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ADVANCES IN CONTROLLED SHEEP BREEDING

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THE TITLE of this paper may be misleading because, regrettably, there have been no major advances in controlled sheep breeding since the breakthrough in 1952 when the role of progesterone in the ovulation-oestrus phenomenon was demonstrated (Dauzier and Wintenberger, 1952; Dutt, 1952; Robinson, 1952). This paper is a report of current work aimed at achieving another advance of comparable importance.

Control of reproductive phenomena in the ewe may be attempted in three major ways, namely:

(1) Induction of a normal ovulation-oestrus phenomenon in anoestrus, accompanied by full fertility.

(2) Synchronization of the time of oestrus and ovulation for controlled artificial insemination in the normal breeding season, again accompanied by full fertility.

(3) Removal of the number of ovulations as a factor limiting fertility in the normal breeding season.

Before proceeding it is necessary to make three points:

(1) Although interested in each of these three aspects, of recent years work has been concentrated upon the former two and this paper deals only with these.

(2) Since many of the practical problems of anoestrous breeding and of controlled A.I. are common, they are discussed together.

(3) This paper does not give details of individual experiments but presents a general picture of the experimental approach and a summary of the more important results obtained during the past several years.

Experimental Approach

BACKGROUND

The background to the work reported here has been reviewed by the author (Robinson, 1959). Broadly speaking it is well established that oestrus and ovulation may readily be induced in the anoestrous ewe by the injection of pregnant mare serum gonadotrophin following a period of preparation with pro-

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gesterone. The efficiency of this treatment as evidenced by the proportion of ewes which exhibit oestrus and ovulation is improved by increasing the duration of treatment from 3 to 12 days. Increasing the period between successive progesterone injections decreases efficiency and the maximum practical interval is 2 days when injections are given in oil. The labour involved, coupled as it has been with low lambing percentages, has precluded large-scale practical application. Generally speaking, the same principles apply to the synchronization of oestrus in the breeding season, when the duration of progesterone treatment is even longer, namely, one complete cycle of 16 days.

The Problems

The major problems requiring solution are:

(1) Simplification of progesterone injection procedure.

(2) Development of a sharp end point to progesterone treatment.

(3) Determination of factors affecting fertility following treatment. The ram and ewe are both involved.

(4) Determination of the maximum use possible for rams by A.I. during a limited period at different times of the year.

Methods of Investigation of these Problems

Three methods are in use, involving three types of sheep, namely:

(1) *Spayed crossbred ewes.* A flock of 80 spayed crossbred ewes was used to determine, among other things, the relative effectiveness of various progesterone priming techniques. The end point was the induction of oestrus following the injection of oestrogen. This work continues on a year-round basis.

(2) *Intact cyclic Merino ewes.* The relative effectiveness of various progesterone injection techniques can be tested using the end points of (a) suppression of oestrus, (b) release of oestrus and ovulation, and (c) subsequent fertility to A.I. This work is confined to the autumn months.

(3) *Intact anoestrous British or crossbred ewes.* The relative effectiveness of various progesterone priming techniques can be tested using the end points, following gonadotrophin injection, of (a) oestrus accompanying ovulation, and (b) subsequent fertility to A.I. or to natural mating. This work is confined to the spring months.

Of recent years efforts have been concentrated on spayed ewes and on intact cyclic ewes. It was found that any progesterone priming technique which was effective with spayed ewes was equally effective with the anoestrous or intact cyclic animal. The first problem to solve was that of obtaining a simple, effective, and above all, precise, method of progesterone priming.

Experimental Design

Multi-factorial experimental designs were used. Factors studied include type, dose, and frequency of progesterone injections, type and dose of oestrogen, type and dose of gonadotrophin.

Experimental Results

The prerequisites of a satisfactory progesterone preparation are:

- Prolonged duration of activity.
- Sharp end point, *i.e.*, a rapid decline to zero in the level of circulating progesterone.
- High degree of fertility following cessation of treatment.

