ON THE OUT-SEASONAL PRODUCTION OF LAMBS II. FURTHER EXPERIMENTS ON THE HORMONAL INDUCTION OF FERTILTY IN THE ANOESTROUS EWES, AND CHANGES OF OESTROGEN CONCENTRATIONS IN THE BLOOD PLASMA OF THUS TREATED EWES, COMPARED WITH THOSE OF OESTROUS CYCLE IN BREEDING SEASON

ON THE OUT-SEASONAL PRODUCTION OF LAMBS
II. FURTHER EXPERIMENTS ON THE HORMONAL INDUCTION OF FERTILTY IN THE ANOESTROUS EWES, AND CHANGES OF OESTROGEN CONCENTRATIONS IN THE BLOOD PLASMA OF THUS TREATED EWES, COMPARED WITH THOSE OF OESTROUS CYCLE IN BREEDING SEASON*1

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In previous papers the results on the hormonal production of out-seasonal lambs with the Corriedale ewes were reported (40—41). To obtain more data, further trials were carried out in 1959*3 and 1960. However, the weakness in oestrous response to the treatment was again observed throughout these trials. In addition, the lambing rate remained not so high, although it was never inferior to those hitherto reported (8, 9, 11, 32, 35). As to possible causes of these obstacles, many problems were pointed out, and some researches have been attempted to improve the results in the hormonal control of reproductivity in the anoestrous ewes (11, 18, 25, 33, 44). Among these problems, the poor response in oestrus of treated ewes, as had been previously noticed (11, 18, 35), was regarded as a factor to be improved. The authors thought, therefore, it was desirable to know the changes of oestrogen concentration in the blood plasma of the treated ewes, compared with the changes during the oestrous cycle in the breeding season*4, since no work had been published on this line.

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*1 Contribtion from the Laboratory of Animal Reproduction, 62-J-3.

*2 Present adress: Department of Animal Science, Tohoku National Agricultural Experiment Station, Morioka, Iwate Prefecture.

*3 The results were preliminarily reported already (42).

*4 The results were read at the 14th Tokoku Meeting on Endocrinology, 16th December, 1961.

In the present paper, i) additional data on the early lamb-production, and ii) the change of oestrogen concentration in the blood plasma of the induced ewes in comparison with those of normal cycling ewes, are described.

Materials and Methods

i) *Animals and experimental groups in the induction of out-seasonal lambing.*

Corriedale ewes and rams of the University Farm were used in two trials (1959 and 1960).

a) *Trial II*

In 1959, a total of 23 ewes was divided into four groups (experimentals and controls, two each) as follows: Experimental I, II were administered with 12.5 mg progesterone (Luteogen-Tomoda) twice daily for three days, followed by 1,000 i.u. PMS (Peamex-Tomoda) after two days intermission. Two hundreds Rb.u. of a gonadotrophin rich in luteinizing hormone (LH), Synahorin-Teikoku Zoki Co., was further given to the Experimental II group on the next day of PMS treatment. The reason of using two control groups was to increase the number of ewes in the control groups, because it had been said that many ewes of the Farm had showed a tendency of early lambing before the end of the year. This would, if it had happened in a control with a small number of ewes, cause confusion of the final judging of the lambing results of the treated ewes. Fifteen ewes were allotted into three groups (Exp. I, II and Cont. I), making allowance for age and previous lambing date. Besides, eight ewes selected at random from the flock were used to set Cont. II. On account of accidents which happened during the summer pasturing, eight ewes were lost; one each from Exp. II and Cont. I and three from Cont. II. Another ewe was omitted from the final score due to the abortion of the foetus.

The treatment started on the 23rd of June. When oestrus appeared, ewes were mated alternatively with two rams once a day at least during the persistence of oestrus. Except for the period of the treatment, the ewes were put on mountain pasture (about 500 m above sea level) with two rams from the begining of June until the end of October, according to the usual way of raising sheep on the Farm. Taking the pregnancy of the ewes as 145 ± 7 days, births given from the 16th to 30th of November were accounted as positive.

b) *Trial III*

In this trial in 1960, three groups were set: i) nine ewes which had been employed in the previous year (group A), ii) five treated freshly (group B), and iii) 14 lactating ewes (group C). This trial started from the 19th of May, more than a month earlier than the last one, so that some ewes, particularly most of which had given birth at a later date, were still in lactation. To mention the differences in the methods from the preceding trial, the duration and total dose of progesterone was increased to five days and 125 mg (11),

instead of three days and 75 mg. In addition, some ewes of each group were given 0.5 or 0.7 mg of oestradiol benzoate in oil (Gynandol-Tomoda) at 48, 72, and 96 hrs, as presented in Table 2.

In this case three rams (two of them had been used in the previous trial) were used, but unexpectedly their desire for mating was not strong enough to serve for all the ewes in heat. Eight ewes were again lost by accident during pasturing.

