THE INFLUENCE OF VARIATIONS IN THE LEVEL OF NUTRITION ON REPRODUCTION IN THE EWE

by

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ABBREVIATIONS

ARGG	anti-rabbit gamma globulin
BSA	bovine serum albumin
CPM	counts per minute
C.V.	coefficient of variation
DF	degrees of freedom
EDTA	ethylenediaminetetra-acetic acid
FSH	follicle stimulating hormone
h	hours
HCG	human chorionic gonadotropin
im	intramuscular
LH	luteinizing hormone
LH-RH	luteinizing hormone releasing hormone
min	minutes
NRS	non-immune rabbit serum
PBS	0,14 M NaCl, 0,01 M $NaPO_4$
PMSG	pregnant mare serum gonadotropin
RGG	rabbit gamma globulin
RH	releasing hormone
SAMM	South African Mutton Merino
S.E.	standard error
TDN	total digestible nutrients
TSH	thyroid stimulating hormone

C O N T E N T S

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GENERAL INTRODUCTION

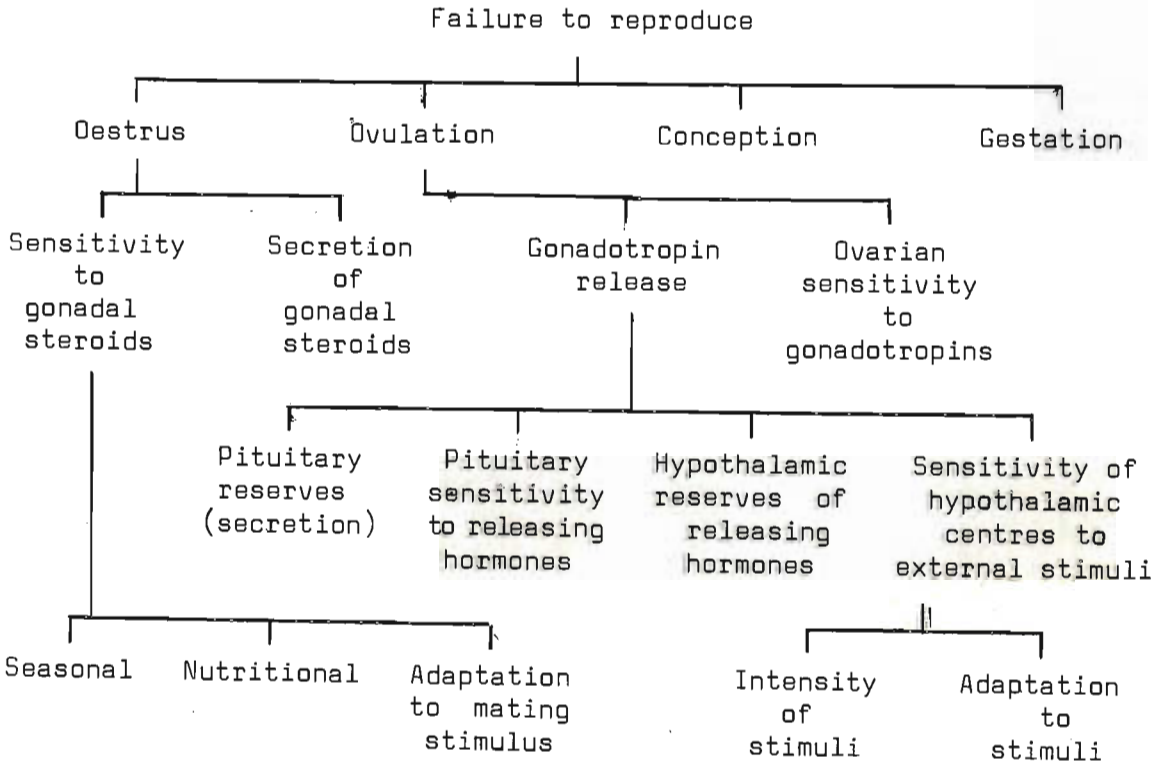
The physiographic features and nature of the vegetation make South Africa one of the pastoral countries of the world (Joubert, 1954); only some 11 percent of the total area of 105 924 159 hectare can be cultivated. Consequently, livestock farming constitutes an important branch of agriculture and over the period 1966 to 1970 the wool produced by the 25 - 30 million sheep (83 percent Merino) contributed 23 percent to the value of agricultural exports. According to figures published by the Division of Agricultural Marketing Research (1971) the latter constituted 33,6 percent of the total value of all exports.

Periodic droughts, extending over a number of years, combined with unscientific farming methods, result in repeated severe reduction in the sheep population. In addition, agriculturists are perturbed by the low reproductive rate of the wool sheep in the extensive farming areas. According to Hunter (1962), lambing percentages of less than 50 percent are not unknown in such areas. These extensive areas, which constitute more than half the total area of South Africa, receive a low and erratic summer rainfall accompanied by dry winters (Joubert, 1954). The results quoted by Adler (1964) show that even in areas enjoying a higher, and more evenly distributed rainfall, there can be little reason for complacency regarding the reproductive rate of both cattle and sheep.

Depending on the interaction between climate and soil, both quantitative and qualitative nutritional problems arise in the natural herbage. Although the vegetative cover is scanty in the low rainfall areas, the feeding value does not exhibit notable seasonal variations (Joubert, 1954). The grazing animal is, however, often underfed because of overstocking. In the more humid grassveld regions, animals maintained exclusively on the natural herbage are likely to encounter both quantitative

and qualitative nutritional deficiencies. This is due mainly to the marked variation between summer and winter climatological conditions (Joubert, 1954). Consequently, where cropping is possible, the nutrition of the ewe, which lambs and lactates during autumn, is based on the production of cereal crops, notably oats. In the summer rainfall areas the distribution of the rainfall is often such that insufficient growth of these crops occurs and undernutrition of the lactating ewe is a common occurrence. The spring rains are also unreliable so that, in spite of the very dubious practice of late-winter burning of veld, the nutritious natural grazing produced is often insufficient to meet the needs of the ewe lambing at this time.

Inadequate nutrition has been shown to be an important factor influencing the reproductive rate of female sheep (Roux, 1936; Hunter, 1962; Smith, 1962; Coop, 1964, 1966). On the basis of the data presented by Dun, Ahmed & Morrant (1960) and Mullaney (1966), the more obvious mechanisms by which a failure to reproduce could be mediated, may be diagrammatically represented as follows:



From this scheme it can be seen that the hypothalamic centres controlling both cyclic gonadotropin release and oestrous behavioural patterns are particularly implicated. A similar dichotomy has been proposed by Symington (1969).

The investigation to be described was initiated in an attempt to examine the productive wastage which is associated with a failure to maintain adequate levels of nutrition during lactation. Attention was focussed on this stage of the productive cycle since the available evidence suggested that female sheep were likely to be exposed to under-nutrition at such times. An attempt has also been made to identify the physiologic mechanisms by which poor feeding reduces the reproductive rate of ewes. In view of the facilities available it was considered advisable to limit the investigation to those aspects which could be accommodated. These were the stimulus applied to the ovary (i.e. the level of gonadotropin in the circulation) and the sensitivity of this target organ to such stimuli.

CHAPTER I

THE INFLUENCE OF VARIATIONS IN THE LEVEL OF NUTRITION DURING LACTATION AND THE PRE-MATING PERIOD ON THE REPRODUCTIVE PERFORMANCE OF THE EWE

INTRODUCTION

In the grassveld regions of South Africa it is common practice to mate ewes in spring and early summer (Adler, 1964) so that the lambs are born at a time when their growth can be optimum (Reyneke, 1969). However, in common with the finding in Australia, a number of factors combine to prevent the maximum lambing potential being attained from such early-season mating.

Fertility can be improved by mating ewes in autumn, which coincides with the peak of sexual activity (Watson, 1953; Dun et al., 1960; Allden, 1956; Shelton & Morrow, 1965; Moule, 1966; Watson & Radford, 1966).

Dun et al. (1960) have accounted for the observed 37 percent greater number of lambs produced by mating during autumn, rather than in spring, on the following basis:-

Incidence of oestrus	11 percent
Pattern of oestrus	5 percent
Incidence of multiple ovulation	57 percent
Conception rate	27 percent

Shelton & Morrow (1965) and Watson & Radford (1966) have also demonstrated the importance of the proportion of ewes being mated at the

different times and Hunter (1964) has discussed this problem.

It has long been thought that the seasonal differences in the reproductive rate of ewes might be associated with variations in the level of nutrition. It has been clearly demonstrated that poor nutrition can extend the anoestrous period (Roux, 1936; Hunter, 1962; Smith, 1964a, 1965), reduce the incidence of oestrus during the breeding season (Hafez, 1952; Allen & Lamming, 1961; Smith, 1966) or precipitate onset of anoestrus (McKenzie & Terrill, 1937; Smith, 1962). Similarly, it is generally recognised that the level of nutrition and attendant changes in bodymass play an important role in determining ovulation rate in the ewe (Clark, 1934; McKenzie & Terrill, 1937; Allen & Lamming, 1961; Wallace, 1961; Hill, Lamond & Godley, 1969; Lamond, Gaddy & Kennedy, 1972). The practice of flushing during and/or prior to the breeding period is usually found to increase the ovulation rate (Wallace, 1961; Allen & Lamming, 1961; Killeen, 1967).

In his searching studies on the practice of flushing, Coop (1964, 1966) has shown that ovulation rate is partly dependent upon bodymass of a ewe at mating (the static effect) and partly upon the rising plane of nutrition at this time (the dynamic effect).

The purpose of the experiments which were undertaken was to investigate the significance of variations in the level of nutrition of woolled sheep exposed to a regular regime of mating and lambing. The conditions likely to be encountered by the commercial producer were approximated as far as possible.

PROCEDURE

Plan of the experiment

In order to study the effects of undernutrition during lactation

on the annual reproductive performance of ewes, over a period of six consecutive years, groups of ewes were placed on rations designed to maintain condition during lactation (high plane) or to result in a loss in bodymass of approximately 20 percent during the same period (low plane). After weaning the lambs, all the ewes received the same ration. Three weeks prior to the introduction of entire rams the treatment groups were further sub-divided and the ration increased in certain cases so as to produce a flushing effect. The basic experimental treatments are represented in Fig. 1, including certain of the modifications which were introduced.

Experimental animals

The experimental animals were drawn from a flock of Merino ewes and a flock of ewes varying from ½ SAMM - ½ Merino to almost purebred SAMM, hereinafter referred to as crossbred ewes. Allocation to the experimental treatments at parturition was by random selection without reference to any previous treatment. The same population of ewes was utilised in consecutive years. During 1970 it was possible to incorporate maiden ewes and in 1972 females which had already produced one lamb joined the flock from which experimental animals were selected. Allocation of the ewes to the high and low planes of nutrition, respectively, during 1972 was in the ratio of 3:2. This procedure was followed in order to obtain approximately the same number of ewes, on each level of feeding, exhibiting oestrus during the seasonal anoestrus period (see Chapter 3). Only ewes with single lambs were utilised in the experiment and in the case of twin lambs, one lamb was removed so that each ewe suckled only one lamb.

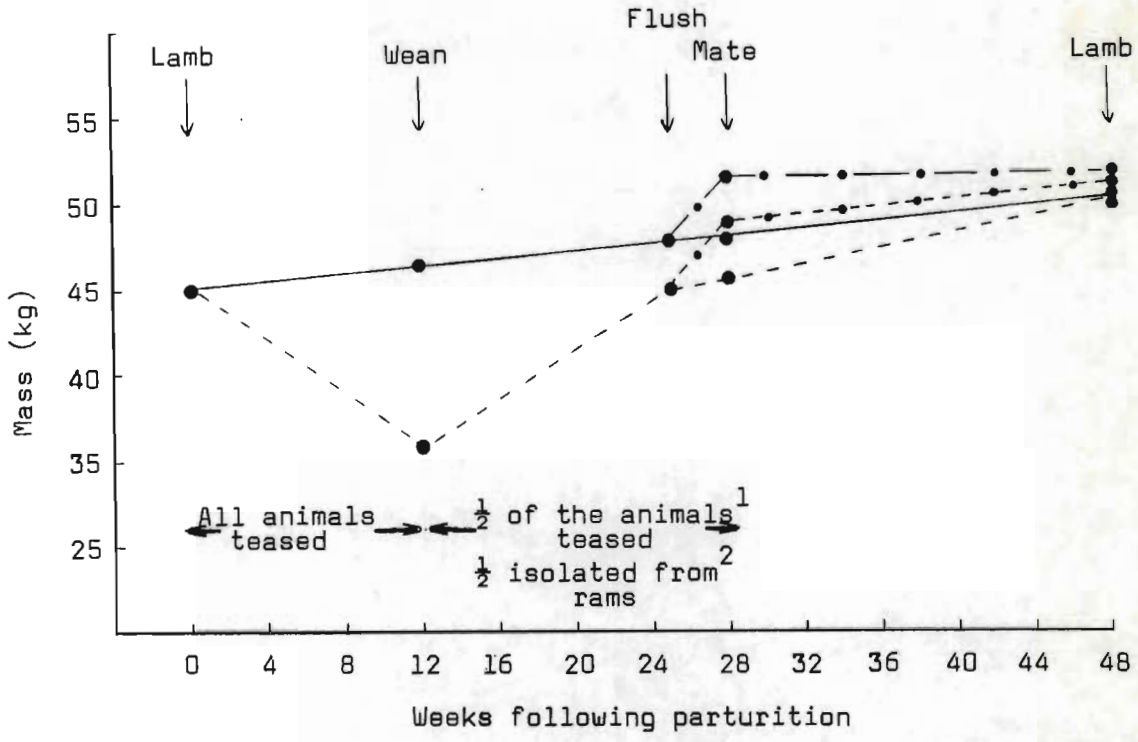


Fig. 1. Plan of the experimental treatments illustrating the expected changes in mass as a consequence of (i) high (——) and low (----) planes of nutrition applied during lactation and (ii) the flushing effect (——·——, ---·---) three weeks prior to joining with fertile rams.

1. Not applied during 1967.
2. Not applied during 1971 and 1972.

Rations fed

(a) Ewes

Immediately after parturition, the ewes and their lambs were allocated to the various treatment groups and placed on dry-lot in partly roofed pens. The ration supplied during the 84-day lactation period was based on maize silage, milled lucerne hay and a concentrate mixture consisting of 90 parts maizemeal and 10 parts whale carcase meal. The relative amounts of the various feeds were selected to meet the feed requirements of the type of animal included in the experiments, as given in Table 1.

Table 1. Nutrient requirement of ewes, estimated nutrient content and composition of basic ration fed to ewes on two planes of nutrition during lactation.

Ration constituent	High plane ration				Low plane ration			
	Quantity (kg)	Dry matter (kg)	Dig. protein (kg)	TDN (kg)	Quantity (kg)	Dry matter (kg)	Dig. protein (kg)	TDN (kg)
Maize silage	1,36	0,32	0,01	0,16	0,68	0,16	0,004	0,08
Lucerne hay	0,91	0,81	0,09	0,45	0,45	0,41	0,04	0,23
Concentrate meal	0,45	0,39	0,04	0,36	0,22	0,19	0,02	0,18
Total		1,52	0,14	0,97	Total	0,76	0,064	0,49
Requirements[1]	45 kg lactating ewe	1,54-1,86	0,13-0,14	1,0-1,18	45 kg non-lactating ewe	1,23	0,07	0,68

[1] According to Morrison (1956), Thomas & Aitken (1959), N.R.C. (1968).

The amounts of feed actually fed were adjusted according to the average mass of the animals selected each year and the quality of the available maize silage, so that the pre-determined changes in bodymass could be attained. Lactation was terminated according to the average date of parturition of groups of ewes in which the range in the dates of lambing

did not exceed seven days. After weaning, all the ewes received the same quantity of feed. The amounts given were sufficient to maintain bodymass and allow for normal wool growth in animals adequately fed during lactation.

Commencing on 11 October of each year, the animals to be flushed prior to mating received increased quantities of concentrate meal, and during the first two years of the experiment (1967, 1968) the daily allowance of lucerne hay was also raised. At this time the daily allowance of maize silage was reduced in the case of the ewes not undergoing flushing. When mating with entire rams was initiated (1 November) the experimental animals were placed on kikuyu pasture (Pennisetum clandestinum). A system of rotational grazing was practised until the subsequent parturition. At this stage pen-feeding was again initiated, and non-lactating animals were placed on winter rations of maize silage and legume hay.

(b) Lambs

(i) Suckling period

At approximately one week of age the lambs were offered free access to the same feeds as supplied to the lactating ewes, except that the concentrate meal was replaced by a creep-ration calculated to contain 17 percent crude protein.

(ii) Post-weaning

After weaning, and until commencement of the first grazing season, the quantity of lucerne hay was limited to 0,45 kg per lamb per day. The creep ration was replaced by maizemeal (0,45 kg/day) and maize silage was supplied free choice. During the second and subsequent winter-feeding periods the daily ration for each animal comprised 0,22 kg maizemeal, 0,45 kg lucerne hay and maize silage ad lib.

Occurrence of oestrus

The period from lambing to 31 October

Once the lactating ewes had been placed on dry-lot, daily observations for oestrus were made at 0700 h and 1500 h using sexually active, vasectomized rams. These teaser rams were changed at least every seven days.

During 1967, observations for oestrus were terminated at the end of the lactation period, this was done in order to minimise the stimulatory effect of the continued presence of males (Lishman & Hunter, 1966, 1967; Lishman, 1969), a situation which would be different to that occurring in commercial flocks. However, the results obtained emphasised the need to continue observations for oestrus until the beginning of the next tupping period. Consequently, the number of animals in each treatment group was doubled in 1968. At weaning, each treatment group was divided into two sub-groups. The ewes in the one sub-group were isolated from rams until 17 days prior to the introduction of entire breeding rams. The normal twice-daily observations for oestrus were resumed in this sub-group on 15 October. In this way it was hoped to synchronise the period of maximum mating with the end of the flushing period. In the remaining sub-group, observations for oestrus continued uninterrupted until 1 November. The experiment was terminated at this stage during 1972.

The ewes which were isolated from rams were housed in pens similar to those used during the lactation period. These pens were situated at least two kilometres from the nearest ram. The results obtained for the sub-groups either continuously associated with or isolated from rams have been combined except for the occurrence of mating after weaning and during the period 15 October until removal of the entire rams.

The period of joining with entire rams

On 1 November, each year, all experimental groups were combined into one flock according to breed. Entire breeding rams (three %) bearing raddle crayons (Radford, Watson & Wood, 1960) were introduced and the ewes marked were recorded daily to the end of the six-week mating period.

Ewe-lambs

The ewe-lambs born during 1969 were joined with raddled, vasectomized rams when they had reached an average age of six months. Three months later this was changed to twice daily teasing and these observations continued until their first joining with entire breeding rams (average age 19 months).

Determination of bodymass

The bodymass of each ewe and lamb were recorded three days after parturition and thereafter on every Wednesday until weaning. For the ewes the interval between recordings was then increased to 14 days until the subsequent parturition. The bodymass of each lamb was recorded at monthly intervals from weaning onwards.

Feed intakes

While the ewes were pen-fed the mass of the different ration components was determined each day. Feed refusals were recorded daily prior to the provision of fresh feed. During the lactation period the lambs were separated from the dams when the latter received their daily concentrate meal allowance.

Yield of wool

At shearing the total mass of the fleece, including belly, was recorded for each ewe. In 1968 an attempt was made to simplify the procedure by determining the bodymass of the ewes prior to and after shearing. This procedure was found to be inaccurate, and was, therefore, abandoned.

General management

Accepted principles of dosing, mineral supplementation, inoculation, castration and tail-docking were applied throughout the course of these experiments, and all the sheep were shorn prior to 1 November each year.

RESULTS

Ewes

Feed intakes

The average daily feed intake during the lactation, post-weaning and flushing periods is presented in Table 2.

Soon after initiation of the experiment during 1967 it became clear that the planned intake of 1,36 kg maize silage for the ewes on the high plane of nutrition would not be attained with the quality of silage available. This could be ascribed mainly to the large size of the feed particles and the high moisture content of the silage. Increasing the quantity of lucerne hay did not produce the desired response of maintaining body condition. Therefore, the daily allowance of concentrate meal was increased during subsequent years. With the introduction of a better quality silage (higher dry matter content) during 1971, the daily intake of this feed was more than doubled.

Table 2. Average daily feed intake of ewes on high and low planes of nutrition during lactation and prior to mating.

Year	Type of ewe	Nutritional plane:-		Feed intake (kg) during different periods:								
				Lactation			Post-weaning			Flushing		
		Lactation	Pre-mating	Concen-trate	Lucerne hay	Maize silage	Concen-trate	Lucerne hay	Maize silage	Concen-trate	Lucerne hay	Maize silage
1967	Merino	High High	High Low	0,51	1,14	1,11	0,30	0,54	0,94	0,57 0,28	0,80 0,45	0,69 0,57
		Low Low	High Low	0,25	0,59	0,66				0,57 0,28	0,79 0,45	0,80 0,58
1968	Cross-bred	High High	High Low	0,62	0,85	0,92	0,34	0,45	1,13	0,57 0,34	0,74 0,45	0,71 0,45
		Low Low	High Low	0,31	0,46	0,59				0,57 0,34	0,81 0,45	0,77 0,45
1969	Merino	High High Low Low	High Low High Low	0,73 0,32	0,91 0,45	0,84 0,49	0,40	0,45	1,13	0,80 0,40 0,80 0,40	0,45	1,13
1970	Merino	High Low	Low Low	0,64 0,25	0,85 0,45	0,72 0,42	0,34	0,45	1,13	-	-	-
	Cross-bred	High Low	Low Low	0,75 0,39	0,88 0,45	1,09 0,62	0,40	0,45	1,13	-	-	-
1971	Merino	High Low	Low Low	0,69 0,34	0,86 0,45	2,19 0,93	0,34	0,45	1,34	-	-	-
1972	Merino	High Low	Low Low	0,77 0,35	0,77 0,45	1,56 0,71	0,34	0,45	1,10	-	-	-

The ewes on the restricted feed intake during lactation were offered approximately 50 percent of the amounts consumed by the animals on the high plane of nutrition. The former ration formed the basis of that applied after weaning, except that the quantity of maize silage was slightly more than one kg per day.

During the flushing periods of 1967 and 1968 an attempt was made to achieve the same feed intake as that shown by ewes on the high plane during lactation. However, the planned intake of roughage was not obtained and during 1969 only the concentrate ration of the ewes flushed was therefore increased.

Changes in bodymass

The variations in average bodymass of the experimental groups of ewes, commencing three days after parturition and continuing until approximately two weeks prior to the initiation of the subsequent lambing season, are summarised in Fig. 2. The average bodymass at certain important stages of the experiment is presented in Table 3. From the data in Fig. 2 it is evident that during the first two years of the experiment the ewes on the high plane of nutrition exhibited a small loss in bodymass during the lactation period. However, after 1968 the modified daily ration (Table 2) resulted in a nett gain in total bodymass, approximately equivalent to the expected normal growth of the fleece viz., one percent per month increase in total bodymass.

The desired loss of 20 percent in bodymass was very nearly realised among the ewes subjected to undernutrition (Table 3). The maiden ewes exposed to this treatment showed a somewhat smaller loss than the older sheep.

After weaning, when all the ewes received the same amount of feed, those subjected to feed restriction during lactation exhibited the

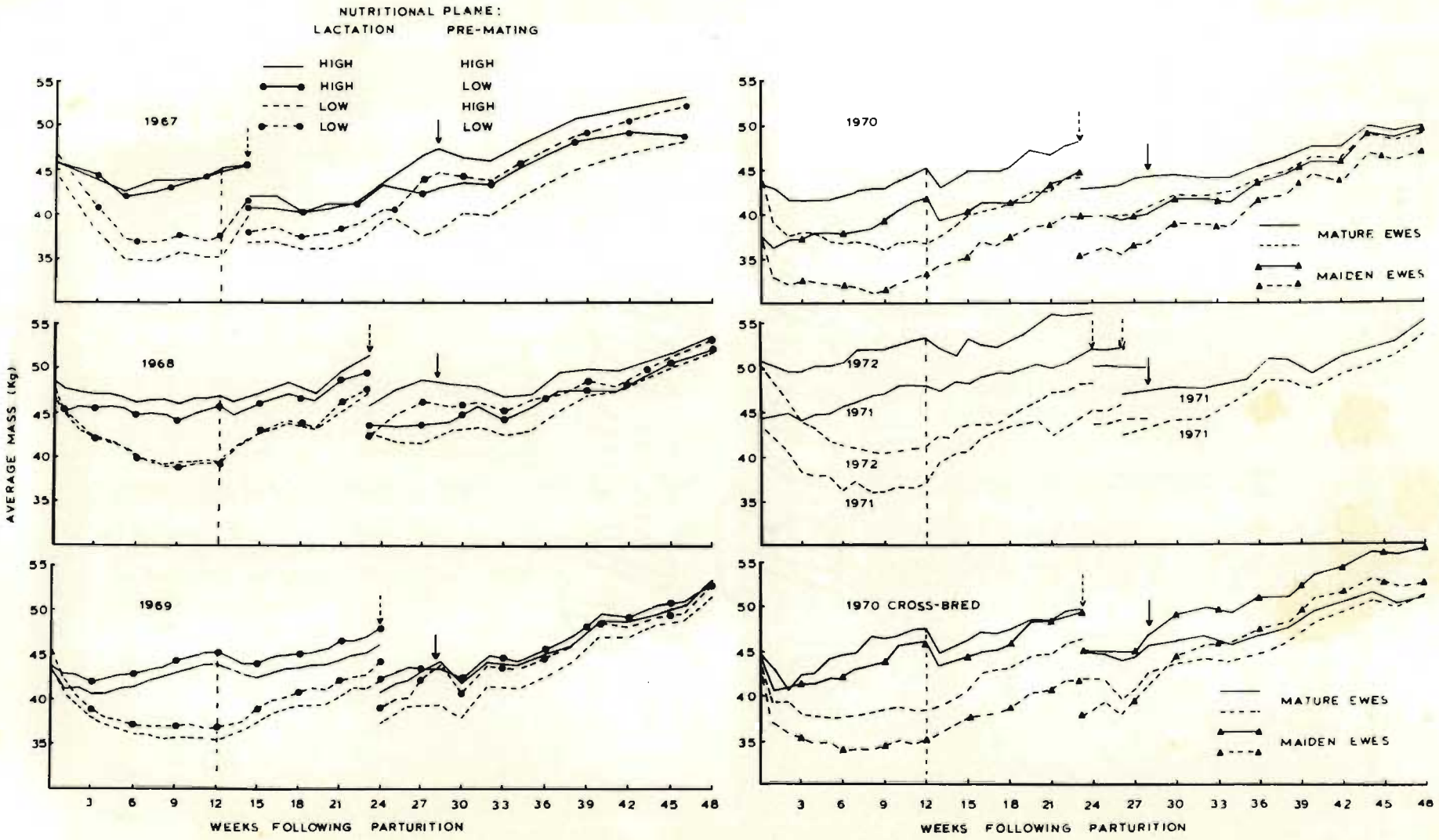


FIG. 2. CHANGES IN BODY MASS OF GROUPS OF EWES SUBJECTED TO HIGH AND LOW PLANES OF NUTRITION DURING LACTATION AND DURING A THREE-WEEK PRE-MATING PERIOD.

↓ DENOTES JOINING WITH ENTIRE RAMS

↓ DENOTES SHEARING

Table 3. Average bodymass and change in bodymass, of groups of ewes on two planes of nutrition during lactation and prior to mating.

Year	Type of ewe	Average Age (years)	Nutritional plain:-		Number of Animals	Average bodymass (kg)								
			Lactating	Pre-mating		Pre-partum	Post-partum	Weaning	Gain to weaning (%)	Pre-flushing	Pre-mating	Flushing gain (%)	Pre-partum	Post-partum
1967	Merino	5	High	High	29	47,3	45,5	44,9	- 1,7	43,1	47,3	9,7	53,4	50,1
			High	Low	29	47,0	45,8	45,3	- 0,9	42,7	42,7	0	48,9	47,5
			Low	High	28	48,4	47,0	37,6	-17,7	40,4	44,8	10,9	52,3	49,8
			Low	Low	28	45,6	44,5	35,4	-19,7	39,5	38,7	-2,0	48,2	45,9
1968	Cross-bred	6	High	High	58	54,7	48,7	46,9	- 3,7	45,6	48,7	6,7	53,7	49,0
			High	Low	58	53,7	47,4	45,6	- 3,8	43,6	43,7	0,2	51,9	46,5
			Low	High	59	54,4	48,4	39,3	-18,8	42,2	46,5	10,2	53,3	48,0
			Low	Low	59	54,4	47,8	39,1	-18,2	42,5	41,1	-3,3	51,8	47,6
1969	Merino	7	High	High	59	46,9	43,4	43,8	0,9	41,6	44,4	6,7	53,5	45,2
			High	Low	55	48,6	43,8	45,4	3,6	43,0	43,3	0,7	52,8	42,9
			Low	High	52	49,3	45,8	36,7	-19,9	40,0	43,8	9,5	52,9	44,6
			Low	Low	55	48,7	44,0	35,5	-19,3	38,5	39,0	1,3	51,4	43,2
1970	Merino	8	High	Low	88	53,2	43,5	45,2	3,9	-	44,0	-	49,7	43,3
			Low	Low	84	52,8	44,5	36,8	-17,3	-	40,6	-	48,9	42,8
		Maiden 2-3	High	Low	35	42,9	37,7	41,8	10,9	-	39,5	-	49,4	42,7
			Low	Low	37	43,3	37,7	33,4	-11,4	-	36,6	-	47,0	41,3
	Cross-bred	8	High	Low	38	58,4	49,7	52,4	5,4	-	50,7	-	55,9	47,6
			Low	Low	37	59,0	49,3	43,3	-12,2	-	47,5	-	56,4	48,8
		Maiden 2-3	High	Low	32	53,7	47,9	51,3	7,1	-	51,6	-	61,4	55,5
			Low	Low	31	53,1	48,8	40,0	-18,0	-	46,2	-	57,5	52,4
1971	Merino	3-5	High	Low	62	50,2	44,3	48,0	8,3	-	47,7	-	56,6	51,6
			Low	Low	65	50,4	43,4	37,3	-14,0	-	44,1	-	55,8	50,6
1972	Merino	2-6	High	Low	53	55,0	50,9	53,7	5,5	-	-	-	-	-
			Low	Low	78	56,6	50,6	41,3	-18,4	-	-	-	-	-

expected compensatory gain. When expressed in terms of increase in bodymass, the overall response to flushing was below expectation. The benefit of flushing was maintained, although at a diminishing rate, until just prior to the subsequent parturition. In only one instance viz., for the crossbred ewes of 1970, were the animals that had been subjected to nutritional stress during lactation able to compensate completely for the loss in bodymass, before the following parturition.

Reproduction

Post-partum anoestrus

The duration of the post-partum anoestrous period, as measured by the interval between parturition and the first oestrus prior to weaning, is given in Table 4. From these results, it appears that there was a tendency for anoestrus to persist throughout lactation in a higher proportion of the ewes subjected to undernutrition during this time than in ewes adequately fed. This situation was accentuated amongst the maiden ewes. However, according to the χ^2-test none of the differences were significant, not even when a pooled χ^2-test was conducted. Regression analyses were also performed by considering the interval to oestrus as the dependent variable "Y" and using six measures of each ewe's bodymass or change in bodymass as the independent variables "X". These parameters were

(i) Bodymass at parturition - initial bodymass.

(ii) Bodymass at weaning.

(iii) The lowest or minimum bodymass recorded during the lactation period - a measure of the maximum stress experienced during lactation.

(iv) The accumulated difference between the post-partum

Table 4. Duration of the post-partum anoestrous period among ewes on two planes of nutrition during lactation. Ewes which did not exhibit oestrus while lactating have been excluded.

Year	Type of ewe	Average Age (yrs)	Nutritional plane during lactation	Number of animals	Parturition to first oestrus (days)	Number of ewes anoestrus	No. of ewes oestrus once only prior to seasonal anoestrus
1967	Merino	5	High	58	39,2 ± 9,5	2	3
			Low	57	42,6 ± 13,0	4	1
1968	Cross-bred	6	High	110	42,9 ± 11,9	8	1
			Low	109	46,8 ± 13,1	11	0
1969	Merino	7	High	113	48,5 ± 15,3	10	1
			Low	109	46,5 ± 13,3	12	4
1970	Merino	8	High	69	52,8 ± 12,9	13	6
			Low	67	49,8 ± 9,6	9	5
		Maiden 2-3	High	30	47,5 ± 9,5	5	7
			Low	20	52,4 ± 11,4	17	4
	Cross-bred	8	High	42	51,7 ± 9,6	2	1
			Low	47	52,2 ± 12,0	7	1
		Maiden 2-3	High	28	46,7 ± 12,0	1	4
			Low	24	48,4 ± 12,2	8	3
1971	Merino	3-5	High	59	50,1 ± 12,6	3	1
			Low	54	50,5 ± 11,4	11	4
1972	Merino	2-6	High	49	50,0 ± 11,0	5	0
			Low	76	51,6 ± 10,3	3	3

bodymass and the bodymass measured each week until cessation of lactation - this parameter was used to assess a particular level of stress and the time for which it continued.

(v) The difference between the intitial bodymass and that recorded at weaning, expressed as a percentage of the initial mass (weaning %) - this could be expected to correct for the possibility that the stress associated with each kg loss in bodymass would depend on the total bodymass of the animal.

(vi) The difference between the initial bodymass and the minimum livemass recorded, again expressed as a percentage of the intitial mass (minimum %).

Little significant association between bodymass or change in bodymass and interval to oestrus was observed. The only relationship to approach significance was the negative correlation between initial bodymass and the post-partum anoestrous period. The "F" value obtained was 3,05 (3,84 required for significance) and this was achieved after pooling all the results, but omitting the data where a positive correlation was obtained viz., 1968 and 1972.

The regression equation was:

$$Y = 51,52 - 0,074X$$

Amongst the maiden ewes (1970) there was a negative correlation between the post-partum interval to oestrus and the maximum loss in bodymass, expressed as a percentage of the post-partum mass ($F = 3,94$, $DF = 98$).

It was found that ewes which lambed later in the year tended to show a shorter anoestrous period. When all the observations were

combined, with the independent variable "X" expressed as the interval from 1 March to the date of parturition, the equation

$$Y = 58,0 - 0,227X$$

was obtained with an "F" value = 33,96. This correlation was negative for every year of this study and the instances in which the regression for a particular year was significant are illustrated in Fig. 3.

Seasonal changes in the incidence of oestrus

The number of ewes, in each treatment group, which accepted service by the vasectomized rams during the period 15 May to 31 October each year and the distribution of matings after introduction of entire rams are presented in Table 5. Where the ewes were continuously associated with rams the results have been expressed as percentages of the number of animals in each group. These data are presented in Fig. 4. The data for the Merino and crossbred ewes (1970) have been combined as there was no apparent difference between these two types.

From Table 5 and from Fig. 4 it is clear that, except for the 1968 results and the five-year-old ewes of 1971, there was a delayed depressing effect of undernutrition during lactation on the incidence of overt oestrus. In general, this effect began to be manifested during the 17-day period commencing 5 July and continued until even after joining with entire rams. The difference in the incidence of oestrus between the ewes which maintained their bodymass during lactation and those fed so as to lose mass varied from year to year and was particularly small amongst the maiden ewes. Differences which were significant, according to the χ^2-test have been indicated in Fig. 4.

When the ewes that were isolated from rams during the post-weaning period were again joined with rams the level of spontaneous oestrus was

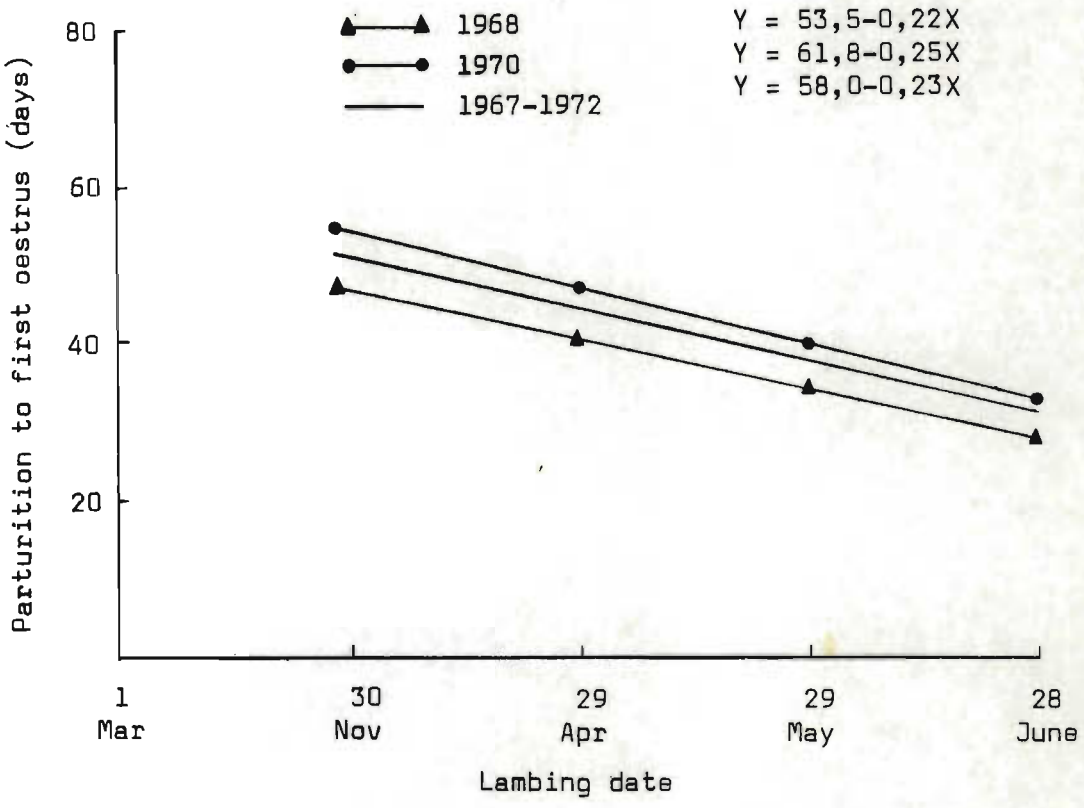


Fig. 3. The influence of date of lambing on the post-partum interval to first oestrus.

