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The Environmental Factors on the Lamb Growth, Analytically Studied with Extra-Seasonal-Lambs

II. Extra-Seasonal-Lamb-production by the Combined Treatment of Artificial Light and Hormone

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(Figs. 1-7; Tables 1-4)

INTRODUCTION

In the growth and development of the Japanese Corriedale lambs, the probable importance of the age of three months old was stressed by MIMURA (1956) [1], and MIMURA, although inconclusive, suggested that the growth rate of lambs might be modified by the light rhythm, nutritional change and sexual maturity at the age. But this will be fully confirmed only, when these environmental factors and the growth pattern will be studied analytically on the lambs which are produced all over the year.

Primary object of our studies was again to find out how much effect the environmental factors had on the growth, as compared with normal lambs and extra-seasonal-lambs.

But in these years many experiments had been conducted mainly with the object of producing some extra-seasonal-lambs. The authors reported in the previous paper in this series [2] that all of ewes under light treatment came into oestrus for 40–50 days intervals after the change-over from increasing to decreasing light, and it might be considered that there are some constant intervals in Corriedale ewes to come in oestrus under short day treatment. But the light treatment presents the problems of housing and management of animals that makes practical application of this method difficult.

In recent years, the effeciency of hormonal therapy for extra-seasonal-lamb-production has been shown by COLE, HART and MILLER (1945) [3], ROBINSON (1951, 1954) [4] [5], DUTT (1953) [6], RAESIDE (1956) [7], GORDON (1958) [8] and others [9] [10], and the literature on the effect of estrogens and progesterones on time of ovulation in anoestrous ewes has been reviewed by DUTT (1953) [11]. By progesterone-P.M.S. therapy, GORDON (1958) showed that the induction of pregnancy in the nonmilking ewe is much hopeful, and a conception rate of 50 % or higher can be expected after treatment in anoestrus.

But it is most essential, from the practical application's viewpoint, that the method should be greatly simplified and the higher percentage of conception might be

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expecte dcertainly. Although there is some uncertainty as to the exact manner in which the light environment exercises its effect on the pituitary, the most widely held view is that the eye acts as a light receptor and, presumably by the transmission of impulses from it along neural pathways, the pituitary is stimulated (YEATES, 1954)[12].

When the head of ewes will be covered with a black hood, therefore, the animal might be controlled to light rhythm. When the changes induced under decreasing lighting will be expected in pituitary activities of ewes, the combination of lighting and hormone therapy might come to practical considerations more effectively than light treatment or hormonal therapy alone.

The experiments described in this paper were designed primarily to examine the combined effect of decreasing light and progesterone-P.M.S. therapy on the oestrous response of anoestrous ewes, and trials were undertaken in 1959–1961.

MATERIALS AND METHODS

1. Experimental animals

Twenty-four Japanese Corriedale ewes and yearlings were experimented in Fukuyama Experimental Farm of Hiroshima University.

In the first trial (July, 1959) five 5-year-old ewes and one 4-year-old ewe, averaging 50*kg* in live weight, were used. In the second trial (Feb., 1960) 8-year-old and 2-year-old ewes, averaging 42*kg* in live weight, were used. In the third (Apr., 1960) one yearling ewe, 53*kg* live weight, was used, and in the fourth (June, 1960) one 6-year-old, two 5-year-old, one 4-year-old and two yearling ewes, averaging 45*kg* in live weight were used.

In the fifth trial (Feb., 1961) one 10-year-old (55*kg* in live weight), two yearling ewes (35*kg* in live weight) were used. In the sixth trial (Mar., 1961) one 7-year-old, two 6-year-old, and two yearling ewes, averaging 45*kg* in live weight, were used.

Details on all experimental animals are indicated in Table 1.