Besides the lambing results, the oestrous response in behaviour was observed, and the oestrogen concentration of the blood plasma drawn at 24 hr after PMS injection was estimated. The procedure for the latter will be described in the next paragraph (ii, b).

ii) *Measurement of oestrogen concentration*

a) *Animal conditions, treatment and responses in ewes, and time of blood sampling*

The animals consisted of eight ewes with normal lambing histories. Blood samples were obtained several times during either the oestrous cycle, mainly at the time of oestrus, or before and after the inducing treatment.

Two ewes were employed twice in successive cycles during the natural breeding season (from the end of October to November).

The inducing treatment was divided into three types, *i. e.*, progesterone-PMS combination, and the individual hormone alone. Due to the absence of a ram in spring and the early summer of 1960, teasing of the ewes could not be performed, but observation of changes in colour and swelling of vulva were carried out, as in the later cases. Response of the ewes in behaviour to the ram were checked twice a day, in the morning and the evening. The day when ewes were found to stand for service was regarded as the first day of oestrous cycle in the cases of the breeding season. Except as mentioned elsewhere, ovarian responses with regard to number of corpora lutea, ovarian follicles and ovulating points were macroscopically examined by the laparotomy performed just before treatment started (A), and at 72 hr after the final injection (B). The operations of laparotomy were conducted under the anaesthesia of Ravonal-Tanabe (400 mg of thiopental sodium/head) and were finished within 20 minutes.

Some of them were used repeatedly either for the same or for different treatments. However, intervals of more than one month were put between the two treatments.

The same preparations of progesterone and PMS were employed as in the previous trials, two modes of progesterone administration being adopted (see in Table 3).

The animal conditions and the time of sampling are presented in Tables 2 and 3, and Figure 1, with the results of the responses.

b) *Preparations and estsmation method of assay samples*

The procedure for preparation of assay materials was adopted after Sulman (43), and 30 or 45 ml of the plasma was used as a starting material. The final products, dried residues, kept in a desiccator until their usage were used in a state of fine-suspension in slightly alkaline distilled water. These samples were assayed by our modification of Sulman's intravaginal method as described in the previous report (39).

The essentials of the modification was as follows: i) mice were used thrice for measurements, being tested for their sensitivity to 0.0003μg oestrone standard before and after the assays for unknown samples, and ii) criteria for the positive were solely carried out by the amount of cornified epithelium in the smear. At one point of the assay, more than three mice were allotted. When a positive result (2 positives out of 3 mice) was obtained, sample, being diluted three or five times, was further tested to know the lower range of concentration. The values of oestrogen concentration were expressed with the upper and lower limits of μg equivalent oestrone per litre of blood plasma. In the calculation, 0.0002μg of oestrone was regarded as the minimum effective dose.

Results

I) *Induction of out-seasonal lambing*

The results of Trials II and III are illustrated in Tables 1 and 2.

a) *Trial II*

i) *Lambing score*: As seen in Table 1, positive cases came out as follows: Exp. I, 3/5; Exp. II, 2/4: Cont. I, 0/3: Cont. II, 0/5. One aborted case (No. 56 in Cont. I, on 17th Oct.) was eliminated from the scoring.

There might exist no clear difference between Exp. I and II, although the final conclusion had to be decided by further experiment. Assuming no differences between I and II groups of experimentals and controls, the positive cases of the treated and non-treated animals were summed up as 5/9 (55.5%) and 0/8, respectively. So that a success percentage of the above half was again demonstrated, as in the previous trial (3/5, 60%).

Negative cases of the experimentals and all of the controls provided later the normal fertility, and some of them showed early lambing (at the end of December), as had been suspected. But they neither exceeded over nor approached to the due date of birth in the treated group.

ii) *Oestrous response in behaviour*: Oestrus appeared from about 48 hr after PMS injection and continued up to 72 hr, but in some cases it extended until 120 hr. The desire for the ram was again not prominent enough to stand still for service. Most of them were, therefore, needed to be lightly held by the head for successful mating, as noted in the proceeding report (40, 41), while the desire of the rams for mating seemed to be quite normal.

Table 1. Animal condition, treatment and results in mating in 1959 (Trial II).