Table 5. Oestrous activity of ewes maintained on two planes of nutrition during lactation and either continuously associated with or isolated from rams after weaning.

ear	Type of ewe	Average Age (yrs)	Nutritional plane during lactation	Post-weaning association with rams	Number of Animals	Number of ewes oestrus during 17-day periods commencing on dates shown:										Number of ewes marked fertile by rams*		Number of ewes not marked
						15/5	1/6	18/6	5/7	22/7	8/8	25/8	11/9	28/9	15/10	Day 1-17	Day 18-42	
967	Merino	5	High	Isolated	58	35	47	-	-	-	-	-	-	-	-	16	42	0
			Low		56	27	46	-	-	-	-	-	-	-	-	10	46	0
968	Merino Cross-bred	6	High	Continuous	58	29	49	41	46	48	42	35	32	22	24	36	21	1
			High	Isolated	58	38	38	-	-	-	-	-	-	-	26	45	13	1
			Low	Continuous	58	35	45	49	43	41	42	41^{++}	31	28	18	38	17	1
			Low	Isolated	60	25	44	-	-	-	-	-	-	-	14^{++}	33	13	0
969	Merino	7	High	Continuous	62	22	42	48^{+}	51^{+}	52	49	41	40	37^{+}	39^{+++}	41	14	0
			High	Isolated	61	31	41^{++}	-	-	-	-	-	-	-	11^{++}	46	11	0
			Low	Continuous	61	24	38	48^{+}	44^{+}	37^{+++}	35	30	16^{+}	9	8	32	20	3
			Low	Isolated	60	33^{+}	42^{+}	-	-	-	-	-	-	-	4^{+++}	30	23	1
70	Merino	8	High	Continuous	63	24	44	51	49	54	49	43	39	39	45	59	13	1
			High	Isolated	65	24	49	-	-	-	-	-	-	-	29	53	12	0
			Low	Continuous	62	26	43	49	41	42	40	26	18	17	19	48^{+}	13	0
			Low	Isolated	69	18	48	-	-	-	-	-	-	-	20	51	13	2
	Cross-bred	Maiden 2-3	High	Continuous	32	9	20	18	17	17	12	9	3	2	5	25	7	0
			High	Isolated	35	15	28	-	-	-	-	-	-	-	4	27	8	0
			Low	Continuous	37	7	17	13	16	17	10	7	5	3	5	21^{+}	15	0
			Low	Isolated	33	6	13	-	-	-	-	-	-	-	2	13	19	1
71	Merino	3-4	High	Continuous	40	17	32	32	36	34	37	34	37	20	21	39^{+}	0	0
			Low		44	13	22	27	27	24	19	15	14	11	15	39	5	0
		5	High	Continuous	22	7	17	17	20	20	19	19	17	13	15	22	0	0
			Low		21	6	15	17	17	16	16	15	13	11	14	20	1	0
72	Merino	2-6	High	Continuous	53	32	39	47	50	45	47	42	39	42	45	-	-	-
			Low		78	42	63	68	63	60	62	49	5	41	52	-	-	-

* First oestrus only; + Indicates one ewe lost due to various causes e.g. death, theft.

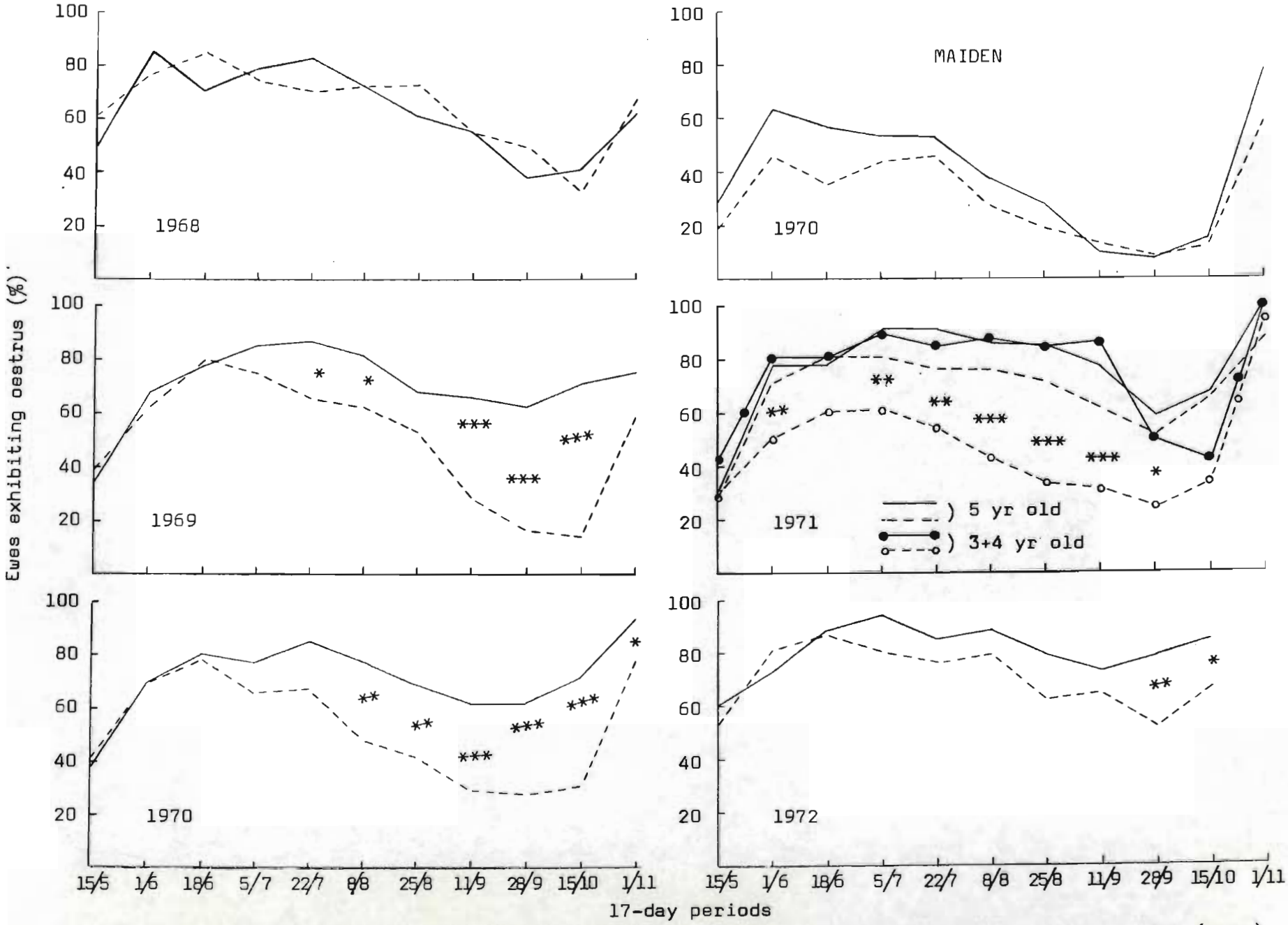


Fig. 4. Seasonal variations in the incidence of oestrous in groups of ewes subjected to either a high (——) or a low (----) plane of nutrition during the lactation period and continuously associated with rams

lower than the incidence of oestrus in ewes continuously associated with rams. However, only amongst the groups on the high plane was this difference significant and it was confined to 1969 ($P \leq 0,001$) and 1970 ($P \leq 0,01$).

For the ewes isolated from rams until 15 October, comparisons made between animals on the different planes of nutrition show the level of spontaneous oestrus (i.e. oestrus observed during the 17-day period commencing 15 October) to be higher on the high plane than on the low plane, both during 1968 ($P \leq 0,05$) and during 1970 ($P \leq 0,01$). It appears that stimulation by joining with rams and/or resumption of the natural breeding season rapidly obliterate the effect of poor nutrition during lactation.

A comparison between the maiden and mature ewes shows that during 1970 the difference in incidence of oestrus was significant from 1 June onwards, in the case of the underfed ewes, and one cycle later for ewes on the high plane. These differences continued until joining with entire rams, the level of significance gradually increasing from $P < 0,05$ to $P \leq 0,001$. During 1971 the older ewes on the low plane of nutrition also exhibited a higher level of oestrous activity than the 3-4 year-old females and the differences were significant from 8 August up to and including 15 October.

During the 17-day period commencing 15 October 1970, similar age differences were noted amongst the ewes isolated from rams until this time (high plane, $P \leq 0,01$; low plane, $P \leq 0,02$).

Relationships between bodymass and the incidence of oestrus

(i) The period 8 August to 17 November

The results presented in Fig. 4 indicate that by the 17-day period commencing 8 August the incidence of oestrus amongst the ewes on the two

nutritional planes during lactation differed significantly in all years (1968 and 1972 excluded).

In view of this finding attention was given to this and subsequent 17-day cycles. Each ewe was categorized according to the parameters of bodymass or change in bodymass, described earlier, and the data for the years 1968, 1969, 1970 and 1971 were pooled. The treatments applied during October 1972 (see Chapter 3) may have stimulated onset of mating and the data for this year has therefore not been included. The bodymass recorded, just prior to joining with entire rams (pre-mating bodymass) was also examined for possible association with the incidence of oestrus recorded during the years 1968-1971. Frequency distributions were then constructed by sub-dividing the range of observations for each of these parameters into classes containing approximately the same number of individuals. The proprotion of the ewes within each class which exhibited oestrus during the individual 17-day periods was determined and the significance of the observed relationship between the various parameters (independent variable, "X") and the proportion of ewes showing oestrus ("Y") was tested by the method of regression analysis. Weighting was applied according to the number of animals within each class. Where the regression was found to be significant the relevant equation is included in Table 6, and the dependence of "Y" on each "X" is described by the results in this table. From these data it is apparent that, when the results for the ewes where no effect of the plane of nutrition was observed (Fig. 4) were omitted, the effect of the post-partum and pre-mating bodymasses were considerably reduced. These two parameters then showed a significant association with incidence of oestrus only during the first and last 17-day periods respectively. The magnitude of the correlation between bodymass and proportion of ewes mating thus appears to depend here on the time interval between the determination of bodymass

Table 6. Variation in the proportion of ewes showing oestrus ("Y") in relation to bodymass or change in bodymass ("X"). ** and * indicate P = 0,01 and P = 0,05, respectively.
A = all data pooled B = omitting data for 1968, maiden Merino ewes 1970 and 5 year-old ewes 1971.

Independent variate	Pooled data	17-day periods commencing								
		8 August			25 August			11 September		
		Regression equation	Signf regr	Depend† Y on X	Regression equation	Signf regr	Depend† Y on X	Regression equation	Signf regr	Depend† Y on X
Bodymass post-partum	A	Y=23,6+0,88X	16.30**	84,4	NS	-	16,3	Y=12,7+0,76X	13,37**	81,6
	B	Y=30,8+0,71X	13,74**	82,1	NS	-	13,3	NS	-	56,8
Bodymass at weaning	A	Y=14,7+1,15X	72,30**	96,0	Y=24,3+0,73X	6,90*	69,7	Y=-10,5+1,36X	6,04*	66,8
	B	Y= 1,9+1,42X	19,82**	86,8	Y=12,9+0,95X	14,65**	83,0	Y=-23,8+1,59X	7,63*	71,8
Minimum bodymass	A	Y= 7,9+1,41X	38,69**	92,8	Y=15,2+1,03X	7,06*	70,2	Y=-15,2+1,55X	6,65*	68,9
	B	Y=-5,2+1,68X	16,12**	84,3	Y=13,8+1,01X	6,06*	66,9	Y=-24,3+1,70X	9,84*	76,6
Bodymass pre-mating	A	NS	-	60,1	NS	-	21,5	NS	-	51,6
	B	NS	-	40,9	NS	-	1,1	NS	-	55,8
Accumulated difference	A	Y=68,2+0,12X	11,79**	79,7	NS	-	50,3	Y=56,9+0,24X	27,41**	90,1
	B	Y=70,3+0,20X	16,49**	84,6	NS	-	47,7	Y=57,7+0,35X	26,66**	89,9
Percent change to weaning	A	NS	-	9,9	NS	-	42,3	Y=52,0+0,72X	9,92**	76,8
	B	NS	-	48,7	NS	-	52,0	Y=51,5+1,36X	25,53**	89,5
Percent change to minimum	A	Y=72,6+0,69X	9,58*	76,2	NS	-	36,8	Y=65,2+1,38X	31,09**	91,2
	B	Y=76,4+1,04X	7,52*	71,5	NS	-	48,5	Y=68,2+1,86X	10,33*	77,5

		28 September			15 October			1 November		
Bodymass post-partum	A	Y= 4,0+0,79X	22,26**	88,1	NS	-	27,7	NS	-	2,3
	B	NS	-	0,2	NS	-	0,1	NS	-	57,4
Bodymass at weaning	A	Y=-16,7+1,32X	8,51*	73,9	Y=-13,0+1,24X	6,47*	68,3	NS	-	42,9
	B	Y=-36,0+1,74X	8,01*	72,8	Y=-25,1+1,50X	8,66*	74,3	NS	-	45,2
Minimum bodymass	A	Y=-18,3+1,45X	26,49**	89,8	Y=-24,4+1,60X	14,87**	83,2	Y=36,2+1,04X	10,65*	78,0
	B	Y=-46,7+2,10X	8,43*	73,7	Y=-51,0+2,26X	17,41**	85,3	Y=24,3+1,40X	8,86*	74,7
Bodymass pre-mating	A	NS	-	42,3	Y= 0,3+0,84X	26,53**	89,8	Y=29,9+1,02X	119,05**	97,5
	B	NS	-	27,6	NS	-	62,8	Y=33,4+0,99X	12,44**	80,6
Accumulated difference	A	Y=47,2+0,20X	14,61**	83,0	Y=50,2+0,26X	48,62**	94,2	Y=84,1+0,16X	9,30*	75,6
	B	Y=51,7+0,34X	16,01**	84,2	Y=51,9+0,31X	89,15**	96,7	Y=88,3+0,18X	61,45**	95,3
Percent change to weaning	A	Y=44,5+0,78X	26,28**	89,7	Y=45,3+1,00X	40,48**	93,1	Y=81,6+0,59X	33,56**	91,8
	B	Y=47,1+1,54X	46,26**	93,9	Y=46,3+1,06X	158,14**	98,7	Y=85,1+0,62X	194,45**	98,5
Percent change to minimum	A	Y=53,3+1,06X	13,36**	81,7	Y=55,4+1,18X	9,00*	75,0	Y=87,4+0,69X	11,42*	79,2
	B	Y=62,0+1,82X	12,97**	81,2	Y=59,7+1,58X	17,40**	85,3	Y=92,5+0,80X	6,48*	68,3

and a particular 17-day mating period.

Although the significance of the remaining parameters was only slightly affected by excluding certain of the observations (Table 6) no particular parameter was consistently more closely associated with occurrence of oestrus than any other. During the first two 17-day periods the proportion of ewes exhibiting oestrus was most closely associated with their bodymass at weaning. During the remaining periods, when the change in mass to weaning was expressed as a percentage of the initial bodymass, a particularly close association with incidence of oestrus was attained. These results are illustrated by the histograms in Fig. 5. It is evident that the greater the bodymass at weaning, or the lower the loss in mass to weaning, the greater the proportion of ewes exhibiting oestrus. There was also some suggestion (Fig. 5) that the response, in terms of the percentage of ewes showing oestrus, changed markedly when the bodymass at weaning fell below 40 kg or the loss to weaning, expressed as a percentage of the post-partum bodymass, exceeded five percent.

(ii) The number of oestrous cycles observed

The incidence of oestrus in the ewes during the period 8 August to 17 November can also be described by the number of occasions on which each ewe exhibited overt oestrus during the six 17-day periods ("Y"). The variates, previously described, were again used in a regression analysis and the results are summarised in Table 7. The pre-mating bodymass showed no significant relationship with the occurrence of oestrus and has thus been omitted. When all the observations were combined, it appeared that the occurrence of oestrus in ewes was as closely associated with bodymass at parturition as with any other variable. However, by limiting the analysis to the ewes which showed an effect of undernutrition

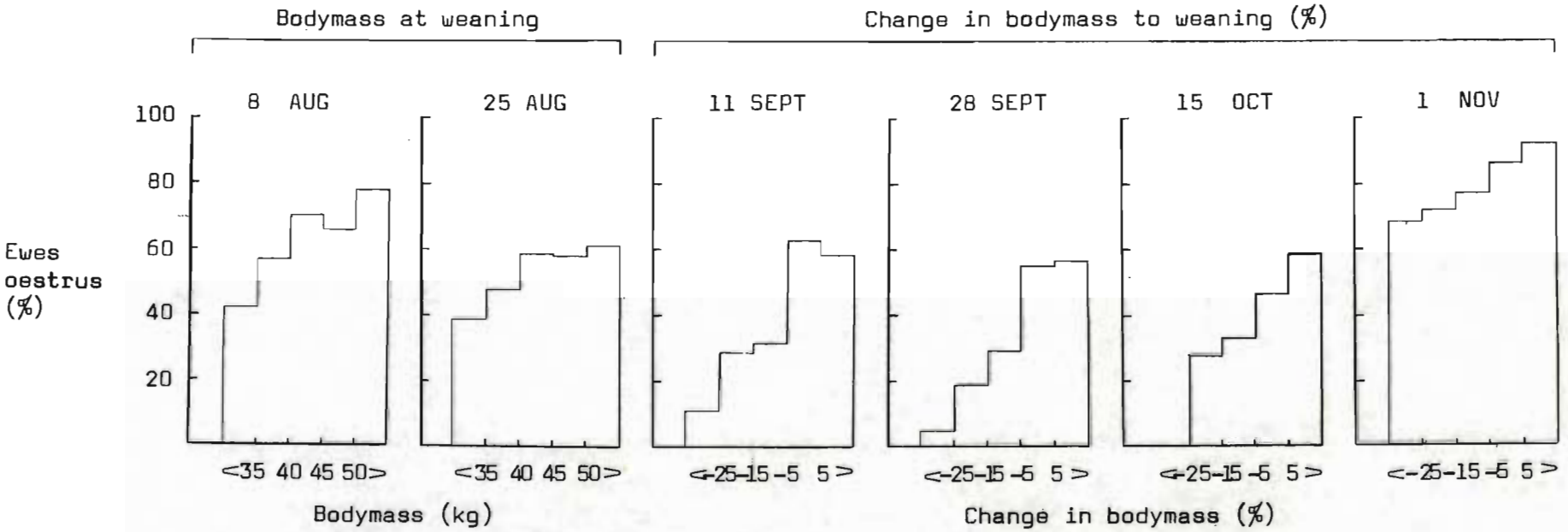


Fig. 5. Association between bodymass or change in bodymass and the % of ewes showing oestrus during 17-day periods commencing on the dates shown. The two parameters presented were more closely associated with oestrous activity during the respective 17-day periods than any other variate (see text for details).

Table 7. Association between bodymass or bodymass change ("X") and number of oestrous cycles exhibited ("Y") during successive 17-day periods (8 August to 1 November) among ewes not isolated from rams after weaning.

Year	Breed	Average age (yrs)	Percent dependence Y on X					
			Post-partum	Weaning	Lowest	Accumulative	Weaning %	Lowest %
1968	Cross-bred[1]	6	46,4	17,7	30,0	0,2	0,02	6,0
1969	Merino	7	0,2	74,4	60,2	65,7	73,1	61,5
1970	Merino	8	8,4	55,8	43,2	73,1	81,1	67,7
	Merino[2]	2	5,3	1,1	11,6	1,4	0,1	0,4
	Cross-bred	8	0,7	17,0	3,4	69,3	54,1	29,5
	Cross-bred	2	2,0	64,0	52,7	89,1	96,4	96,5
1971	Merino	3-4	71,0	94,7	85,7	77,1	72,9	67,0
	Merino[3]	5	1,3	1,0	7,5	20,3	11,6	11,6
All ewes			86,8	86,4	89,8	83,2	78,1	85,4
All ewes excluding 1, 2 & 3			29,3	90,9	89,0	95,4	95,5	94,8

during lactation (Fig. 4) it became clear that the incidence of overt oestrus was highly dependent on the bodymass or bodymass changes recorded during, or at the conclusion of the lactation period (Table 7). The results are illustrated in Fig. 6. The change in bodymass to weaning again produced the most consistent relationship with oestrus, and the instances where a significant association was attained are illustrated in Fig. 7.

The failure to show a significant relationship for the data obtained during 1968 and also for the maiden Merino ewes in 1970 is probably due to the relatively high incidence of oestrus during the anoestrous season in 1968, and the low incidence in the maiden ewes during 1970. Few of the maiden Merino ewes exhibited more than four oestrous cycles during the period under consideration. During 1971 the five-year-old ewes did not show a depression in the level of oestrous activity following under-nutrition during lactation (Fig. 4) and hence any relationship between bodymass and oestrous activity was obscured.

(iii) The occurrence of oestrus prior to and during the "fertile" mating period

The results in Table 5 demonstrate that certain of the ewes, isolated from rams after weaning, exhibited spontaneous oestrus when rejoined with rams on 15 October while others mated only one or two cycles later. Furthermore, among the ewes continuously associated with rams there was a notable increase in the proportion showing overt oestrus during the 17-day period commencing 1 November.

In an attempt to account for the distribution of matings the ewes were classified according to the 17-day period during which mating first occurred, beginning on 15 October. It was concluded that the results differed for the two levels of nutrition applied during lactation and

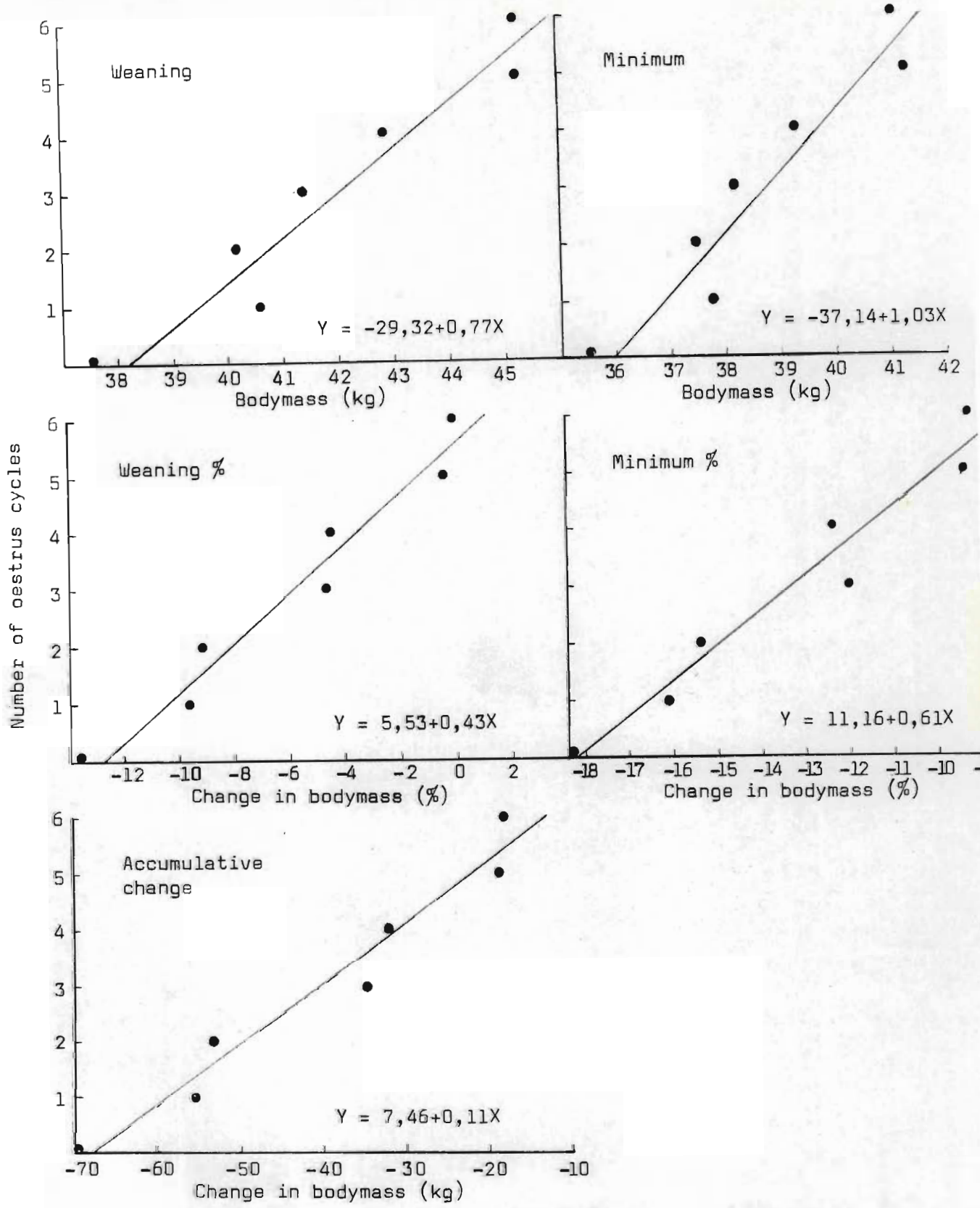


Fig. 6. Association between number of oestrous cycles (Y) and bodymass or change in bodymass (X), measured at various stages during the lactation period.

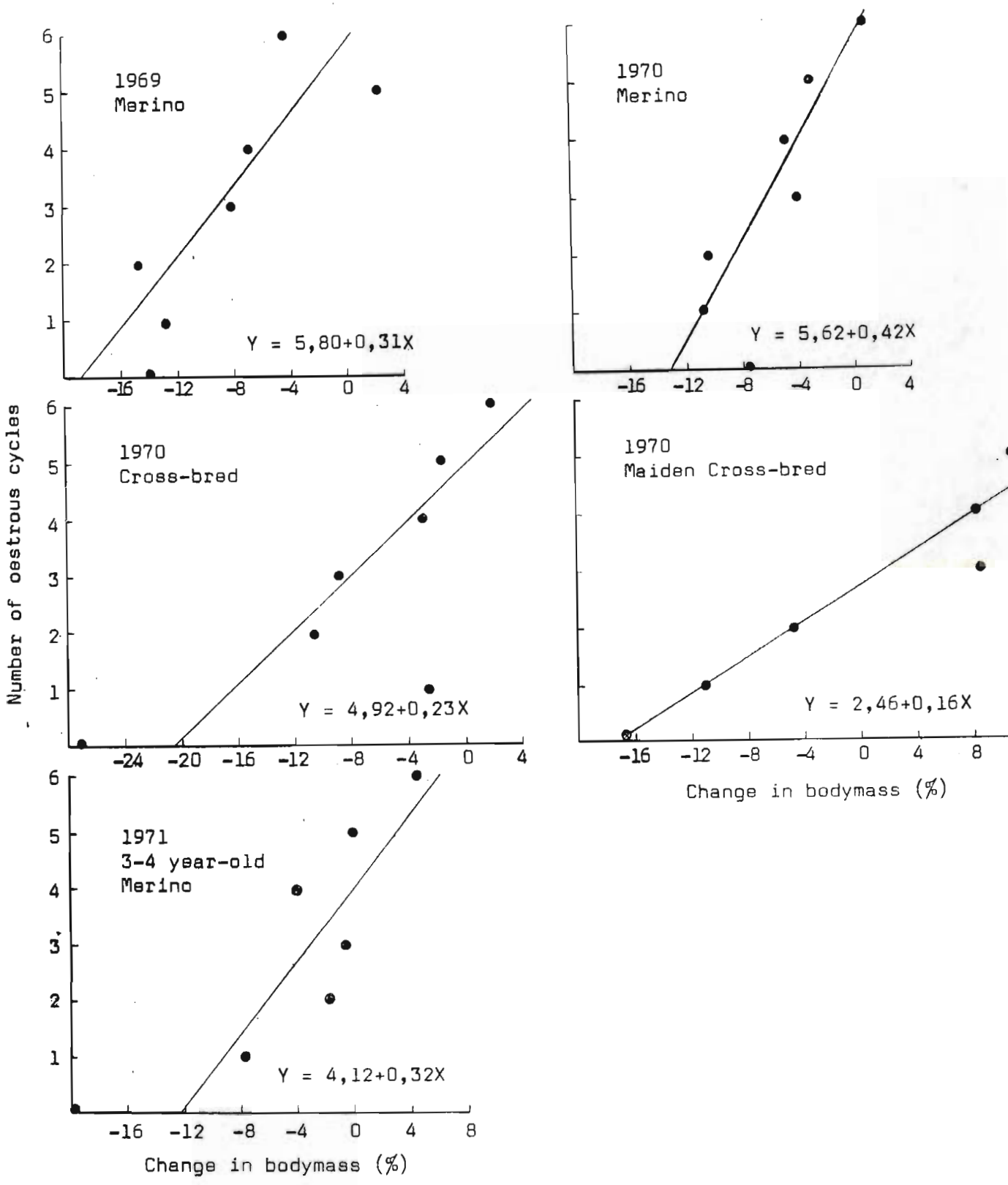


Fig. 7. Relationship between the incidence of oestrus and bodymass change to weaning, expressed as a percentage of the bodymass post-partum, amongst ewes classified according to breed, age and year of study.

Y = number of oestrous cycles.
X = change in bodymass to weaning (%).

that the data for the ewes isolated from or continuously associated with rams could be pooled.

During 1967 the ewes were joined with rams only on 1 November and consequently these results have been omitted. It is clear from the results, which are summarised in Table 8, that the ewes which mated early possessed a greater bodymass and also underwent less reduction in mass during their preceding lactation than those which mated later. There were a greater number of significant differences between ewes which mated in the three 17-day periods when classified according to the pre-mating bodymass (Table 7). This agrees with the results presented in Table 6. The results in Table 8 also showed that, when each parameter is considered in turn, the mean of the ewes mated in a particular 17-day period varied according to the nutritional level applied during lactation.

Mating with entire rams

The daily pattern of mating, after joining with entire rams on 1 November, is presented in Fig. 8 and Fig. 9. From Fig. 8 it appears that the nutritional stress during lactation had little effect on the pattern of mating or on the total number of ewes mated during the first 17 days. However, a pooled χ^2 for the 1968-1971 data indicates that amongst the ewes continuously associated with rams significantly more ewes on the high plane than on the low plane were mated during the first 17-day period after joining with entire rams ($P \leq 0,05$). Amongst the ewes isolated from rams the effect of the nutritional plane was significant only during 1969. None of the other differences shown were significant. The results depicted in Fig. 9 indicate that the effect of association with rams was minor and varied with the year or age of the ewes considered.

Table 8. The mean bodymass or bodymass change measured at different times, and the mating of ewes during the 17-day period prior to, and the two 17-day periods following joining with entire rams. The dates on which these periods commenced are given in the table. Figures which differ significantly are joined by a line and the level of significance is indicated by: *P = 0,05; **P = 0,01; ***P = 0,001. The S.E. associated with a particular set of means is given below each set of means.

Plane of nutrition during lactation	Number of ewes mated:			Post-partum bodymass (kg) of ewes mated:			Bodymass (kg) at weaning of ewes mated:			Lowest bodymass (kg) during lactation of ewes mated:			Pre-mating bodymass (kg) of ewes mated:			Accumulative bodymass change (kg) of ewes mated:			Percent bodymass change to weaning of ewes mated:			Percent change to lowest bodymass of ewes mated:		
	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11	15/10	1/11	18/11
High	205	205	79	45,6	44,7	44,5	47,1	46,1	44,9	42,6	41,3	40,9	48,3	46,5	43,2	-3,5	-6,3	-12,6	3,5	3,8	1,1	-6,4	-7,2	-8,0
					*			*			*			***			*			*			*		
					±6,63			±6,68			±6,32			±8,99			± 30,65			±8,20			±5,78		
Low	111	222	136	46,5	45,2	44,2	39,4	37,6	36,2	38,0	36,1	34,8	46,7	43,7	40,7	-70,7	-80,1	-83,8	-15,3	-16,8	-17,7	-18,5	-20,3	-21,1
					**			***			***			***			**			**			**		
					±6,59			±6,30			±6,19			±8,17			± 31,51			±7,6			±6,46		

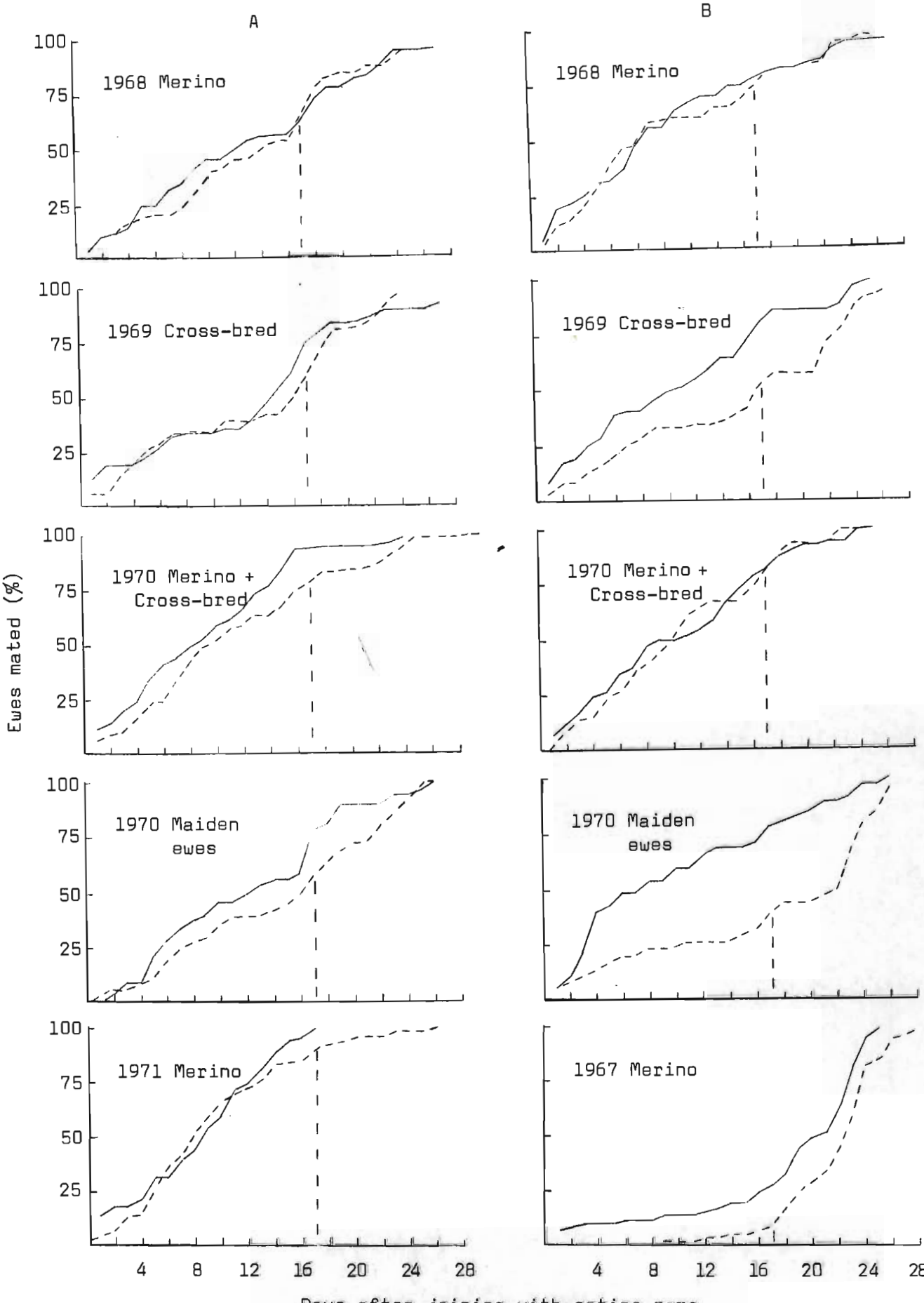


Fig. 8. Daily cumulative total number of ewes mating with entire rams following either a high (——) or a low (----) plane of nutrition during lactation and either continuously associated with (A) or isolated from (B) rams after weaning.