Table 1. Details of animals and treatmens

Trial No.	Date of onset of treatment	No. of animals	Light treatment			Hormonal therapy	
			Decreasing period(days)	Daylight length from onset to final (hours and minutes)	Period of constant daylight after decreasing treatment (days)	Progesterone injection (mg, days)	P. M. S. injection (i. u.)
1	July 21,'59	5(0)	7	14:09→ 9:48	7	25*mg* × 5	750
2	Feb. 18,'60	2(1)	7	11:01→ 9:12	7	〃	〃
3	Apr. 12,'60	1(1)	7	12:56→ 9:37	5	25*mg* × 3	〃
4	June 9,'60	6(2)	7	14:31→10:53	5	〃	〃
5	Feb. 10,'61	3(2)	7	10:46→ 8:38	5	〃	〃
6	Mar. 17,'61	4(1)	7	12:00→ 8:54	5	〃	〃
6	〃	3(1)	—	—	—	〃	〃

Remark: Parenthesized number is the number of yearlings.

In all experiments one fertile Corriedale ram was employed.

The sheep were in good health, and they were maintained under normal housing condition described in previous paper.

Fig. 1. Ewes under decreasing daylight were covered with a black hood, and then were housing in a pen.

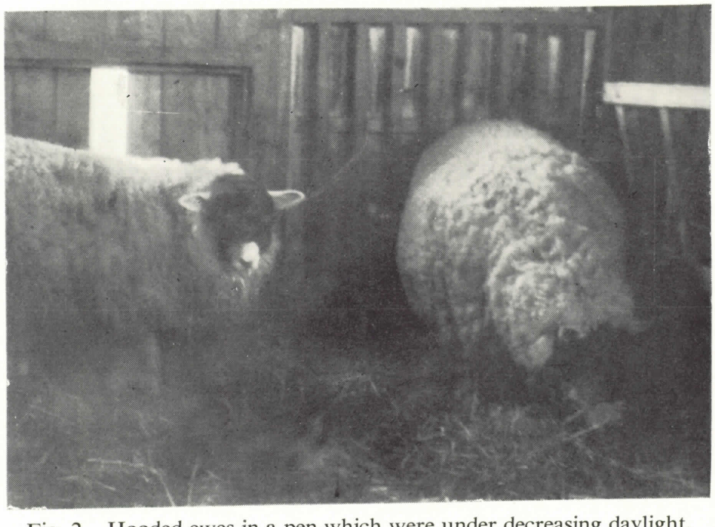

Fig. 2. Hooded ewes in a pen which were under decreasing daylight.

2. Light treatment

Before the light treatment all experimental ewes were teased by aproned ram day by day for five weeks to ascertain their anoestrous condition.

For seven days the ewes were housed in one pen and their head was covered at the calculated time with a black hoods which were prepared from thick cloth and touched in the same way indicated in Fig. 1 and 2.

In the sixth trial, three of seven ewes (No. 55–50, No. 60–2 and No. 60–8) were not under artificial darkness.

Decreasing hours of day light are described in Table 1.

3. Hormonal therapy

Ewes were intramuscularly injected with progesterone 25*mg* for five days (in the first and second trials) and three days (in the third～sixth).

After final light treatment two forms of progesterone were employed, these being an oil solution using a dilution rate of 12.5*mg* progesterone per 1 *ml* of oil prepared by the Teikoku Hormone MFG. Co., Ltd. P.M.S. was available in purified form commercially prepared by Teikoku Hormone MFG. Co., Ltd. The purified serum was supplied in vials containing 1,000 i. u., and it was diluted in 0.6 % saline solution a few minutes before injection. P. M. S. solution containing 750 i. u. was administered by intramuscular injection following 2 days after the final injecton of progesterone. From one day after P.M.S. injection, all the ewes were running with a fertile ram in a pen twice daily. At this running, inspection for evidence of oestrus was generally made in the same way described in previous paper. Details of treatments were indicated in Table 1.

Thirteen of twenty-four ewes were slaughtered after mating, and their ovaries were examined for the presence of corpora lutea and the state of follicular development to determine whether oestrus or ovulation had occurred in ewes.