Group	Anim. No.	Previous lambing date (No. of birth)	Age	Mating VII*1	VIII	IX	X	XI (3/VII)	Lambing result Date (litter no.)	Positive score
Exp. I (PMS)	20	27/I/59 (3)	4		+	+	+	+	19/I (♀)	
	34	27/I/59 (3)	3		+		+		19/II (♀)	
	9	16/XII/58 (5)	6		+	+	+		**29/XI** (♂♀)	
	47	5/XI/58 (1)	3	+	+	+			**18/XI** (♂)	
	69	···	1		+	+			**25/XI** (♀)	3/5
Exp.II (PMS+ LH)	21	25/XII/58 (4)	5		+				30/XII (♂)	
	38	30/I/59 (3)	3		+	+			3/I (♂)	
	10	24/XI/58 (5)	6		+		+		**30/XI** (♀)	
	71	···	1		+				**26/XI** (♂)	2/4
Cont.I	22	25/XII/57 (3)	5						21/XII (♂)	
	271	2/I/58 (5)	7						12/I (♂)	
	56*2	14/XI/58 (1)	3						17/X abortion*2	
	73	8/II/59 (1)	2						26/II (♀♀)	0/3
Cont II	12	29/XI/58 (4)	5						2/I (♂)	
	54	22/III/58 (1)	3						31/XII (♀)	
	79	29/III/59 (1)	1						25/XII (♀)	
	55	24/IV/58 (1)	3						28/II (♀)	
	42	4/II/59 (2)	3						6/II (♂)	0/5

*1 Roman numerals indicate the day after the start of the treatment. : I — III : 12.5mg × 2 × 3 progesterone, IV — V : intermission, VI : 1,000 i.u. PMS, VII : 200 Rb.u. LH rich gonadotrophin (only for Exp. II).

*2 Omitted from the score.

b) *Trial III*

The resuts obtained in Trial III are given in Table 2.

i) *Oestrous response in behaviour*: Weak oestrous signs started from 24 hrs. after PMS treatment. It became gradually stronger from that time, but not so strong as seen in the breeding season. As a result, the help could not be avoided at the time of mating. On the contrary, some treated with oestrogen showed very clear oestrus and were easily mated.

In this trial performed in May, however, the intensity of male desire was found to be unexpectedly weak and then it was dfficult to mate every ewe with enough times during the time the oestrus existed.

No marked differences in the oestrous response of ewes were detected among the groups of different conditions (A—C, Tab. 2).

ii) *Lambing results*: Very disappointing lambing results were yielded this time; 0/6 in A, 1/4 in B, and 1/10 in C groups. However, as seen in Table 2, all ewes proved their sound fertility, giving birth at a later date. In addition,

Table 2. Animal condition and results in oestrus, mating and oestrogen concentration in 1960 (Trial III).

Group	Anim. no.	Previous date of lambing (no. of birth)	Age	Oestrus*2 and Mating VIII*1	IX	X	XI	XII	XIII	(1/VI) XIV	Date of birth*3 (no. and sex of young)	Oestrogen conc'n. in blood plasma*4
A	9	29/XI/59 (6)	7	−	⊥	+	++	+	+	+	8/I/61 (♂♀)	
	10	30/XI/59 (6)	7	−	−	+++	+	+	+	−	21/III/61(♂♂)	
	34	19/II/60 (4)	4	−	⊥	+	+	+	+	+	14/I/61 (♂♀)	+
	38	3/I/60 (4)	4	−	−	⊥	⊥↙	+++	+++	−	···	+
	20	19/I/60 (4)	5	−	⊥	++	+	+	+	−	25/I/51 (♂)	
	21	30/XII/59 (5)	5	−	⊥	++	++	−	−	−	16/I/51 (♂♀)	
	47	18/XI/59 (2)	3	−	⊥	++	++	++	+	−	···	
	69	25/XI/59 (1)	2	−	⊥	++	+	++	⊥	⊥	···	
	71	26/XI/59 (1)	2	−	⊥	⊥	+↙	+++	+++	+++	10/II/61 (♀)	
B	12	2/I/50 (4)	6	−	⊥	⊥	+	+	⊥	−	···	
	22	21/XII/59 (3)	5	−	⊥	+++	++	++	+	−	30/V/ (♀)	+
	42	18/I/60 (3)	4	−	−	++	++	++	++	+	29/I61 (♀)	
	54	31/XII/59 (2)	4	⊥	⊥	+	++	++	⊥	⊥	11/XI/69 (♂)	
	56	16/X/59 (3)	4	−	⊥	+++	++	++	⊥	−	**21/X/60** (♀)	
C	55	28/II/60 (2)	4	−	−	↘++	↘+++	++	++	++	6/II/61 (♀)	
	73	26/II/60 (2)	2	−	⊥	+	++	++	+	−	12/II/61 (♂♂)	
	7370	22/III/60 (1)	1	−	⊥	+	++	++	+	−	**21/X/60** (♀)	
	12724	27/III/60 (1)	1	−	⊥	↘++	↘+++	+++	+++	+++	···	+
	9534	31/III/60 (1)	1	−	−	+	++	++	++	⊥	···	
	7378	10/IV/60 (1)	1	−	⊥	⊥	++	−	⊥	⊥	17/II/61 (♀)	
	12651	11/IV/60 (1)	1	−	−	++	↘++	↘+++	+++	⊥	3/I/61 (♂)	
	7942	15/IV/60 (1)	1	−	−	+++	++	++	+	++	5/II/61 (♀)	
	6023	15/IV/60 (1)	1	⊥	−	−	⊥	+++	+++	+++	10/II/61 (♂)	
	7834	18/IV/60 (1)	1	−	−	⊥	+	+	⊥	−	16/II/61 (♀)	
	13033	21/IV/60 (1)	1	−	−	++	⊥	−	−	−	5/II61 (♀)	+
	13027	21/IV/60 (1)	1	−	−	⊥	⊥↙	++	++	+++	···	+
	7971	28/IV/60 (1)	1	−	−	⊥	⊥	++	⊥	−	27/II/61 (♀)	
	7081	1/V/60 (1)	1	−	⊥	+	++	++	+	−	···	