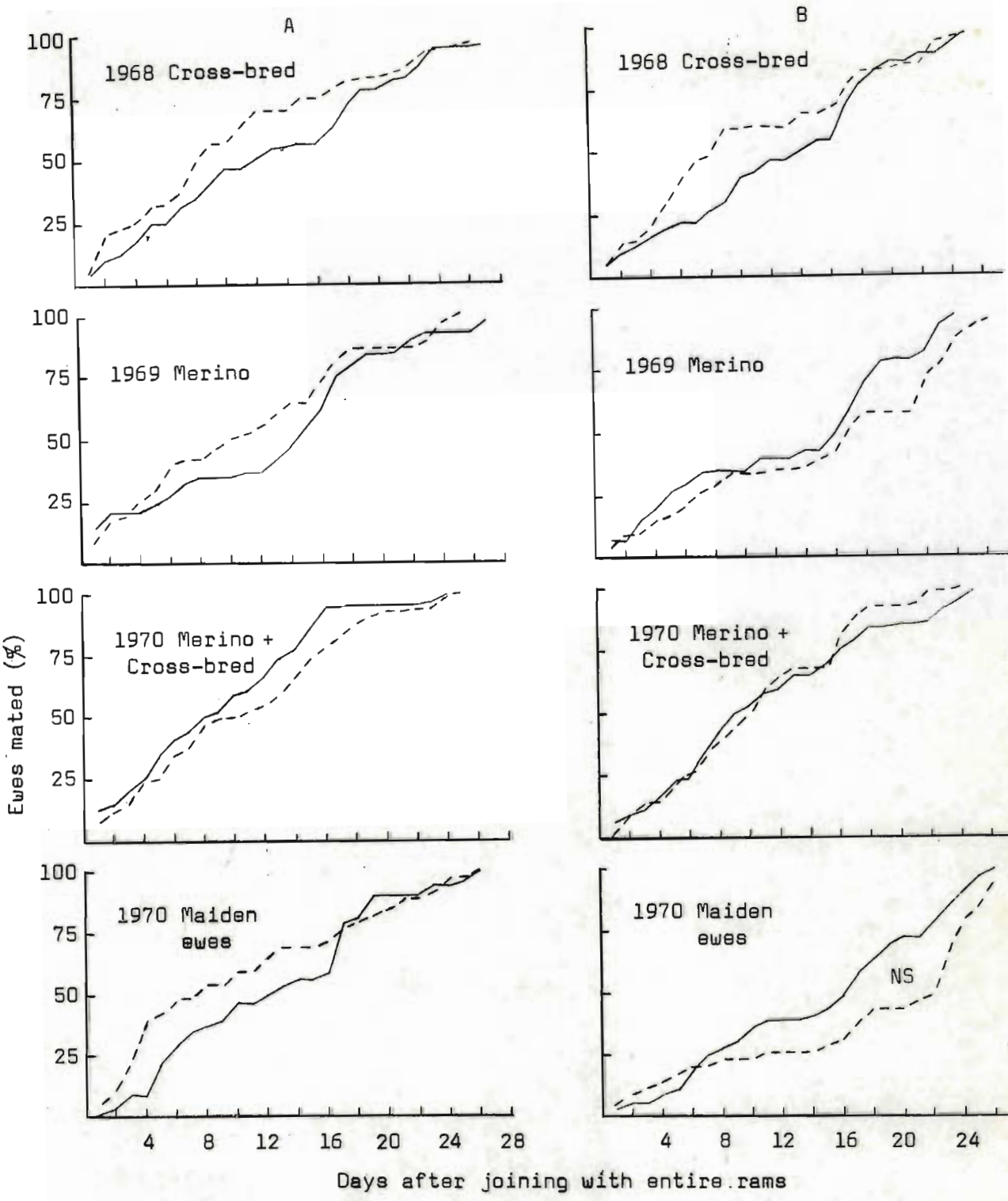


Fig. 9. Daily cumulative total number of ewes mating with entire rams after either continuous association with (——) or isolation from (----) rams after weaning and maintained on high (A) or low (B) planes of nutrition during lactation.

Lambing performance

(i) The number of lambs produced

The number of lambs produced by the ewes which survived until the lambing season during each yearly replication of the experiment, and the proportion of barren ewes, is depicted in Table 9. Although there was a tendency for a greater proportion of barren ewes to occur when the plane of nutrition was restricted during lactation the difference was not significant, even when a pooled χ^2-test was performed. A similar situation applied to the proportion of twin lambs. Flushing had little effect on the proportion of twin-births. The total number of lambs produced per 100 ewes lambing was greater following the high plane of nutrition than following the low plane during the previous lactation. Exceptions to this trend occurred during 1968 and 1969.

(ii) Relationships between bodymass and the number of lambs produced

Classification of the ewes according to the number of lambs produced during the lambing season and the various measures of stress (Table 10, Fig. 10) again showed that the individuals with the greatest bodymass, or those undergoing the smallest change in bodymass, were likely to produce the most lambs. Isolation of the ewes after weaning appeared to have no consistent effect on the responses observed. When all the results were combined, it was again evident that the change in bodymass occurring during lactation had a marked effect on the lambing performance of the ewes. Since there were only three classes of lambings, viz. 0, 1 and 2 lambs per ewe, regression analyses using the number of lambs as dependent variable are of dubious validity. However, the results obtained, showing the dependence of the number of lambs on the bodymass (Table 10), support the conclusion that the number of lambs born per ewe

Table 9. Lambing performance of groups of ewes maintained on two levels of nutrition during the preceding lactation and in certain cases flushed prior to mating.

Year	Type of ewe	Average Age (yrs)	Nutritional plane:-		Number of animals	Number of ewes:						Lambs/100 ewes mated	Lambs/100 ewes lambing
						Served and not lambing		Producing singles		Producing twins			
			Lactation	Pre-mating		No.	%	No.	%	No.	%		
1967	Merino	5	High	High	28	1	6,9	23	82,0	4	14,3	110,7	114,8
			High	Low	29	2	10,7	23	79,3	3	10,3	100,0	107,4
			Low	High	28	3	10,7	24	85,7	1	3,6	92,8	104,0
			Low	Low	24	5	33,3	18	75,0	1	4,2	83,3	105,3
1968	Cross-bred	6	High	High	58	9	15,5	39	67,2	10	17,2	101,7	120,4
			High	Low	57	8	14,0	41	71,9	8	14,0	100,0	116,3
			Low	High	58	8	13,8	38	65,5	12	20,7	106,9	124,0
			Low	Low	55	9	16,4	36	65,4	10	18,2	101,8	121,7
1969	Merino	7	High	High	56	3	5,3	50	89,3	3	5,3	100,8	105,6
			High	Low	52	5	9,6	40	76,9	7	13,5	103,8	114,9
			Low	High	51	9	17,6	36	70,6	6	11,8	94,1	114,3
			Low	Low	54	5	9,2	44	81,5	5	9,2	100,0	110,2
1970	Merino	8	High	Low	89	15	16,8	65	73,0	9	10,1	93,2	112,2
			Low	Low	82	13	15,8	65	79,3	4	4,9	89,0	105,8
		Maiden 2-3	High	Low	35	4	11,4	27	77,1	4	11,4	100,0	120,7
			Low	Low	37	7	18,9	29	78,4	1	2,7	83,7	103,3
	Cross-bred	8	High	Low	38	6	15,8	22	57,9	10	26,3	115,8	131,2
			Low	Low	36	8	22,2	21	58,3	7	19,4	97,2	125,0
		Maiden 2-3	High	Low	32	1	3,1	24	75,0	7	21,9	118,7	116,1
			Low	Low	31	3	9,7	25	80,6	3	9,7	100,0	110,7
1971	Merino	3-5	High	Low	60	8	13,3	42	70,0	10	16,7	103,3	119,2
			Low	Low	64	12	18,7	44	68,7	8	23,5	93,7	115,4

Table 10. Relationship between number of lambs born (Y) and bodymass or change in bodymass (X) of ewes either continuously associated with or isolated from rams after weaning. Results which differ significantly are joined by a solid line and the level of significance is designated as: * P = 0,05; ** P = 0,01; *** P = 0,001.

Association with rams after weaning	Number of ewes in each category			Classification of ewes according to bodymass or change in bodymass during lactation and subsequent lamb production:																	
				Post-partum			Weaning			Lowest			Accumulative difference			Percent change to Weaning			Percent change to Lowest			Pre-mating		
	Lambs			Lambs			Lambs			Lambs			Lambs			Lambs			Lambs			Lambs		
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Isolated	59	404	66	44,1	44,8	49,2	40,0	41,0	46,9	36,9	38,3	43,2	-57,6	-49,1	-38,0	-9,1	-8,3	-4,3	-16,4	-14,5	-12,0	40,2	42,2	48,7
				***			***			***			*			*			**			*		***
	S.E.			±6,22			±7,50			±6,50			± 47,69			± 12,54			±8,83			±7,47		
Continuous	89	366	70	44,4	44,4	49,2	40,2	41,7	47,0	37,8	38,6	44,0	-49,4	-37,7	-31,3	-9,2	-5,7	-4,1	-14,8	-12,9	-10,4	44,9	46,4	53,4
				***			***			***			*			*			*			***		
	S.E.			±6,35			±7,49			±6,63			± 47,14			± 12,58			±8,97			±8,69		
All ewes	148	770	136	44,3	44,6	49,2	40,1	41,3	47,0	37,4	38,4	43,6	-52,7	-43,7	-34,6	-9,1	-7,1	-4,2	-15,4	-13,8	-11,2	42,9	44,2	51,1
				***			***			***			**			**			**			***		
	S.E.			±6,28			±7,49			±6,56			± 47,64			± 12,59			±8,93			±8,38		
Dependence Y on X percent				63,25			76,43			74,43			99,99			98,14			96,78			73,65		

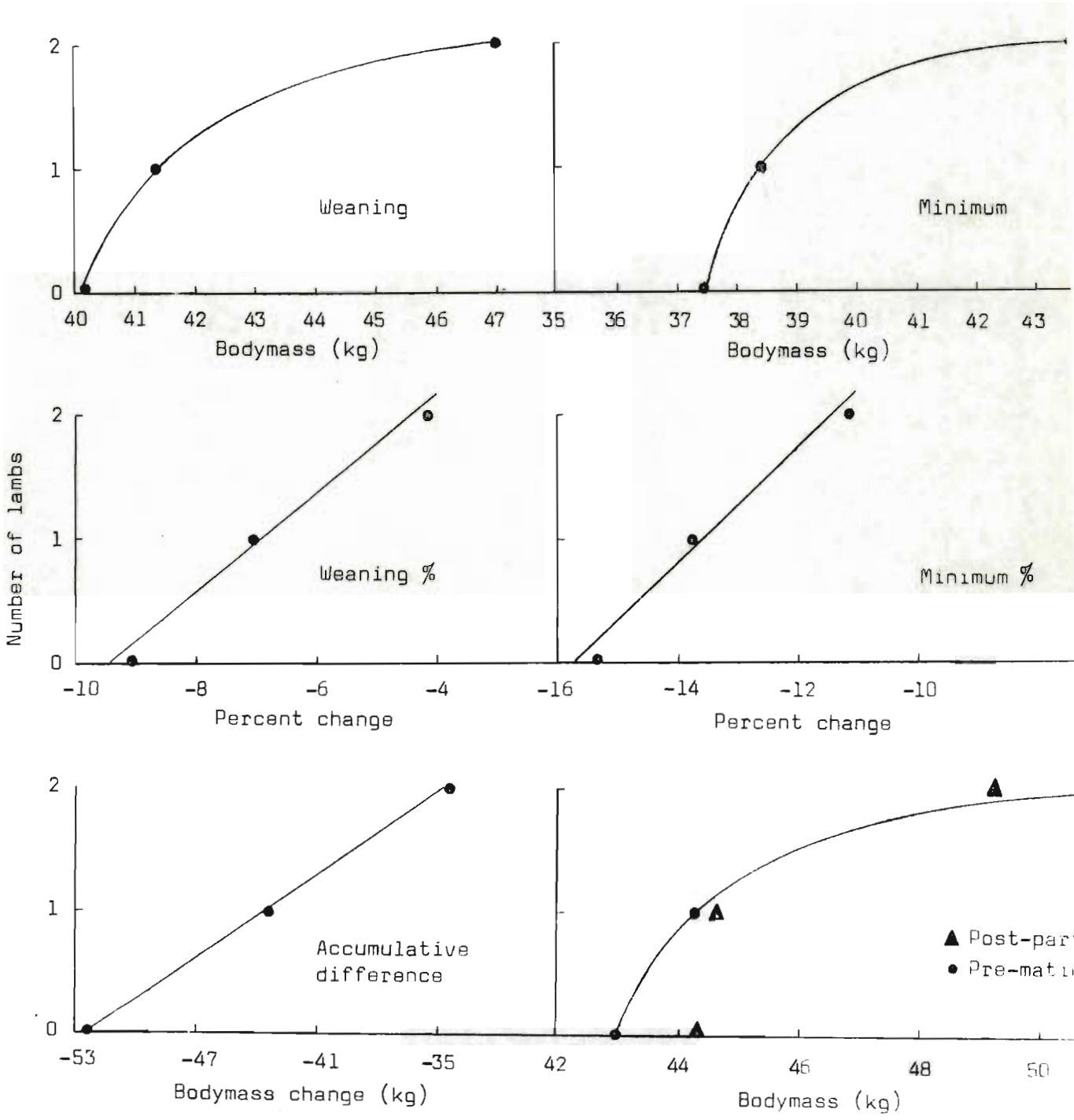


Fig. 10. Relationship between bodymass or change in bodymass and number of lambs born.

was more closely associated with the change in bodymass of the ewe prior to weaning than with her bodymass per se. Virtually all the results in Fig. 10 suggest curvilinear relationships, but with only three points available a straight line was found to be adequate in certain cases (Table 10).

Wool production

The average wool yield of the Merino ewes on the two planes of nutrition during lactation is given in Table 11. The data for 1968 and for the crossbred ewes have been omitted.

Table 11. Average wool production of ewes subjected to two planes of nutrition during lactation.

Year	Type of ewe	Nutritional plane during lactation	Number of animals	Average wool weight (kg)
1967	Merino	High Low	58 56	4,73 ± 0,69 3,96 ± 0,67
1969	Merino	High Low	122 120	5,54 ± 0,90 4,99 ± 0,86
1970	Merino	High Low	124 121	5,18 ± 0,85 4,77 ± 0,74
1971	Merino	High Low	62 65	5,52 ± 1,01 3,47 ± 0,79

In all cases the restriction in feed intake during lactation resulted in a significant reduction in the wool yield of the ewes ($P = 0,001$). However, there was no apparent break in the wool and the distribution of tender fleeces did not vary between the two planes of nutrition.

Lambs

Feed intake

During 1967 the housing facilities were such that the suckling lambs could not be confined in their relevant treatment groups, except during short periods of the day. It was thus not practicable to measure the daily intake of maize silage and lucerne hay. During subsequent years this was achieved. For the results in Table 12 it appears that the lambs reared by the ewes on the restricted feed intake were unable to compensate for the reduced milk intake by increasing their consumption of other feeds offered. The apparent lower intake of roughage shown by the lambs of ewes on the high plane of feeding, as compared to those on the low plane can probably be ascribed to consumption by the lambs of part of the ration offered to the ewes. However, among the animals on restricted feeding, the intense competition by the ewes for the available feed effectively excluded the lambs.

Self-feeders were used during 1972 and the feed intake was not recorded.

Growth

Pre-weaning

It is abundantly clear that sub-maintenance feeding of the lactating ewes resulted in a severe retardation of the pre-weaning growth of their lambs (Fig. 11). In all cases there was a significant difference in the weaning weights of the two groups of lambs ($P = 0,001$). There was no significant difference in the weaning bodymass between wethers or female lambs.

Post-weaning

The data in Table 13 demonstrates that the advantage in bodymass

Table 12. Feed intake of lambs classified according to the level of nutrition of the dam during lactation.

Year	Nutritional level of ewe during lactation	Average daily intake (kg) per lamb					
		Total pre-weaning period			7 days prior to weaning		
		Concentrate	Lucerne hay	Maize silage	Concentrate	Lucerne hay	Maize silage
1967	High	0,11	-	-	0,27	-	-
	Low	0,11	-	-	0,27	-	-
1968	High	0,12	0,07	0,19	0,27	0,17	0,21
	Low	0,12	0,09	0,21	0,23	0,21	0,23
1969	High	0,11	0,06	0,08	0,25	0,10	0,16
	Low	0,09	0,07	0,10	0,22	0,10	0,19
1970	High	0,12	0,12	0,18	0,24	0,22	0,30
	Low	0,13	0,12	0,23	0,23	0,24	0,31
1971	High	0,12	0,12	0,18	0,23	0,22	0,30
	Low	0,13	0,12	0,23	0,23	0,21	0,29

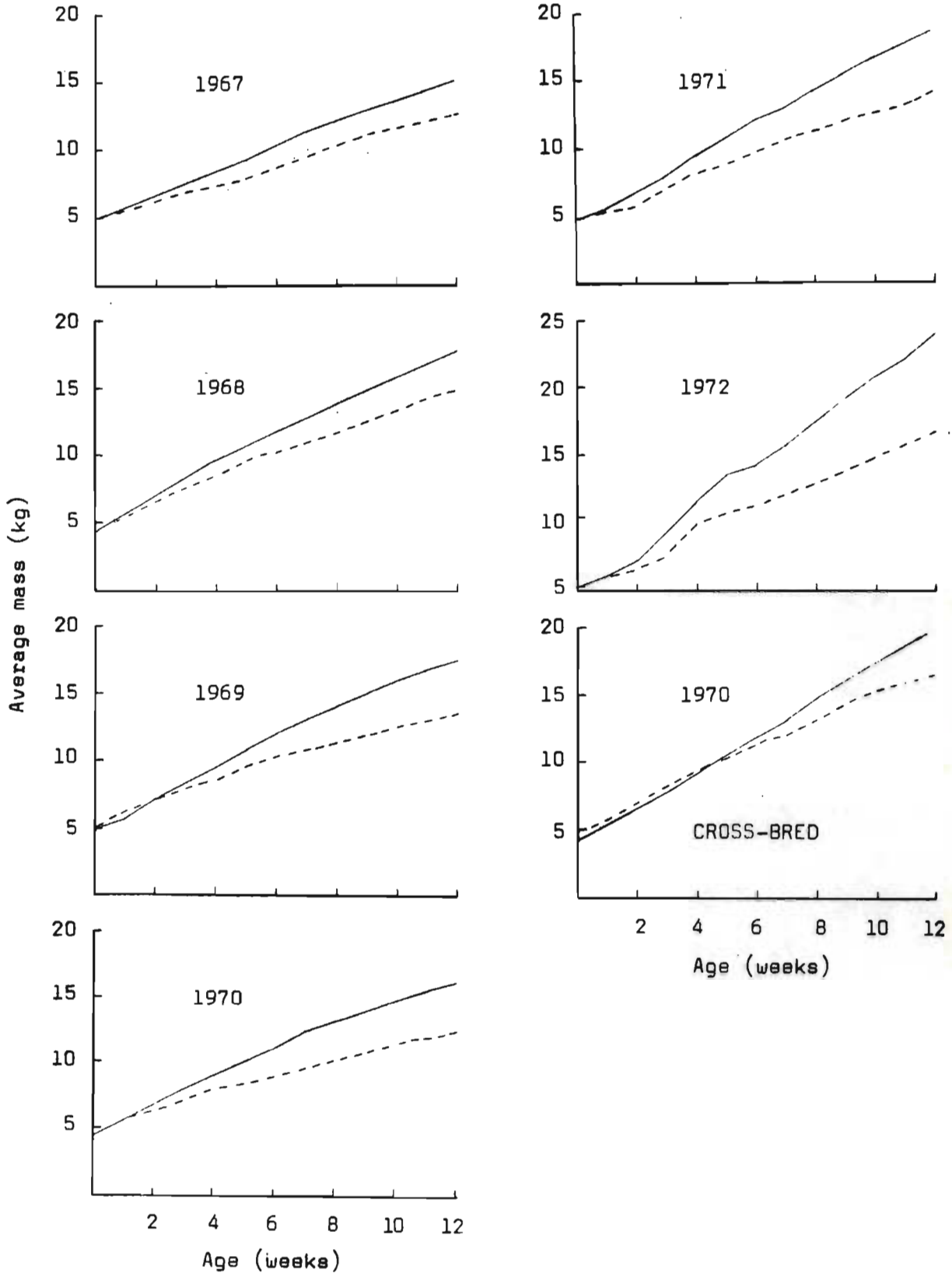


Fig. 11. Pre-weaning growth of lambs suckled by ewes on either a maintenance (——) or a sub-maintenance (----) ration.

Table 13. Pre- and post-weaning growth of ewe lambs reared by dams on two planes of nutrition.

Year of Birth	Nutrition of dam	Bodymass (kg) at various ages:															
		n	Birth	n	12 weeks	% difference High > Low	n	6 months	% difference High > Low	n	12 months	% difference High > Low	Mating n	Mating 19 months	% difference High > Low	Post-partum n	Post-partum 24 months	% difference High > Low
1967	High	28	5,0	28	14,6	18,6	40	20,6	7,3	34	31,4	4,7	38	31,9	1,5		-	-
	Low	27	4,9	27	12,3		38	19,2		23	30,0		34	31,4			-	
1968	High	69	4,4	69	17,7	21,2	67	23,9	13,8	67	32,6	6,2	49	43,0	8,3	33	47,9	4,6
	Low	68	4,3	68	14,6		64	21,0		60	30,7		37	39,7		23	45,8	
1969	High	51	4,4	51	16,1	31,9	22	21,3	11,5	21	31,5	5,7	20	42,8	4,4	13	41,7	2,7
	Low	69	4,6	69	12,2		24	19,1		24	29,8		21	41,0		15	40,6	

Table 14. Incidence of oestrus and lambing performance of ewe-lambs reared by dams maintained on two planes of nutrition during lactation.

Nutrition of dam	Percent ewes oestrus during 17-day periods commencing at average ages shown:										First fertile mating 1970			Lambing 1971		
	9	12	15 months								% mated days:		Not	Number of ewes producing		
	months	months	15 Apr	3 May	21 May	7 June	24 June	11 July	28 July	14 Aug	1-17	18-42	mated	No lambs	Singles	Twins
High	25,0	60,0	45,0	45,0	60,0	65,0	50,0	0	10,0	15,0	25,0	65,0	10,0	4	12	2
Low	19,2	28,8	38,4	19,2	70,0	66,4	28,6	0	14,3	4,8	38,1	52,4	9,5	4	15	0

which existed at weaning among the ewe-lambs, suckled by dams which received adequate feeding, gradually diminished in time, but was still evident at the first parturition.

Reproduction

The attempt to determine the age of puberty of the ewe-lambs born during 1969 was not a success, primarily because of the protracted period over which this appeared to occur. The data in Table 14 therefore reflect results obtained once the ewe-lambs had attained an average age of nine months. When these females were first exposed to fertile rams no animal showed a bodymass of less than 34 kg, and culling on the basis of inadequate mass was not necessary.

The results in Table 14 indicate a higher level of oestrous activity amongst the ewe-lambs which experienced no severe growth restriction prior to the age of 84 days. There was also no apparent effect on the number of lambs born during the first and second lambing seasons. After shearing in the spring of 1969 approximately 50 percent of the ewe-lambs were lost due to exposure during cold, rainy weather. Consequently, the number of animals involved is small, and no definite conclusions are warranted.

DISCUSSION

The value of the results presented here depends upon the extent to which they may find application in situations encountered by the majority of sheep farmers. It is therefore necessary to examine critically the two planes of nutrition applied to the ewes and the resulting changes in their bodymass. Every effort was made to ensure that the animals in these experiments were in an optimum condition at lambing time and, with few exceptions, the ewes on the high plane of nutrition during lactation

did not deteriorate in condition while suckling lambs.

There is a notable dearth of reliable data describing the change in bodymass of lactating ewes in commercial flocks. However, experimental studies have shown that it is unlikely that mature lactating ewes will improve in condition even when fed liberally during lactation (Wallace, 1948; Papadopulos & Robinson, 1956; du Toit, Nel & Cronje, 1971). Furthermore, in considering the effects of nutritional deprivation on productivity in sheep and cattle, Allden (1970) draws attention to the fact that the data relating to experimental studies are usually confined to the short-term effects of undernutrition on immature livestock. In the case of the grazing animal the situation is even more serious, and it is recognised that such animals are subjected to greater stresses than pen-fed animals (Allden, 1970).

Smith (1962) noted that during a two-month period in spring ewes lost up to 23 percent of their bodymass and he was of the opinion that this loss was similar to that observed in central western Queensland during spring and early summer. In later experiments the loss over six- and 12-week periods was somewhat smaller and varied from 7,9 to 14,3 percent, both during autumn and spring (Smith, 1966). In pen-feeding experiments Hunter (1962) recorded a loss of approximately 17 percent in bodymass of non-lactating animals maintained on a low plane of nutrition for six months. In the present experiments, the average reduction in mass during lactation varied from 11,4 to 18,0 percent for maiden ewes and 12,2 to 19,9 percent for older ewes (Table 3). A considerable effort has been made to focus attention on each individual ewe and it has been possible to relate the reproductive performance to a relatively wide range of changes in bodymass.

The relationship between bodymass or bodymass change and reproductive activity, as recorded in the present experiments, (Figs. 5 to 7),

represents an advance in the understanding of the phenomenon of anoestrus in ewes which have an extended breeding season, such as the Merino and allied breeds. These findings confirm the suspicion that size, and therefore bodymass, is an extremely important factor in selection programs. Large animals do not only cycle more regularly, but they are also more likely to be served early in the breeding season and to produce more lambs per parturition than small individuals. For example, a ewe with a bodymass exceeding 45 kg at weaning is more likely to exhibit continued oestrous activity during the accepted non-breeding period (July-Sept), to be mated within one oestrous cycle after exposure to breeding rams and to produce twin-lambs, than a ewe with a lesser bodymass. Where Merino ewes received supplementary feeding (drought rations) at the time of mating, White & Ternouth (1970) concluded that fewer than 40 percent of the ewes having a bodymass of less than 38 kg held to service during the first 17 days of the breeding period. Furthermore, Coop (1966) concluded that the degree of barrenness was influenced by bodymass alone and not by an increase in mass shortly before mating. The results in Fig. 10 agree with the findings obtained by these workers.

In reviewing the different parameters used to quantitate the nutritional stress which occurred it cannot be concluded that any particular parameter adequately described all situations. In general, the change in bodymass which occurred during the lactation period appears to provide an acceptable description of the stress encountered, provided this change is viewed in relation to the bodymass three days after parturition. The apparent significant effect of the latter, when all the data are pooled (Table 7), appears to be merely fortuitous. This relationship may depend to a large extent upon (i) the 1968 results, where the incidence of oestrus was not markedly depressed and the ewes showed a relatively high post-partum bodymass and (ii) the low incidence of oestrus amongst the maiden Merino

ewes which in turn exhibited the lowest initial bodymass. The former is supported by the results obtained during 1972. During this replicati of the experiment the ewes showed the highest post-partum bodymass recorded throughout this investigation. Although the loss in bodymass during lactation was similar to that recorded earlier the occurrence of oestrus was not markedly depressed and the pattern exhibited was similar to that recorded during 1968 (Fig. 4).

From the results in Table 8 it is apparent that although the ewes mated during the 17-day period commencing 15 October possessed a higher bodymass, particularly at the time of mating, than those bred later, the average mass differed according to whether the ewe was on a high or low plane of nutrition during lactation. This confirms the findings of Smith (1966) viz., that both the level of nutrition and bodymass, independent of the level of nutrition influence the incidence of oestrus in sheep during spring. Although the seasonal changes in occurrence of oestrus are related to bodymass (Figs. 5, 6 and 7) the overall effect on the number of ewes mating to entire rams is negligible. This agrees with the findings of Smith (1965, 1966), but is somewhat contrary to the results obtained by Hunter (1962) and by Smith (1962).

Smith (1966) maintains that a notable reduction in the incidence of oestrus amongst Merino ewes apparently occurs only where the quality of the pasture deteriorates during late summer, autumn or winter, resulting in reduced bodymass. The data presented here does not contradict this hypothesis, but suggests that the age of the animal also merits consideration. Although Smith (1965) studied ewes from the age of 12 months until they were more than two years of age, he made no direct comparison with mature individuals.

Smith (1965) and Lishman & Hunter (1966, 1967) suggested that, when studying the factors influencing oestrus under practical farming conditior

it is necessary to isolate the ewes for some time before they are rejoined with rams during the normal mating period. This opinion has been substantiated by the present results. Furthermore, rejoining with rams is probably the main stimulus which obliterates the deleterious effects of poor nutrition. A similar situation has been shown to exist in female mice subjected to undernutrition and stimulated by joining with males (Cooper & Haynes, 1967; McNeilly, Cooper & Crighton, 1970). The absence of the male stimulus may account for the results obtained by Hunter (1962) and by Smith (1962) where the anoestrous period was markedly prolonged by undernutrition. In attempting to account for the failure to observe a ram stimulus in certain instances, Hunter, Belonje & van Niekerk (1971) suggested that this could perhaps be related to the nutrition of the ewes at some earlier stage. The results in Figs. 4 and 5 indicate that this is unlikely to be the case when underfeeding occurs in autumn, although poor nutrition may sometimes slightly delay mating, particularly in maiden ewes.

The data obtained in this study confirms earlier findings (Lishman & Hunter, 1966, 1967; Hunter, 1969; Lishman, 1969) that isolation of ewes from rams leads to a cessation of sexual activity, while continuous association with rams reduces the proportion of ewes experiencing a period of anoestrus.

It is also evident from the data in Table 5 and Fig. 4 that some factor or factors operate to initiate breeding in a large proportion of anoestrous ewes which had been continuously associated with rams. Such individuals are mated soon after being joined with entire rams. The stimulatory effect of the presence of males on the occurrence of oestrus in mice is well illustrated by the work of Whitten (1959), Lamond (1959), Marsden & Bronson (1965) and Bronson & Desjardins (1969). This influence of the male gradually wanes (McNeilly *et al.*, 1970; Cooper, Purvis &

Haynes, 1972), but can be restored by removing a particular male, followed by re-introduction at a later stage (McNeilly et al., 1970). Such findings have led to the conclusion that the unfamiliar provides a stronger stimulus than the familiar (Cooper et al., 1972). It is thus possible that substitution of the entire rams for the vasectomized rams used for some six months may constitute a new stimulus. Alternatively, the twice daily teasing with vasectomized rams may not have constituted as strong a stimulus as the presence of entire rams throughout a 24-hour period. The typical lag phase prior to a high incidence of mating, following joining of ewes with rams after a period of isolation, was not observed among the ewes continuously with rams. Hunter & Lishman (1967a,b) and Hunter, Belonje & van Niekerk (1970) have shown that overt oestrus can occur in the absence of the corpus luteum of a previous cycle and furthermore, it has been observed (Lishman, 1968; unpublished) that oestrus can more easily be induced in both entire and ovariectomized ewes previously isolated from rams than those continuously associated with the same group of rams. By introducing new stimulus animals (entire rams), short heats and those of a low intensity, which may normally not be noted under conditions of twice-daily teasing (Hunter, 1968) may have been detected by the entire rams present throughout the day and night. The possibility also exists that such sub-normal heats may have been converted to normal overt oestrus by the new stimulus. In this context Sinclair (1950) has suggested that introduction of rams can transform silent heat to full heat. Hunter (1968) has suggested also that the mating of ewes under certain conditions could be more a problem of silent ovulation, or heats of varying duration and intensity, rather than one of ovulation failure. This hypothesis warrants careful consideration in attempting to account for the results presented here.

A further possible explanation of the results is that the ewes may have become synchronised with an annual mating-lambing rhythm, as suggested by Lyle & Hunter (1967). Accordingly, the new breeding season commences spontaneously during late October to early November of each year. Where isolated ewes have been rejoined with rams one cycle later than in the present investigation, viz., 1 November, the level of spontaneous oestrus has been comparable to that observed when similar animals were joined on 15 October. Lishman & de Lange (1967) have shown also that anoestrus persisted in ewes until January, if the ewes were not joined with rams until this time. Lishman (1969) also noted that the new breeding season commenced only in late January when ewes were continuously associated with the same group of rams. The work of Joubert (1962) indicated that, in most of the breeds or crosses studied, the increase in sexual activity commenced gradually during October and continued to increase for six months. The data obtained by Hunter (1962, 1964) showed a similar tendency, but the period of increasing oestrus was given as about three months.

It must be conceded, however, that the ewes incorporated in this study had a greater opportunity to adapt to the annual rhythm described by Lyle & Hunter (1967) than those involved in the earlier studies.

Considering the situation as a whole then it is suspected that breeding is initiated by a change in the male stimulus coinciding with the early stages of the "natural" breeding season.

The duration of the post-partum ancestrous period is influenced by factors such as the breed of the ewe, the season of lambing, nutritional factors, the time of weaning and stimulation by rams (Granger, 1955; Hunter, 1968). Ewes which lamb when the seasonal stimuli for the initiation of oestrus are strong, resume normal breeding within three to six weeks after parturition (Sayed, Blakeslee & Nelson, 1952; Hafez,

1952; Granger, 1955, Williams, Garrigus, Norton & Nalbandov, 1956; Lees, 1964; Miller & Wiggins, 1964; Smith, 1964b). The interval reported here agrees closely with that observed by Granger (1947), Smith (1964b) and Fletcher (1971), but is considerably shorter than that reported by Joubert (1962) for Merino ewes lambing in autumn.

Kirillow (1944) has reported that poor nutrition prolongs the period of post-parturient anoestrus. van Niekerk & Mulder (1965) are of the opinion that the plane of nutrition is the greatest single factor affecting the duration of post-partum anoestrus. The results obtained by Smith (1964b) support this contention. The delayed onset of oestrus, associated with an extended suckling period (Barker & Wiggins, 1964), may also be at least partly due to the nutritional stress involved.

The results presented here fail to show a consistent significant relationship between the change in bodymass during lactation and the delay to first oestrus (except amongst maiden ewes), but there appears to be a tendency for the ewes with the greatest bodymass at parturition to exhibit oestrus sooner than lighter animals. This supports the findings of Hunter & Lishman (1967b) and Vosloo, Hunter & Carstens (1969) where a similar, but significant, effect was observed regarding the interval to first ovulation.

Both Barker & Wiggins (1964) and Lees (1967) detected a clear tendency for the mean day of first service to be progressively delayed as the date of parturition became later during autumn and winter (northern hemisphere). Where observations were made covering lambing seasons extending over several months (Hunter et al., 1970), or less than one month (Hunter, 1971), a negative correlation was obtained. The results of the investigation reported here show the length of the lactational anoestrous period to be negatively correlated with the date of lambing, in spite of the fact that the lambing season did not exceed seven weeks

in duration. Hunter (1968) has reviewed the effect of seasonal factors on the length of the post-partum anoestrous period. He concluded that the interval to first oestrus after lambing can be expected to be greatest in ewes lambing during the late stages of the breeding season. However, the data in Fig. 3 do not agree with this hypothesis since it is commonly accepted that in the Merino the breeding season reaches a peak during the months of March and April. Relationships such as these are of practical significance only where re-breeding soon after lambing constitutes an important aspect of the production system. For such systems the recommendation would be to restrict lambing to the month of June and thereby limit the post-parturient anoestrous period to an average of 35 days.

The results in Table 9 and Fig. 10 demonstrate that bodymass <u>per se</u> is correlated with the number of multiple births. This confirms the hypothesis proposed by Coop (1962), and also demonstrated by Wallace (1961), Tribe & Seebeck (1962), Coop (1966) and Lino & Braden (1968). In Merino ewes the ovulation rate has been reported to be 105 percent for ewes of 35,0 to 37,5 kg at the time of ovulation. The rate increased at least five percent for every additional 2,5 kg up to 53,5 kg, and at least 10 percent per 2,5 kg increase in the range 40,4 to 48,4 kg (Edey, 1968). The apparent curvilinear relationship between the majority of parameters relating to bodymass (Fig. 9) agrees with these findings, but the data suggest that the bodymass at mating is perhaps less closely related to lambing rate than changes in mass which may have occurred at an earlier stage. Guerra, Thwaites & Edey (1972) concluded that size and condition, the two components of bodymass, each have a significant and independent influence on ovulation rate. Their findings suggested that bodymass was more useful in predicting ovulation rate than either of its two components. The findings presented here should not be construed as a contradiction of

the results obtained by Coop (1966), since he concentrated on changes which occurred very much nearer the time of fertile mating than was the case in this study.