RESULTS

The response of ewes is given in Table 2. Nineteen of the twenty one ewes receiving the combined treatment came into oestrus, therefore the percentage is about

Table 2. The response of ewes to the treatment

Trial No.	No. of animals (No. of yearling parenthesized)	No. of oestrous ewes induced by the treatment					No. of ewes examined for ovaries
		Oestrous ewes observed by inspection	Pregnant ewes in first service (1)	Oestrous ewes observed by return of normal oestrus (2)	Oestrous ewes regarded by examination of ovaries (3)	Total oestrous ewes ((1)+(2)+(3))	
1	5(0)	5	2	2	—	4	0
2	2(1)	1	1	—	1***	2	1
3	1(1)	1	—	—	1**	1	1
4	6(2)	6	1	2	2*	5	2
5	3(2)	3	—	—	2*+1***	3	3
6	4(1)	4	1	—	3*	4	3
6	3(1)	2	—	—	1*+1***	2	3

Remarks. * : Presence of corpora lutea present on their ovaries.
** : Presence of Graafian follicle, but no corpora lutea.
*** : Presence of large follicle, but no corpora lutea and no Graafian follicle.

90. When the oestrous ewes were limited to one which was ascertained by pregnancy, return of normal oestrus after induced oestrus and presence of corpora lutea and Graafian follicle in the ovaries at slaughter time, the number of ewes showing ovulation is seventeen and the percentage is about 81. Within those receiving hormone alone 2 ewes were observed in oestrus by inspection, but upon examination at slaughter only one had corpora lutea present on its ovaries. The cytological observations on ovaries are given in Table 3 and Figs. 3–6.

Table 3. Results of the examination for ovaries

Trial No.	Ewe No.		Presence in ovaries			Remarks
			Corpora lutea	Graafian follicle	Large foll. over 5*mm* in diameter	
2	58-2	L	0	0	0	
		R	0	0	1	Fig. 3
3	59-4	L	0	1	0	
		R	0	1	0	Fig. 4
4	59-2	L	1	0	0	
		R	1	0	0	
	59-6	L	0	0	0	
		R	1	0	2	
5	52-021	L	1	0	1	
		R	1	0	0	
	2471	L	1	1	0	
		R	0	0	0	
	60-4	L	0	0	0	
		R	0	0	1	Fig. 5
6	56-41	L	1	0	0	
		R	0	0	0	
	323	L	0	0	0	
		R	1	0	0	
	60-6	L	0	0	0	
		R	2	0	0	
	°55-50	L	0	0	0	
		R	0	0	0	
	°60-2	L	0	0	1	Fig. 6
		R	0	0	0	
	°60-8	L	1	1	0	
		R	0	0	0	

Remarks. 1) °: Ewe treated only with hormone.
2) L: Left ovary.
3) R: Right ovary.

Table 4. Number of lambs produced by the treated ewes

Trial No.	Pregnant ewes			Total number of lambs	Lambs per 100 ewes
	In first service	In following service	Total		
1	2	2	4	7	175
2	1		1	2	200
4	1	2	3	3	100
6		1	1	1	100
Total	4	5	9	13	144

Although, of nine ewes that produced extra-seasonal-lamb, only four were

conceived in first service. Thirteen lambs were produced and total percentage of production per ewe was about 144. The number of lambs produced is given in Table 4.

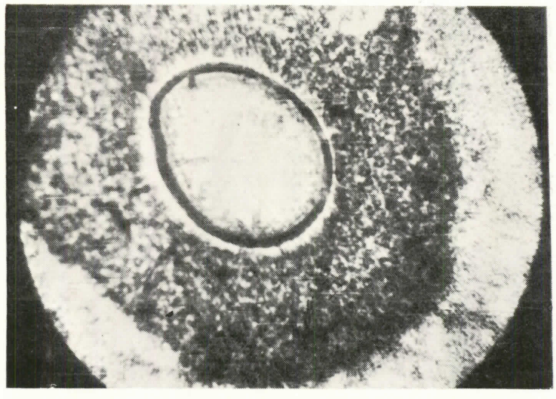

Fig. 3. Large follicle in right ovary of No. 58-2 (x400).
This would not develop to Graafian follicle.