*1 Roman numerals indicate the day after the start of the treatment;
I−V : 12.5 mg×2×5 progesterone, VI−VII : intermission, VII : 1,000 i.u. PMS for A, 750 i.u. for B and C, Some of each group were treated further with oestrogen (↘) 0.7mg and (↙) 0.5mg.

*2 Oestrous intensity is expressed as follows : +++indicates ewes which stand still to accept ram, ++ ewes need help by holding lightly at mating, + not strong desire, and — slight indication of oestrus, +++ and ++ underlined means successful mating.

*3 Date in bold letter show positive cases.

*4 Blood samples were drawn at 24 hrs.(IX) after PMS injection, and + indicates the oestrogen concentration 2.2> >0.44 μg oestrone equivalent / l of blood plasma.

no differences of birth date between the three groups seemed to exist. The present trial was a complete failure.

iii) *Measurement of oestrogen concentration in the bloon plasma*

Blood samples taken at 24 hr after PMS injection were determined with the bioassay. The results will be presented later, together with other results.

II) *Responses in oestrus and ovary, and changes of oestrogen concentration in the blood plasma either during breeding season or at the time of induction treatment*

a) *Response in oestrous behaviour and in the ovary*

The results obtained are shown in Table 3.

Oestrus continued for 24 or 48 hr in two cycles during the mid-breeding season when the blood sample was drawn.

Four out of six ewes were treated with two hormones in conjugation, progesterone was given for three days (No. 5—8), but for five days to the rest two (No. 9, 10). The grade in congestion and swelling of vulvae, and mucin content of uterine excrete changed gradually to some extent, but were not so distinguished as those of the breeding season. The teasing of the other ewes (No. 9, 10) failed to obtain any sign of mating throughout four days after PMS treatment. This response is thought to be one of the poorest including the last three trials.

Ovulating points ranging from 1 to 2 were found in the ovary in all six cases by the examinations performed at 72 hr after PMS treatment. A few large follicles (more than six mm in diameter) were seen in No. 7—10 and 13, at the beginning of the treatment, despite the time of the year being in mid-anoestrum. Also, considering from the existence or non-existence of the corpora lutea, some changes in the ovary, were probably happening at the time of the year, even not regularly.

With separate treatment of either progesterone or PMS, all four were negative in oestrous behaviour, except one of progesterone group (No. 11) showed a positive on the fourth and fifth day after the final injection. As regards the ovulation of these cases, all of progesterone treated and one out of two in the PMS group showed the positives.

In No. 13 and 14 those were treated with progesterone one month ago, relatively fresh corpus luteum was presented at the beginning of PMS treatment.

b) *Changes in oestrogen concentration of the blood plasma*

i) The time of blood sampling is given in Table 3. The results of the concentration are illustrated in Figure 1 and express the range of upper and lower limits of the individual measures. Besides Figure 1—I of the changes according to the time course of natural oestrous cycle, the results of 1960 and 1961, these two differed with regard to the duration and total dosage of

Table 3. Conditions of animals at the time of sampling

i) Ewes during breeding season

Case No.	Animal name	Date of onset of oestrus	Behaviour to ram (+, −) (=Date of sampling) I	II	III	VIII	Remarks
1	A	30/X/60	++	++	−−	−	Teasing was carried ont twice daily: 9 AM and 5 PM, and the day when ewes were found to stand for service was regarded as the 1st day.
2	B	3/XI/60	++	++	+−	−	
3	A	17/XI/60	++	+−	−−		
4	B	18/XI/60	++	++	−−		