Duration of Activity

Studies on the efficiency of oestrus suppression in cyclic ewes are particularly valuable for the determination of satisfactory Interval × Progesterone × Dose interactions.

TABLE 1: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES

Progesterone in Oil

	1957				1959 *a*			
	n	*S*	*R*	*L*	*n*	*S*	*R*	*L*
Every day	36	33	32	13	6	6	6	4
Every 2 days	36	31	30	12	6	6	4	2
Every 4 days	36	20	12	4	6	4	2	1
Every 8 days	—	—	—	—	6	1	0	0

n = Number
R = Released oestrus within 10 days (A.I.)
S = Suppressed oestrus
L = Lambed to A.I.

TABLE 2: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES

Progesterone in Benzyl Benzoate

	1959 *b*			
	n	*S*	*R*	*L*
Every day	16	13	11	4
Every 2 days	16	15	14	6
Every 4 days	16	7	6	3
Every 8 days	16	1	1	0

n = Number
R = Released oestrus within 10 days (A.I.)
S = Suppressed oestrus
L = Lambed to A.I.

(1) *Type of Progesterone Preparations*

Preparations tested were progesterone in:
(a) Peanut oil.
(b) Benzyl benzoate.
(c) A commercial medium (16476 b, Farbwercke Hoechst).
(d) Benzyl alcohol emulsion.
(e) Aqueous microcrystalline suspension.
(f) "Proluton" (17α-oxyprogesterone-17-capronate; Schering).

(a) *Progesterone in peanut oil.* Effective spacing is limited to 2 days, and the end point is moderately precise (Table 1). Lambing rate to a single artificial insemination was generally reduced to about 40 per cent. from the normal figure to 60 to 70 per cent.

(b) *Progesterone in benzyl benzoate.* Results were essentially similar to those obtained with oil (Table 2).

(c) *Experimental preparation 16476 b (Farbwercke Hoechst).* This solution has a more prolonged action than those in peanut oil or benzyl benzoate. Ovulation was suppressed in 90 per cent. of cases when injections were spaced at 4-day intervals. This was the effective limit. Release of oestrus and ovulation was slightly less efficient than with oil. Lambing rates appeared somewhat lower but this was not statistically significant (Table 3).

TABLE 3: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES
Progesterone in Experimental Preparation 16476 b (Farbwercke Hoechst)

	1959 a				*1959 b*			
	n	S	*R*	*L*	*n*	S	*R*	*L*
Every day	6	6	4	1	16	16	11	4
Every 2 days	6	5	4	1	16	13	11	3
Every 4 days	6	5	4	2	16	15	13	5
Every 8 days	6	1	1	1	16	5	4	1

n = Number
S = Suppressed oestrus
R = Released oestrus within 10 days (A.I.)
L = Lambed to A.I.

TABLE 4: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES
Progesterone in Benzyl Alcohol Emulsion

	1957			
	n	S	*R*	*L*
Every day	36	33	19	3
Every 2 days	36	28	11	2
Every 4 days	36	31	16	0
Every 8 days	—	—	—	—

n = Number
S = Suppressed oestrus
R = Released oestrus within 10 days (A.I.)
L = Lambed to A.I.

(d) *Progesterone in benzyl alcohol emulsion.* Emulsion injections have been tested only up to intervals of 4 days, at which interval they were quite effective. Release of oestrus was considerably delayed and this suggests that an interval of at least 8 days would be effective (Table 4).

(e) *Progesterone in aqueous suspension.* Microcrystalline suspensions were moderately effective in suppressing oestrus when given at intervals up to 8 days. Oestrus release was quite unsatisfactory and when oestrus did occur, it was usually infertile (Table 5).