The response to flushing, both in terms of increase in mass and in the incidence of multiple births, was disappointing. Compared to the 1,4 kg maximum true gain per week due to flushing grazing ewes, obtained by Coop (1966), the present gains are perhaps acceptable. The increase in the proportion of ewes twinning as a result of flushing varied between 2,5 and 4,0 percent, and the increase in total number of lambs born was 2,3 to 7,4 percent (Table 8). Increases of between seven and 13 percent have been reported by Tribe & Seebeck (1962), between eight and 16 percent by Hulet, Blackwell, Ercanbrack, Price & Humphrey (1962) and 15 percent (Coetzee, 1964) when ewes were flushed for not more than three weeks prior to mating. On the average, flushing for 17 days prior to conception has been found by Coop (1966) to result in a 10 percent increase in twinning; the flushing response was no better for ewes in good condition than for those in poor condition. The latter finding is confirmed by the present data. Coop (1966) emphasised the fact that the sensitivity to flushing may be different in populations exhibiting a bodymass and lambing percentage different to that of the animals incorporated in his investigations.

It is possible that selection against twinning has been rigid in the population from which the experimental animals were obtained. This means that little response to flushing should be expected. The genetic basis for a high twinning rate has been demonstrated by Turner, Hayman, Triffit & Prunster (1962), Young, Turner & Dolling (1963) and Packham & Triffit (1966). Darlow (1942) has suggested that in ewes having a low genetic potential for increased ova production, the response to flushing will be small. The results of Lamond (1963) and Bellows, Pope, Meyer,

Chapman & Casida (1963a,b) support this contention.

It would appear that the standard of management, including level of nutrition, on the average farm in South Africa does not favour the production and rearing of more than one lamb per ewe. Until the level of farming improves very little can be gained by recommending a lamb drop (number of lambs born per number of ewes lambing) of more than 100 percent.

The data of Drinan (1968), McLaughlin (1966) and Stevenson (1968) illustrate the conclusion that differences in the bodymass of lambs at weaning, due to twinning or resulting from the time of birth within the lambing season, are still evident at 18 months of age. In addition, Schinckel & Short (1961) noted that although severe undernutrition during the first 16 weeks of post-natal life reduced bodymass by 48 percent, two years later the difference had shrunk to 10 percent. In grazing animals, Reardon & Lambourne (1966) and Allden (1968) concluded that undernutrition during the early stages of post-natal life did not significantly affect final mature size. The results pertaining to the ewe-lambs produced in the experiments described here are in accordance with the foregoing.

Some reports indicate that the reproductive rate of maiden ewes (Esplin, Madsen & Phillips, 1940; Davies, 1950), but not the lifetime production of lambs (Bradford, Weir & Torrell, 1961; Reardon & Lambourne, 1966; Giles, 1968) is influenced by the plane of nutrition during their early post-natal life.

The findings reported here suggest that even in maiden ewes the influence of poor nutrition during early life is negligible (Table 14), but further work is required before definite conclusions can be drawn.

At first glance it might be said that the results presented suggest that undernutrition during lactation has no marked effect on the performance of the ewe. Similarly, Coop (1966) has drawn attention to

the fact that the post-weaning period is commonly considered not to constitute a critical stage in the annual cycle of the ewe and that ewes may be subjected to nutritional stress without evidence of a notable effect, except for a loss in bodymass. He warned, however, that loss of bodymass was associated with a reduction of 0,22 to 0,45 kg in wool production, a lowering of the twinning rate and an increase in the incidence of barrenness. In a similar vein, the data obtained in the present investigation demonstrate that feed restriction during lactation reduces the wool production by 0,41 - 2,50 kg, the total number of lambs born by 2,1 to 6,4% (up to 17,4% in the case of maiden ewes) and severely retards the growth of suckling lambs. Some of these effects can be modified by the stage of maturity of the ewe and are likely to be particularly severe in young ewes which have not reached their mature size. van der Westhuyzen (1971) has also reported that maiden ewes are more sensitive to an inadequate level of nutrition than older animals. Clearly, undernutrition of the lactating ewe is highly undesirable, and this factor will play a major role where the frequency of lambing is increased to more than once every 12 months.

The analysis of the data presented here has again emphasised that studies where the frequency of oestrus and occurrence of parturition are measured can be considerably complicated by the statistical methods available. Although treatment differences may follow a similar pattern over several replications of an experiment the use of the χ^2-test commonly indicates significant differences only where the treatments applied produce widely divergent responses. Coop (1966) has outlined the problems encountered and has indicated that large populations are required to show significant treatment effects. Since the number of ewes was limited the alternative of repeating the experiment over a number of years was followed in the experiments described here. Although more than 1 200

ewes were incorporated over a period of six years the results showed considerable variation from year to year. The practice of conducting an investigation over several seasons is no doubt sound, particularly when seasonal factors are likely to play a significant role. However, such a procedure does not necessarily simplify interpretation of the findings and can very often further complicate the conclusions. A factor which created additional problems was that the population studied changed over the years.

The necessity to utilise relatively large groups of animals (usually not less than 25 per treatment) where the χ^2-test is used to test for treatment differences also seriously limits the number of factors that can be varied at any one time. This has precluded the investigation being expanded to include variations in the level of nutrition at other stages of the productive cycle e.g. late gestation or post-weaning. A situation has thus arisen in which the results are applicable perhaps only to a limited set of circumstances.

The type of study that has been described is also hampered by the necessity to use a sexually active ram to detect oestrus in the ewe. This automatically introduces a stimulus which should be excluded under certain conditions. Since the ram appears to rely to a certain extent on the olfactory sense to detect ewes in oestrus the possibility of training a sheepdog to follow a similar procedure is perhaps not as unrealistic as may initially appear.

Investigations in this field must place emphasis on the long-term effects of feed restriction, especially as regards the influence on the progency of poorly fed ewes. In this respect particular attention should be given to the immature dam. Only when the information from studies of this nature becomes available can the principles governing efficient commercial practice be placed on sound foundations.

CHAPTER 2

SENSITIVITY OF THE OVARY TO GONADOTROPINS

INTRODUCTION

The ewe has been shown to experience seasonal variations in ovulation rate (Dermody, Foote & Hulet, 1970), ovarian response to exogenous gonadotropins (Bundig, Schooley, Bock & Steelman, 1953; Braden, Lamond & Radford, 1960), sensitivity to progesterone (Lamond & Bindon, 1962; Lamond, 1964a) and its response to gonadal steroids (Raeside & McDonald, 1951; Reardon & Robinson, 1960). The finding that the response is usually greatest at the peak of the breeding season suggests that these factors may be involved in the seasonality of breeding. Long-term variations in the level of nutrition of ewes may also affect reproduction via an alteration of one or more of these ovarian mechanisms.

On the basis of work done by Werner (1939), Maddock & Heller (1947), and by Rinaldini (1949), it has been concluded that under conditions of poor nutrition the gonads and accessory sex structures atrophy due to a relative lack of gonadotropins reaching the target organs, and not because of the development of a refractory state in the gonads (Ershoff, 1952). This hypothesis is also favoured by Ratner & Meites (1963).

In order to study variations in ovarian sensitivity to gonadotropins, it would be advisable to subject test animals to equal stimuli, for example, equal doses of a gonadotropin. The administration of exogenous gonadotropins usually results in a concomitant release of endogenous gonadotropins (Lamond & Emmens, 1959; Lamond & Bindon, 1966; Stevens, Jackson & Nalbandov, 1968; Sugawara, Umezu & Takeuchi, 1969; Sugawara & Takeuchi, 1970; Cumming, Brown, Blocky & Goding, 1971a; Christenson &

Eleftheriou, 1972) and the ovarian response then represents the effect of both exogenous and endogenous stimuli acting in concert. The obvious method for excluding the unknown quantities of endogenous gonadotropins is to remove the pituitary gland, but the problems associated with hypophysectomy on a large scale, and the low survival rate of operated animals, serve as major deterents to work of this nature.

It is generally accepted that progestogens suppress ovarian cycles by blocking the acute release of LH by the pituitary gland (Makepeace, Weinstein & Friedman, 1937; Astwood & Fevold, 1939; Dempsey, 1939; Gordon, 1963 ; Hoffman, 1967; Nalbandov, 1966; Pincus, 1966; Arimura & Schally, 1969; Stevens, Spies, Hilliard & Sawyer, 1970; Hilliard, Schally & Sawyer, 1971). A series of experiments was, therefore, conducted to determine the ovarian response following the administration of physiological levels of exogenous gonadotropins when progestogens were used to suppress pituitary release of LH. It was hoped to develop a technique whereby the sensitivity of the ovary to gonadotropins, under varying conditions, could be tested, particularly where animals had been subjected to under-nutrition.

These studies involved a series of preliminary experiments which were modified progressively in accordance with the results obtained from each completed trial.

DEVELOPMENT OF THE EVALUATION TECHNIQUE

General procedure

Where progestogens are used for the induction of out-of-season breeding, or for the suppression of oestrous cycles during the breeding season, the usual practice is to apply progestational therapy for a period varying from 10 to 16 days, followed by the administration of

gonadotropins in certain cases. Smith & Robinson (1969) have shown that a 12-day treatment period with progestogen is sufficient to synchronise all ewes. Plotka, Erb & Harrington (1970) demonstrated that, in the cycling ewe, luteal function begins to decline 12 to 14 days after oestrus. Therefore, throughout the preliminary experiments described in this section, progestogens were administered for an initial period of 12 days. The day on which treatment commenced was taken as day one. Where applicable, gonadotropins were administered on day 13, and progestogen therapy continued, uninterrupted, until ovarian examination four days later (day 17). The laparotomy technique of Lamond & Urquhart (1961) was followed.

Two progestational compounds were included in this study viz., crystalline progesterone and 17α-acetoxy-9αfluoro 11β -hydroxy-4-pregnene-3,20 dione (SC-9880 or Cronolone). The crystalline progesterone was dissolved in arachis oil (10 mg/cm^3) and injected daily (intramuscularly) at 0800 hours. SC-9880 was applied intravaginally through the medium of impregnated, intravaginal pessaries (Synchro-mate, G.D. Searle & Co., Ltd.).

Each treatment group of ewes was usually sub-divided, and the day on which treatment commenced was staggered so that examination of the ovaries could more easily be accomplished. Wherever the date of commencement of a treatment is quoted, this refers to the first sub-group. Observations for oestrus were made only in the initial experiments and vasectomized rams were introduced only on day 15 so as to avoid ovarian stimulation and ovulation.

The experimental animals were housed in partly-roofed pens and were fed lucerne hay ad lib. Water, and a salt-bonemeal lick, were freely available.

Ovarian response to exogenous gonadotropins during uninterrupted progestational therapy

Experiment 1

Daily injection of crystalline progesterone

Lamond (1964a) concluded that when progesterone is administered daily, the optimal dose to suppress oestrous cycles does not fall below 10 mg per day. Consequently, this dosage was administered daily (im) to 24 mature Merino ewes which had been randomly allocated to the six treatment groups listed in Table 15.

Two gonadotropins, PMSG and HCG, were used in these experiments. In view of its FSH-like characteristics, PMSG has been used to induce out-of-season breeding and ovulation (Hunter, 1968) and to increase the ovulation rate during the breeding season (Gordon, 1958a). The dosages of PMSG used vary between 500-1000 IU. Robinson (1951a) has suggested that the threshold level for the induction of ovulation during anoestrus is 400 IU, although ovulation has been induced by dosage rates as low as 250 IU (Robinson, 1951b; Gordon, 1958a).

Procedure

The purpose of this experiment was to establish the dose of PMSG which would be just sufficient to induce ovulation. The level of PMSG used was based on a rate of 250 IU. A similar procedure was adopted for HCG where the main interest lay in the ovulating capacity of this gonadotropin.

Both PMSG and HCG were freeze-dried preparations which were reconstituted in distilled water prior to use. Administration was by subcutaneous injection.

At laparotomy, the number of corpora lutea (indicating recent

ovulations) and the number and size of ovarian follicles were recorded.

Results

The results obtained after examination of the ovaries are summarised in Table 15. Follicles with a diameter of 5 mm or more were classified as large.

Table 15. Ovarian response to PMSG and HCG in progesterone-blocked ewes.

Breed of ewe	Date commen-ced	Number per group	Type of progesto-gen	Gonado-tropin	Dose IU	No.of ewes ovula-ting	Ovulations		Total no. of large follicles
							Total	Range /ewe	
Merino	27 Sept 1967	4	None	None	-	3	3	0-1	0
			Progeste-rone	None	-	0	0	-	3
				HCG	250	1	1	0-1	1
					500	0	0	-	4
				PMSG	250	0	0	-	1
					500	1	2	0-2	2

The results in Table 15 suggest that very few of the ewes had shown spontaneous ovulation prior to the commencement of the experiment. Only two animals, one which underwent progesterone therapy but received no gonadotropin, and the other which received 500 IU PMSG, possessed a corpus luteum of a previous cycle. In the control group (no progesterone) ovulation was apparently induced by introduction of teaser rams, and the progesterone therapy was clearly able to block such ovulations in ewes which received no exogenous gonadotropin. Although few ovulations were induced by either PMSG or HCG, the occurrence of large follicles in ewes treated at the 500 IU level suggest that this dose was only slightly below the threshold for ovulation. This applies particularly to HCG, where the follicles appeared to be close to rupture.

Experiment 2

Intravaginal pessaries containing SC-9880

Robinson (1964, 1965) developed an effective method for controlling oestrus and ovulation in the ewe by administering progestogens intravaginally, using polyurethane sponges. Work on a number of steroids has shown that in the cyclic, entire ewe the steroid SC-9880 (Cronolone) is highly effective as an inhibitor of ovulation and comparable to progesterone as regards interval to oestrus and ovulation (Shelton & Robinson, 1967). At dosage rates of between 10 and 40 mg, oestrus and ovulation were blocked for at least 15 days (Robinson, Moore, Holst & Smith, 1967).

Procedure

The treatments applied in Experiment 1 were repeated, but the blocking agent in this case was intravaginal pessaries impregnated with 30 mg SC-9880. Thirty-six maiden and parous Dorper ewes were randomly allocated to six treatment groups (Table 16) and the pessaries were removed only at laparotomy.

Results

The occurrence of recent ovulations and the total number of large follicles in all ewes exposed to the same treatment are presented in Table 16.

Table 16. Ovarian response to PMSG and HCG in ewes bearing intravaginal pessaries impregnated with progestogen.

Breed of ewe	Date commenced	Number per group	Type of progestogen	Gonadotropin	Dose IU	No.of ewes ovulating	Ovulations		Total no. of large follicles
							Total	Range /ewe	
Dorper	30 Sept 1967	6	None	None	-	1	1	0-1	2
			SC-9880	None	-	0+	0	-	4
				HCG	250	1*	1	0-1	4
					500	2	2	0-1	3
				PMSG	250	2+	6	0-4	2
					500	4	10	0-4	2

* One and + two ewes respectively lost the pessaries and have been omitted.

The Dorper ewes which were not receiving injections of gonadotropins appeared to be in a deeper stage of anoestrus, as judged by the number ovulating following introduction of rams, than the Merino ewes in Expt. 1. However, the former ewes showed a greater response to gonadotropins than the latter. Administration of PMSG resulted in a greater proportion of the ewes ovulating and also more ovulations per animal than HCG. The possibility that the lag phase to ovulation may not be the same for the two gonadotropins used should be borne in mind when considering the respective responses. Since ovarian examinations were conducted at a fixed interval after gonadotropin injection (four days) this would result in fewer ovulations being observed where the latent period exceeded 96 h than where a more rapid stimulation was produced.

In addition to the great variability between animals in the response to PMSG, this gonadotropin also resulted in marked stimulation of follicle enlargement and a high incidence of cystic follicles, at both dose levels. Robinson (1951b) has reported similar findings. HCG, on the other hand, produced only minimal stimulation of the ovaries as regards ovulations

and growth of follicles. In ewes treated only with progestogens the ovaries seldom contained more than one medium to large follicle. These results are illustrated in Plate 1.

Experiment 3

Variations in the level of exogenous gonadotropin

Procedure

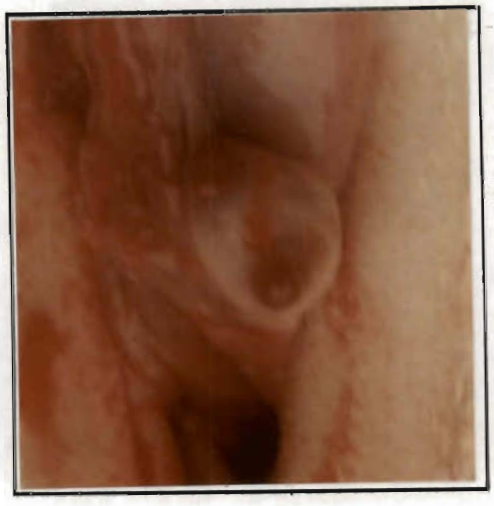
The results obtained in Expts. 1 and 2 suggested that the dose of gonadotropin was perhaps too low to induce ovulation in most of the ewes. It should be borne in mind in this connection that the initial trials were conducted during the anoestrous period, and Robinson (1950) has stated that both the breed of ewe and stage of anoestrus influence the results from a given dose.

In Experiment 3 increased levels of gonadotropin were used (Table 17). The Dorper ewes previously incorporated in Experiment 2 were again randomly allocated to the six treatment groups. Progesterone was administered daily (10 mg in oil, im) for an initial period of 12 days, gonadotropins injected on the 13th day and progesterone treatment continued until laparotomy on day 17.

Results

Over the dose-range of 500-1000 IU HCG the number of ewes ovulating and the ovulation rate per ewe did not change with increase in dose rate (Table 17). With PMSG, the number of ewes ovulating followed a similar trend, but the ovulation rate showed a higher variation than with HCG.

A



B

C

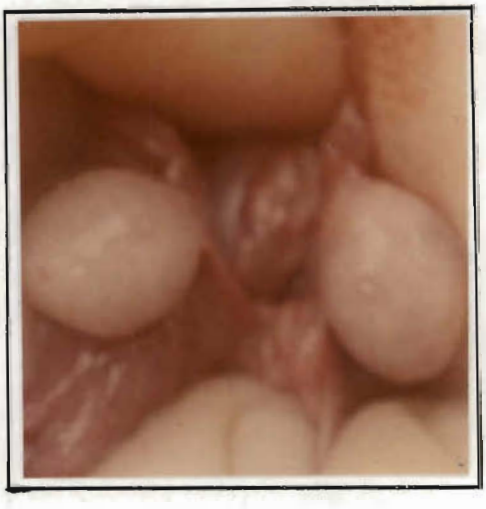


Plate 1. Examples of the limited stimulation by HCG (A), the high incidence of cystic follicles following treatment with PMSG (B) and the ovaries of a ewe receiving progesterone treatment, but no exogenous gonadotropin (C).

Table 17. Ovarian response to increasing levels of PMSG and HCG in progesterone-blocked ewes.

Breed of ewe	Date commen-ced	Number per group	Type of progesto-gen	Gonado-tropin	Dose IU	No.of ewes ovula-ting	Ovulations		Total no. of large follicles
							Total	Range /ewe	
Dorper	20 Nov. 1967	6	Progeste-rone	HCG	500	4	7	1-2	4
					750	4	7	1-3	12
					1000	4	6	1-2	9
				PMSG	500	2	9	4-5	10
					750	2	5	1-4	26
					1000	2	4	1-3	26

In this experiment the response to exogenous gonadotropins was generally greater than in Expt. 1 and is perhaps related to the later stage of the breeding season during which Expt. 3 was conducted. Where the ewes were treated with PMSG the high incidence of cystic follicles was again evident.

The observation that administration of gonadotropins in excess of the 500 IU level did not increase the number of ewes ovulating, but stimulated additional follicular development suggested that the lower dose approximated the threshold level for the induction of a single ovulation. It appeared also that 500 IU HCG produced a reasonably predictable response. Consequently, it was decided to base future trials on levels of gonadotropin not exceeding 500 IU and to concentrate on the use of HCG.

Form of progestational therapy and the dose required

Experiment 4

Daily injection of progesterone or the use of progestogen pessaries

When this investigation was initiated it was not anticipated

the Dorper and Merino ewes would show a differential response to exogenous gonadotropin treatment. However, the data obtained in Expts. 1 and 2 suggested that the lesser response in the former trial could have been due to breed differences or to the different progestogen therapies.

Procedure

The Merinos used in Expt. 1 were randomly allocated into four groups and treated either with progesterone or SC-9880. The pessaries were removed at the time of laparotomy after 16 days of insertion and daily progesterone injections (10 mg) were conducted for the same period. Gonadotropins were injected on day 13 as before.

Results

The results in Table 18 indicated that there was little difference between progestogen treatments as regards the proportion of ewes possessing large follicles. Among the ewes injected with HCG there was a greater number of ovulations after progesterone than after SC-9880 pre-treatment. This suggests that the latter progestogen may have more effectively blocked endogenous LH release than progesterone.

Table 18. The type of progestational therapy and the ovarian response to exogenous gonadotropins.

Breed of ewe	Date commen-ced	Number per group	Type of progesto-gen	Gonado-tropin	Dose IU	No.of ewes ovula-ting	Ovulations		Total no. of large follicles
							Total	Range /ewe	
Merino	22 Nov. 1967	6	Progeste-rone	HCG	500	5	5	0-1	1
				PMSG		1	1	0-1	5
			SC-9880	HCG		1	2	0-2	5
				PMSG		0	0	-	6

Experiment 5

The level of progestogen treatment

Although the available literature suggests that a daily dose of 10 mg progesterone is sufficient to suppress oestrous cycles (Lamond, 1964b), there appears to be no information on the minimum level which will effectively inhibit pituitary release of LH in the presence of the positive feedback resulting from administration of exogenous gonadotropins

Procedure

In order to test whether the dosage levels of progestogen used in the earlier experiments (10 mg progesterone per day for 16 days or pessaries containing 30 mg SC-9880) were adequately suppressing pituitary release, 18 of the Merino and 18 of the Dorper ewes previously used in these investigations were each randomly allotted to three groups. Commencing on 15 January, 1968, they were treated with either pessaries containing 30 mg SC-9880 or were given daily injections of 10 or 20 mg progesterone. On day 13, 500 IU HCG was administered. At laparotomy eight cases of hydrops uteri were observed and the results of this experiment have been discarded. A second attempt was initiated on 23 April when 24 Merino ewes that had not previously been used in these experiments were randomly divided into three groups and treated as described in Table 19. Approximately one year later the experiment was repeated and the levels of progestogen modified further (Table 19).

Results

The data presented in Table 19 suggest that as the level of progesterone rises there may be slight reduction in the response to HCG, as shown by either the total number of ovulations or the number of ewes ovulating. However, the number of large follicles did not always support this conclusion.

Table 19. The ovarian response to 500 IU HCG in ewes receiving different levels of progestational therapy.

Breed of ewe	Date commen-ced	Type of progesto-gen	Dose	No. of ewes	Ewes ovula-ting	Distribution of ovulations					Total large follicles
						1	2	3	4	To-tal	
Merino	23 April 1968	Progeste-rone	10 mg per day	8	7	4	3	0	0	10	6
			20 mg per day		5	4	2	0	0	8	10
		SC-9880	30 mg initial		7	4	2	1	0	11	7
	9 May 1969	Progeste-rone	10 mg per day	10	9	2	5	1	1	19	15
			40 mg per day		7	3	3	1	0	12	13
		SC-9880	30 mg initial		10	4	4	2	0	18	16
			40 mg initial		10	3	7	0	0	17	18

Redmond (1968) concluded that progesterone decreased the sensitivity of follicles to LH, but Hixon & Armstrong (1971) consider the evidence for an antiovulatory effect of progesterone, at the ovarian level, to be only circumstantial.

In general, it appears from Table 19 that progesterone does not have a direct effect upon the ovarian response to exogenous gonadotropins. This agrees with the findings of Spies, Stevens, Hilliard & Sawyer (1969) and Naqvi & Johnson (1970).

Stilboestrol suppression of pituitary gonadotropin release

Experiment 6

It has been suggested that while progesterone inhibits pituitary release of LH, oestrogen leads to an accumulation of FSH in the pituitary gland (Martini, Fraschini & Motta, 1968; Saunders, 1970). Oestrogen undoubtedly suppresses ovarian function in domestic (Nalbandov, 1958) and other animals (Everett, 1961). It was, therefore, decideded to use the synthetic oestrogen diethylstilboestrol in an attempt to suppress pituitary

release of gonadotropin prior to treatment with exogenous gonadotropins.

Procedure

Twenty-eight Merino ewes were randomly divided into four treatment groups and injected (im) daily with 1 mg diethylstilboestrol. This treatment was continued for 10 days and the dose was then increased to 2 mg for the following four days. Gonadotropins were injected on day 11, and laparotomies were performed three days later.

Results

The results presented in Table 20 suggest that the stilboestrol treatment did not block pituitary release of gonadotropins since ovulation was induced in the majority of animals, irrespective of their treatment with exogenous gonadotropin.

Table 20. Ewes ovulating following treatment with diethylstilboestrol and exogenous gonadotropins.

Breed of ewe	Date commenced	Gonadotropin	Dose IU	No. of ewes		Total number of ovulations
				per group	Ovulating	
Merino	18 August 1970	None	-	7	6	6
		HCG	500		4	6
		PMSG	500		7	9
		HCG + PMSG	250+250		5	5

Tervitt & Welch (1970) have reported that 2 mg stilboestrol administered early in the oestrous cycle can induce ovulation 56 h later, and Foote (1964), and Howland, Kirkpatrick, Woody, Pope & Casida (1968) hav recorded a similar response with oestradiol-17β . The results in Table 20 do not contradict the belief that short-term administration of oestrogens stimulates secretion of pituitary gonadotropins while long-term application inhibit such secretion (Everett, 1961; Greep, 1961; Barraclough & Haller, 1970).

THE INFLUENCE OF UNDERNUTRITION ON THE OVARIAN RESPONSE TO GONADOTROPIN

Experiment 7

The series of preliminary experiments showed that ovulation could be induced while maintaining a progestational blockage of the endogenous release of gonadotropins. It was also demonstrated that HCG produced a fairly predictable result and that this technique could possibley be applied to test for differences in ovarian sensitivity to gonadotropin. A further two experiments were conducted to observe the ovarian response to HCG after ewes had been subjected to a period of undernutrition continuing for approximately five months.

Procedure

On May 27, 1970, Merino ewes (two years of age) were randomly divided into two equal groups of 30. Commencing on this date they were placed on high and low levels of nutrition respectively such that a bodymass difference of at least 20 percent would be produced at the end of a five-month period. The high plane ration consisted of 0,45 kg mille lucerne hay, 0,1 kg maize meal and maize silage ad lib. The animals on the low plane were restricted to half the intake of those on the high plane and the maize meal was replaced by Eragrostis curvula hay, fed ad lib. The bodymass of the ewes was recorded weekly and the ewes were kept isolated from rams at all times.

Commencing on October 27 (day 1) each ewe was injected daily with 10 mg of progesterone in 1,0 cm^3 arachis oil. Administration continued at this rate for a further nine days and the dose was then doubled for a further three days. On day 11 the ewes on each plane of nutrition were further randomly subdivided into two sub-groups and either 350 or 500 IU HCG in 1,0 cm^3 aqueous solution was injected subcutaneously at 0800 hours.

At laparotomy, three days later, the ovaries were excised, trimmed and placed in 0,9 percent saline. Examinations were then made for corpora lutea, indicating recent ovulations, and also for follicular development. The ovaries were then dried on filter paper and weighed prior to being sliced into small sections. The ovarian fluid was absorbed onto filter paper and the mass of the remaining ovarian tissue again determined. The quantity of ovarian fluid was therefore equal to the total mass of each ovary minus the mass after release of the follicular fluid.

Results

The change in bodymass of the ewes on the two planes of nutrition is illustrated in Fig. 12A; the results of the ovarian examinations are summarised in Table 21. Since the presence of a corpus luteum markedly increased the mass of the ovary the data in Table 21 includes only ovaries in which no corpora lutea were present.

Table 21. The ovarian response to exogenous gonadotropin in ewes maintained on two planes of nutrition.

Plane of nutri-ion	Dose of HCG (IU)	Number of ewes		Total number of		Average mass of ovarian fluid (g)	Average mass (g) of ovaries in which no ovulation occurred	
		Per group	Ovula-ting	Ovula-tions	Large follicles		Left ovary	Right ovary
High	350	15	13	20	16	0,616	0,3739 —*—	0,5371
	500	15	13	17	20	0,596	0,4562	0,5147
Low	350	15	11	16	20	0,466	0,4413	0,4474
	500	15	12	15	18	0,548	0,4926	0,4908

* Indicates difference significant, P = 0,05.

During the 22-week period prior to the commencement of progesterone therapy the difference in bodymass between the ewes on the low and those

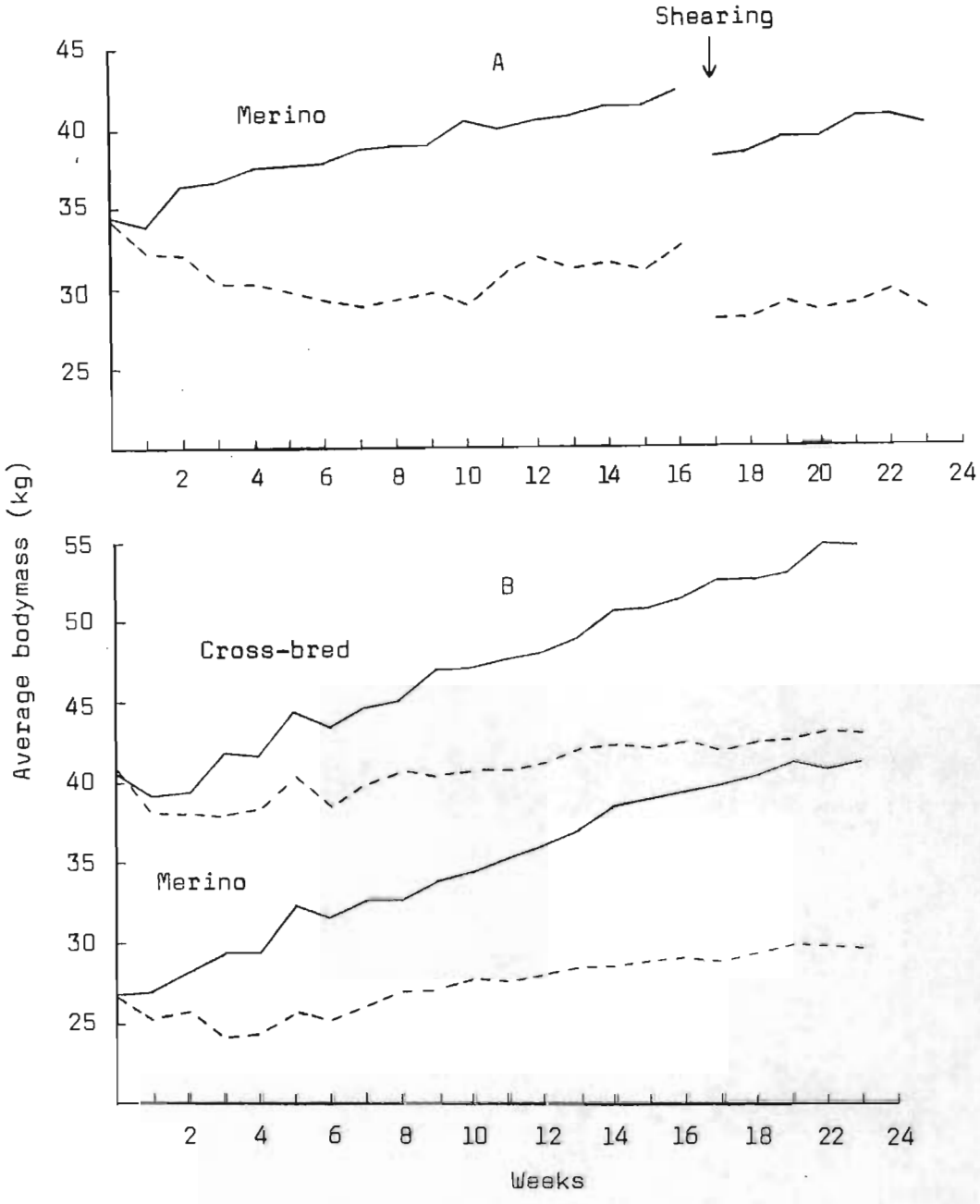


Fig. 12. Changes in bodymass of groups of ewes on high (——) and low (----) planes of nutrition.
A - commenced 27 May, 1970
B - commenced 25 Nov, 1970

on the high plane of nutrition gradually increased until the difference was 39,5 percent. Most of this difference is due to increases in the mass of the well-fed ewes; the low-plane ewes lost relatively less mass than was gained by the high-plane animals.

The data in Table 21 show that there was some tendency for the ovaries of the well-fed ewes to contain more ovarian fluid than was present in the ovaries of the ewes subjected to nutritional restriction. This was particularly evident when 350 IU HCG was administered. The left ovary in this group had a significantly lower ($P = 0,1$) mass than the right ovary. No other differences were significant and it is clear that undernutrition had no marked influence upon the ovulatory response of the ovary.

Experiment 8

The data obtained in Expt. 7 suggested that the ovulatory response of the ovary was, perhaps, too insensitive a measure of ovarian reaction to exogenous gonadotropin. On the other hand, the quantity of ovarian fluid appeared to warrant further attention.

Procedure

Expt. 7 was repeated, but the interval between the administration of the gonadotropin and the removal of the ovaries was reduced to less than 24 h. By this means it was hoped to attain maximum ovarian stimulation with a minimum of ovulations occurring. The dose of HCG was also reduced in order to avoid masking possible differences in ovarian sensitivity.

A total of 38 Merino and 44 Cross-bred (SAMM x Merino) ewes (18 months of age) were randomly allocated to two groups and, commencing on 25 November, 1970, they were placed on high and low planes of nutrition.

The rations fed were identical to those used in Expt. 7. Each group of ewes was sub-divided prior to the commencement of progesterone administration. The date on which the latter commenced was staggered so as to limit the number of ovariectomies to be performed on any one day. Daily injections of progesterone (10 mg/day) commenced in the first sub-group on 15 April, 1971 and continued for 10 days in all animals. On day 11, the daily dose was doubled, as in Expt. 7, and either 0, 250 or 350 IU HCG was injected on this day. Initially, the gonadotropin was administered at 0800 h and ovariectomy took place within 24 hours. A number of ewes had already ovulated when the laparotomies were performed. Therefore, in the second, third and fourth sub-groups the injection of HCG was timed so that ovarian examination took place within 22, 21 and 19 h respectively, after gonadotropin administration.

Results

The data presented in Fig. 12B show that after 21 weeks the average bodymass of the ewes on the high plane of nutrition exceeded that of the underfed animals by 26,7 percent in the case of the cross-breds and by 27,0 percent amongst the Merinos.

Even though ovariectomy was performed not more than 24 h after administration of HCG a large proportion of the cross-bred ewes had already ovulated by this time (Table 22). Consequently, the mass of the ovary is again presented only where ovulation had not occurred at the time of ovariectomy. Since the left and right ovaries showed very little difference in mass, the results in Table 22 represent the average of all ovaries in which no ovulation had occurred.

When the ovarian response to HCG is measured in terms of ovarian fluid, the ewes on the high plane of nutrition tended to show a greater reaction to increasing levels of HCG than animals maintained on a low

Table 22. Ovarian response in ewes subjected to two planes of nutrition prior to HCG treatment. Means which differ significantly have been joined by a line. *P = 0,05; **P = 0,01.

Breed	Plane of nutrition	Ewes ovulating			Mean ovarian fluid (g)			Mean ovarian mass (g)		
		HCG			HCG			HCG		
		0 IU	250 IU	350 IU	0 IU	250 IU	350 IU	0 IU	250 IU	350 IU
Merino	High	0/6	2/6	2/6	0,2914	0,2909	0,4777	0,5012	0,4966	0,4745
	Low	0/6	1/8	0/6	0,2646	0,2554	0,2839	0,3796	0,3157	0,3709
Cross-bred	High	0/6	5/8	2/8	0,4615	0,6018	0,4575	0,7729	0,7078	0,6005
	Low	0/6	6/8	6/8	0,3090	0,4669	0,5147	0,5645	0,5980	0,6599

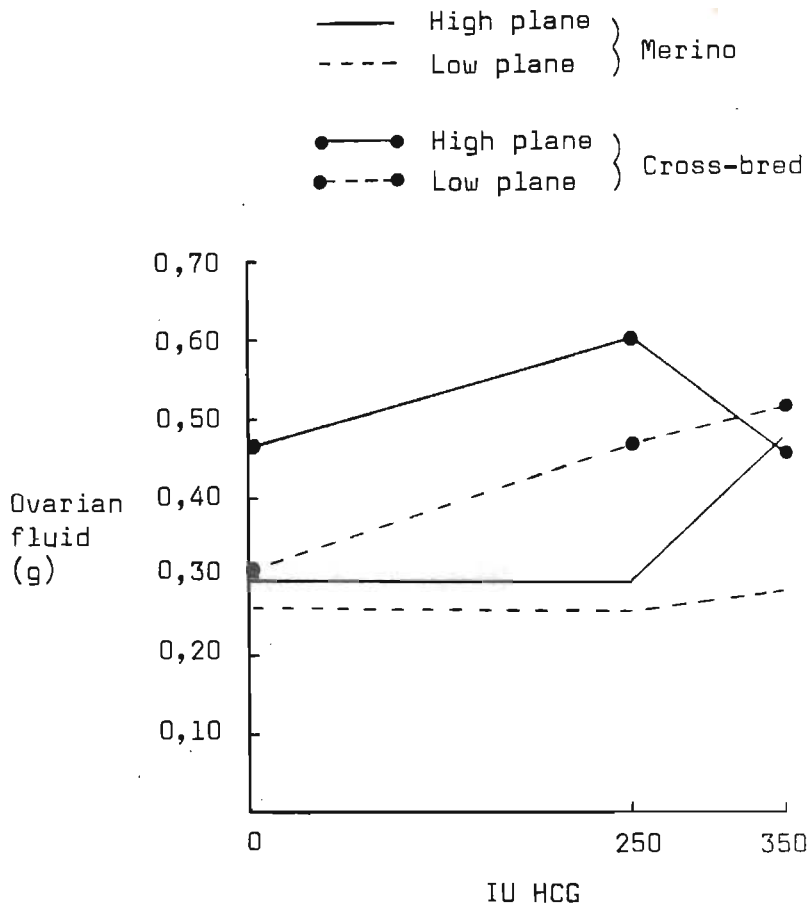


Fig. 13. Ovarian response to HCG in ewes on two planes of nutrition.

plane of feeding (Fig. 13). The gonadotropin treatment had no measurable effect on the mass of the ovary (Table 22). Where no gonadotropin was administered, the high plane of nutrition increased the ovarian mass without markedly influencing the fluid content of such ovaries (Table 22, Fig. 13).