ii) Ewes treated during non-breeding seasan

Case no.	Anim. name	Treatment Date	Dose*1	Behaviour to ram*2 I	II	III	IV	Date of sampling*2	Ovarian response*4 A(0) C.L.,	L.M.,	O.P.	B(72) CL,	L,	M,	O.P
5	C	28/IV～ 2/V/60	12.5×2 ×3,(2),1,000						L*3	0	2	0	0	1	1	1
									R	0	3	0	0	1	1	1
6	D	Ibid							L*3	0	2	0	0	0	5	0
									R	0	1	0	0	0	1	1
7	C	15～ 20/VI/60	12.5×2 ×3,(2),1,000					(−120)0,12,24	L	2	0	0	0	0	1	1
									R	1	3	0	0	0	2	2
8	D	Ibid						48, 72	L	1	3	0	0	0	2	2
									R	0	4	0	0	1	3	0
9	E	12～ 19/VI/61	12.5×2 ×5,(2),1,000	−	−	−	−	(−168),0,12,24	L	1	3	0	0	0	2	1
									R	2	0	0	0	0	5	1
10	F	Ibid		−	−	−	−	48, 72	L	1	4	0	1	1	0	0
									R	0	4	0	0	1	2	1
				I	II	III	IV	V VI								
11	G	12～26/V/61	12.5×2×5	−	−	−	+	+ − 0, 24,72	L				1	1	1	1
									R				1	0	4	0
12	H	Ibid		−	−	−	−	− − 360	L				0	0	2	1
									R				0	1	2	0
13	G	26/VI/61	1,000	−	−	−	−	0, 24, 48,	L	0	1	0	0	1	0	1
									R	1	0	1	0	1	0	1
14	H	Ibid		−	−	−	−	72	L	0	0	0	0	2	0	1
									R	1	0	0	0	5	3	0

Note: *1 mode of hormone administration (see in Text)

*2 Behaviour and data of sampling show day and hrs after the final injection, respectively, and (−x) hrs. before, *i.e.*, the sampling at the start of treatment.

*3 AO of No. 5, 6 indicates exceptionally the ovarian response at 24 hr after PMS treatment.

*4 Abbreviations in the column of ovarian response, C.L., L., M., O.P. mean numbers of corpus luteum, large follicles (>6mm in diameter) middle follicles (3—6mm) and ovulation points.

progesterone, was presented in Figure 1—II and 1—III, separately.

In two cycles of two ewes, the oestrogen concentration rose at the second and first days of each cycle, after the finding of oestrous behaviour by the teasing. The values of the concentration were 6> >2*, 2> >0.66 $\mu g/l$, respectively. The other determinations, including that of the eighth day of the first cycle, showed the values less than 0.66 $\mu g/l$.