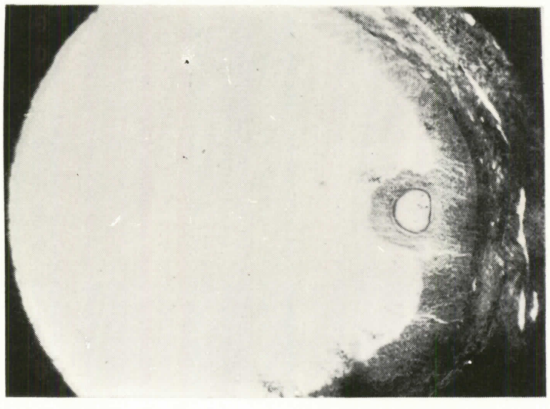

Fig. 4. Graafian follicle in right ovary of No. 59-4 (x100).

In the eighteen ewes that were injected with P.M.S. oil solution and came to oestrus, the daily induce of matings relative to the time of P.M.S. injection was recorded and the data are given in Fig. 7. The highest response was observed two days after the time of P.M.S. injection, followed by the next day.

DISCUSSION

The authors showed in previous paper that all treated ewes came into oestrus as a result of light treatment, and that the results confirmed perfectly the major role of light in regulating the reproduction of sheep.

The mechanism by which the gonads may be influenced by environmental light changes had been discussed by YEATES(1954)[12] and others, and although it is agreed

Fig. 5. Large foll. in right ovary of No. 60-4 (x400).
This would not develop to Graafian follicle.

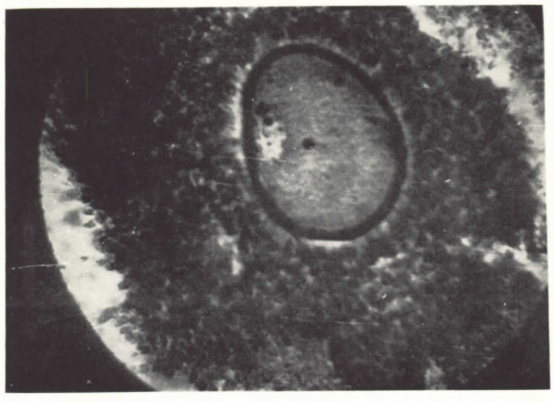

Fig. 6. Large foll. in left ovary of No. 60-2 (x400).
This would not develop to Graafian follicle.

that pituitary is the regulator, there is some uncertainty as to the exact manner in which the light environment exercises its effect on this organ.

Dr. DUTT, in his report of 1953[6], proposed that in ewes during the nonbreeding season the ovaries pass through transitional periods from the breeding season to complete quiescence and from complete quiescence to the onset of the breeding season, during which time they follow a rhythm pattern similar to that during the breeding season. He further proposed that failure of all but a low percentage of anoestrous ewes treated only with gonadotrophic hormone respond with synchronous oestrus and ovulation may be due to treating during this transitional period.

It has been suggested by many workers (HAFEZ, YEATES, MARSHALL) that there is a certain level of threshold in pituitary activity required to induce the ovarian response. Therefore it may be probably explained that intense decreasing of light prior to hormonal therapy will reduce the threshold to much lower level, and thus