(f) *"Proluton" (17α-oxyprogesterone-17-capronate).* Table 6 does not give a full picture of the effect of "Proluton". Four of the five ewes in which oestrus was not suppressed were served within 2 days of their first injection. Presumably the initial rate of absorption was too slow to inhibit ovulation in some ewes in which follicular maturation was initiated. Exhibition of oestrus after cessation of treatment was delayed for up to 48 days.

(2) *Dose*

Marked increase in the daily dose of progesterone did not result in a corresponding increase in the duration of activity, particularly when the spacing of injections exceeded 4 days

TABLE 5: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES
Progesterone in Aqueous Suspension

	1957				*1959 a*			
	n	*S*	*R*	*L*	*n*	*S*	*R*	*L*
Every day	36	32	12	0	6	6	3	0
Every 2 days	36	26	10	2	6	5	2	0
Every 4 days	36	25	12	1	6	4	1	0
Every 8 days	—	—	—	—	6	5	4	0

n = Number　　*R* = Released oestrus within 10 days (A.I.)
S = Suppressed oestrus　　*L* = Lambed to A.I.

TABLE 6: SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES
"Proluton" (17α-oxyprogesterone-17-capronate)

	1959a			
	n	*S*	*R*	*L*
Every day	6	6	0	0
Every 2 days	6	6	0	0
Every 4 days	6	4	1	0
Every 8 days	6	3	0	0

n = Number　　*R* = Released oestrus within 10 days (A.I.)
S = Suppressed oestrus　　*L* = Lambed to A.I.

(Table 7). At intervals of up to 4 days, and using daily dose levels between 6 and 16.7 mg progesterone, a dose-response effect was observed. For all practical purposes a daily rate of 10 mg appeared adequate and there was no point in exceeding it.

Sharpness of End Point

The distribution of date of first oestrus after cessation of progesterone treatment was bimodal, with an interval approximately equivalent to one oestrous cycle (Tables 8, 9). There was

TABLE 7: EFFECT OF PROGESTERONE DOSE ON SUPPRESSION AND RELEASE OF OESTRUS IN CYCLIC EWES

All forms of preparation

Rate of Daily Dosage (mg)		*1957*				*1959*			
1957	*1959*	*n*	*S*	*R*	*L*	*n*	*S*	*R*	*L*
6.0		108	74	45	8				
	7.8					32	18	16	4
10.0		108	83	54	14				
	15.6					32	21	16	5
16.7		108	102	55	10				
	31.2					32	23	20	8
	62.5					32	23	19	9

NOTE: Intervals between injections:
1957 — 1, 2 and 4 days; 1959 — 1, 2, 4 and 8 days.

TABLE 8: DISTRIBUTION OF DAY OF FIRST OESTRUS

Interval from last Progesterone (Days)	*Oil solution (n=17)*	*Benzyl benzoate (n=35)*	*16476 b (n=48)*	*Aqueous suspension (n=20)*	*B. alcohol emulsion (n=46)*	*"Proluton" (n=19)*
1 - 4	10	25	32	2	29	
5 - 8	2	7	7	3	17	1
9 - 12				7	*	
13 - 16				1		4
17 - 20	3	3	6	4		3
21 - 24			2	3		
25 - 28						
29 - 48						11
>48	2		1			

*Observations for oestrus were not continued beyond 8 days.

TABLE 9: MEAN INTERVALS—PROGESTERONE CESSATION TO OESTRUS

	Oil solution	*Benzyl benzoate*	*16476 b*	*Aqueous suspension*	*B. alcohol emulsion*	*"Proluton"*
Intervals	3.7±1.6	3.5±1.4	3.1±1.4	8.4±2.7	4.5±1.3	14.8±4.5
(days)	18.7±1.6	19.7±2.0	20.1±2.0	23.8±3.2	N.A.	37.0±5.9

N.A. = Data not available

a clear tendency for a higher proportion of ewes to fall into the second population with increase in the duration of activity of the preparation used. It appeared, therefore, that "suppression of oestrus" was not synonymous with "suppression of ovulation". Fourteen per cent. of ewes in which oestrus had been blocked by injection with progesterone in oil, benzyl benzoate, or 16476 b may be presumed to have ovulated without oestrus within 8 days of cessation of treatment. As would be expected, the spread was greater with longer-acting preparations but the bimodal nature of the curves was clearly evident. With suspensions, 40 per cent. fell into the second curve, and with "Proluton", 58 per cent. Data were not available for emulsions. In the case of "Proluton", the day of the first oestrus more nearly approached a random distribution.