The cross-bred ewes appeared to show a greater response to HCG stimulation, both in terms of ovulation and ovarian fluid, than the Merinos. This difference may be influenced to a certain extent by the greater ovarian mass of the former animals. This, in turn, appears to be correlated with the bodymass of the ewes.

DISCUSSION

Progestational inhibition of pituitary release

The experiments described here were originally thought to be the firs in which progesterone therapy was employed to prevent the release of natura pituitary hormones augmenting the response to exogenous gonadotropins. However, Bellows et al. (1963a) have used a similar procedure to study the mechanism whereby flushing increases the ovulation rate in sheep. Woody, Ginther & Pope (1967) successfully induced ovulation in the ewe after administering pituitary extracts on the fourth and fifth days of a 12-day progesterone treatment period (25 mg/day). The units used to measure gonadotropin activity (gram equivalents of pituitary extract) complicate comparison with the commonly accepted international units. In pigs, Polge & Day (1967) concluded that it required about twice the dose of PMSG to induce the ovulatory response during treatment with the progestogen, ICI 33828, than when administered during the normal follicular phase of the cycle. These results support the belief (Allen & Lamming, 1960) that when exogenous gonadotropins are administered, this stimulates release of

pituitary reserves of gonadotropins.

Although it is commonly accepted that both progesterone (Astwood & Fevold, 1939) and synthetic progestogens suppress LH release (Pincus, 1965) this theory is by no means universally accepted. In discussing the nature of the luteotrophic process Rotchild (1966) maintains that progesterone does not block tonic LH release, but that the ovulatory surge is suppressed. He bases this conclusion on the fact that even with 10 mg progesterone per day in rats follicles continued to grow as far as the pre-ovulatory stage. In the same discussion Nalbandov disagreed with these conclusions and stated that progesterone completely blocks LH in the pig and to a large extent also in the sheep and the guinea-pig. Nalbandov maintained that in such animals the ovaries are completely devoid of anything but the smallest type of follicles, none of which approach ovulatory size. The observations made in the present study agree with this conclusion. Both species differences, as well as the levels of progesterone used, may account for differences of opinion reported in the literature. In reviewing the subject, McDonald & Clegg (1967) are of the opinion that inconclusive results have been obtained. The recent development of sensitive assay methods for determining circultating levels of gonadotropins must eventually throw light on the controversy. Where progesterone has been applied at the rate of 20 or 40 mg per day for 10 days (McDonald & Clegg, 1967) the serum LH levels were not reduced below the normal basal levels. From four to six days after the withdrawal of progesterone, serum LH levels again rose. This agrees with the earlier findings of Hoffman & Schwartz (1965) where the pituitary level of LH was found to decrease sharply after termination of progesterone treatment. Similarly, Labhsetwar (1967) reported a significant increase in hypophysial LH stores when norethyndrel was administered for 10 days to female rats.

After more than 30 years of research, the locus of action of progestogens in blocking ovulation remains undecided. Hilliard et al. (1971) have reviewed the evidence suggesting either a suppression of LH-releasing hormone or direct action on the adenohypophysis. These workers have obtained results favouring action outside the central nervous system. It should also be borne in mind that progesterone, even in massive doses, is essentially inactive in blocking FSH release (Desaulles & Krähenbühl, 1964) and that continued growth of ovarian follicles (Ulberg, Christian & Casida, 1951; Zimbelman, 1963; Johnson & Ulberg, 1965) up to the pre-ovulatory stages can thus be expected. In the present experiments the ewes which received progesterone, but no exogenous gonadotropin did not ovulate during the treatment period. However, there were signs of continued follicular growth, agreeing with the findings of Desaulles & Krähenbühl (1964).

The possibility that exogenous gonadotropin administration may have induced release of endogenous gonadotropins in this investigation cannot be entirely excluded. This applies particularly to the pessaries containing SC-9880. Morgan, Loch & Robinson (1967) estimate that, on the average, 16 percent of the SC-9880 present in a pessary at any time would be absorbed each day. Therefore, in pessaries containing an intial dose of 30 mg, during the 15th day (i.e. two days after gonadotropin administration) 0,4 mg could be absorbed and the fully effective dose of 0,3 mg (Morgan et al., 1967) would be maintained during the 17th day. In contrast, Robinson (1970) suggests that, regardless of the initial dose impregnated, the amount of SC-9880 absorbed over the last four days, of a 16-day insertion period, is minute.

Comparison of gonadotropins

The results obtained here show that under the prevailing circumstance

HCG produces a reasonably predictable result, both in terms of ovulation rate and appearance of the ovary and corpus luteum (Plate 1). On the other hand, PMSG results in a large number of cystic follicles. This is further circumstantial evidence to indicate a progesterone block to pituitary LH release, since PMSG is said to possess follicle stimulating and luteinizing actions in the approximate ratio of 5 : 1 (Lamond, 1962). Robinson (1962) is of the opinion that PMSG produces more consistent responses than HCG. He found that the latter produced more ovulations and that the incidence of cystic follicles was higher with HCG. In contrast, Chumming & McDonald (1967) have noted a marked variation in the response of Romney ewes to PMSG. It has been stated that PMSG is unlikely to cause ovulation in the ewe with a functional corpus luteum (Robinson, 1950, 1951b). The findings described here are contrary to this belief. Betteridge (1971) has concluded that HCG requires no endogenous pituitary hormones in order to induce ovulation and that it need not cause their release to be effective. This is probably due to the fact that, like PMSG, HCG also has LH and FSH activities (Ashitaka, Tokura, Tane, Mochizuki & Tojo, 1970). It would appear that under the conditions of these experiments HCG is a better balanced source of gonadotropins than PMSG. The data obtained in Expt. 8 confirm the findin that ewes ovulate within 20 to 28 h after HCG treatment (Ortavant, Thibaul & Winterberger, 1949; Braden, Lamond & Radford, 1960; Dzuik, Hinds, Mansfield & Baker, 1964), but it was considered probable that under conditions of continued pituitary suppression ovulation would be further delayed. It is obviously necessary to delay examination of the ovary as much as possible in order to obtain the maximum response to administered gonadotropins. The apparent shortening of the interval to ovulation, which was observed in the cross-bred ewes, in Expt. 8, suggests that this latent period could be a useful measure of the degree of stimulation

by gonadotropins.

Occurrence of hydrops uteri

No reasonable explanation can be offered for the incidence of hydrops uteri when the ewes were subjected to repeated hormone therapy. This condition has not previously been reported under these conditions, but has been observed in ewes treated with clomiphene (Lindsay & Robinson 1970).

In the present experiments no particular sequence of treatments was found to be common to the development of hydrops uteri, and the condition could not be induced where ewes were subjected to three successive 18-day periods of progesterone therapy, separated by rest periods of one month. Hulet & Foote (1969) reported that ewes subjected to repeated PMSG treatment rapidly develop a refractory reaction. In the data reported here, those animals which developed hydrops uterus had received at least two previous treatments with gonadotropins.

Sensitivity of the ovary

Bindon, Chang & Turner (1971) have examined the response of Merino ewes to PMSG in animals which had been bred for high and low fecundity. They recorded 100 percent ovulation when 375 IU PMSG were administered during the normal oestrous cycle. The dose-response curve was found to vary between the two genetic types. The thrice greater response of the animals with the greater inherent fecundity was ascribed to either higher levels of endogenous gonadotropin or to more sensitive ovaries (Bindon et al., 1971). Similar genetic differences in the ovarian response of ewes to exogenous hormones have been reported by Robinson (1950) and by Larson, Banbury & Spaeth (1970). In comparable studies

with rats, genetic differences have been explained in terms of ovarian sensitivity (McLaren, 1962; Land & Falconer, 1969), while females selected for fecundity have been shown to increase the pituitary output of gonadotropins (Mauleón & Pelletier, 1964). Besides being more sensitive to injected FSH, the ovaries of such animals have also been found to contain a greater number of developing and primordial follicles (Mauleón & Rao, 1963). These studies, therefore, lend some support to the hypothesis that sensitivity of the ovary can vary (Table 22, Fig. 13).

Investigations using hypophysectomized mice have demonstrated unequivocally that under conditions of restricted feeding both the proportion of mice ovulating and the number of ova per ovulating female are lower following gonadotropin treatment than in similarly treated animals fed ad libitum (Fielden & Brumby, 1962). An association between bodymass and ovulation rate following treatment with PMSG has been observed in ewes (Guerra, Thwaites & Edey, 1971). However, Tait (1971) concluded that the plane of nutrition did not modify the response to PMSG. The occurrence of multiovulation in anovulatory human subjects treated with gonadotropins suggests some ovarian involvement since such subjects probably possess deficient endogenous levels of gonadotropins. Cases of over-stimulation regularly occur in humans (Seddon, 1970), despite preliminary testing with graded levels of gonadotropins (Crooke, Butt & Bertrand, 1966).

Using intact sheep, Bindon et al. (1971) found that the response to increasing doses of gonadotropin differed between groups differing in inherent fecundity. This finding agrees with the results presented in Fig. 13. The results suggest further, that the ovaries of the cross-bred ewes were more sensitive to gonadotropic stimulation, in terms of both ovulation and mass of ovarian fluid, than those of the Merino ewes. This may, perhaps, be correlated with the greater ovarian mass of the

former animals, but the possibility of an incomplete progesterone block can again not be ignored. Land & Falconer (1969) have concluded that the size of the ovary has little or no effect on the ovulation rate of mice.

Howland (1972a) reported that underfed rats experience a progressive decline in ovarian mass during a 34-day experimental period and that the response of the hypothalamo-hypophysial mechanism to hemiovariectomy was also impaired. Good nutrition showed a tendency to increase the ovarian mass of the ewes in Expt. 8 (Table 22), but the results in Expt. 7 (Table 21) were less conclusive. Varying the dose of gonadotropin appeared to have little effect on the ovarian mass.

Conclusions

In view of the limited number of animals involved in any single experiment of this series, only tentative conclusions are warranted. The need for large numbers of animals in experiments of this nature is fully appreciated, but available facilities and technical assistance imposed limitations on the practical size of the individual experiments. The problems encountered can perhaps be gauged from the fact that during the course of this investigation a total of 390 laparotomies and 142 ovariectomies were performed on the 294 ewes studied. Further investigations are clearly indicated and attention should be focussed on animals which have been subjected to greater stresses than the animals on the low planes of nutrition in these experiments. The technique developed appears to hold promise, particularly as the numerous hormonal imbalances which must follow hypophysectomy are apparently not as severe when progesterone is used to inhibit acute pituitary release of LH. The limitations of hypophysectomy are evidently realised by Betteridge (1971) who transplanted the ovaries from oestrous rabbits into hypophysectomized individuals. In

this way he sought to avoid the regressive changes within the follicles which, in turn, could inhibit the response to exogenous gonadotropins. This raises the possibility that the variation in response to HCG was not due to differences in ovarian sensitivity, but the result of a deficiency of some extra-ovarian factor or factors required to mediate the effect of this exogenous gonadotropin. A further possibility is that under high nutrition the number of vesicular ovarian follicles that can respond to gonadotropins increases (Hammond, 1952).

The technique developed in this study can probably be improved upon by incorporating a combination of oestrogen and progesterone, since this appears to be a more effective inhibitor of pituitary release. It may then be possible to study various facets of the ovarian response simply by changing the proportion of these two gonadal steroids. The use of drugs such as chlorpromazine to block ovulation (Robertson & Rakha, 1965) could also yield interesting results, but the greatest potential appears to lie in the application of antisera. Such substances are highly specific and likely to produce no side effects. Incorporation of sensitive radioimmunoassays will then demonstrate the inactivation of the endogenous hormone being studied. This principle could also be applied to determine whether gonadal steroids successfully suppressed pituitary release of gonadotropins after application of exogenous stimuli. However, the absence of a cross-reaction between endogenous and exogenous hormones is vital to the successful application of such tests.

CHAPTER 3

PLASMA LUTEINIZING HORMONE LEVELS IN MERINO EWES

INTRODUCTION

In sheep, undernutrition is associated with a cessation of oestrous cycles (Hunter, 1962; Smith, 1962). Ershoff (1952) is of the opinion that atrophy of the gonads under similar conditions (Rinaldini, 1949) is at least partly due to a reduction in the quantity of gonadotropins reaching the target organs. The anoestrous condition which follows periods of feed restriction in ewes is similar to the normal seasonal anoestrus and often involves a prolongation of (Hunter, 1962) or a precipitated onset of (Smith, 1962), the seasonal period of sexual quiescence. A satisfactory explanation of the factors involved in the cessation of sexual activity during certain seasons of the year may, therefore, assist in clarifying the events which follow a period of undernutrition.

The commonly accepted, simplified concept of the mechanism of ovulation includes the release of FSH from the pituitary during the late dioestrous and pro-oestrous stages of the cycle (Robertson & Hutchinson, 1962). In the presence of small amounts of LH, follicular growth and oestrogen secretion are induced (Greep, van Dyke & Chow, 1942; Lostroh & Johnson, 1966; Eschkol & Lunenfeld, 1967), to be followed by the acute release of LH some hours prior to ovulation (McCracken, Baird & Goding, 1971). The belief has thus arisen that the breeding and non-breeding seasons may be related to the rate of synthesis and/or release of LH from the adenohypophysis (Dutt, 1960; Robertson & Hutchinson, 1962). This is supported by the finding that during anoestrus in sheep the ovaries are not quiescent and follicles continue to grow and regress as during the normal oestrous cycle (Grant, 1934; Cole & Miller, 1935; Roux, 1936;

Kammlade, Welch, Nalbandov & Norton, 1952; Watson, 1952).

There is evidence to show that long-term undernutrition results in degeneration and atrophy of the gonads and secondary sex structures (El-Sheikh, Hulet, Pope & Casida, 1955; Wiltbank, Rowden, Ingalls, Gregory & Koch, 1962).

However, in the underfed rat, the cessation of oestrous cycles and ovarian atrophy have been associated with elevated levels of total pituitary gonadotropin (Rinaldini, 1949; Srebnik & Nelson, 1963). In sheep the total gonadotropic potency of the pituitary has also been reported to be higher during anoestrus (Kammlade et al., 1952) and during the onset of the breeding season (Warwick, 1946; Raeside & Lamond, 1956) than during the breeding season. In contrast, Lamond, Radford & Wallace (1959) and Roche, Foster, Karsch, Cook & Dzuik (1970) have been unable to demonstrate increased pituitary LH reserves in the sheep during anoestrus.

Interpretation of these early findings has been particularly hampered by the fact that no conclusive information could be obtained on the quantity of hormone released by the pituitary. This was due mainly to the inability of the existing bioassay techniques to detect the small quantities of gonadotropin in the circulation. Progress in this field was slow until the development of more sensitive assay techniques.

The radioimmunoassay, first developed by Yalow & Berson (1960), has greatly simplified the study of endocrine secretions and the successful application of this assay has depended, in no small way, on the isotopic labelling technique of Greenwood, Hunter & Glover (1963). Radioimmunioassay methods, besides being considerably more sensitive than the accepted bioassay techniques (Berson, 1968), are extremely useful in that circulating levels of the various pituitary hormones can

be detected without sacrificing the test animal.

Following the development of a sensitive and specific immunoassay for LH (Midgley, 1966; Odell, Ross & Rayford, 1966) it became possible to examine the quantities of this gonadotropin reaching the target organs. The purpose of the present study was to examine such hormonal levels in plasma, using the radioimmunoassay, in animals subjected to undernutrition during lactation, in the expectation that a better understanding of the hormonal mechanisms leading to a reduction of oestrous activity would result from the investigation.

PROCEDURE

Experimental animals

The experimental animals used in these studies were Merino ewes that had been maintained on either a high or a low plane of nutrition during lactation. The experimental rations, changes in bodymass and reproductive activity of the ewes have been described in Chapter 1. The following stages of the breeding season, or categories of animals, were studied:-

(i) Ewes anoestrus during lactation

During the period June to July, 1970, blood samples were obtained, at weaning, from all the ewes which had not exhibited oestrus during the lactation period. Ewes which were showing oestrus regularly, served as controls and sampling in these animals was limited to the interoestrous period.

Five consecutive daily samples, each of 10 cm^3 blood, were obtained by jugular veni-puncture and collected into heparinized test tubes. The plasma was separated by centrifugation and the samples stored at $-15^{o}C$

until assayed.

(ii) Late anoestrus

Just prior to the expected commencement of the new breeding season (10 October, 1970) i.e. when the incidence of oestrus had declined to a minimum in ewes continuously associated with teaser rams, at least five anoestrous animals were randomly selected from the two age groups (maiden or eight-year old) and from the two planes of nutrition. A similar procedure was adopted for the ewes isolated from rams at that time, except that the reproductive state was unknown in these ewes.

Blood samples were collected and stored as before.

(iii) Ewes exhibiting oestrus

During 1969, it was noted that ewes underfed during lactation showed a lower incidence of oestrus than ewes on a high plane of nutrition; this difference began to appear during August. In an attempt to elucidate the mechanisms involved in a cessation of breeding, it was decided to study the pre-ovulatory release of LH with the object of identifying any abnormalities in the pattern of hormone release. The aspects considered likely to exhibit aberrations were the timing of the LH surge in relation to the onset of oestrus (latency to release), the duration of the surge, the maximum concentration of LH in the circulation and the shape of the curve derived from graphic representation of the pre-ovulatory LH release. Consequently, during the months of August, September and October of 1970 and 1971, blood samples were obtained from ewes at the time of oestrus. Observations for mating were made using vasectomized teaser rams. The rams were placed with the ewes at intervals of not more than two hours, but at the peak times of commencement of oestrus viz., sunrise and sunset, continuous observations were made, the rams being frequently interchanged

between the various groups of ewes. Oestrus was taken to commence when a ewe would permit service by the vasectomized ram. At this stage the ewe was immediately removed from the presence of the ram.

Blood samples were obtained from the first five ewes on each plane of nutrition which exhibited oestrus after the commencement of an observation period (i.e. August, September or October). Samples were drawn into heparinized syringes, from an indwelling silastic cannula (Portex) inserted into the jugular vein. During 1970, 10 cm^3 of blood was taken at two-hour intervals, commencing as soon as a ewe stood for service by the ram and continuing for a further 36 hours. The following year the sample size was reduced to 5 cm^3 and the interval between collections to one hour. After centrifugation, the plasma samples were stored at $-15^{o}C$ in two equal portions so as to avoid repeated freezing and thawing of the same sample.

The procedures which were adopted were such that ewes exhibiting their last oestrus prior to a period of anoestrus were likely to be included in the sample. At the same time the sample of ewes would include those showing their first oestrus of the new breeding season.

The results obtained during 1970 and 1971 suggested that the release of LH occurred prior to oestrus in some ewes. Consequently, during October 1972 blood samples were collected at four-hour intervals, commencing approximately 12 hours prior to the expected time of oestrus. As soon as mating had occurred the samples were taken at hourly intervals for the next 24 hours. Ewes that had been on either the high or the low plane of nutrition during lactation were again studied.

(iv) Anoestrous ewes injected with oestradiol-17β

The pre-ovulatory release of LH can be induced in the anoestrous ewe by the administration of exogenous oestradiol-17β (Goding, Catt, Brown,

Kaltenbach, Cumming & Mole, 1969; Radford, Wheatley & Wallace, 1969).

The ability of the ewes to respond to oestrogen injection was tested during October, 1972. Of the ewes which had been on the high plane of nutrition during lactation 11 had not exhibited oestrus for between one and eight oestrous cycles. On the low plane of nutrition 33 ewes were judged to be anoestrus at this time. The latter animals were randomly divided into three groups and injected with either 5, 10 or 40 μg oestradiol-17β . The ewes on the high plane received either 5 or 10 μg injections.

Goding et al. (1969) administered the same oestrogen in 2,0 cm^3 0,9% NaCl by intramuscular injection and detected a release of LH eight to 12 hours later. In view of the insolubility of oestradiol-17β in aqueous media, 20 mg was dissolved in 1,0 cm^3 acetone and then added to 50 cm^3 0,9% NaCl. This suspension was then further diluted to provide solutions containing either 5, 10 or 40 μg in 2,0 cm^3.

Immediately after intramuscular injection of the oestradiol-17β an intravenous cannula was inserted into the jugular vein of each ewe and a sample of blood collected. Four hours later a further sample was drawn and thereafter at two-hour intervals for the following 26 hours. Radioimmunoassay of the samples obtained showed that none of the ewes had responded by exhibiting a release of LH.

The experiment was repeated two weeks later at which stage six high plane and 21 low plane ewes were judged to be anoestrus. On this occasion the oestradiol-17β was dissolved in arachis oil. The dose administered was either 40 (3 high plane, 11 low plane ewes) or 20 μg (3 high plane 10 low plane ewes) in 0,5 cm^3 oil. Blood samples were collected at 0 and 6 hours after oestrogen administration and thereafter at two-hour intervals for the following 34 hours.

Immunoassay procedure

Radioiodination

Purified ovine LH (G3-206; 2,02 x NIH-LH-S14, Papkoff) was iodinated at 19°C, using the method of Greenwood et al. (1963) as modified by Niswender, Reichert, Midgley & Nalbandov (1969). An aliquot containing 2,5 µg LH in water (1,0 mg/cm^3 solution) was transferred to a small glass test tube (0,7 cm^3 capacity) and 25 µcm^3 of 0,5 M sodium phosphate buffer (pH 7,5) was mixed with the LH. One half millicurie, carrier-free ^{125}I (Radiochemical Centre, Amersham) was added and after gentle agitation, 20 µg of the oxidising agent chloramine-T in 40 µcm^3 0,05 M phosphate buffer was introduced. The reaction was allowed to proceed for two minutes while the contents of the vial were agitated by finger-tapping. The reaction was then stopped by the addition of 120 µg sodium metabisulphite in 50 µcm^3 0,05 M phosphate buffer. A transfer solution (100 µcm^3) consisting of 16 percent sucrose and 1,0 µg KI was added and purification of the iodination mixture accomplished by gel filtration using disposable 20 x 1 cm Bio-Gel P60 columns (Niswender et al., 1969). The columns were quilibrated for at least 24 h with 0,05 M phosphate buffer before the iodination mixture was added. Approximately one hour prior to use, 50 mg bovine serum albumin (BSA) was passed through the column to reduce non-specific binding of the LH. The excess albumin was washed from the column with 30 cm^3 0,05 M phosphate buffer.

The iodination mixture was layered beneath the buffer on the column and a further 70 µcm^3 of transfer solution (8 percent sucrose, 0,5 µg KI) added to the iodination vial, recovered and in turn layered on the column.

The ^{125}I-LH was eluted from the column with 0,05 M phosphate buffer.

Successive aliquots of 1,0 cm^3 each were collected in glass test tubes containing 1,0 cm^3 0,14 M NaCl, 0,01 M $NaPO_4$ (PBS) pH 7,0, with five percent lyophilized egg white (EW). The tubes were immediately capped with parafilm, and after gentle mixing a 50 μcm^3 sample was drawn for counting in a Beckman Liquid Scintillation Counter. Further samples were taken from the tubes containing the protein peak (usually tubes no. 3 or 4) for testing of the immunoreactivity. The remaining ^{125}I-LH was divided into aliquots sufficient for approximately 500 assay tubes and snap-frozen in a mixture of dry ice and alcohol. These aliquots were stored at -15^{o}C until required. If storage exceeded seven days, the iodinated LH was purified by re-chromatography on Bio-Gel P60 columns, prepared as before.

Immunoreactivity of ^{125}I-LH

In order to avoid the use of an inferior iodinated product in a large scale assay, it was considered essential to obtain a rapid assessment of the immunoreactivity of the labelled LH. Initially, the dextran-coated charcoal method of Herbert, Lau, Gottlieb & Bleicher (1965) was applied. Dextran 200 was used in view of the successful application of a similar dextran in the assay of somatotropin (Lau, Gottlieb & Herbert, 1966). However, the initial results appeared unrealistically high (90-96 percent undamaged hormone) and it was decided to use the talc method of Rosselin, Assan, Yalow & Berson (1966) as an alternative test. Subsequently, after high titre anti-LH from rabbits which had been challenged with ovine LH, became available in reasonable quantities, the pre-precipitation procedure using excess anti-LH was utilised in combination with various incubation periods. In all cases the ^{125}I-LH to be tested was diluted to approximately 10000 CPM using 0,1 percent EW-PBS. The details of the various test procedures are as

follows:

(i) Charcoal-dextran test

To a series of glass culture tubes (12 x 75 mm) were added:

1. 500 μcm^3 Veronal-0,25% bovine albumin buffer (pH 8,6)
2. 100 μcm^3 ^{125}I-LH
3. either 1,0 cm^3 charcoal dextran or distilled water.

The contents of the tubes were mixed for 30 seconds by touching on a Vortex mixer, allowed to stand for 15 minutes and then centrifuged at 1000 x g for 30 minutes. The supernatant was carefully decanted into counting bottles and the radioactivity counted. This technique is based on the observation that undamaged hormone enters the pores of the charcoal-dextran while free iodide and damagedhormone remain in the suspension (Herbert, 1968). The undamaged fraction of the labelled hormone was therefore estimated by comparing the radioactivity of the assay tubes containing charcol-dextran with the "total count" tubes i.e. those in which water replaces the charcoal-dextran.

(ii) Talc test

A series of culture tubes were prepared by addition of:

1. 1,0 cm^3 buffer (0,05 M phosphate; 0,25% BSA)
2. 300 μcm^3 ovine plasma
3. 100 μcm^3 ^{125}I-LH
4. either 150 mg or no talc.

Procedures for mixing, incubation, separation, counting and estimation of undamaged ^{125}I-LH were the same as for the charcoal-dextran technique.

(iii) Pre-precipitation test·

Tubes were prepared containing:-

1. 500 μcm^3 PBS-1% EW
2. 200 μcm^3 ARGG diluted 1:2 in PBS
3. either 200 μcm^3 NRS diluted 1:400 in 0,05 M EDTA-PBS, pH 7,0 or 200 μcm^3 anti-LH serum diluted 1:400 in EDTA-PBS.

   The assay tubes were incubated at 4^oC for three days and were then either used immediately or stored at -15^oC after freezing in a dry ice-alcohol mixture.

4. The ^{125}I-LH to be tested was added to all tubes in a volume of 100 μcm^3 and the incubation continued at 4^oC for varying periods.
5. Cold PBS (1,0 cm^3) was added to each tube. After centrifugation for 20 minutes, the supernatant was decanted and the radioactivity counted. The percent ^{125}I-LH precipitated by the anti-LH serum was calculated, using the tubes containing NRS as a measure of the total radioactivity added.

(iv) Post-precipitation procedure

This formed part of the normal assay procedure (see "Conduct of a typical assay") and was essentially the same as the pre-precipitation technique. In this test the ^{125}I-LH was allowed to react with the anti-LH serum for 48 hours prior to the addition of ARGG. The incubation was then continued for a further three days.

Production of anti-sera

(i) Anti-sera to rabbit gamma globulin

Anti-rabbit gamma globulin was produced in adult wethers by subcutaneous injection of 100 mg RGG emulsified in 2,5 cm^3 saline and 2,5 cm^3 Freund's complete adjuvant. An additional two immunizations were applied at two-week intervals and after a further 14 days 250 cm^3 blood was removed by jugular veni-puncture. The blood was allowed to stand at 37°C for one hour in order to facilitate clotting and then stored overnight at 4°C to complete clot retraction. The serum was separated, centrifuged, snap-frozen and stored at -15°C until required.

Additional supplies of ARGG were obtained by bleeding at monthly intervals after a booster dose of 50 mg RGG in 2,5 cm^3 saline had been injected seven days previously. The ARGG was tested at various dilutions in the immunoassay and the dilution which optimally precipitated the RGG in assay tubes containing 200 μcm^3 filler ovine plasma was used in the routine assay.

(ii) Anti-serum to ovine LH

On days 1, 11 and 21 each of five, random-bred rabbits were injected subcutaneously with 1,0 mg NIH-LH-S16 emulsified in 1,0 cm^3 0,14 M NaCl and 1,0 cm^3 Freund's complete adjuvant. Two weeks after the last LH injection (day 35) approximately 15 cm^3 blood was drawn from the ear vein and allowed to clot. The serum was decanted, centrifuged and an aliquot of 100 μcm^3 removed for testing. The remaining serum was snap-frozen and stored at -15°C for future use. The serum to be tested was diluted 1:400 with 0,05 M EDTA-PBS, pH 7,0. Further dilutions (up to 1:40 000) were made, using 1:400 NRS-0,05 M EDTA-PBS, pH 7,0. The ability to precipitate ^{125}I-LH was then tested in the immunoassay.

Booster injections of 0,5 mg LH in saline and adjuvant were given on days 47, 71, 116 and a final injection of hormone in saline medium, on day 128. Blood was again collected on day 136 and treated as before. Following the second bleeding, and initial testing, the sera from three rabbits were selected for further examination. Various quantities of standard LH were incubated with the anti-serum at a dilution which bound approximately 50 percent of the ^{125}I-LH, viz., 1:100 000 in all three cases.

The anti-serum selected for use in the assay was further tested with a plasma high in LH and also examined for cross-reaction with ovine FSH and TSH.

Conduct of a typical assay

Glass culture tubes (12 x 75 mm) were used for the radioimmunoassay and the reagents were added in the following order: PBS-1% EW buffer to give a final tube volume of 1,0 cm^3, 0,0625 to 0,2 cm^3 of the plasma to be assayed; and 0,2 cm^3 of anti-LH serum previously diluted to 1:100 000 in 1:400 NRS-0,05 M EDTA-PBS, pH 7,0. The standard ovine LH (NIH-LH-S16) was dissolved in PBS-1,0 percent EW and included solutions ranging in concentration from 0 to 32 ng/cm^3. These standard solutions were stored frozen in 2,0 cm^3 aliquots. After rapid thawing 0,5 cm^3 aliquots were added to the appropriate assay tubes.

All tubes were capped with parafilm, mixed by shaking and incubated at 4^{o}C for 24 h. An aliquot of ^{125}I-LH was thawed rapidly and diluted with PBS-0,1% EW so that 0,1 cm^3 gave 10 000-13 000 CPM. This volume of diluted ^{125}I-LH was added to each culture tube and incubation continued for a further 24 hours. Separation of bound and free fractions was achieved by the addition of 0,2 cm^3 of a dilution of ARGG previously shown

to optimally precipitate the RGG. The ARGG was diluted with cold PBS, the usual rate of dilution being 1:2. After a further three days of incubation 1,0 cm^3 cold PBS was added to dilute the unbound ^{125}I-LH. The assay tubes were centrifuged at 1 000 x g for 30 min in a cold-room at 4°C. The supernate was carefully decanted into counting vials and the radioactivity determined. The CPM contained in buffer control tubes containing anti-serum and ^{125}I-LH, but no unlabelled hormone, was set equal to 100 percent and the counts in all tubes containing standard or unknown plasma expressed as a percent of this value. The schedule for a typical assay is detailed in Table 23.

Table 23. Essential features of a typical assay.

Tube No.	Reagents added (μcm^3)							Purpose
	Buffer	^{125}I-LH	Standard	Ovine plasma	NRS	Anti-LH	ARGG	
1, 2	900	100	-	-	-	-	-	Total count
3, 4	500	100	-	-	200	-	200	Non-specific binding
5, 6	500	100	-	-	-	200 of 1:400	200	Maximum binding
7, 8	500	100	-	200 or < [1]	-	200 of 1:400	200	Plasma damage
9-39	-	100	500 [2]	-	-	200	200	Standard dose response curve
40-51	300	100	-	200	-	200	200 of 1:0-1:4	Assay to assay variation and incomplete precipitation
52→	<500	100	-	200 or <	-	200	200	Unknown samples

[1] Volume equal to that of unknown samples.

[2] Standard LH in concentrations varying from 0 to 32 ng/cm^3.

Initially, all plasma samples were assayed using a 0,2 cm^3 sample. Smaller samples were subsequently included for plasmas found to contain a

high level of LH. A particular effort was made to eliminate the error resulting from assay to assay variation by incorporating in the same assay all the samples from the experimental animals associated with a particular series of comparisons. All determinations were performed in duplicate, except for the dilutions of the standard-response curve where three samples were used in each case.

RESULTS

Elution patterns after radioiodination and rechromatography

A typical elution pattern from the Bio-Gel P60 column, showing separation of ^{125}I-LH and free iodide is shown in Fig. 14A. The pattern resulting from the re-purification of a sample of the protein peak, stored frozen, is depicted in Fig. 14B.

Immunoreactivity of iodinated hormone

The immunoreactivity of the freshly iodinated LH as measured by the charcoal-dextran, talc, pre-precipitation and post-precipitation double-antibody tests is presented in Table 24.

Table 24. Immunoreactivity as assessed by various procedures.

Fraction tested	Percent ^{125}I-LH intact according to:			
	Charcoal/Dextran	Talc	Pre-precipitation	Post-precipitation
3*	68,5	69,5	58,0	-
3*	76,5	74,8	68,9	77,5

* Separate iodinations on the same day.

A more detailed comparison of the latter two tests is summarised in Table 25.

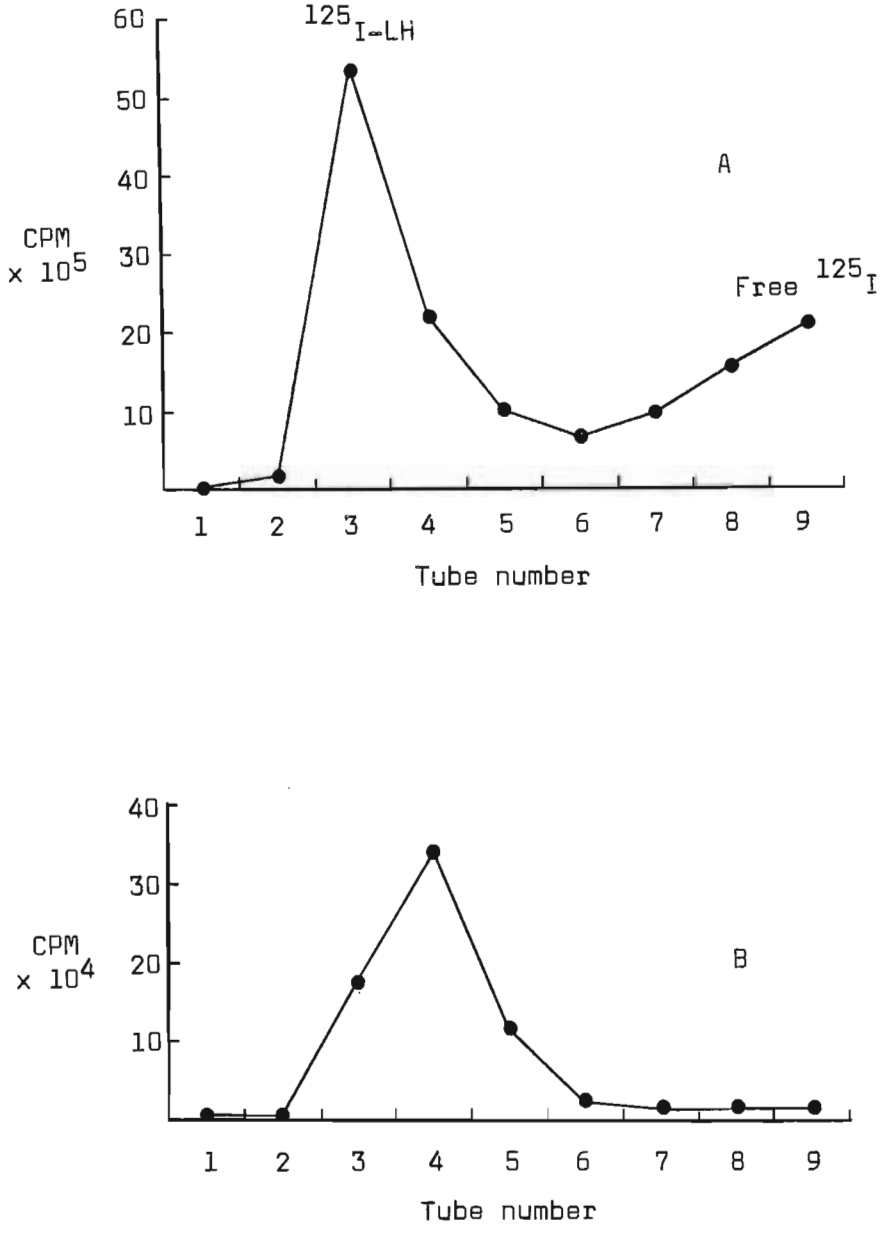


Fig. 14. Elution diagram from a 1 x 15 cm column of Bio-Gel P60. Each point represents the radioactivity in successive 1,0 cm^3 aliquots as estimated by counting 0,1 cm^3 samples.