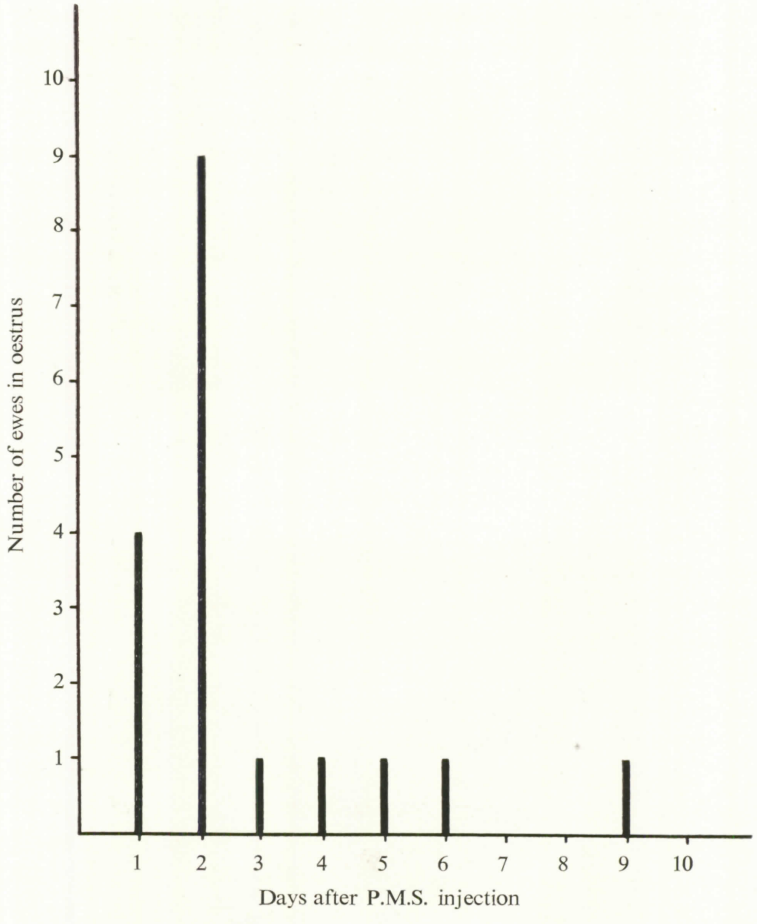

Fig. 7. Daily incidence of oestrus in ewes treated with the combined method in relation to the date of P.M.S. injection.

further stimulating of hormone will be sufficient to induce oestrus even under unfavourable conditions.

Results in present experiment gave assurance of higher percentage (about 81 %) of induced oestrus under combined treatment than hormonal therapy alone described in GORDON. In the sixth trial two of three ewes under hormonal therapy alone would not come to oestrus or not to ovulation at least, although all four ewes under combined treatment came to oestrus. This is a definite proof that an effect of the light treatment took place at the time before the hormonal stimulation. From the results in the first and second trials, in relation to progesterone administration, successive injections daily for five days were not supposed preferable compared with injections daily for three days.

DEMPSEY, HERTZ & YOUNG (1936) advanced the hypothesis that oestrus and ovulation are brought about by the immediate action of progesterone (reviewed by DUTT, 1953)[11] and DUTT (1953) concluded that the action of progesterone is not

merely augmenting the effect of P.M.S. But in the progesterone-P.M.S. therapy the effect must be expected as the combination of treatment, and GORDON's results (1958)[8] were in general agreement with those of ROBINSON (1954)[5] who reported that a pretreatment period of 7 days and longer is necessary for the induction of a reliable oestrous response. In relation to progesterone, it is apparent from the present datum that a convenient treatment is three successive daily injections of 25*mg* progesterone.

Total number of lambs per ewe was not large (1.44/ewe), but the datum shown in Table 4 indicated that there are many ewes ovulating 2 ova or more. Therefore it is of practical importance that the method should be studied on the problem of embryonic loss.

In recent years, RADFORD (1961)[13] showed that a question of photoperiodic control of sexual activity requires further clarification and also that factors other than lighting may play equally as important a role, at least in Merino ewes in south-eastern Australia. YOSHIOKA and SAKAI(1961)[14] have also indicated that in she-goats duration of light treatment need for induction of oestrus and ovulation ranged from 48 to 113 days, and the effect of light treatment was influenced by various additional factors---environments, individualities, animal condition etc.

