gestation lengths on each farm were as follows:

| | Number of ewes | Mean (days) | Standard deviation |
|---|---|---|---|
| Farm A . . . . . | 16 | 151.8 | 3.5 |
| Farm B* . . . . . | 34 | 149.0 | 1.8 |

* Includes Merinos in another experiment carried out concurrently and inseminated over the period 24-29th May.

The difference between the means is highly significant ($t = 3.08$, $P < 0.01$) and is probably related to the crossbreeding which took place in Farm A.

Ten extra sheep were available on farm B and these were given progesterone and 600 IU of PMS but were injected with HCG at 4 p.m. on Day 2 and inseminated on the morning of Day 3. Four of these ewes lambed.

**Experiment 2.**

The dates of the final progesterone injections for each group of ewes is shown in Table II. The ewes used over the six month period were all from the same property but were of mixed ages. The first forty were aged sheep, all of which had had at least one lamb, whilst the next twenty were maiden ewes. The ewes were generally in good condition and were brought into yards and fed lucerne chaff during the 2½ weeks of injections.

TABLE II.

**Results of Experiment 2.**

All ewes received daily I/M injection of 10 mg progesterone in oil for 12-14 days; 500 IU of PMS was given S/C on Day 0.

| Date of Final Progesterone (Day 0) | 500 IU of HCG I/V and Artificial Insemination (Day 2) | | Natural Service (Days 2 and 3) | | |
|---|---|---|---|---|---|
| | Pregnant | Non-Pregnant | Pregnant | Non-Pregnant | Not Served |
| 8/6/59 | 1 | 4 | 5 | 0 | 0 |
| 13/7/59 | 0 | 5 | 3 | 1 | 1 |
| 18/8/59 | 0 | 5 | 2 | 2 | 1 |
| 22/9/59 | 0 | 4 | 3 | 1 | 1 |
| 1/11/59 | 0 | 5 | 2 | 2 | 1 |
| 13/12/59* | . | 4 | 4 | 0 | 1 |
| Total | 2 | 27 | 19 | 6 | 5 |

* 1000 IU of HCG I/M.

**Analysis of Variance**

| Source of Variation | DF | Mean Square |
|---|---|---|
| Times | | |
| Quadratic | 1 | 2446*** |
| Remainder | 4 | 101 |
| Treatments | 1 | 9453*** |
| Interaction | 5 | 38 |
| Error Variance | ∞ | 164.1 |

*** P < 0.001.

Two raddled rams were placed with five ewes on the morning of Day 2. It was not possible to collect from these rams for the insemination of the other five ewes later in the day, hence semen of good quality was obtained from two additional rams and mixed prior to insemination. Each ewe received at least 0.2 ml. of the mixed semen.

During October-November results of an experiment became available which seemed to confirm that there is less ovulating hormone produced by ewes in anoestrus than during the breeding season (Lamond, 1960a). The doses of HCG used in our experiment were relatively large and high doses of HCG inhibit ovulation in the mouse (Lamond, 1960b) and decrease fertility in the cow (Lamond, unpublished). The December group of ewes was therefore given 1000 IU of HCG intramuscularly on the morning of Day 2; insemination took place 8 hours later. To give comparable results to intravenous injection, in the rabbit, at least, a 3-4 times increase in dose of HCG is required if given intramuscularly (Braden, personal communication).

In the ewes placed with the rams, oestrus generally occurred in the period 48-72 hours after the final progesterone injection.

The results of this experiment are shown in Table II. A summary of the analysis of variance after conversion of the data to angles is appended to the Table. Fertility following artificial insemination was practically negligible whereas that following natural service compared favourably to what is normally expected in the field (Sinclair 1957). Of the ewes served 75% lambed. The number of ewes which lambed as a proportion of those served was significantly greater during June and December.

## DISCUSSION

The significant difference in fertility between the two farms in Experiment 1 may be due to one of the following factors:

(a) On farm A the ewes received two inseminations at an approximate three-hour interval. It is difficult to see how the double insemination could have influenced results. Ovulation is unlikely to occur less than 48 hours after a final injection of 10 mg of progesterone even when PMS is given (Braden, Lamond and Radford, 1960). However, sperm transport in ewes in which ovulation is hormonally induced may be impaired. One insemination may bring about a response in the tract which, in some way, improves transport of a second batch of sperm.

(b) Little is known of breed differences in fertilising capacity of sperm. On Farm A Cheviot semen was used but no differences were observed between their fertility and that of the Merino,

(c) Little is known of the differences in hormonal levels in ewes throughout the year. It seems likely that there are differences in endogenous production of gonadotrophic hormone (Nalbandov, 1958; Lamond, 1960a) and changes in sensitivity of the tract to oestrogen (Raeside and McDonald, 1959; Robinson and Reardon, 1960) and possibly progesterone. The significant interaction between farms and HCG may give a clue. If, due to effect of external factors such as light, temperature, nutrition and management, and internal factors such as strain of sheep, there are marked differences in ovulating hormone production, it is only necessary to assume that endogenous levels of ovulating hormone in the sheep on Farm B were fairly high and that additional HCG delayed ovulation. The fact that four of 10 sheep on Farm B lambed after insemination on Day 3 instead of Day 2, seems to support this hypothesis.

There is some evidence supporting the idea that dosage level of progesterone may be an important factor in fertility following synchronised ovulation (Davies, 1960; Lamond and Lambourne, 1960). Thus Lamond and Lambourne (1960) demonstrated that the dose and the interval between injections influence time of oestrus. They also obtained evidence in favour of an hypothesis that fertility was highest when the ewes were served on Day 2. The ewes in Experiment 2 in this paper mated within a period 48-72 hours after the final progesterone injection and they proved relatively fertile. If in fact only those actually marked by the rams are taken into account the fertility level is very high. This seems to indicate that 10 mg. of progesterone daily cannot in itself be a factor limiting fertility under our conditions.

Significant differences in fertility following constant doses of progesterone—PMS and natural mating were observed during the period June to December. The differences, however, were not large and suggest that out-of-season breeding studies should be initiated with this technique as the basis for further investigation. One might well visualise the following steps in an investigation of this nature.

1. Determine the optimal dosage levels of progesterone and PMS, when followed by natural mating,
2. Using the optimal method, examine seasonal differences in ram fertility.
3. Examine the possibilities for A.I.

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## DISCUSSION

**Dr. E. A. Campbell (Qld.).**—Implantation occurs as a result of interaction of oestrogen and progesterone. Perhaps the reason why A.I. was unsuccessful is because the balance of these two hormones was upset.

**Answer.**—The time of administration of P.M.S. in relation to the final injection of progesterone undoubtedly influences the oestrogen-progesterone ratio and hence egg and sperm transport.

**Dr. R. B. Dun (N.S.W.).**—Recent work has shown that teasing after insemination gives a considerable lift in fertility to those ewes which are late in oestrus at the time of insemination.

**Professor T. J. Robinson (N.S.W.)** commented that results were poor when inseminations were made a fixed time after injection. However, with teasing, he obtained results from 40-56% lambing for single inseminations.

**Dr. R. B. Dun (N.S.W.).**—There appeared to be no difference in lambing rates for ewes using hand service and normal artificial insemination when the ewes came into oestrus naturally.

**Professor T. J. Robinson (N.S.W.)** commented that spring joining gave 15% higher lambing percentage than autumn joining in a well-controlled experiment using the same rams by A.I. on ewes from the same flock. He asked what time of the year Dr. Lamond's experiments were carried out.

**Answer.**—In May with later experiments from June to December.