Degree of Fertility

There was a clear relationship between the sharpness of the end point of progesterone activity and fertility to insemination at next oestrus (Table 10). This is a particularly important observation and is the clue to past failures, particularly if viewed in the light of evidence of "silent" ovulations following treatment in a fairly high proportion of ewes.

Failure to conceive was due in many cases to failure of fertilization owing probably to an imbalance of the normal progesterone-oestrogen levels at the time of ovulation and fertilization. Progesterone, though rapidly metabolized, is a compound highly insoluble in water and body fluids. Small amounts capable of suppressing oestrus, but incapable of suppressing ovulation, may be released in some cases from injection sites or from adipose tissues for many days even where injections have been made in oil. A tubal and uterine environment sufficiently abnormal to result in impaired fertilization is easily visualized.

Reliable data on rates of fertilization, and on subsequent embryonic survival, after endocrine treatment are lacking for obvious reasons of expense and time. Tables 11, 12 and 13 sum-

Table 10: Percentage Ewes Lambed to a Single Artificial Insemination

		Progesterone treated					
	Controls	*Oil solution*	*Benzyl benzoate*	*16476b*	*Aqueous suspension*	*B. alcohol emulsion*	*"Proluton"*
Inseminated	62	125	32	52	44	46	1
Lambed	46	46	13	18	3	5	0
Per cent.	74	37	41	35	7	11	0

marize results of two experiments carried out in successive years on anoestrous crossbred ewes in which an oestrus-ovulation response was induced by progesterone-PMS treatment.

The basic objects of the experiment were to determine in the anoestrous ewe:

(1) The fertilization rate after progesterone-PMS induced ovulation and oestrus.

(2) The survival rate of fertilized ova.

(3) The effect of subjecting the ewe to 1 or 2 artificial cycles before permitting service, as compared with a 6-day progesterone priming treatment.

Ewes were slaughtered 2 or 21 days after service for ovum and embryo recovery. Service was by Suffolk rams which were producing semen of good quality.

The most obvious point arising from Table 11 is the great discrepancy between the total number of ovulations and the survivals at 21 days (56/168 = 33.3% survivals).

It is not possible accurately to break down this loss into loss at fertilization and loss in the early embyro stage but loss at the fertilization stage is obviously abnormally high (50 cleaving ova of 107 recovered = 47 per cent.). In view of the general ease of recovery of ova from the oviduct, it is probable that, in many cases where recovery has not been effected, ovum transport has been abnormally fast, or alternatively that ovulation-oestrus time relationships have been abnormal, and that such ewes have not been fertilized. The lack of significant difference between ewes classed as fertilized at 2 days and 21 days—approximately 50 per cent. of possible cases—clearly indicates failure of fertilization or of first cleavage as being more important than post-fertilization failure.

TABLE 11: FERTILITY AND SURVIVAL OF OVA IN ANOESTROUS EWES
Oestrus and ovulation induced by progesterone-PMS treatment

					Total ova or embyros			
Year	*Days after service*	*n*	*Ewes ovulated*	*Total ovulations*	*Recovered*	*Fertilized**	*Ewes from which recovered*	*Ewes fertile*
1954	2 days	32	30	68	48	25	22	16
1954	21 days	32	30	79	24	21†	17	15
1955	2 days	48	41	65	59	25	36	18
1955	21 days	48	48	89	38	38	24	24

*Cleaving or with sperm obviously attached.
†3 cases of embryonic mortality in 2 ewes, terminating pregnancy.