A - Crude reaction mixture.
B - Repurification of peak tube - 16 days old.

Table 25. The immunoreactivity of freshly iodinated LH as determined by the pre- and post-precipitation test procedures.

Date of test	Aliquot no.from Bio-Gel P60 column	Percent ^{125}I-LH bound by 1:400 anti-LH	
		Test procedure:	
		Pre-precipitation (16h incubation)	Post-precipitation (3 day incubation)
29 Nov 1971	4	66,4	71,3
3 Dec 1971	4	61,5	68,4
9 Dec 1971	4	67,7	74,3
22 Feb 1972	3	81,3	84,3
21 March 1972	3	82,6	81,8
21 March 1972	4	75,8	73,7

After 9 December 1971, the pre-precipitated preparations were stored frozen and thawed immediately prior to use. From the results in Table 24 it is clear that the various rapid test procedures provided a close approximation of the expected performance of the labelled hormone in a normal assay. Such results were, however, obtainable only after considerable practice. With care and meticulous attention to detail, an acceptable degree of proficiency can be attained. In view of the speed with which results can be made available the charcoal-dextran and talc tests are obviously the methods of choice.

The data in Table 25 suggest that an incubation period of 16 h, when using preparations stored frozen, provides a good assessment of the immunoreactivity likely to be obtained in a normal post-precipitation assay.

When attention was focussed on the length of the incubation period and the effect of ageing on the estimated immunoreactivity, it became clear that a short, two-hour incubation period seriously underestimated the potential of a particular labelled product. This procedure was, therefore, abandoned and the results omitted. This test may be found

to be acceptable under different circumstances than those pertaining in the present experiments. The rather poor agreement between the early results obtained by pre-precipitation and those achieved with the post-precipitation procedure (Table 25) reflects the use of somewhat inferior anti-LH and anti-rabbit gamma globulin sera for the former test.

Although ^{125}I-LH appears to undergo denaturation during storage (Table 26), it is evident from the results in Table 27 that the quality of the iodinated hormone can be maintained for some weeks by refiltration on the Bio-Gel column.

Table 26. The effect of ageing on the immunoreactivity of ^{125}I-LH.

Batch of ^{125}I-LH	Date of test	Days after iodination	Percent ^{125}I-LH bound after 16h by 1:400 anti-LH
1	9/11/71	0	61,9
	19/11	10	60,2
	29/11	19	55,8
2	29/11	0	66,4
	3/12	4	61,5
	9/12	10	51,8
3	22/ 2/72	1	65,4
	9/ 3	17	45,6

Table 27. Maintenance of immunoreactivity by rechromatography on Bio-Gel P60 prior to incorporation in a three-day post-precipitation assay.

Date of test	Days after iodination	Percent ^{125}I-LH bound	
		Initial	After-Rechromatography
9 November	1	84,8	-
19 November	10	-	87,4
22 February	1	84,3	-
9 March	17	-	83,7
10 March	18	-	80,8
21 March	1	81,8	-
4 April	15	-	82,4
15 April	26	-	78,5

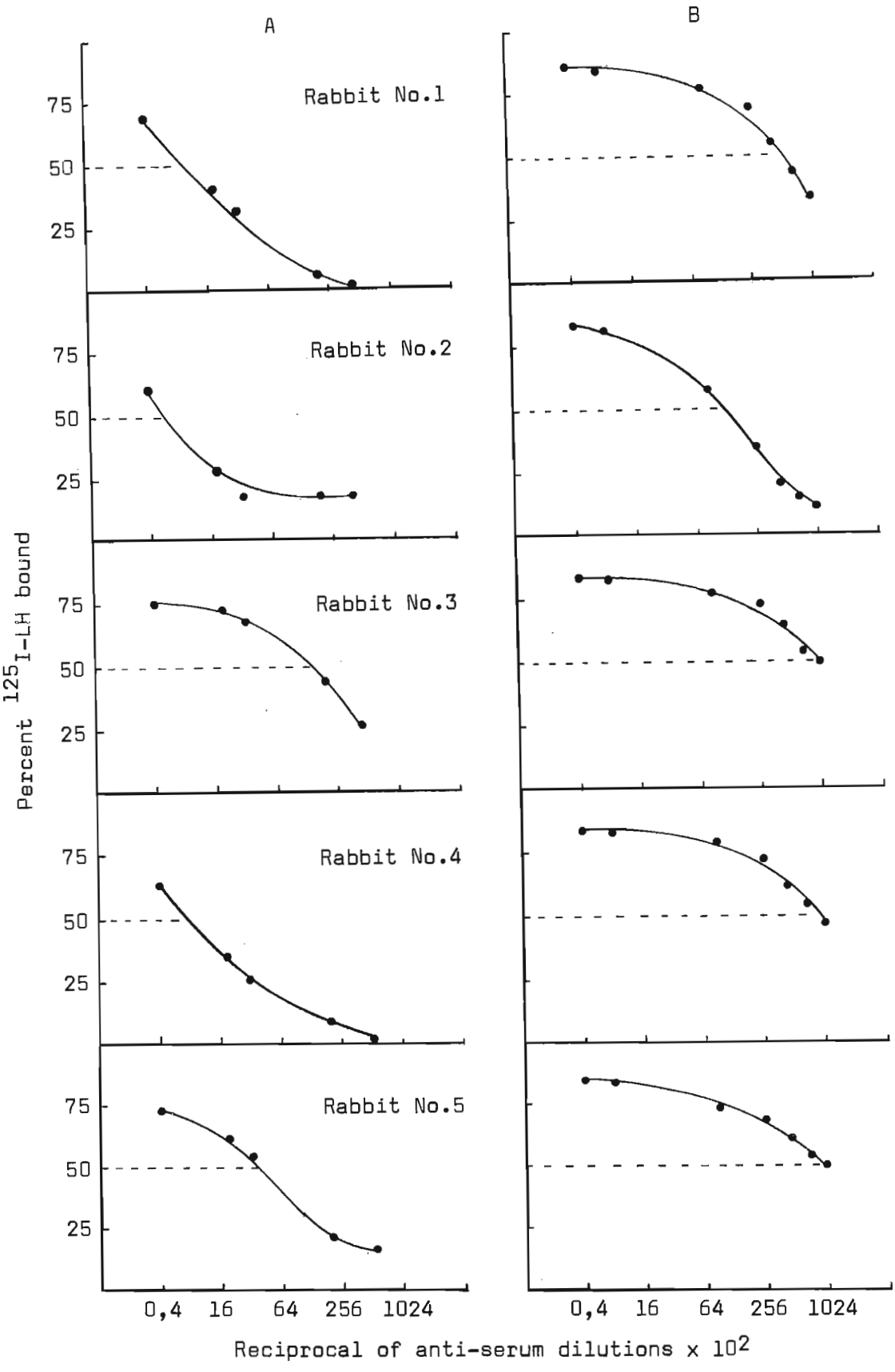


Fig. 15. Anti-serum dilution curves of sera obtained from five rabbits following a preliminary immunization schedule (A) and after booster injections of antigen had been applied (B).

Anti-LH sera

Titre

The percent ^{125}I-LH bound by increasing dilutions of the sera obtained from the five rabbits is presented in Fig. 15A. From this figure it is evident that, of the sera obtained at the first bleeding, only that produced by rabbit no. 3 resulted in acceptable precipitation of the radioiodinated LH when the anti-serum was used at a dilution exceeding 1:20 000. However, subsequent immunization resulted in a dramatic improvement in all sera, particularly in the case of rabbit no. 4 (Fig. 15B). This consideration, together with the appearance of the standard dose-response curves (Fig. 16) suggested that the anti-serum from this particular rabbit could be successfully used for routine assay purposes. When the anti-LH serum obtained from rabbit no. 4 was used to compare the dose-response curve of a plasma previously shown to contain a high level of LH with that obtained for the standard LH preparation (NIH-LH-S16) it appeared that the two curves were parallel. By expressing the percent ^{125}I-LH bound ("Y" axis) as a probit and by fitting a line to the standard values between 0,5 ng/cm^3 and 16,0 ng/cm^3 it was possible to convert the semi-logarithmic sigmoid response-curve to a straight line (Fig. 17). Tests of linearity and parallelism indicated that there was no significant difference between the two lines. The probit transformation was thus used for all standard-dose responses and straight lines fitted by unweighted regression to that section of the curve representing 20-80 percent binding.

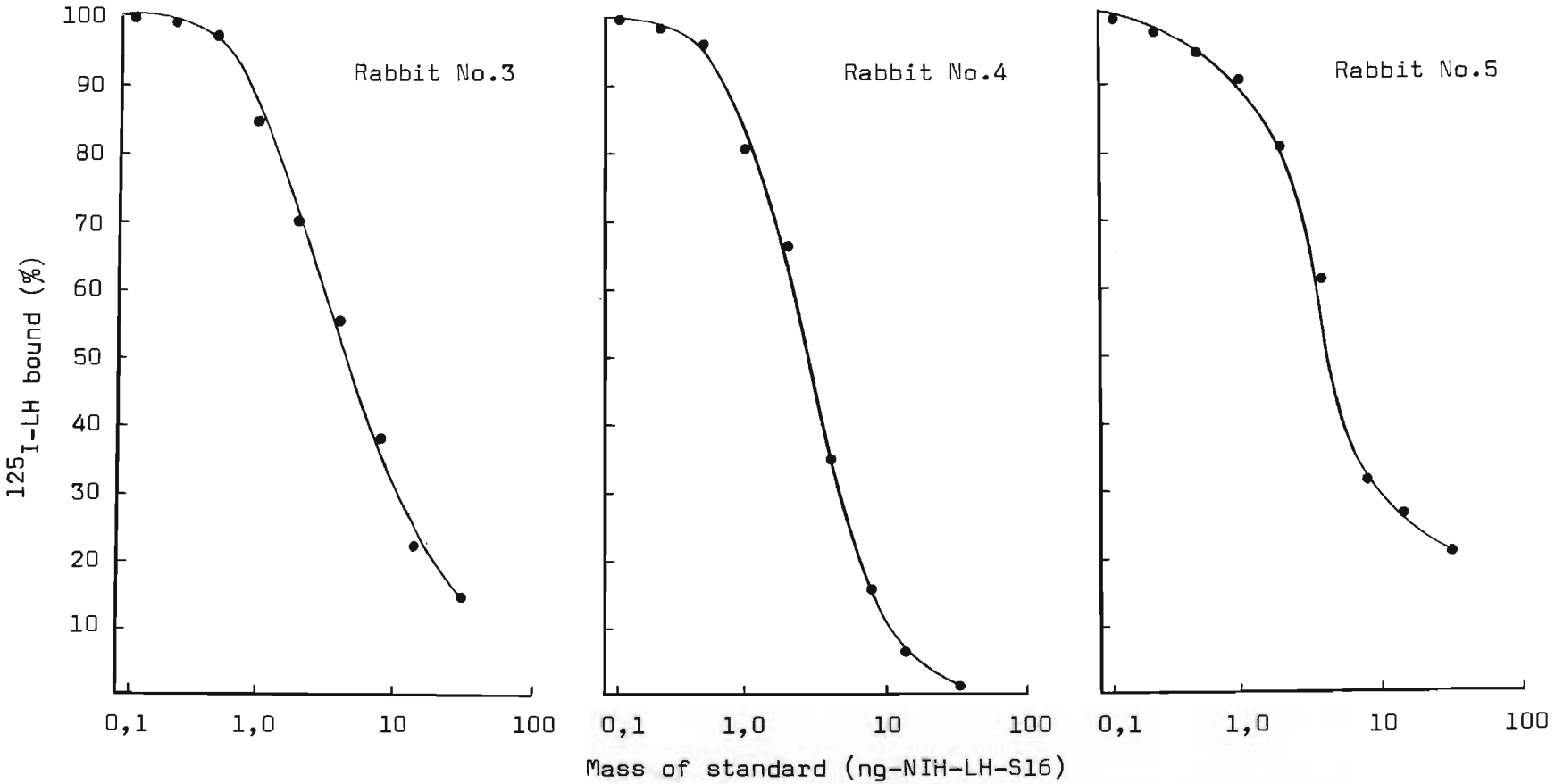


Fig. 16. Semi-logarithmic plot of dose response curves for NIH-LH-S16 using anti-sera from three different rabbits. The amount of ^{125}I-LH bound in the absence of unlabelled LH was set equal to 100 percent.

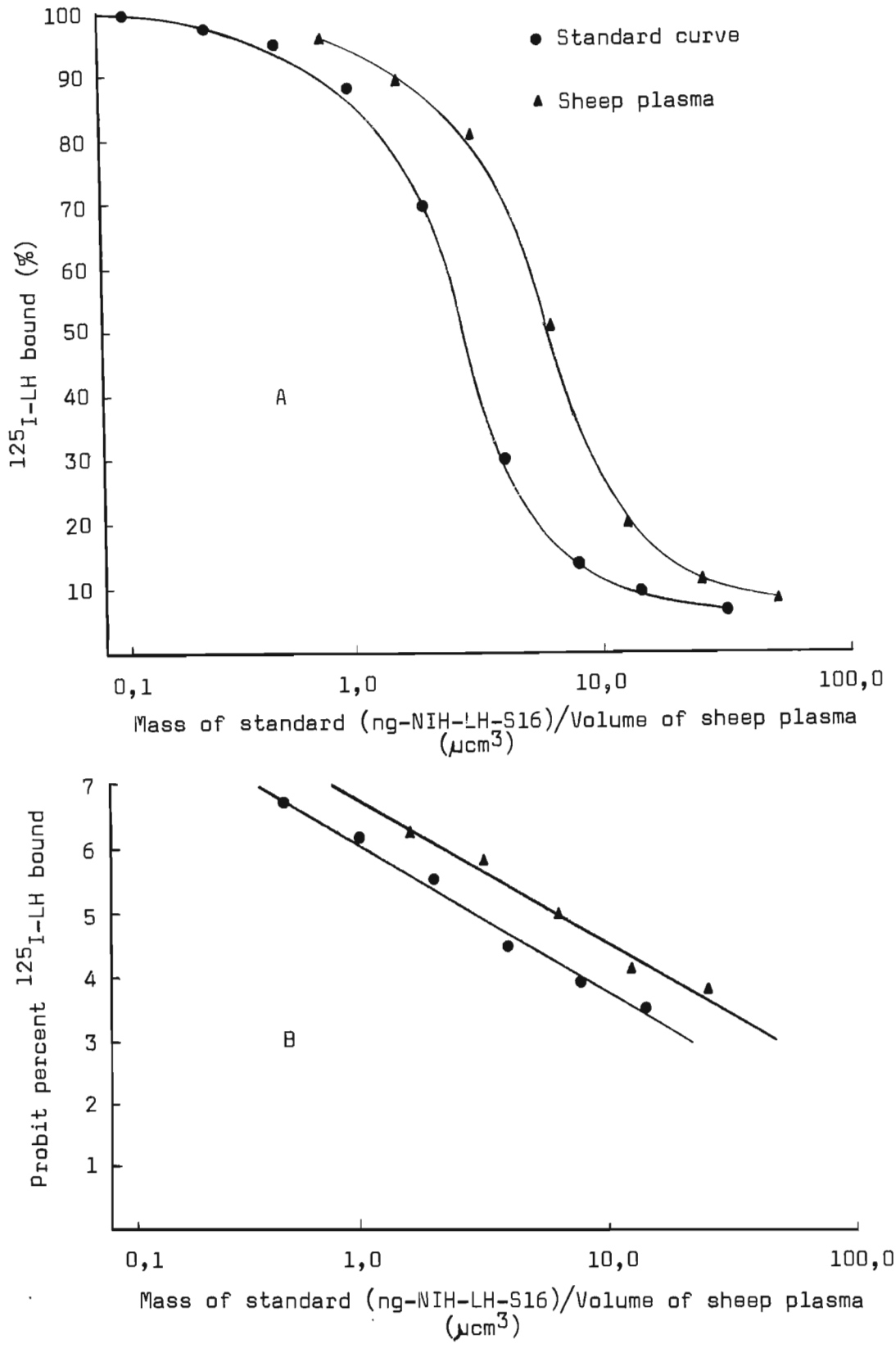


Fig. 17. Dose response curves for ovine NIH-LH-S16 and an ovine plasma sample. The amount of ^{125}I-LH bound in the absence of unlabelled LH was set equal to 100 percent.

A - semilogarithmic
B - probit

Cross-reaction with TSH and FSH

The results obtained after testing of the anti-serum for cross-reaction with ovine FSH and ovine TSH are shown in Fig 18. From the lines fitted using the probit transformation the cross-reaction at 50 percent ^{125}I-LH bound was calculated to be 15,94 percent. The cross-reaction of FSH with the LH anti-serum was negligible.

Assay to assay variation

The repeatability of the LH content of two plasmas, respectively low and medium in LH, as estimated from 16 separate assays, is shown in Table 28 and the need to eliminate this type of variability is evident.

Table 28. Reproducibility of LH levels from plasma pool.

Date of assay	Plasma low in LH		Plasma medium in LH	
	No. of determin-ations	LH content (ng NIH-S16/cm^3)	No. of determin-ations	LH content (ng NIH-S16/cm^3)
January 18, 1972	3	2,5	2	16,0
January 19	2	2,1	2	14,4
February 22	5	2,1	6	16,1
February 23	4	2,2	4	14,5
February 24	5	2,4	5	15,1
March 2	6	3,1	4	13,2
March 3	6	2,3	5	14,3
March 5	6	2,5	6	15,0
March 8	3	2,6	6	14,7
March 9	5	2,8	2	15,2
March 23	6	2,0	6	13,1
March 24	6	2,2	5	13,9
March 27	6	2,6	3	18,1
April 4	5	3,1	6	12,4
April 5	3	2,2	4	12,4
April 10	4	2,6	3	18,0
	Mean = 2,45 $\pm$ 0,34 C.V. = 13,7 percent		Mean = 14,77 $\pm$ 1,69 C.V. = 11,4 percent	

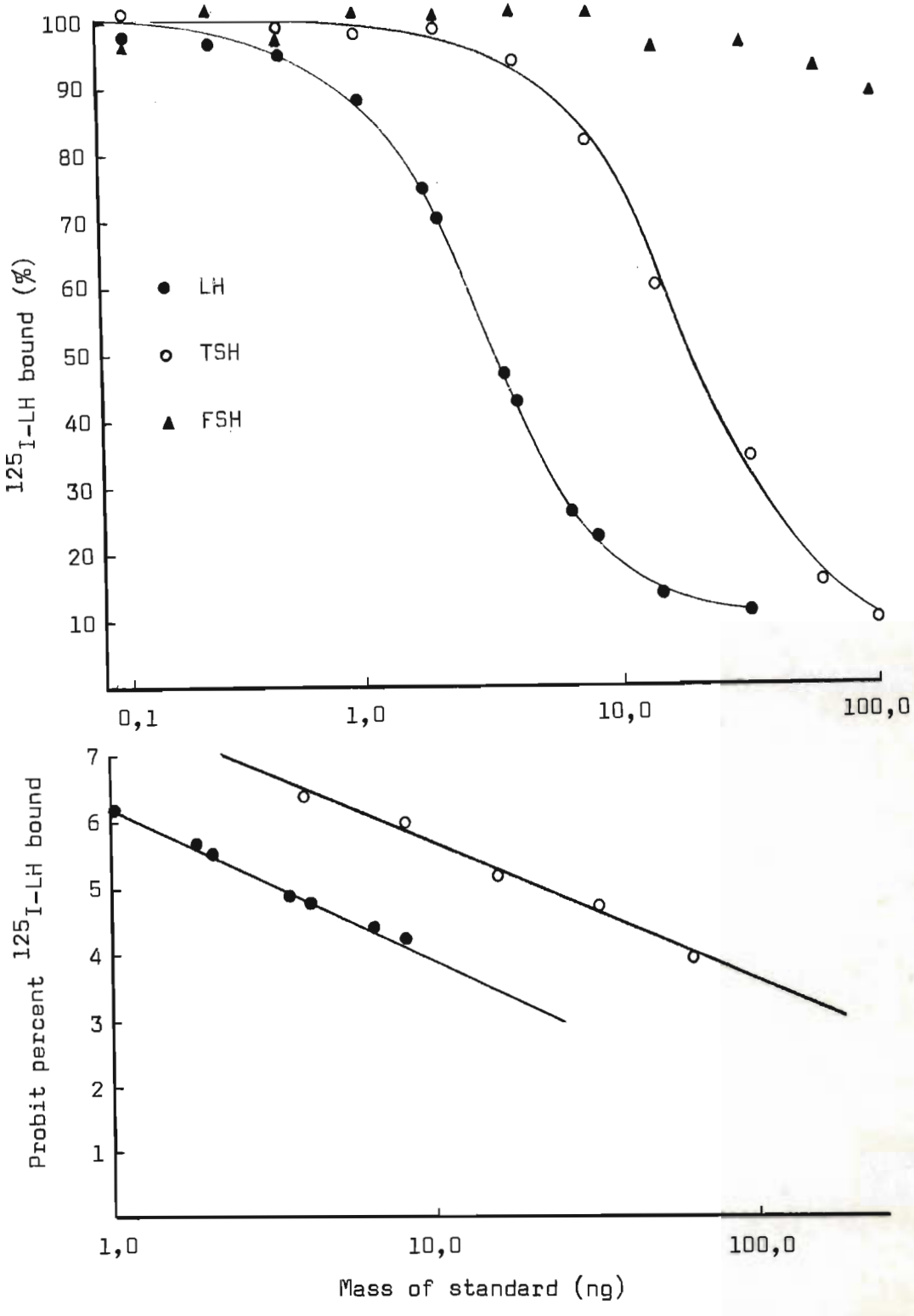


Fig. 18. Dose response curves for ovine LH, ovine FSH and ovine TSH using an anti-serum to ovine HIH-LH-S16.

Additional quality control checks

In order to designate which assays were producing abnormal results a number of parameters were considered and these are presented graphically in Fig. 19. The parameters utilized were:

(i) Slope of the regression line as computed after probit transformation of the percentage ^{125}I-LH bound.

(ii) The 50 percent intercept - a measure of the sensitivity of the assay.

(iii) B/T - counts precipitated in the absence of unlabelled LH (B) as a percent of the total counts added (T).

The 95 percent fiducial limits were then calculated for each parameter and are included in Fig. 19. In no case were these limits exceeded, although the advisability of repeating certain assays, particularly those of the 4th and 5th April, was clear. Wherever doubt existed the entire assay was repeated.

Plasma LH levels in ewes

In this aspect of the study a total of 3 890 plasma samples were collected and the determination of the LH content involved 14 460 determinations.

Ewes anoestrus during lactation

The mean plasma LH levels recorded for the five consecutive daily samples have been averaged for the various classes of experimental animals, and the data is presented in Table 29. In certain individuals one of the daily samples showed a marked deviation from the remaining values (Table 29) and the more striking examples are illustrated in

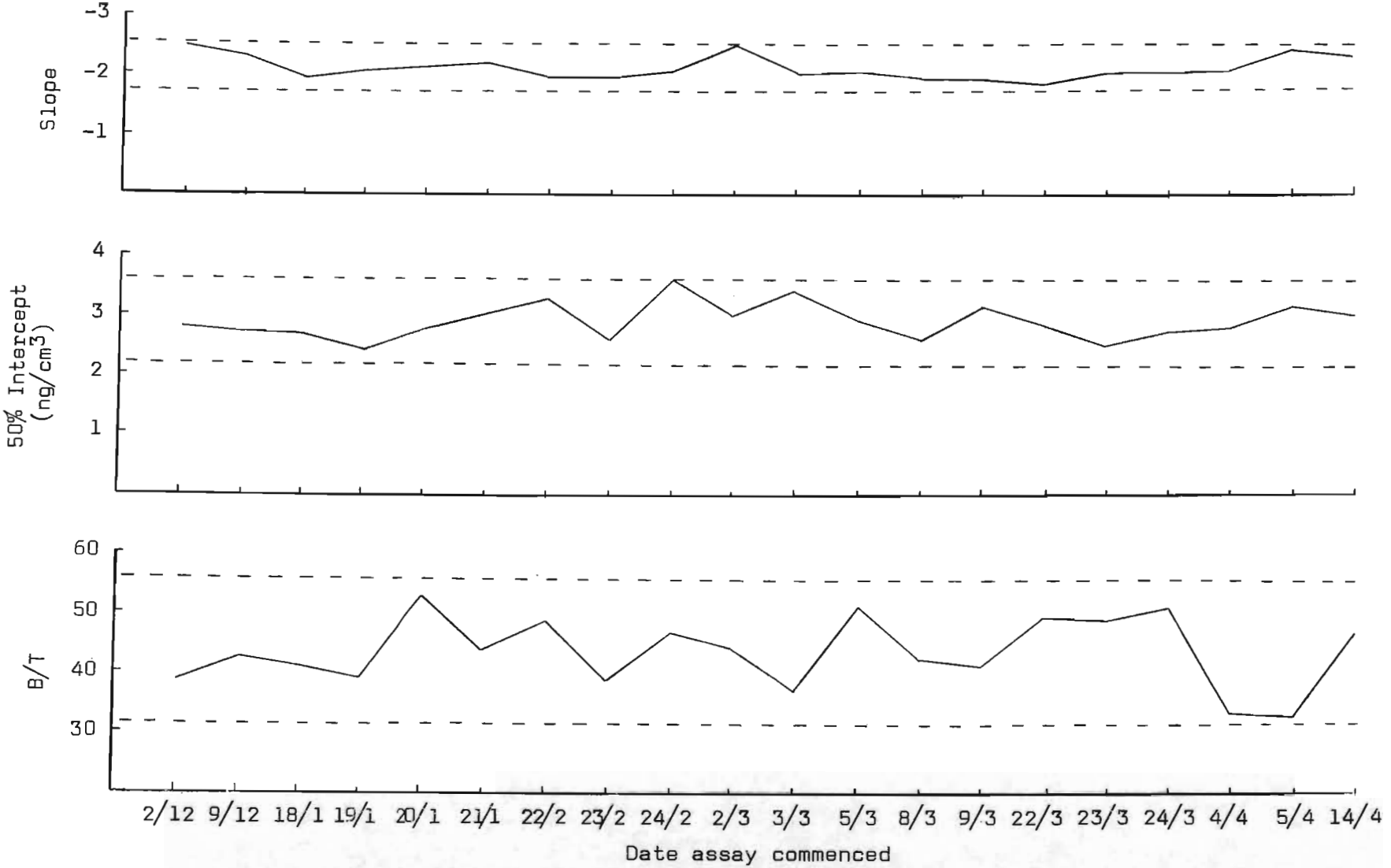


Fig. 19. Quality control chart for 20 LH assays. The area within the broken line indicates the 95 percent fiducial limits for each parameter. See text for description of parameters used.

Fig. 20. This phenomenon was not observed amongst the ewes exhibiting regular oestrus and the one exception listed in Table 29 involved the sample obtained from a ewe the day after oestrus.

Table 29. Plasma LH levels in ewes not exhibiting oestrus during lactation compared to interoestrous samples from cycling ewes.

Plane of nutrition during lactation	Reproductive state during lactation	Age of ewe	Number of animals	Average plasma LH level for five daily samplings ng/cm^3	No. of ewes in which a single daily sample exceeded 6,0 ngLH/cm^3
High	Oestrus	Maiden	5	2,95	0
		Mature	6	3,75	0
	Anoestrus	Maiden	3	4,32	1
		Mature	6	4,19	2
Low	Oestrus	Maiden	3	3,13	0
		Mature	6	3,61	1
	Anoestrus	Maiden	13	3,96	5
		Mature	10	3,27	1

The data in Table 29 showed a tendency for the LH level to be lower amongst the maiden ewes that were exhibiting oestrus regularly than for similar eight-year old sheep. However, according to least squares analysis none of the means listed in Table 29 differed significantly from each other.

Late anoestrus

The plasma samples obtained during late anoestrus (10 October, 1970) show that the ewes which were isolated from rams at this time exhibited a lower plasma LH level than similar animals continuously associated with rams (least squares analysis, P = 0,01). The occurrence of suddenly elevated level of LH was also evident among these ewes (Table 30).

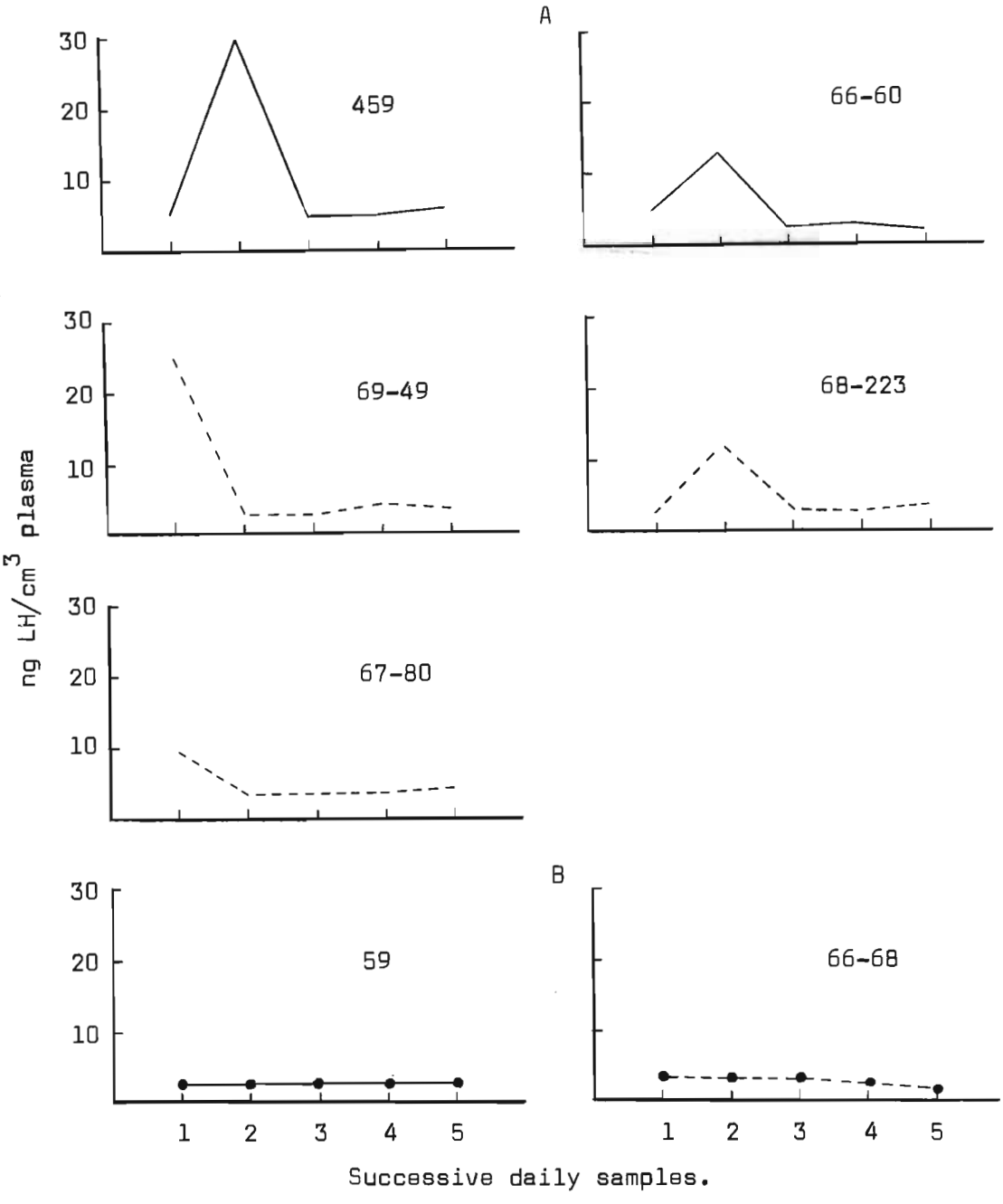


Fig. 20. Plasma LH levels illustrating marked daily variation in ewes not exhibiting oestrus during lactation (A) compared to dioestrous levels of two randomly selected cycling ewes (B).

High plane of nutrition during lactation.

Low plane of nutrition during lactation.

Table 30. Plasma LH levels in anoestrous ewes continuously associated with rams and similar ewes isolated from rams.

Plane of nutrition during lactation	Association with rams following weaning	Age of ewe	Number of animals	Average plasma LH level for five daily samplings ng/cm^3	No. of ewes in which at least one daily sample exceeded 6,0 ngLH/cm^3
High	Continuous	Maiden	5	3,35	1
		Mature	5	4,09	2
	Isolated	Maiden	7	2,76	1
		Mature	8	2,95	2
Low	Continuous	Maiden	5	3,85	1
		Mature	7	4,66	4
	Isolated	Maiden	7	2,94	1
		Mature	8	3,19	1

The mean LH level of the maiden ewes appeared to be lower than that recorded for the mature sheep (Table 30), but this difference was again not significant.

Ewes exhibiting oestrus - 1970 and 1971

For each ewe studied, at the various sampling times, the changes in plasma LH concentration after the onset of oestrus have been presented graphically (Appendix Figs. 1A-2C). An examination of the various graphs suggested that the plasma LH level rose slowly until the concentration approached 10 ng/cm^3. Thereafter, the blood concentration increased sharply. A sharp decline was exhibited for the descending segment of the curve. In order to simplify calculations and presentation of the results the pre-ovulatory surge is defined as the stage during which the plasma level equalled or exceeded 10 ng/cm^3. A similar procedure has been adopted by Thimonier & Pelletier (1971) and Cumming, Brown, Blockey, Winfield, Baxter & Goding (1971b). The duration of the LH-surge has been determined from the graph for each ewe and the total output of hormone is represented by the area under the curve.

The stage at which the LH-surge commenced (in relation to the onset of oestrus), the duration of the release and the time of day when oestrus commenced, are presented in Fig. 21 and in Appendix Table 1. The occurrence of overt oestrus one cycle prior to the oestrus during which blood samples were collected and one cycle subsequent to sampling are also included in Fig. 21.

From the data in Fig. 21 it is obvious that the timing of the LH-surge, in relation to the onset of oestrus, varied greatly from animal to animal. Similar variations were noted for the maximum LH level recorded and for the quantity of hormone released (Appendix Figs. 1A-2C, Appendix Table 1). These results are summarised in Table 31. Only the duration of the pre-ovulatory LH-release appeared to show any measure of consistency. Statistical analysis of the results (analysis of variance) indicated that there was no significant effect of either the plane of nutrition or the year of study on the latency to LH release. The results obtained during 1970 suggested that LH was released for a longer period on the low than on the high plane of nutrition. However, the 1971 results indicated the reverse and the sampling procedure (at two- or one-hour intervals) may have influenced the accuracy of the determinations. In view of this source of error comparisons were limited to the results obtained within single years.

The data pertaining to the maximum LH level also showed a trend during 1971 opposite to that recorded during 1970 (Table 31). Both the 1970 ($P = 0,01$) and 1971 ($P = 0,001$) differences were significant. Although undernutrition appeared to reduce the total quantity of LH released during the pre-ovulatory surge, the coefficient of variation was high (Table 31). The log transformation was applied in order to reduce the variance and although this was achieved (C.V. = 7,2 percent) no significant trends could be demonstrated.