There is no doubt that temperature, humidity, wind, teasing by rams, and other factors will have a part on spontaneous oestrus in anoestrous season of sheep, but the experimental induction of oestrus support of the theory is meagre.

The unreliability of hormonal therapy may be improved by the method described in this paper, and using the black hood method for short period the light treatment may be in practical consideration.

The problem of weak lamb produced in early autumn which was described in previous paper is not resolved in present experiment.

SUMMARY

Following decreasing daylight treatment for 7 days, twenty four anoestrous ewes of Japanese Corriedale were injected with P.M.S. 750 i. u. after 3-5 injections of progesterone 25*mg* daily. The ewes in pretreatment of light were hooded with black thick-cloth, and were under control hours of daylight.

This combined treatment was studied over three years 1959-1961, and the incidence of oestrus, presence of corpora lutea, and the state of follicular development was studied.

Total 13 lambs were produced by nine of twenty-one ewes treated with the method, and average percentage of lamb-production per ewe was 1.44.

The presence of corpora lutea and Graafian follicle was observed cytologically in 8 of 10 ewes when their ovaries were examined at slaughter time. Seventeen of twenty-one ewes were induced oestrus and the percentage is about 81%.

In the sixth trial, three ewes were under progesterone-P.M.S. treatment alone, and only one cytologically was observed in fertile. The percentage is 33%. Therefore, the hypothesis may be probably explained that the light treatment prior to

hormone administration will reduce the threshold in pituitary activity to induce the ovarian activity to much lower level.

There is no difference between the method of three days injection daily and the method of five days injection. Thus the method combined the pretreatment of decreasing daylight by a black hood with progesterone $25mg \times 3$ and P.M.S. 750 i. u. may be considered of practical application.

The highest response was observed two days after the time of P.M.S. injection, followed by next day, and this is agreed with GORDON's result.

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季節外生産羊を利用しためん羊発育に及ぼす環境要因の解析的研究

Ⅱ. 短日処理とホルモン処理併用法による子羊の季節外生産

三村　耕・朝日田康司・森田　遙

第1報において筆者らは，非繁殖期のコリデール種雌羊を短日処理すると，長日から短日に変化してからほぼ40～50日で人工的に発育を誘起できること，しかし第1回の発情では多くの場合妊娠しないこと，しばしば弱い子羊を生産することなどを報告した．

短日処理によるめん羊の人工発情誘起法は，長期間を要する，管理に手数を要するなどの問題を有するが，反面最近数多く報告されているホルモン処理法では，高価な薬品を相当量使用する他，その効果が比較的確実でない欠点が指摘される．

筆者らは，両方法の併用法，すなわち強度の短日処理1週間（以後ホルモン処理終了まで同一日照時間維持）——黄体ホルモン25㎎/日×3～5筋注—2日後 P.M.S. 750 i. u. 筋注法の効果を試験した．またこの場合短日処理は，厚い黒頭巾を雌羊の頭に被せ，明るい羊舎内に放置する簡便法によった．

1959～1961，6回に亘り21頭を試験したが，発情と認められたもの19頭（90%），妊娠—分娩，卵巣所見—黄体・グラーフ卵胞の存在—により確実に発情と認められたものは17頭，81%に達した．

また第6回試験では併用法による4頭をホルモン処理法による3頭と比較したところ，確実に発情したもの前者100%に対し，後者は33%であった．

黄体ホルモン処理日数を3日に短縮しても5日間の処理となんら差異を認めなかった．

強度の短日処理により，下垂体機能の亢進乃至反応閾値の低下など，本併用法による人工発情誘起のメカニズムについても論議した．

なお処理羊は P.M.S. 筋注後2日目に最も多く発情し，Gordon (1958) の報告と一致した．

本報の一部は「三村・岡本他．肉綿羊の研究．興文社，東京，1961」および昭和34年度，35年度文部省科学研究費による研究報告集録農学編（Ⅱ）．肉綿羊造成に関する試験（代表者：三村耕）にそれぞれ速報している．