Failure of fertilization can be due to:

(1) Intrinsic faults in the ova.
(2) Intrinsic faults in the spermatozoa, or
(3) Faulty uterine and tubal environment for sperm transport, or fertilization.

Table 12 shows the effect on fertilization of the uterine environment. At first sight, the 1955 experiment suggested that conditioning of the uterus played an essential part in fertilization, an effect not evident in the 1954 experiment. In all cases, the percentage of fertile eggs was subnormal.

TABLE 12: OVA FERTILIZED
(Detail from Table 11)

Year	*Treatment*	*n*	*Ewes ovulated*	*Total corpora lutea*	*Total ova re-covered*	*Total ova Ferti-lized*	*Ewes from which re-covered*	*Ewes fertile*
1954	Conditioned	16	15	38	25	13	12	8
1954	Not conditioned	16	15	30	23	12	10	8
1955	Conditioned	16	15	22	22	14	15	10
1955	Semi-conditioned	16	14	19	18	10	13	7
1955	Not conditioned	16	12	24	19	1	8	1

Conditioned = 30μg ODB-Progesterone 16 days — 30μg ODB-Progesterone 16 days — 1,000 iu PMS
Semi-conditioned = 30μg ODB-Progesterone 16 days — 1,000 iu PMS
Not conditioned = Progesterone 6 days — 1,000 iu PMS
(ODB = oestradiol benzoate)

TABLE 13: EMBRYOS SURVIVING 21 DAYS
(Detail from Table 10)

Year	*Treatment*	*n*	*Ewes ovulated*	*Total corpora lutea*	*Total embryos re-covered*	*Sur-viving em-bryos*	*Ewes from which embryos recovered*	*Ewes fertile*
1954	Conditioned	16	15	39	16	15	10	10
1954	Not conditioned	16	15	40	8	6	7	5
1955	Conditioned	16	16	29	12	12	7	7
1955	Semi-conditioned	16	16	33	16	16	9	9
1955	Not conditioned	16	16	27	10	10	8	8

Conditioned = 30μg ODB-Progesterone 16 days — 30μg ODB-Progesterone 16 days — 1,000 iu PMS
Semi-conditioned = 30μg ODB-Progesterone 16 days — 1,000 iu PMS
Not conditioned = Progesterone 6 days — 1,000 iu PMS

Survivals of embryos to 21 days are presented in Table 13. The 1954 experiment suggested that embryonic mortality in unconditioned ewes was highly important. The 1955 experiment completely belied this and, indeed, suggested that many ova classed as infertile at 2 days were in fact fertile.

The high incidence of one-cell, presumably fertile, eggs recovered from the unconditioned ewes in 1955 suggests a difference in the ovulation-oestrus time relationships between the three treatments, which raises again the question of endocrine balance at this critical stage.

The data in Tables 12 and 13 suggest that prior conditioning of the uterus by progesterone and oestrogen may be less important for fertilization and survival than might have been expected. The most important factor affecting the environment for fertilization and early cleavage is probably the progesterone-oestrogen balance at that time.

Conclusion

The major problems are the simplification of the progesterone priming technique and the raising of the degree of fertility following treatment. These two may well go together. In attempting to simplify, attempts have been concentrated on injections of long-acting preparations, with disastrous results on subsequent fertility, owing probably to the continual release of small amounts of progesterone at the time of fertilization. The correct approach should be to ensure a continuous intake followed by a controlled and sudden cessation. Two possible approaches are being explored, namely: (a) Intra-vaginal application followed by douche and (b) Intra-aural injection followed by excision.

The use of intra-vaginal pessaries and oil injection have not been successful, but preliminary results of oil injections into the ear suggest that a physiological rate of absorption is practicable. The next step is the use of long-acting preparations administered by this route followed by excision.