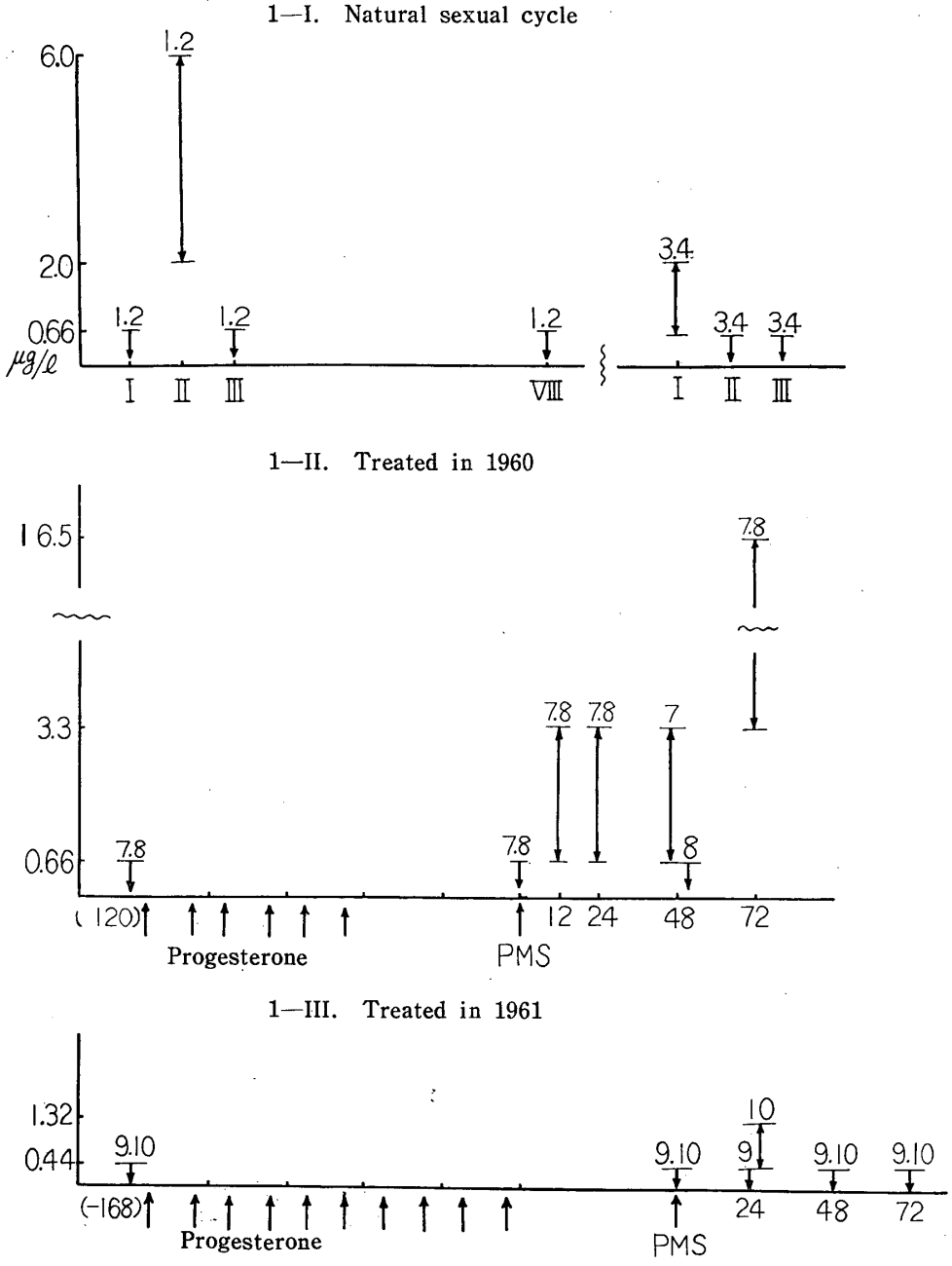

Fig. 1. Changes of oestrogen concentration in the blood plasma of ewes in the breeding season and the inducing treatment (no. in the figures indicates case no.).

* $x> >y$ means the oestrogen concentration is between x and y μg equivalent to oestrone/l.

The results of the combined treatment appeared to be greatly different among groups, of which could only be distinguished by the progesterone treatment, and less so among the individuals within the groups. Looking into Figure 1—II, the two values of No. 7, 8, those treated for three days with progesterone at 12 and 24 hrs. came up to 3.3> >0.06 μg/l. Then at 48 hrs., one remained at the same level, but the other fell below 0.66 μg/l, and both went up to 16.5> >3.3 μg/l at 72 hr. Two in Figure 1—III, which were treated with progesterone for five days, showed rather less marked changes in the concentration, and only one positive case (No. 10) of 1.23> >0.44 μg/l at 24 hr, all of the rest measurements at −72, 0, 48 and 72 hrs including 24 hr of No. 9, counted less than 0.44 μg/l. So that No. 9 stood with no changes throughout the course of the determination undertaken.

Neither progesterone nor PMS treated alone caused any positive result at all so far as measured by the present method, and remained unchanged with the values less than 0.44 μ/l.

ii) Here the authors wished to show the results from Trial III in which the blood plasma was taken at 24 hr after PMS injection.

Only six out of 28 cases resulted in the positive response of 2.2> >0.44 μg/l: two out of nine repeated group(A), one out of five newly treated (B) and three out of 14 lactating—newly treated (C). The other negatives were smaller than 0.66 or 0.44 μg/l. No close correlation between the positives and the intensity of oestrous response in behaviour at 24 or 48 hr could be found.

Although the oestrogen concentration at the oestrus of the ewes induced with the combined hormone were not consistent, if the authors dare to compare these values with those of the natural oestrous cycle, roughly speaking, it could be said that the oestrogen concentration at the oestrus of the two types increased to a similar extent. But a very high value of No. 7, 8 at 72 hr was exceptionally different.

Discussion

i) Further data with the same grade of successful percentage in the out-seasonal lamb production were obtained (Trial II). Summing up the results of this trial together with our previous data, eight out of 14 ewes yielded early lambs by means of the hormonal treatment in the anoestrum. Although the case number is still not enough to draw any final conclusion, the percentage in lambing score (57 %) obtained in our experiments with Corriedale in Japan could not be regarded as unsatisfactory, compared with those obtained by other breeds in Australia, Britain, France and U.S.A. (7, 10, 12, 32, 35). However, the present technique is not considered to be an absolutely sufficient one to apply to the practical field, so as to expect gratifying results in the lambing rate. In addition, a poor response in oestrous behaviour was observed in almost all of our cases.

It is unknown at the present stage whether the weak oestrus is connected with the incomplete lambing results, and whether this is due to the breed (Corriedale). The weakness in oestrus is thought to be improved, at any rate, since it is undesirable at the time of practical application.

Introducing LH into the techique, using LH rich gonadotrophin, is unlikely to be effective in increasing the lambing percentage, although LH is principally thought to be useful in securing ovulation (3, 24, 31).

ii) In trial III some attempts (repeated treatment, treatment with wet ewes and addition of oestrogen after PMS injection) were made to obtain progress in lambing result. Whereas, on account of unexpected weakness in the sexual activity (libido and probably spermatogenesis) of the rams, as noticed by some workers (4, 11, 45, 46), the lambing results were extremely bad. In this case, once more, not so prominent oestrus was demonstrated in the treated ewes.

As regards oestrogen concentration of the blood plasma, discussions will be given in the next paragraph.

iii) Before discussing on the oestrogen concentration, let us consider in general the possible factors which may influence the results in lambing. Some attempts have been made both to cover the more basal aspects of reproductive physiology in sheep, and to provide informations concerning to the practical usage of the procedures. In other words, since the beginning of this study, many experiments have been devoted to reproduce the same condition of ewes as natural oestrus. But promising way for the improvement has not thoroughly been established.