DATE SAMPLED	ANIMAL NUMBER	INITIATION AND DURATION OF LH RELEASE HOURS AFTER ONSET OF OESTRUS 1.0 2.0 3.0	TIME OESTRUS COMMENCED h. mn	OVERT OESTRUS PRE-SAMPLING	OVERT OESTRUS POST-SAMPLING
AUGUST 1970	68-46		5.55	+	+
	66-45		14.00	+	+
	66-72		14.00	+	+
	66-104		13.30	+	$-^{3}$
	232		3.00	+	$-^{3}$
	441		13.45	+	+
	7		7.22	$-^{2}$	$-^{6}$
	67-69		13.45	+	+
	67-76		9.45	+	+
	67-82		5.55	+	$-^{6}$
	246		5.55	+	+
SEPTEMBER 1970	59		11.56	+	+
	63		4.08	+	+
	66-86		17.53	+	$-^{2}$
	459		11.54	$-^{1}$	+
	441		12.15	+	+
	67-69		13.50	+	$-^{1}$
	67-76		6.10	+	+
	141		10.03	+	+
	308		15.53	$-^{1}$	$-^{1}$
	495		9.50	+	$-^{1}$
OCTOBER 1970	64		15.17	$-^{1}$	+
	66-104		2.50	+	+
	68-284		20.55	$-^{2}$	+
	306		2.50	$-^{3}$	+
	441		17.50	+	+
	67-2		6.20	+	+
	66-56		5.40	+	+
	68-132		20.30	$-^{1}$	+
	67-76		18.40	+	+
	152		14.10	$-^{3}$	+
	67-68		5.10	0	+
	67-71		16.10	0	+
	68-85		16.00	0	+
	89		20.50	0	+
	288		13.35	0	+
	66-99		5.35	0	+
	66-6		14.12	0	+
	66-7		12.00	0	+
	66-134		19.55	0	+
	157		9.35	0	+

DATE SAMPLED	ANIMAL NUMBER	INITIATION AND DURATION OF LH RELEASE HOURS AFTER ONSET OF OESTRUS 1.0 2.0 3.0	TIME OESTRUS COMMENCED h. mn	OVERT OESTRUS PRE-SAMPLING	OVERT OESTRUS POST-SAMPLING
AUGUST 1971	67-25		16.25	+	$-^{1}$
	66-39		11.55	+	+
	66-38		10.40	+	−
	68-90		16.30	+	+
	68-222		7.35	+	+
	68-295		13.30	+	$-^{3}$
	67-10		5.45	+	+
	67-11		13.40	$-^{1}$	−
	66-58		17.30	+	−
	67-69		6.15	+	+
	68-134		10.50	+	+
SEPTEMBER 1971	67-49		5.20	+	+
	66-70		5.10	+	+
	68-81		13.30	+	+
	66-142		5.10	+	+
	68-269		5.15	$-^{2}$	+
	68-287		15.45	+	−
	66-20		5.10	+	+
	66-46		5.20	+	+
	67-68		10.30	+	−
	68-169		8.22	+	−
	68-259		5.25	+	−
OCTOBER 1971	66-137		14.12	+	+
	68-49		11.16	$-^{1}$	+
	66-57		9.18	+	+
	67-81		16.30	+	+
	68-81		7.10	+	+
	68-85		9.05	+	+
	66-139		9.25	+	+
	66-12		4.30	$-^{1}$	+
	66-58		9.05	+	+
	66-103		9.05	+	+
	68-173		4.30	+	+
	68-211		4.15	$-^{1}$	+
	68-276		16.30	$-^{5}$	+
	68-296		7.10	$-^{3}$	+

FIG 21 TIME OF COMMENCEMENT, IN RELATION TO THE ONSET OF OESTRUS, AND THE DURATION OF THE PRE-OVULATORY RELEASE OF LH IN EWE MAINTAINED ON HIGH (——) AND LOW (- - -) PLANES OF NUTRITION DURING LACTATION

\+ DENOTES OVERT OESTRUS RECORDED ONE CYCLE PRIOR TO OR FOLLOWING SAMPLING

− DENOTES OVERT OESTRUS NOT RECORDED AND SUFFIX NUMBER INDICATES NUMBER OF CYCLES FOR WHICH OESTRUS WAS NOT OBSERVED

o EWES ISOLATED FROM RAMS FROM WEANING UNTIL 15 OCTOBER

Table 31. The variability of parameters associated with the pre-ovulatory release of LH in ewes on high or low planes of nutrition during lactation.

Date sampled	Latency to release (h min)		Duration of release (h min)		Maximum level (ng/cm^3)		Total released (ng)		No. of observations in which LH release (ng) equalled:				
	High plane	Low plane	High plane	Low plane	Range	Mean	Range	Mean	< 250	251-500	501-750	751-1000	>1000
Aug 1970	6.44	6.35	10.33	11.15	60,7-386,6	High plane = 175,6	293,8-1460,0	High plane = 743,5	0	1	2	3	2
Sept 1970					51,8-220,9	Low plane = 166,8	178,3-885,8	Low plane = 677,0	2	0	1	1	0
Oct 1970					69,0-273,1		335,2-967,4		0	2	6	7	0
Aug 1971	11.48	7.52	10.07	9.18	156,2-320,3	High plane = 218,9	511,4-1519,0	High plane = 852,1	0	0	1	4	1
Sept 1970					170,3-326,3	Low plane = 239,1	554,2-1120,9	Low plane = 804,9	0	0	1	3	2
Oct 1971					100,5-306,8		299,4-1196,8		0	2	2	2	2
S.E.	±2.00		±1.42		± 7,59		±296,92						
C.V.(%)	24.02		16.26		3,86		39,13						

A careful examination of the data suggests the following:

(i) The timing of the pre-ovulatory surge is not related to the time of day or the onset of oestrus. In several instances the acute release of LH was initiated prior to the commencement of oestrus, but also occurred in some ewes several hours after mating commenced. The results obtained during 1971 (Fig. 21) illustrate this point.

(ii) The LH-surge recorded at the "last" oestrus of the one breeding season and at the "first" oestrus of the new season did not appear to be different from that observed in animals which cycled regularly. Ewe no. 7 did not show oestrus for some 53 days prior to or 103 days subsequent to the oestrus studied during August, 1970. In this case there was evidence to suspect an inadequate secretion of LH.

(iii) The incidence of oestrus without an LH-peak being demonstrated was highest when anoestrus was deepest i.e. mid-September to mid-October. A total of 11 animals exhibited no LH-surge within 36 h (1970) or 24 h (1971) after the onset of oestrus and the total quantity of LH released was particularly low during September, 1970.

(iv) Slightly more than 70 percent of the values obtained for the total release of LH were above 500 and below 1 000 ng.

(v) The hormonal patterns could not be correlated with the bodymass at weaning or the weight change during lactation (Appendix Table 1).

During 1970, ewe no. 441 was included in all three sampling periods (August, September and October). On each occasion the latency to the rise in the plasma LH concentration was very close to 6 h, but the other

parameters studied exhibited relatively wide variation.

Ewes exhibiting oestrus - 1972

The changes in plasma LH concentration of the ewes which exhibited oestrus at the expected time are presented in Fig. 22. The results were similar to those obtained during 1970 and 1971 (Appendix Figs. 1A-2C) and it was also shown that in six of the 14 examples studied the pre-ovulatory surge of LH commenced prior to the initiation of mating. This confirmed the pattern suggested by the earlier results.

The data in Fig. 22 also suggest some tendency for the ewes on the low plane of nutrition to exhibit a lower maximum level of LH than the high plane animals. One ewe (no. 69-14) did not exhibit an LH surge within the period 12 hours before and 24 hours after the commencement of oestrus. The pattern of LH secretion at the previous oestrus is depicted in Fig. 22 and it can be seen that ewe no. 69-14 showed a relatively low peak LH concentration and a surge which continued for only seven hours.

Release of LH following oestrogen administration

When observations ceased on 31 October, 1972, of the 27 ewes injected with oestradiol-17β in arachis oil 10 (5 high plane, 5 low plane) had exhibited oestrus 2-9 days after oestrogen administration. Ovarian cycles had apparently commenced spontaneously in these individuals by the time oestradiol-17β was injected and these animals have been excluded from the results.

The results for the four ewes (all low plane) which exhibited an LH surge within the observation period are presented in Fig. 23. The release of LH appeared to commence within a relatively short period in all animals and the peak concentration exceeded 300 ng/cm^3 plasma in three

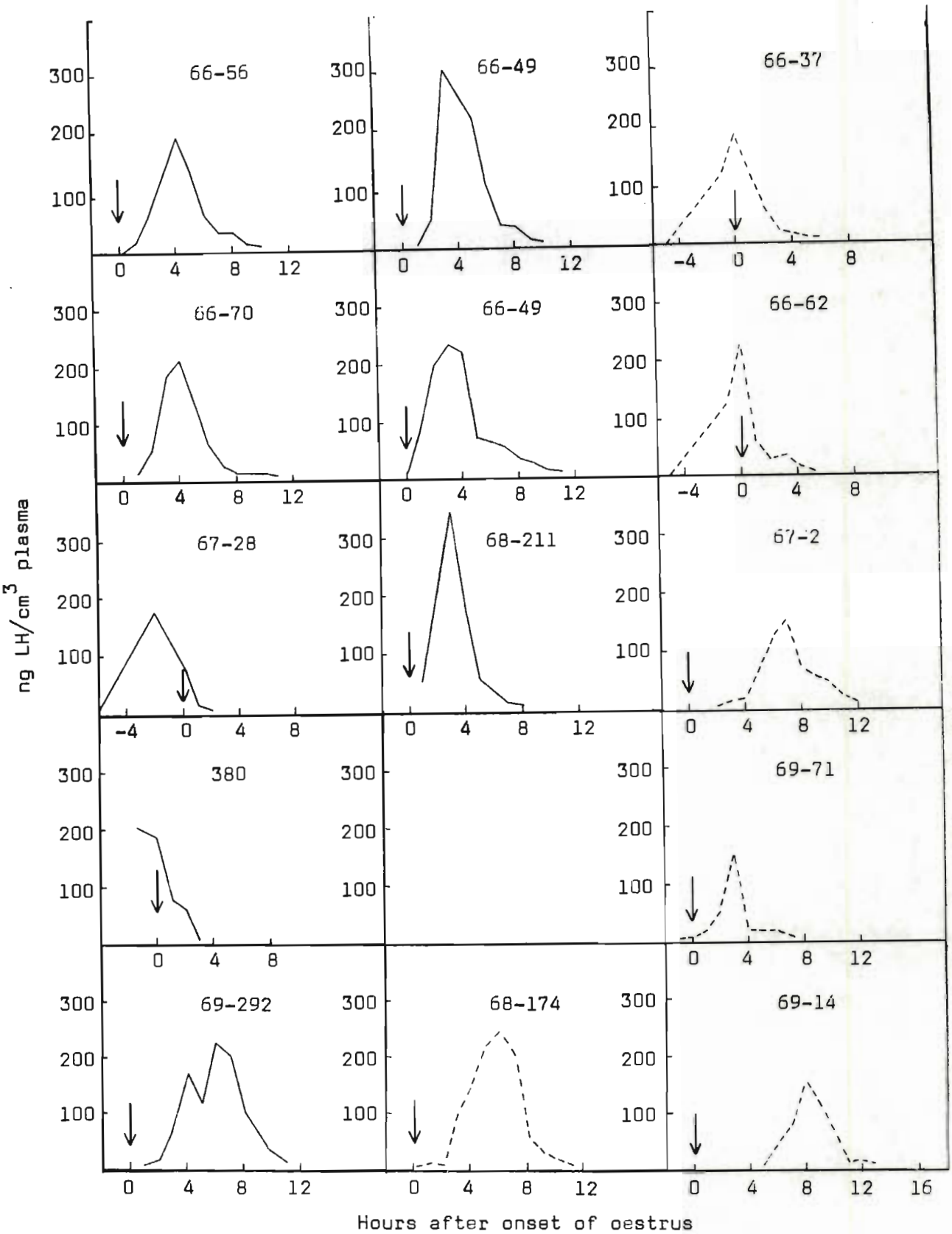


Fig. 22. Plasma LH concentration measured at four-hour intervals prior to, and at intervals of one hour during, oestrus in ewes, maintained on either high (——) or low (----) planes of nutrition during lactation.

↓ indicates onset of oestrus.

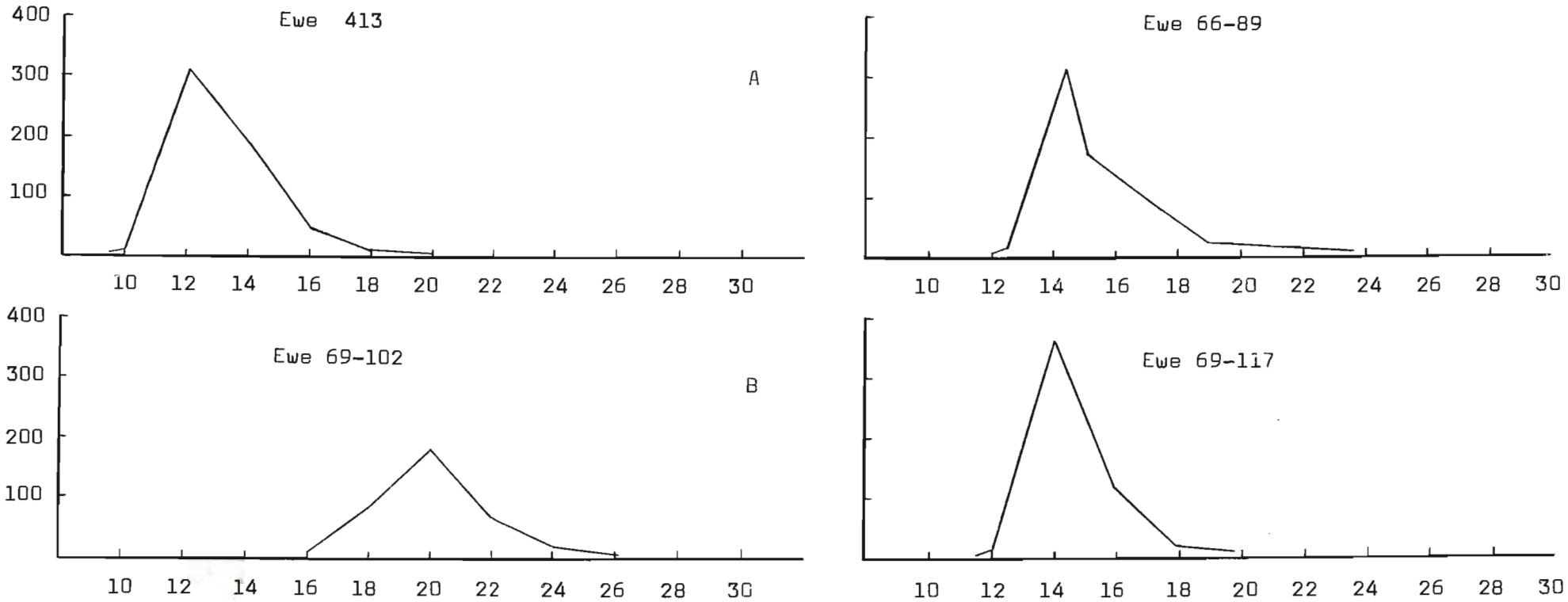


Fig. 23. Changes in the plasma concentration of LH following administration of either 20 µg (A) or 40 µg (B) oestradiol-17β to anoestrous ewes.

individuals. Only two of the ewes which were exhibiting regular oestrous cycles showed a comparable concentration (Fig. 22). The duration of the LH surge i.e. the period during which the concentration equalled or exceeded 10 ng/cm^3, was close to 10 hours in all four cases.

A number of factors militated against determining whether the plane of nutrition during lactation or the duration of the anoestrous period influenced the release of LH following oestrogen injection. These factors were:

(i) The failure of the oestradiol-17β in acetone and saline solution to induce a release of LH.

(ii) The small number of ewes anoestrus at any stage after being maintained on a high plane of nutrition during lactation.

(iii) The spontaneous initiation of the new breeding season prior to the administration of oestradiol-17β in arachis oil.

The response of the ewes to oestrogen in oil was also below expectation.

DISCUSSION

With the advent of radioimmunoassay methods it was predicted that the study of the endocrine secretions would make rapid progress. While this cannot be denied, a disturbing tendency has been noted for these techniques to be applied to the study of processes relating to the normal animal without much attention being given to the application of experimental situations. Midgley, Niswender, Gay & Reichert (1971) also subscribe to this view and have presented evidence in support of such claims. There can be no doubt that research on normal animals is essential in order to establish norms (Midgley et al., 1971), but there appears to be a notable lack of application of these findings.

Validity of the assay

(i) Reproducibility

Rodbard, Rayford, Cooper & Ross (1968) have developed an empirical quality control system in order to measure, amongst others, the reproducibility of the radioimmunoassay. This system permits sequential monitoring of the performance of separate assays and is most useful in pin-pointing the reason for results which may exceed predetermined tolerance limits (see Fig. 19). These workers indicate that since the graph of percent ^{125}I-LH bound vs. log X is sigmoidal in character, the logit, probit or arc-sine transformations may be used to achieve linearization. In considering the use of either weighted or unweighted regression analysis for fitting a straight line, Rodbard & Lewald (1970) are not convinced that the weighted regression is superior. In fact, Midgley and co-workers find weighting unnecessary (Rodbard & Lewald, 1970).

In general, the results of the assays performed in this investigation conformed to the tolerance limits suggested by Rodbard et al. (1968). The reproducibility of the determined LH level of two control plasmas, as measured by the coefficient of variation viz., 13,7 and 11,4 percent, agrees with the figure of approximately 20 percent (Goding et al., 1969), 10,35 percent (Scaramuzzi, Caldwell & Moor, 1970) and that of between 18 and 20 percent given by Scaramuzzi, Blake, Papkoff, Hilliard & Sawyer (1972).

(ii) Cross-reaction with TSH and FSH

It is not the intention to review in detail the implications of the observed cross-reaction with TSH, when using antibodies prepared against LH, since Samli & Geschwind (1967), Geschwind & Dewey (1968),

Goding et al. (1969) and Scaramuzzi et al. (1970) have discussed this problem.

The cross-reaction of 15,94 percent reported here agrees closely with the theoretical figure of 16 percent, calculated by Scaramuzzi et al. (1970). Other figures reported are 14 percent (Goding et al., 1969), 17 percent (Geschwind & Dewey, 1968) and 18 percent (Scaramuzzi, et al., 1970).

Baron, Terterin & Jutisz (1967) and Geschwind & Dewey (1968) concluded that FSH did not cross-react in their assay for LH. Scaramuzzi et al. (1970) observed that as much as 10,0 ng of FSH was required to cause notable precipitation of LH. In a later study, Scramuzzi et al. (1972) showed that up to 50,0 ng FSH did not cause significant displacement of ^{125}I-LH from the antibody.

The foregoing suggests that an acceptable anti-serum to ovine LH has been produced. The fact that the anti-serum can be successfully used at a dilution of 1:100 000 demonstrates that this is one of the best anti-sera to LH currently available.

Gonadotropin levels following feed restriction

On the basis of the results obtained with bioassay Piacsek & Meites (1967) concluded that the pituitary concentration of LH was unaffected in female rats fed a ration restricted to 50 percent of the normal intake. However, the total content of LH was found to be reduced. A marked increase in gonadotropic potency of the pituitary gland after chronic under-feeding has also been recorded by Rinaldini (1949). In a study similar to that of Piacsek & Meites (1967), but using radioimmunoassay, Howland (1971) and Howland & Ibrahim (1971) noted an increased pituitary FSH level in underfed rats, but the concentration

of LH in the pituitary gland was apparently again not altered. The general consensus of opinion is that inanition (either chronic or acute) affects pituitary size rather than the concentration of FSH and LH within the pituitary (Bellows et al., 1963a; Howland, Kirkpatrick, Pope & Casida, 1966; Symington, 1969).

Upon studying the circulating levels of these gonadotropins, Howland (1971, 1972b) and Howland & Ibrahim (1971) observed that the serum LH level was reduced in the underfed rat, but Howland (1971) could not demonstrate a similar effect for FSH. The sheep included in the observations reported here did not show a comparable effect of underfeeding on the plasma LH level when ewes in anoestrus were compared with those undergoing regular oestrous cycles. There were also no notable differences in the basal LH levels of ewes which resumed breeding during lactation compared to those which remained anoestrus during this time. In young ewes Squires, Scaramuzzi, Caldwell & Inskeep (1972) could also detect no influence of plane of nutrition during rearing on the plasma LH level following treatment with exogenous hormones.

Recent work by Roche et al. (1970) tends to support these findings since they could not detect changes in the pituitary LH content during anoestrus as compared to the breeding season. The explanation offered by Howland & Ibrahim (1971), implicating impaired production of releasing hormones in underfed rats, appears attractive but does not explain all the results obtained by these workers. Although Roche et al. (1970) have demonstrated that the plasma LH level changed during various stages of anoestrus they concluded that the endocrine basis for the seasonality of oestrus in sheep had not been elucidated.

Day to day variations in the basal LH level

The interoestrous LH concentration in the blood of sheep appears

to vary somewhat in the different studies reported. Thus Niswender, Roche, Foster & Midgley (1968) and Roche et al. (1970), have observed that the level varied from undetectable amounts to 2 ng/cm^3 serum. Geschwind & Dewey (1968), Goding et al. (1969), Niswender et al. (1969) and Reeves, Arimura & Schally (1970a) have obtained similar values. Somewhat higher concentrations of 3,9 to 6,7 ng/cm^3 (Pelletier, Kann, Dolais & Rosselin, 1968) and from 5 to 15 ng/cm^3 (Wheatley & Redford, 1969) have been recorded. Similar variability in the reported basal levels for cattle have been discussed by Snook, Saatman & Hansel (1971).

The general consensus of opinion has been that the blood-level of LH does not vary markedly between the ovulatory peaks and the results obtained in the present study confirm this. However, during anoestrus minor fluctuations in the day to day level do occur (Fig. 20) and comparable results have been reported by Butler, Bolt & Malvern (1971). Similar cyclic surges have been observed in ovariectomized ewes (Roche et al., 1970; Reeves, O'Donnell & Denorscia, 1972a) with the peaks approximately one hour apart (Butler et al., 1971; Reeves et al., 1972a). Irregular variations in the serum LH levels have been reported in other ovariectomized females such as hamsters (Goldman & Porter, 1970), rats (Gay, Niswender & Midgley, 1970), monkeys (Dierschke, Battacharya, Atkinson & Knobil, 1970) and rabbits (Scaramuzzi et al., 1972).

Cumming, Brown, Blockey & Goding (1971a) suggest that even during the oestrous cycle, spontaneous surges in the LH level do occur, with the level rising from 2 to 7 ng/cm^3.

Factors influencing the basal LH level

(i) Age

The plasma LH level of both anoestrous ewes and those exhibiting

regular oestrous cycles tended to be lower in the maiden than in the eight-year old ewes (Tables 29 and 30). A similar effect of age has been reported for both pituitary LH content and the serum LH level in rats (Howland, 1971).

(ii) Association with males

Lishman & Hunter (1966, 1967) have shown that when ewes are isolated from rams the females tend to cease exhibiting oestrus and an examination of the ovaries (Hunter, 1969) has shown a reduced incidence of ovulation. It appears that the secretion of LH may be implicated since the present study has shown that in ewes isolated from rams the plasma LH level is lower than in similar animals continuously associated with rams. This finding provides further support for the scheme proposed by Lishman & Hunter (1967) and suggests that the presence of the ram can result in an increased release of LH from the pituitary. It is tempting to implicate the hypothalamus as the centre through which such a stimulus is mediated, but there is little evidence suggesting that the hypothalamic gonadotropin releasing hormones are involved in the tonic release of gonadotropins from the pituitary. The current contention is that these substances are responsible only for the acute release occurring prior to ovulation.

The pre-ovulatory surge in plasma LH concentration in ewes exhibiting oestrus spontaneously

(i) Time of onset

Goding et al. (1969) and Wheatley & Radford (1969) presented evidence suggesting that the LH surge which resulted in ovulation in the ewe commenced after the onset of oestrus. This agrees with the observed time of release from the pituitary (Robertson & Rahka, 1966). Although

Goding et al. (1969) presented data obtained from only four ewes, they concluded that the plasma LH level began to rise 4 to 16 h after oestrus was first detected. In the 40 oestrous periods studied by Wheatley & Radford (1969) the latency to the LH surge was remarkably constant at 4,6 $\pm$ 4,3 hours. The data presented here indicates that during the seasonal anoestrous period, at least, there is a marked variation in the timing of the LH peak in relation to the onset of overt oestrus. In the discussion following papers by Eik-Nes (1971), and others, Goding confirms that in the normal ewe the LH surge can commence prior to the onset of overt oestrus or even as late as 16-18 h after oestrus is first detected. This variation is further illustrated by the results obtained by Cumming, Blockey, Brown, Catt, Goding & Kaltenbach (1970). Their data showed that the plasma LH level rose prior to the onset of oestrus only following progestogen synchronisation of oestrus. Marked variations in the release of pituitary LH in relation to the initiation of mating have also been demonstrated by the earlier studies incorporating bioassay techniques (Santolucito, Clegg & Cole, 1960; Robertson & Hutchinson, 1962; Dierschke & Clegg, 1968).

There can be little doubt that the procedure adopted for detecting the onset of overt oestrus and the frequency with which blood samples are taken can influence the conclusions made. However, it would be expected that where numerous observations are made, such as by Wheatley & Radford (1969), the variation would be similar to that observed here. Goding et al. (1969) suggested that the results obtained by Wheatley & Radford (1969) may represent an evoked release of LH caused by the frequent introduction of rams. In view of the results obtained by Parsons & Hunter (1967) such a situation would apply only if rams were frequently associated with oestrual ewes and not if rams are present only until oestrus was first detected.

Even with daily sampling in 20 ewes, Goding et al. (1969) did not entirely miss the LH surge, but these authors indicated that with such sampling procedures there was a greater than 50 percent chance of completely missing the LH peak. This contention is supported by the data presented by Niswender et al. (1970). The great variation in the timing of the LH surge, as illustrated by the results reported here, complicates interpretation of the findings. Either the surge was missed entirely or no peak occurred. Until now the former has been presumed, probably on the basis of the infrequent sampling procedure. In view of the fact that in several ewes only the decreasing phase of the LH surge was detected and since in the early experiments (1970 and 1971) sampling was initiated only after overt oestrus had been detected, and then continued for a maximum of 36 h, no definite conclusions can be made. However, the possibility that no peak may occur, particularly at the end of the breeding season, should not be entirely discounted.

(ii) The maximum LH level in the plasma

Published data on the greatest concentration of LH in the blood at oestrus shows considerable variation. In many cases, perhaps, this may be ascribed to extended intervals between samplings. Where observations were made every two days, Pelletier et al. (1968) reported a maximum of 80 ng/cm^3. The values obtained for daily sampling were 95-175 ng (Reeves et al., 1970a), 6,0-82,5 ng (Niswender et al., 1968) and 15-67 ng (Niswender, et al., 1969). Sampling at intervals of eight hours, of two hours, of four hours to 30 minutes and every 30 minutes yielded peak values of 40-100 ng (Geschwind & Dewey, 1968), 120-200 ng (Goding et al., 1969), 46-460 ng (Wheatley & Radford, 1969) and 80 to more than 200 ng (Goding, et al., 1969) respectively. Where each animal was sampled only once, but at 6-15 h after the initiation of oestrus, levels as high as

250 ng were observed (Bjersing, Hay, Kann, Moor, Naftolin, Scaramuzzi, Short & Younglai, 1972).

It may again be expected that where a number of animals or oestrous periods are studied the range in LH concentrations would be similar, irrespective of the period between samplings. The results presented by Goding et al. (1969) and Wheatley & Radford (1969) suggest that the detected maxima of serum LH increase as sampling becomes more frequent The present results (Table 31) demonstrate that the level for Merino ewes can vary between 51,8 and 386,6 ng/cm^3 and that the variation was similar where hourly or two-hourly sampling was applied. These results and those obtained by Wheatley & Radford (1969) for Merino and cross-bred types and those noted for the Corriedale (Goding et al., 1969) provide circumstantial evidence that breeds which exhibit an extended breeding season also release high levels of LH at oestrus. It should be noted, however, that different radioimmunoassay techniques were used in each of the three studies.

(iii) The quantity of LH released during the pre-ovulatory surge

Goding et al. (1969) reported that the magnitude of the LH release varied by less than two-fold in four animals, but Reeves, Arimura, Schally Kragt, Beck & Casey (1972b) concluded that, following administration of releasing hormone, there was considerable variation in the magnitude as well as the shape of the curve. The results presented in Table 31 show that in Merino ewes the quantity of LH released, as measured by the plasma levels in samples obtained from the jugular vein, may vary between 293,8 and 1 518,9 ng - more than a five-fold variation.

On the basis of data obtained from immunoassays (Geschwind & Dewey, 1968) and pituitary release of LH (Robertson & Rahka, 1966; Dierske & Clegg, 1968) it was calculated that if the pituitary release extended over

three hours the peak value would be about 80 ng/cm^3 and the total duration less than six hours (Geschwind & Dewey, 1968). Subsequent work has shown that the duration is close to 10 h (Goding et al., 1969; Cumming et al., 1971a; present results) with a peak considerably higher than postulated. An underestimate of pituitary reserves/release or breed differences may account for the conflicting results.

The release of LH following oestrogen administration

As long ago as 1940 D'Amour observed that the urinary content of oestrogen in women increased prior to the elevated excretion of gonadotropin and Catt (1969) has confirmed this finding. D'Amour (1940) therefore postulated that increased levels of oestrogen in the circulation are responsible for ovulation. Several reports have shown that injection of oestrogen can result in ovulation in the ewe (Hammond, Hammond & Parkes, 1942; Hammond, 1945; Casida, 1946; Vandernoot, Reece & Skelley, 1949; Döcke & Dörner, 1965; Howland et al., 1968; Piper & Foote, 1968). The oestrogens probably act by inducing a release of LH since the latter hormone has been concluded to be the ovulatory factor in sheep (Cumming et al., 1971b). In fact it is well documented that administration of physiological levels of oestrogen result in an increase in the blood level of LH (Brown, Catt, Cumming, Goding, Kaltenbach & Mole, 1969; Goding et al., 1969; Radford et al., 1969, 1970; Goding, Blockley, Brown, Catt & Cumming, 1970; Radford, Wallace & Wheatley, 1971). The mechanism of action of oestrogen appears to be through a sensitization of the hypophysis to LH-releasing hormone (Reeves, Arimura & Schally, 1971). Current theories on the regulation of ovarian cycles in the ewes thus incorporate the belief that the increased secretion of oestrogen prior to oestrus (Moore, Barrett, Brown, Schindler, Smith & Smyth, 1969; Scaramuzzi

et al., 1970; Obst, Seamark & Brown, 1971; McCracken et al., 1971) results in the pre-ovulatory release of LH (Radford et al., 1969; Cumming et al., 1971a; McCracken et al., 1971; Obst et al., 1971).

It is generally found that oestrogen produces an elevated blood concentration of LH within 24 hours after administration (Goding et al., 1969; Radford et al., 1969, 1970, 1971; Akbar, Howland & Stormshak, 1970; Restall, Radford & Wallace, 1972; Squires et al., 1972). The latent period to LH release is not substantially influenced by the injection medium (oil or saline solution) or the route of administration (intramuscular or intravenous). The response to oestradiol-17β depicted in Fig. 23 thus agrees with other findings particularly those of Goding et al. (1969, 1970). It would seem advisable to interpret published results on the release of LH following oestrogen administration with caution since some workers conclude that a blood concentration of less than 40 ng/cm^3 indicates an acute release of LH (Akbar et al., 1970; Radford et al., 1969; Squires et al., 1972). In contrast, Goding et al. (1969), Scaramuzzi, Tillson, Thorneycroft & Caldwell (1971) and Restall et al. (1972) have demonstrated that the blood level is similar to that occurring in naturally cycling ewes. Serum LH levels as low as 20 ng/cm^3 have been found to result in ovulation (Reeves et al., 1972b), but the surge of FSH following the LH-RH administered may have augmented the effect of LH. The data in Figs. 22 and 23 suggest that the LH level following oestrogen administration to anoestrous ewes may in fact be higher than that usually found in cycling ewes.

Possible reasons for variation in the quantity of LH released

It has been noted that following intra-carotid administration of LH-releasing hormone (LH-RH) the plasma LH level rises 2,5 min later and

reverts to basal levels 2 h later (Reeves, Arimura & Schally, 1970c). It was also found that in the intact ewe the dose response to LH-RH in terms of circulating LH was poor (Reeves et al., 1970c). Furthermore, the reaction to the same dose of LH-RH varies between individuals (Reeves et al., 1972b). In view of these findings it would appear that the total quantity of LH released may be only partly related to either the quantity of LH-RH and/or the sensitivity of the pituitary to releasing hormone. In support of a more generalised hypothesis Reeves et al. (1970c) concluded, amongst others, that the pituitary responsiveness as measured by the LH release, was greatest at oestrus. This conclusion ignores the fact that the pituitary reserves show a linear increase from day one to 15 of the oestrous cycle (Kammlade et al., 1952; Santolucito et al., 1960; Roche et al., 1970). Only minor importance appears to be attached to the finding that increasing quantities of LH-RH do not induce greater pituitary release of LH (Reeves et al., 1970c).

The great variability in timing of the pre-ovulatory LH surge as well as of the concentrations in the circulation reduce the possibility of demonstrating statistically significant treatment differences, if such differences exist. Using only 35 ewes Thimonier & Pelletier (1971) concluded that two genetically different flocks exhibited different peak LH levels. These workers also related the timing of the LH surge to the incidence of single or twin ovulations within each genetic population. In view of the fact that Thimonier & Pelletier (1971) applied a log transformation to the area under the curve, presumably to reduce variation, it cannot be agreed that the pooling of data from 6-12 animals can be justified.

The need to study large groups of animals has again been amply demonstrated by the results obtained in this study. This seems to be a necessary condition unless the variation can be reduced by some other

means. With the recent development of a radioimmunoassay for ovine FSH (Bjersing et al., 1972; Reeves, et al., 1972b) this hormone must also receive closer attention in attempting to account for differences in breeding behaviour. The demonstration of a prolactin-surge which synchronises with that observed for LH at oestrus (Kann, 1971; Bjersing et al., 1972) suggests that this hormone may be more closely involved in the reproductive process than hitherto thought and research in this sphere is urgently required.

GENERAL DISCUSSION

The experiments incorporated in this study were designed to investigate two main objectives:

(i) To what extent undernutrition and consequent loss in bodymass influence the reproductive rate of woolled sheep?

(ii) By what mechanisms the plane of nutrition reduces the fecundity? This incorporated an evaluation of the sensitivity of the ovary to gonadotropic stimulation and the quantitation of the endogenous levels of gonadotropin in the circulation.

In reviewing the results obtained the question arises as to whether or not the findings lead to a better understanding of the problem of low fertility in sheep? In the absence of reliable data pertaining to the situation as it exists in commercial flocks, particularly as regards the nutritional status and the resultant changes in bodymass, it is difficult to draw conclusions which are not unequivocal. Clearly this aspect requires investigation, since by merely changing one set of conditions, for example, the post-weaning plane of nutrition, a completely different picture could emerge. Therefore, unless accurate, quantitative data from a variety of flocks and farming conditions is obtained, research in this field will continue to be based on assumptions which, no matter how honest, may eventually be shown to be completely false.

In planning this study the aim was to create a situation which would parallel that found in practice and thus to establish circumstances permitting the direct application of the findings to agriculture in the field. At all times, except during lactation, the nutritional level employed was that which would allow the potential of the animal to be

expressed without undue limitation. Only those restrictions dictated by sound economic principles, e.g., limiting the use of expensive concentrates, were entertained in the trials described. However, it is somewhat unlikely that in practice the period of feed-scarcity will be limited only to periods as sharply defined as under the experimental conditions. Although there can be no doubt that the feed requirements of the ewe are greatest during lactation, the practical situation is more likely to be characterised by a gradual change from adequate to inadequate feeding. This would be superimposed on similar gradual changes in feed requirement from early pregnancy, through peak lactation, to the post-weaning period. After weaning in winter, the dry ewe is commonly expected to survive on the natural vegetation with little or no supplementation. In the summer rainfall areas there is therefore a distinct likelihood of the ewe being unable to meet her requirements after lactation is terminated. In this discussion a broader approach than that applied in the experimental situation will be adopted and the luxury of extrapolating the findings to other situations will be indulged. Accordingly it is assumed that inanition, regardless of when this occurs, is likely to produce most of the effects observed in the current investigation. In view of the well documented delayed effect of undernutrition on reproduction (Hunter, 1962; Smith, 1962; present experiments) the net effect on reproductive performance will therefore depend on when feed scarcity occurs in relation to the period of exposure to breeding rams.

If, in farming practice, the breeding ewes are adequately fed, both pre-partum and post-weaning, then it would appear from the results obtained that undernutrition during lactation is unlikely to reduce seriously the incidence of mating during the subsequent breeding season. The results presented by Adler (1964) show that the proportion of barren

ewes in the average flock was over 20 percent when mating occurred in early spring. The data in Table 9 are in agreement with this finding. Adler (1964) concluded that seasonal variations in the sexual activity of the ewe during the period of joining with breeding rams was the major factor responsible for variations in the proportion of ewes which lambed. In the study reported here only a very small proportion (never more than five percent) did not mate during spring (Table 5). Therefore, either Adler's conclusions are in error or different circumstances apply under practical farming conditions.

The current results appear to be the first in which a functional relationship has been shown to exist between bodymass or change in bodymass and the incidence of oestrus. The concept established complements the observed association between bodymass and ovulation rate (Cumming & Blockey, 1971; Guerra et al., 1972). Such conclusions support the contention (Guerra et al., 1972) that when the plane of nutrition is high, bodysize may limit the reproductive rate. The evidence is therefore strongly in favour of culling on the basis of substandard bodysize. It has been suggested that the critical bodymass at joining for Corriedale, Romney Marsh (Coop, 1962) and Border Leicester x Merino ewes (Killeen, 1967) is between 41-45 kg. A somewhat lower bodymass of below 38,7 kg has been proposed for Merino ewes (McInnes & Smith, 1966). The commonly accepted figure of 36 kg for maiden Merino ewes at first mating therefore appears acceptable. The additional advantages of higher wool yield and heavier lambs (Ray & Smith, 1966) further amplifies the advantage to be gained by selecting heavy breeding females.