Literature Cited

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DISCUSSION

Dr D. S. Flux: As Professor Robinson said, the importance of this work can be gauged from the fact that the whole future of large-scale artificial breeding and out-of-season breeding in sheep depends upon development of successful techniques for controlling the reproductive mechanism. He and his colleagues are to be congratulated on the very valuable contributions they have made to knowledge on this subject, and he himself for the clear picture he has given us of their work and the difficulties which have still to be overcome.

There are two main approaches to endocrinological work of this type with farm animals. The first involves estimation of levels and changes in levels of circulating hormones in animals reproducing normally. Progress depends on development of suitable techniques for making these measurements and these are generally costly in terms of materials, time and skilled labour. Handling the numbers of samples necessary to follow even a few animals is costly. When you know what you wish to imitate, you have still the problem of working out a suitable system of treatments.

The second, that chosen by Professor Robinson, is to use exogenous treatments to see whether any will produce the desired results. Even when prior knowledge has been used to eliminate all but the most likely treatments, and combinations of treatments, the number to be tested may be very large. The ideal experimental designs to use are likely to be massive factorials which the experimentalist cannot contemplate using, so that he has to compromise by testing fewer treatments and spreading his work over a considerable period of time. An added difficulty is that, because the balance of factors (endocrine, neural and reproductive) concerned in reproduction is likely to differ between individuals, it is unlikely that the experimentalist will find a single dosing system that will work for all the animals he treats.

In connection with the difficulty of giving adequate progesterone therapy with infrequent animal handling, yet having a sharp end point, I wonder if Professor Robinson has thought of trying progestins such as 20α and 20β hydroxypregn-4-en-3-ene which are found in peripheral blood of some species and might have better excretion characteristics than progesterone?

As an alternative, is there any possibility of using substances which would oppose whatever interfering effect progesterone has on fertilization?

I wonder whether the sheep which do not ovulate until about one normal oestrous cycle after treatment ceases have really had an ovulation without oestrus earlier? There is evidence from work with mice that the oestrous cycle is basically a neural phenomenon, and both oestrus and ovulation may have been suppressed for a period following the cessation of treatment.

A.: The purpose of this paper has been to point out those unprofitable avenues which have been explored so that other workers in this field are aware that they have been examined. One of the frustrating aspects of this work is the knowledge that we are half-way there and have been for a long while, and final solution can be reached only when we have a complete understanding of factors controlling sperm and ova transportation, fertilization and implantation.

In answer to the three specific questions raised by Dr Flux, I can only state that the possible use of other progestins or of anti-progestins has been considered, but not very seriously as yet. I grant that I have not conclusively demonstrated a "silent heat" in some cases after cessation of treatment, but have merely presented suggestive evidence.

Q.: *I was surprised to hear the speaker dismiss as of no practical significance techniques which he perfected for inducing superovulation in the ewe. Is the time interval between injection of the gonadotrophin and ovulation important?*

A.: Superovulation techniques are of value but in my opinion are of less value, particularly in Australia, than the potential worth of controlling the time of ovulation and oestrus for purposes of artificial insemination.

Q.: *It was stated that it is a fact that ovulation can be induced in young animals and in hypo-phy-sectomized animals. Can the speaker therefore speculate why the endocrine balance is important in ovulation?*

A.: Ovulation can readily be induced in the anoestrous ewe by gonadotrophin, and if this is preceded by progesterone, oestrus accompanies ovulation. It seems certain, too, that release of L.H. by the pituitary of the ewe plays a part in the ovulation phenomenon. Endocrine inbalance, which quantitatively may be relatively slight, is probably involved in the poor fertilization results.

Q.: *The presence of the ram before the onset of the breeding season has been used to advance the date of mating in Australia. Can the speaker indicate the extent to which this information is being used under commercial flock conditions?*

A.: It is being used but only to a limited extent.

Q.: *Has the speaker considered drenching ewes with a "progesterone bomb" which would release an appropriate amount of progesterone?*

A.: Yes, but the practical problems are enormous.