As noticed above, the weak response in the behaviour of induced ewes was observed throughout our trials, as some authors have already reported (11, 18, 35). Consequently the authors have estimated the oestrogen concentration in the blood plasma of ewes treated in the due technique, and compared this with that of the oestrous cycle during the breeding season, expecting to bring some lights on the problems. There has been no report on the oestrogen concentration in the blood plasma of ewes either of the ones treated with hormones or of the oestrous cycle.

The results obtained in the four cases of the combined administration, though all of them ovulated, showed different changes of oestrogen concentration during a few days after the treatment, and seemed to indicate no relation between the change of oestrogen and the intensity of oestrous behaviour. The latter, however, was again indistinct. In one group in which progesterone was given for three days, 75 mg in total, oestrogen rose after 12 hrs., whilst only a small increase was detected in one case in the other group treated with progesterone for five days, 125 mg in total. It is uncertain at this stage whether the differences of the changes in oestrogen concentration between the two groups could be attributed to the difference with regards to the modes of

progesterone treatment. On this problem a further discussion will be given later.

It is well known that the sexual cycle appears by the alternative actions of oestrogen and progesterone. Many evidences were presented on the quantitative relationship of these two hormones needed to induce oestrous responses both in behaviour and in cervical mucus of the ovariectomized ewes. They proved that progesterone given in advance made much smaller the amount of oestrogen required for the responses than that of oestrogen given alone (17, 21, 27, 29, 33, 31, 37). In our present experiment, endogenous oestrogen was estimated after the treatment of progesterone and PMS in combination. The results in the changes of oestrogen as mentioned in the results indicated generally the same level of rise around 24 and 48 hrs., both after PMS administration and after the finding of oestrus in the cycle, except the negative response and the very high rise at 72 hr, both of which happened in the induced group. Accordingly, the accurate interpretation of quantitative aspects of these hormones as to oestrous behaviour could not be done here.

Two modes of progesterone injection employed in the present experiment are principally not different as the procedure of the induction, as Gordon and others (11, 32, 35) demonstratad that a longer treatment of progesterone was effecetive in obtaining higher percentage of oestrous response than a shorter one. Furthermore, as was demonstrated, the disappearence of injected progesterone was considerably fast (5, 10, 28). If we assume that the acting progesterone in the ewe were to be at a constant and normal level after the two day's intermission, no or weak oestrus in our and other results is likely to come from the unfavourable ratio of oestrogen and progesterone in body fluid, *e.g.*, the oestrogen amount produced endogenously is abnormally high or low. Some previous authors discussed on the occurrence of the abnormal oestrus in connection with 'silent heat' which is often seen at the beginning of the breeding season (6, 30, 37).

At the same time there are other factos to be considered. Robinson proved (33, 36, 37) the effectiveness of a small dose of oestradiol (15—25 μg) given together with PMS in the induction of both oestrus and ovulation. Comparing these doses with the amount of oestrogen required to induce oestrus in spayed ewes which were pretreated with progesterone, he supposed (35) that a very minute different of oestrogen would decide the appearance of oestrus and that the amount necessary for oestrous response might differ depending on breeds and ages of ewes. Especially the breed difference in responses to the usual treatment for the induction, as McDonald also claimed (18), appeared to be taken into consideration. At any event, a method of hormone treatment to correct the unbalance of the ratio mentioned above, must be found by further studies.

Although oestrus and ovulation caused alone or altogether by progesterone treatment has already been reported (8, 9, 34), ovulation occurred before 72 hr, after the final injection, and oestrus on the fourth and fifth day in one of the two treated were found in our experiment. But oestrogen of the plasma did not show any rise as far as our determination went (up to 72 hr).

Many papers have shown the change of plasma oestrogen in women during mentrual cycle and pregnancy. So far as the authors know, there is no report on the oestrogen concentration in the blood plasma of ewes either in the cycle of breeding season or in the inducing treatment during anoestrum. Only Basset *et al.*(1, 2) measured the oestrogen excretion in the urine of ewes, and concluded that no definite change due to the stage of sexual cycle took place. Although a slightly lower value was obtained during the earlier half to the cycle (1.1—1.2 μg oestrone equivalent/total urine of day), compared with 1.1 or 1.3—2.4 μg during oestrus and later half of the cycle, respectively.

In cows, Ogasa ahd Yamauchi demonstrated the curve of oestrogen with two peaks at oestrus and at mid-luteal phase during the cycle, respectively; and further, they could find no clear relation between the oestrogen content and the intensity of mating desire (23). In the mean time, Higaki and his collaborators carried out a more detailed determinetion, and showed the change of oestrogen with the same trend. In the same paper they clarified, in addition, the appearance of a very high ratio of oestrogen and progesterone at oestrus (15, 16). Despite the coincidently increase of oestrogen at oestrus stage of the cycling ewes, no rise seemed to occur at a mid-luteal stage in our results.

As regards does, Nagasawa and his coworkers obtained (22) considerably variable concentrations of oestrogen, as the initial values in the experiment of lactation induction caused by hormone during anoestrus (June).

It is widely believed, though informations given are not sufficient, that the ovarian activity of ewes and does moved gradually and irregularly in the non-breeding season, differing depends on the month, geographical location and breeds (7, 13, 14, 30, 37, 47). On this point, the fact obtained from the experiment on the hormonal induction of lactation seems to be interesting: that is, the ovarian state of does when treated with oestrogen or oestrogen plus progesterone influenced greatly on the degree of development of the mammary gland and on the subsequent lactation (12, 20).

Consequently the significance of hormones, especially of progesterone in the inducing technique, although the latter had been established in a sense, *e.g.* in relation to the changes of endogenous or exogenous oestrogen must be brought to light by future investigation.

Conclusion

Two experiments with Corriedale were conducted by i) field trials of out-seasonal production of lambs, and ii) determination of changes of oestrogen concentration in the blood plasma of ewes treated with progesterone—PMS, compared those of the oestrous cycle in the breeding season.

I) Further results were added to the out-seasonal lamb production attempted in late June with 23 ewes in total at the University Farm. Two experimentals and two controls were set up, but seven ewes were ommitted from the final count due to either loss or abortion. Hormones were administered as follows: 12.5 mg of progesterone twice daily for three days followed by 1,000 i.u. PMS, putting two day's interval between them. One of the experimentals (Exp. II) were further given 200 Rb. u. LH rich gonadotrophin on the day after PMS. Besides a control group consiting of ewes of the same condition as the experimental, another eight (3 of them lost) was chosen from the flock, because the ewes of the Farm had showed a tendency to early lambing within the year, and this, if had happened, might make confusion in the results. Except 11 days for treatment, the ewes were raised, putting them on the mountain pasture together with rams from the beginning of June till the end of October. By such treatment, oestrus, though it was so weak that the ewes needed help at mating, began to appear since 48 hrs. after PMS injection and lasted for two to four days. From the date of birth, the positive results obtained were 3/5, 2/4 in Exp. I and II; 0/3, 0/5 in Cont. I and II, respectively. Assuming no differences among the two experimentals and controls, the total came to 5/9 and 0/8. It approved again that the trial was effective to induce success of nearly half. Addition of LH into the procedure was unlikely to show any apparent advantage, judging from the final score.

II) The third field test was carried out with 28 ewes in May in the year after the preceding trial, and was expected to yield additional information on the subject: i) the effects of repeated employment of ewes for the treatment, ii) of lactating, and iii) of oestrogen injection later than 48 hrs. of PMS treatment to intensity oestrus. On account of the unexpectedly weak interest of three rams (two of which had been used in the preceding year) to the ewes in May, nearly complete failure in lambing results happened. But the ewes' fertility in the breeding season was provided by normal lambing. Subsequently this trial supplied only data on oestrous behaviour and oestrogen cencentration in the blood plasma of the ewes induced.

Again, the intensity of oestrous behaviour of these groups was not satisfactory, though progesterone treatment extended from three to five days.

The results of oestrogen determination will be persented in the next paragraph.

III) Changes of oestrogen concentration in the blood plasma of the ewes treated with Progesterone—PMS were measured by our method which is slightly modified from Sulman's. The changes were compared with those either of the breeding season or of progesterone and PMS alone.

Generally speaking, there were two types of oestrogen changes possibly due to the way of progesterone administration: in one set (75 mg progesterone during 3 days)oestrogen increase during 12—24 or 48 hrs., and then further rose at 72 hrs after PMS treatment. On the other hand, the other set (125 mg, 5 day) hardly showed changes in oestrogen, except for a slight increase at 24 hrs. in one of two ewes. But the higher level of oestrogen at 12—48 hr after PMS injection in the combined treatment seemed to be the same as that of oestrus during the breeding season. Oestrous behaviour in this experiment was again weak, although all ewes provided positive ovulation.

In single treatments, oestrogen remained at a low level unchangeably.

Measurement of oestrogen in the plasma 24 hr after PMS (trial III) demonstrated a few positive cases with the value of $2.3> >0.44\ \mu g/l$, but most cases remained below $0.44\ \mu g/l$. The positive cases showed no close relation to either the type of treatment or to the intensity of oestrous desire.

Our results including that of our preceding experiment were discussed, referring to those of other reports on this line. Further fundamental researches, *i.e.*, on the relation of oestrogen and progesterone in the body fluid to correct the oestrous behaviour, are required. Besides, it may be remarked here that breed differences in the responses to the inducing technique should be clarified.

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