An aspect which has not received much attention in this investigation is that relating to failure of conception and death of the embryo/foetus. Chopping & Lindsay (1970) have concluded that ewes which

are mated, but do not lamb, contribute greatly to the wastage of potential lambs. The present findings are in agreement with this conclusion. The problem of pre-natal loss of the fertilised ovum in sheep is well documented (Hammond, 1921; Henning, 1939; Brambell, 1948; Dutt, 1954; Bellows et al., 1963b; Quinlivan, Martin & Taylor, 1966a,b) and the results presented by Moule (1966) and Quinlivan et al. (1966a,b) indicate the need to pay greater attention to this problem since a high percentage of the ewes fail to lamb after not returning to service. In the present study the figure varied from 3,1 to 33,3 percent, which agrees closely with the results obtained by Mullaney (1966).

The incidence of barrenness in a flock appears to be negatively correlated with bodymass (Coop, 1962, 1966) and body condition (Gunn et al., 1969). The present data also support the conclusion (Guerra et al., 1972) that barrenness is more common among small than large ewes. Since body condition will be influenced greatly by loss in bodymass the results in Figs. 6 and 10 support the contention that body condition has an important additional effect on reproductive rate.

A further factor which reduced the number of lambs in this investigation was the low incidence of multiple births. According to the survey conducted by Adler (1964) the incidence of twinning in commercial flocks very seldom exceeded four percent (calculated on the basis of ewes mated). In a similar study, covering a period of 10 years, Moule (1966) found that the frequency of multiple births, as a percentage of the ewes lambing, ranged from nil to 60 percent and that selection played an important role in the occurrence of twin births. According to Moule (1966) the advisability of selecting for multiple births depends mainly upon the availability of feed for the pregnant and lactating animals. This is probably the most important factor which influences the South African farmer to select against twinning, although Parsons & Hunter

(1965) concluded that this was not consciously practised. The inherently low potential for multiple births, in spite of adequate nutrition, in the population studied here is illustrated by the results in Table 9.

The second facet of this investigation concerned some of the underlying mechanisms involved in a failure to reproduce. It has been suggested that ovulation rate can be influenced by the concentration of circulating gonadotropins as well as by the sensitivity of the ovary to these hormones (Land & Falconer, 1969). When the ovulation rate increases following selection for bodysize the principle contribution appears to be a change in FSH secretion (Fowler & Edwards 1960; Edwards, 1962), whereas changes in ovulation rate following selection for increased size of the litter have been correlated with ovarian sensitivity (McLaren, 1962). Thus Land (1971) concluded that the high reproductive activity of Finnish Landrace sheep is probably due to greater gonadotropic activity than to increased gonadal sensitivity. This is supported by the finding that Blackface ewes having a greater litter size than Merino x Blackface ewes also exhibit a higher release of LH during the oestrous cycle, but not at oestrus (Land, Crighton & Lamming, 1972). The difference betweeen cross-bred and Merino ewes in their reaction to the same dose of HCG (Fig. 13) suggests that ovarian sensitivity cannot be excluded. Since Cumming & Blockey (1971) were unable to demonstrate differences in pre-ovulatory release of LH due to plane of nutrition or liveweight the possibility of changes in the responsiveness of the ovary should be considered.

The results presented in Chapter 2 suggest that ovarian sensitivity, as measured by certain types of ovarian response, can be reduced by poor feeding. The finding that the ovulation rate is not affected is perhaps of minor importance since this is apparently not a major contributor to

low reproductive rates in commercial production. However, under more severe conditions of food restriction the ovulatory process may well become impaired. There is also the possibility that the latent period to ovulation may be influenced by the nutritional plane. A situation in which the times of mating and ovulation are unfavourable to conception may thus develop. In view of the finding that restricted feeding can reduce the amount of ovarian fluid produced following gonadotropin stimulation, it can be expected that the production of gonadal steroids, particularly oestrogens, will be reduced. This, in turn, could influence the release of LH by the pituitary gland and thereby affect the ovulatory process. Other aspects which could exhibit malfunction are the uterine environment and the secretory activity of the corpus luteum. Ultimately the survival of the foetus may be implicated. Much of the foregoing is simple conjecture and only detailed investigation will provide some answers.

Evidence currently available suggests that underfeeding may affect the reproductive process mainly through the failure of the pituitary gland to release gonadotropins. Piacsek & Meites (1967) have shown that restricted feeding reduces the hypothalamic content of LH-RH, while the pituitary reserves of LH are unaffected. Current hypotheses concerning the control of pituitary function incorporate the belief that plasma levels of the gonadal steroids regulate the release of LH-RH. This hormone, in turn, stimulates the synthetic processes within the anterior pituitary gland (McCann & Ramirez, 1964) and the release of LH (Guillemin, 1964; and others). Recent findings suggest, however, that only the release of LH is controlled by releasing hormone (Samli & Geschwind, 1967) and that oestrogen sensitises the pituitary gland to the releasing factor (Döcke & Dörner, 1965; Reeves et al., 1970b). This is perhaps the very process through which the responsiveness of the

ovary could play a role since the level of oestrogen may be inadequate to effect sensitization of the pituitary gland. Seasonal differences in the sensitivity of the pituitary gland to RH have been noted by Domanski & Kochman (1968) and it has also been demonstrated that the oestrous response of ovariectomized ewes to oestrogen is reduced by undernutrition (Gibson & Robinson, 1971). The hypothalamic centres controlling mating behaviour are apparently involved in the latter response and it is therefore possible that similar nervous centres influencing the production of releasing factors may be deleteriously affected by feed scarcity.

At least one other facet requires clarification. This is whether undernutrition influences the secretory capacity of the pituitary gland, particularly that on day 16 of the cycle when the content of LH increases markedly (Roche et al., 1970).

From a review of the current literature and the results described in Chapter 3 the impression is gained that the pre-ovulatory release of LH is an "all or nothing" process and that the explanation for ovarian atrophy and anoestrus following poor feeding should perhaps be sought at the level of the hypothalamus. Mechanisms which are likely to show a rapid response (e.g. gonadotropin secretion and release) may be involved in the increased ovulation rate related to practices such as flushing. The hypertrophy of the pituitary gland, associated with increased ovulation rate in ewes fed high-energy rations (Memon, Antoniewicz, Benevenga, Pope & Casida, 1969) appears to favour this hypothesis. It would be valuable to examine the components of the reproductive mechanism in the populations developed by Turner et al. (1962). The work done by Thimonier & Pelletier (1971) is clearly motivated by such considerations and they have implicated a hitherto unsuspected facet viz., the timing of LH release, in multiple ovulation.

Ershoff (1952) has drawn attention to the fact that gonadotropins are not the only pituitary hormones which are secreted in reduced quantities during underfeeding. Meites & Fiel (1965) have in fact been able to demonstrate that starvation reduces both the synthesis and release of somatotropin releasing hormone. This in turn resulted in depressed production and release of somatotropin by the pituitary gland. It is therefore evident that a broader approach would involve the study of thyrotropin, somatotropin and prolactin.

Moule (1966) is clearly in favour of long-term studies involving a number of criteria likely to influence the reproductive rate. While this cannot be criticised, the findings presented here illustrate the considerable variation which may be encountered when only a small number of factors are varied. Additional aspects which are relevant to a study of the factors limiting sheep production are the losses due to death of the lamb prior to weaning (Bosman, 1959; Adler, 1964; Moule, 1966) and seasonal fluctuations in the fertility of breeding rams (Lamont, 1964; Skinner & van Heerden, 1971). The importance of these factors cannot be ignored.

In beef cattle maintained under extensive feeding conditions it is now generally recognized that the reproductive rate of the herd as a whole can be seriously reduced by the low proportion of first-calvers reconceiving (Bauer, 1965; Harwin, Lamb & Bisschop, 1967). The nutritional level has been implicated by these workers. Stuedemann, Ewing, Guenther & Odell (1966) are of the opinion that the beef cow requires additional feeding while it is growing and developing, which usually means the first three calf-crops. A similar situation may apply in sheep. Venter (1968) has shown that the ewe increases rapidly in size to 2,5 years of age and reaches the maximum bodymass at 4,5 years. Similarly, Bosman (1959) has observed that the reproductive

rate increases to eight years of age. The results obtained during 1970 and 1971 show a trend as regards the resistance to nutritional stress amongst ewes differing in age, the older ewe being more tolerant to nutritional stress than the young ewe.

In view of the availability of synthetic releasing hormone (Reeves et al., 1972b) there can be no doubt that a study of the endocrinological aspects of reproduction holds exciting possibilities and rapid advances may be expected. Such findings must be exploited in practice since there is a great need for improving many of the facets of commercial sheep production.

It has become increasingly evident that the work described here comprises only the initial stages of a much wider field of investigation. The first essential now appears to be to define as accurately as possible the conditions occurring in farming practice and then to undertake experiments designed to elucidate the problems encountered. The temptation to limit research to investigations where the conditions can be rigidly controlled and the results are reasonably predictable must be strongly resisted. This does not imply that the more basic problems do not play an important role in providing the foundation for more practical investigations, but a reasonable balance should be maintained between so-called basic and applied research. In this way the problems must eventually be solved and the research worker make an honest contribution to the advancement of mankind.

SUMMARY

Commencing in 1967 and continuing for a period of six years experiments were conducted to investigate the effect of (i) undernutrition during lactation and (ii) flushing prior to mating on the reproductive performance of Merino and SAMM x Merino ewes. The ewes, which lambed in autumn, were fed so as to either maintain their body condition during the 84-day lactation period or to lose approximately 20 percent of their bodymass during this time. During 1968, 1969 and 1970 the effect on the reproductive performance of isolation from, or association with, vasectomized rams after weaning was also investigated. The wool production of the ewes, and growth and reproduction of the lambs were also recorded.

The main observations and conclusions were:

1. On the high plane of nutrition during lactation the change in bodymass of the mature ewes ranged between an average of -3,8 to 8,3 percent and for the ewes subjected to undernutrition the average loss was -14,0 to -19,9 percent.

2. The duration of the post-partum anoestrous period did not depend on the bodymass or change in bodymass of the mature ewes. The loss in bodymass (X), as measured at weaning, was negatively correlated with the duration of anoestrus (Y) in the case of the maiden ewes. The regression equation was:

$$Y = 58,0 - 0,227X$$

When all the data were pooled a negative correlation between duration of anoestrus and the date of lambing was obtained.

3. The delayed effect of undernutrition on the incidence of oestrus began to be manifested during the 17-day period commencing 5 July.

It was also observed that isolation of the ewes from rams at weaning reduced the incidence of mating during the first 17 days after rejoining with rams on 15 October.

4. The data regarding proportion of ewes oestrus during 17-day periods were examined for possible association with the bodymass post-partum, the minimum bodymass during lactation, bodymass at weaning, the maximum loss during lactation, the loss to weaning and the weekly accumulated change in bodymass. The latter three parameters were considered in relation to the post-partum bodymass. During the period 8 August to 17 November no parameter was more consistently associated with the proportion of ewes oestrus than any other. However, during the 17-day periods commencing 8 August and 25 August the number of ewes oestrus was closely related to their bodymass at weaning. The loss in bodymass to weaning (%) greatly influenced the incidence of oestrus during the remaining 17-day periods. The incidence of oestrus fell markedly when the bodymass was below 40 kg or the loss to weaning exceeded 5%.

5. When the sexual activity was measured by the number of times each ewe exhibited oestrus from 8 August to 17 November (maximum 6, minimum 0) it was again concluded that bodymass at weaning or change in bodymass, to weaning exerted a significant effect.

6. The mean bodymass of the ewes which mated during the 17-day period prior to introduction of entire rams was higher than ewes which exhibited oestrus after joining breeding rams. A similar trend was noted regarding the change in mass during lactation.

7. Nutritional stress had little effect on the pattern of mating to entire rams. Apparently, stimulation by breeding rams and/or resumption of the new breeding season **oblit**erated the effect of poor

nutrtition.

8. Undernutrition did not significantly reduce the number of ewes lambing or the incidence of twin births. Flushing prior to mating was of little benefit. In general, the ewes that were adequately fed during lactation produced the most lambs.

9. Classification of the ewes according to the number of lambs produced (0, 1 or 2) showed that the lambing rate was more closely associated with change in bodymass during the lactation period than with bodymass per se.

10. The restricted feed intake significantly reducedthe wool yield from an average ranging between 4,73 and 5,54 kg to between 3,96 and 4,99 kg.

11. Submaintenance feeding of the lactating ewe significantly reduced the bodymass of the lambs at weaning (84 days of age); an effect which waned during the post-weaning period. A small difference in favour of the ewe-lambs reared by dams adequately fed during lactation was still evident at the first lambing (24 months of age).

The re-initiation of breeding in spring is discussed and a genetic limitation to twinning suggested.

In order to study the sensitivity of the ovary to stimulation a technique incorporating progesterone suppression of endogenous gonadotropin release in ewes was evolved. Exogenous gonadotropins in the form of PMSG and HCG were administered and it was concluded that the latter gonadotropin produced the most predictable response. Progesterone did not appear to reduce the response of the ovary to stimulation.

The test procedure was applied to non-lactating Merino ewes which had been maintained on high and low planes of nutrition for 22 weeks.

Although the average bodymass of the ewes on the two planes of nutrition differed by 39,5% the ovulation rate after administration of 350 or 500 IU HCG was not affected.

In a further experiment similar rations produced a 26,7 percent difference in the case of Merino and 27,0 percent for SAMM x Merino ewes. Treatment with 0, 250 or 350 IU HCG produced a greater quantity of ovarian fluid amongst the ewes on the high than on the low plane of feeding. The cross-bred ewes exhibited a greater response to gonadotropic stimulation, both in terms of ovulations and quantity of ovarian fluid than Merinos.

Evidence suggesting that ovarian sensitivity may constitute a component of fecundity is reviewed.

A study of the gonadotropic influence on the ovary involved the development of a radioimmunoassay to measure ovine LH. A senstivie double-antibody method using rabbit anti-ovine LH serum and radioiodinated ovine LH was developed. In the assay, ovine plasma and an ovine pituitary LH standard produced parallel dose-response curves. Within physiological ranges, the system was free from interference by ovine FSH and TSH and appeared specific for ovine LH. Quality-control checks, including reproducibility estimates, were within acceptable limits.

The assay procedure was used to measure the plasma LH level in ewes which did not exhibit oestrus during lactation. LH titres in these animals were no lower than the basal level of cycling ewes. Sudden surges in the LH content of daily samples obtained from anoestrous ewes were observed. Maiden ewes tended to have a lower plasma LH content than mature animals.

During late anoestrus ewes that were isolated from rams after weaning exhibited a significantly lower plasma LH level than similar individuals continuously associated with vasectomized rams.

At oestrus the timing of the pre-ovulatory surge of LH varied greatly between animals and frequently commenced even prior to oestrus. Marked variations in the peak LH level (51,8 to 386,6 ng/cm^3) and the total quantity of hormone released during the surge were also obtained. The duration of the pre-ovulatory LH-release ranged between an average of 9.18 and 11.15 hours.

Neither the plane of nutrition applied during lactation, the time of day when oestrus commenced nor the incidence of oestrus could be related to the characteristics of the LH surge investigated. Abnormalities of LH release were greatest during mid-anoestrus. Although not significant, there were indications that the total quantity of LH released at oestrus (1970 and 1971 observations) and the maximum concentration in the circulation (1972) were reduced by underfeeding during lactation.

Contrary to expectation, administration of oestradiol-17β induced an acute release of LH in only four of the 27 anoestrous ewes treated. However, the pattern of LH release was similar to that observed in cycling ewes at the time of oestrus.

Possible reasons for a variation in the quantity of LH released into the circulation are discussed. It is concluded that the considerable variability between animals in the pattern of LH secretion creates the need to study large numbers of animals.

The contribution of the findings to a clarification of the problem of low fecundity in commercial ewe-flocks is discussed and possible lines of research indicated.

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Appendix Table 1. Parameters describing the release of LH at oestrus in ewes on two planes of nutrition during lactation.

Date sampled	Plane of nutrition	Ewe No	Latency to release h min	Duration of release h min	Maximum level ng/cm^3	Total released ng	Bodymass at weaning kg	Loss to weaning %
Aug 1970	High	66-45	~	-	34,3+	-	45	-9,2
		68-46	4.24	10.06	386,6	1460,0	41	12,3
		66-72	4.10	8.00	136,2	506,5	43	4,4
		66-104	6.50	10.18	174,2	843,7	44	10,3
		232	~	10.24*	106,4	-	46	4,2
		441	6.20	14.00	249,8	1115,0	40	14,1
	Low	7	2.40	14.00	60,7	293,8	37	-8,0
		67-69	12.20	14.24	160,9	790,8	35	-14,4
		67-76	~	9.00*	158,7	579,9	37	-10,9
		67-86	~	6.42*	218,3+	-	32	-18,6
		246	16.20	11.36	168,4	932,6	38	-18,7
Sept 1970	High	59	-	-	-	-	53	-2,5
		63	-	-	-	-	49	8,0
		66-96	-	-	-	-	45	8,7
		459	-	-	-	-	48	8,1
		441	5.51	9.24	163,1	647,8	40	14,1
	Low	67-69	-	-	-	-	35	-14,4
		67-76	7.57	10.30	220,9	885,8	37	-10,9
		141	3.57	9.18	51,8	178,2	35	-6,1
		308	~	-	-	-	31	-15,9
		495	0.20	8.54	65,0	214,6	43	-23,6
Oct 1970	High	64	8.46	10.54	159,4	725,9	48	4,0
		66-104	3.35	13.18	148,9	714,5	44	10,3
		68-284	3.19	8.48	182,8	654,0	37	6,6
		306	~	12.00	157,4	629,9	43	1,1
		441	6.08	11.06	116,9	546,7	40	14,1
	Low	67-2	1.49	11.30	273,1	904,7	34	-23,5
		66-56	2.29	13.36	213,8	967,4	48	-15,4
		68-132	7.47	8.42	238,1	864,6	29	-17,9
		67-76	11.33	9.36	192,2	815,9	37	-10,9
		152	3.53	9.42	86,8	350,3	43	-7,8
	High*	67-68	12.54	10.18	69,0	335,2	50	13,4
		67-71	4.14	9.06	235,9	851,0	43	-5,9
		68-85	~	-	15,2+	-	48	11,7
		89	~	-	-	-	47	2,0
		288	14.34	9.48	171,5	636,0	46	9,8
	Low*	66-99	-	-	-	-	42	4,5
		66-6	8.8	13.12	138,0	796,1	38	-17,8
		66-7	~	4.54*	187,0+	-	39	-18,1
		66-134	~	7.48	307,3	902,6	38	-5,6
		157	~	4.00*	113,6+	-	38	-5,7

Appendix Table 1 contd.

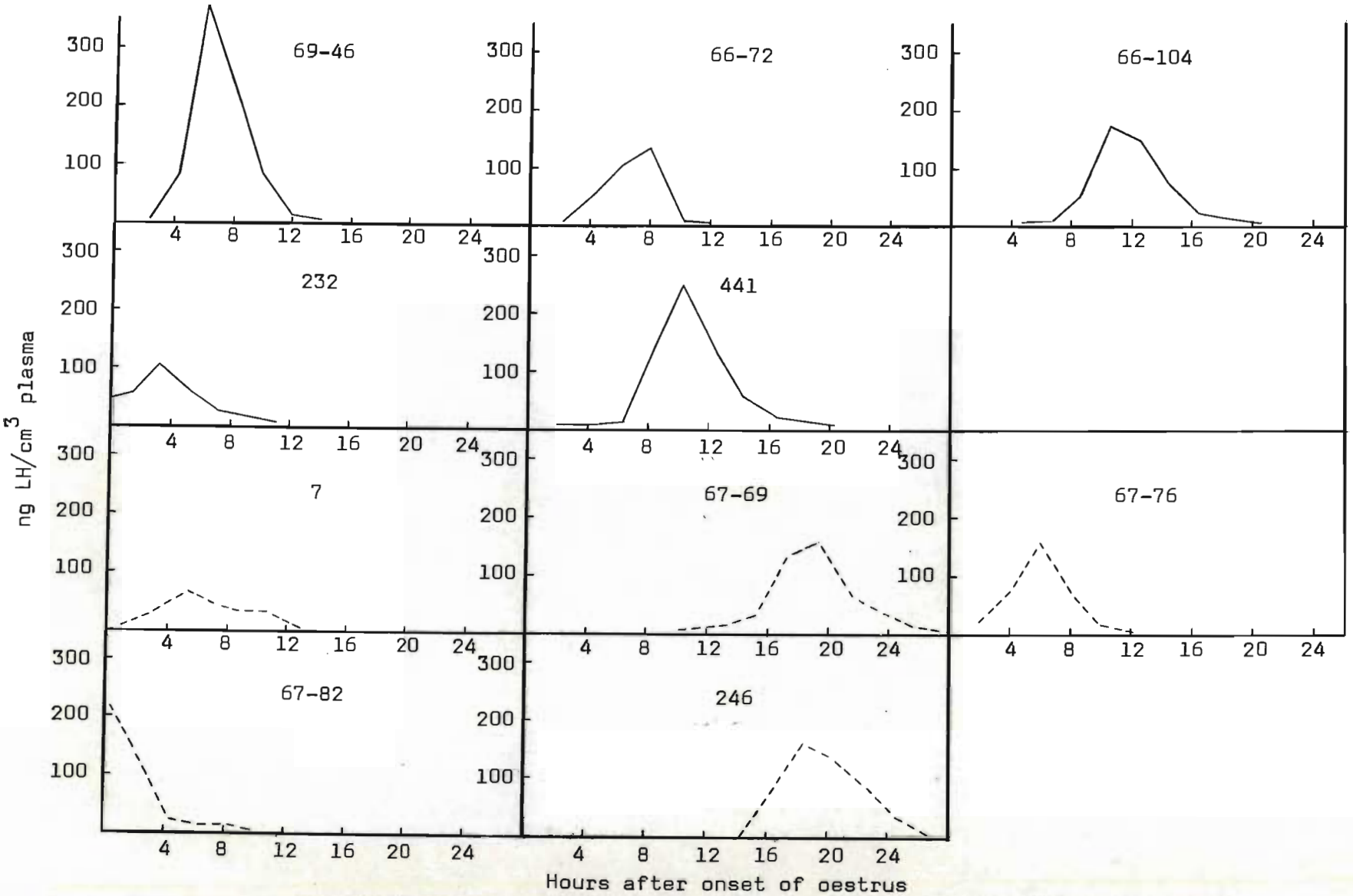
Date sampled	Plane of nutrition	Ewe No	Latency to release h min	Duration of release h min	Maximum level ng/cm^3	Total released ng	Bodymass at weaning kg	Loss to weaning %
Aug 1971	High	67-25	6.37	11.42	320,3	1518,9	52	18,5
		66-39	13.12	10.30	313,4	867,0	45	11,0
		66-68	25.20	-	23,5$^+$	-	52	3,6
		68-90	2.38	9.18	236,3	768,2	53	11,3
		68-222	8.31	10.00	224,6	800,2	43	6,7
		68-295	20.32	5.36*	294,8$^+$	-	45	6,4
	Low	67-10	10.16	7.18	156,2	511,4	41	-14,3
		67-11	~	3,24*	30,9$^+$	-	38	-4,5
		66-58	8.30	9.12	253,5	962,3	32	-17,4
		67-69	23.56	2.00*	147,5$^+$	-	37	-11,0
		68-134	~	4.00*	98,7$^+$	-	42	0,0
Sept 1971	High	67-49	4.47	10.00	217,8	1032,0	50	16,8
		66-70	2.57	9.54	191,2	950,5	48	-0,9
		68-81	22.35	3.36*	290,2$^+$	-	47	3,9
		66-142	-	-	-	-	50	13,4
		68-269	-	-	-	-	45	13,5
		68-287	-	-	-	-	44	16,9
	Low	66-20	~	6.00*	252,7$^+$	-	37	-9,0
		66-46	2.51	8.18	326,3	978,2	30	-25,8
		67-68	10.40	10.30	279,8	752,6	41	-15,9
		68-169	1.39	7.42	170,3	554,2	48	12,6
		68-259	4.49	12.00	259,6	1120,9	40	-18,5
Oct 1971	High	66-37	~	3.18*	156,9$^+$	-	47	0,8
		68-49	18.48	7.12*	101,5	-	49	16,1
		66-57	~	6.54*	232,1	532,5	42	2,0
		67-81	~	5.30*	172,1$^+$	-	46	9,7
		68-81	2.55	9.36	226,5	900,2	48	3,9
		68-85	2.57	10.00	125,2	299,4	53	8,4
		66-139	21.37	5.00*	111,7$^+$	-	43	4,4
	Low	66-12	~	2.36*	34,8$^+$	-	46	-4,7
		66-58	2.54	8.48	232,6	811,1	32	-17,4
		66-103	15.02	7.36	100,5	323,3	34	-22,4
		68-173	2.31	8.54	336,0	1196,8	36	-14,1
		68-211	-	-	-	-	36	-18,5
		68-276	4.32	11.54	306,8	1057,7	33	-8,6
		68-296	6.50	9.48	209,1	585,7	35	-18,7

~ Indicates plasma LH level exceeded 10 ng/cm^3 when sampling commenced.

* Isolated from rams from weaning until 15 October

* Release not fully characterised

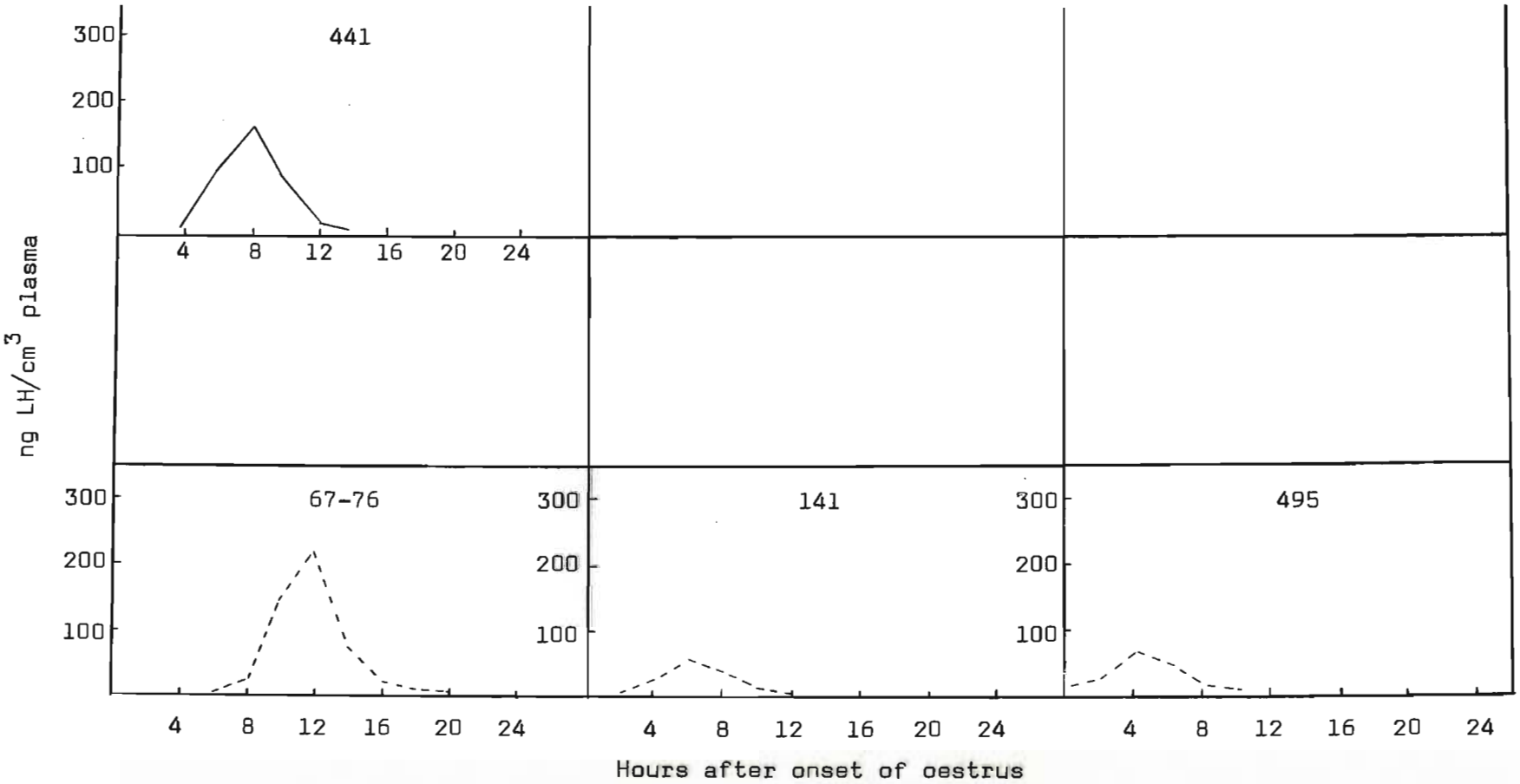
$^+$ Observations incomplete



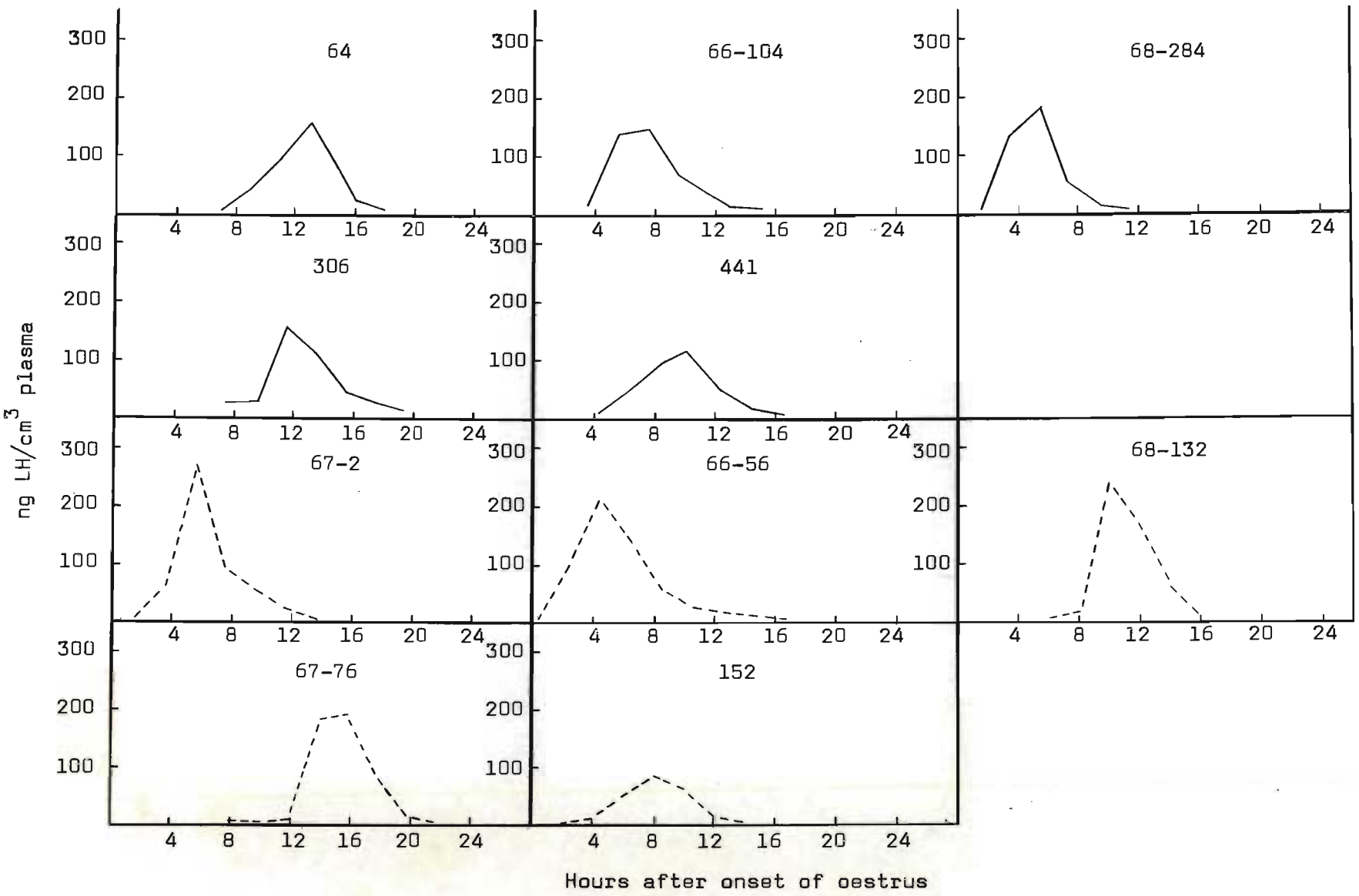
Appendix Fig. 1. LH concentration of jugular plasma measured at oestrus during 1970 in ewes maintained on high (———) or low (----) planes of nutrition during lactation.

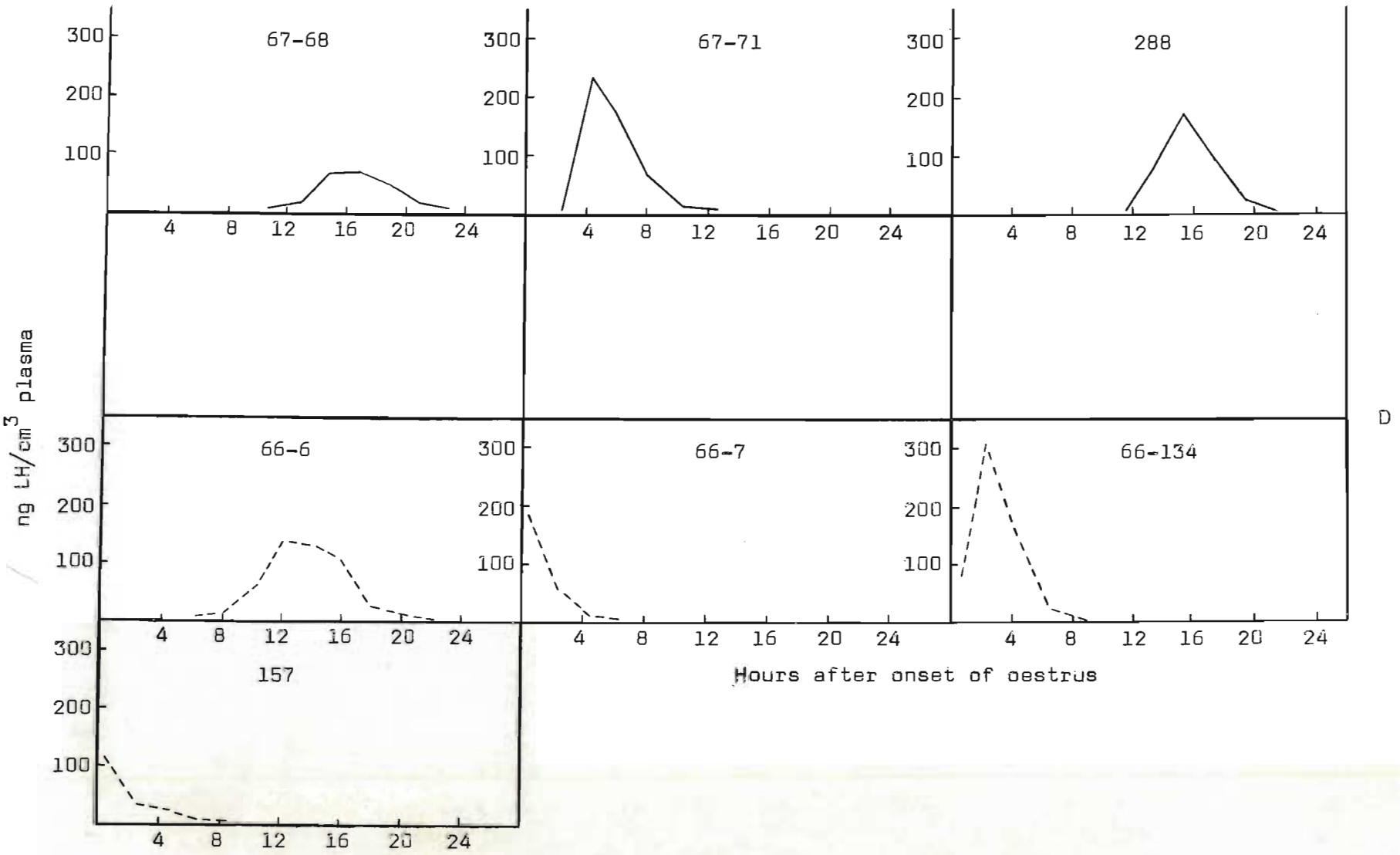
A = August C = October
B = September D = October, isolated from rams until 15 October

A

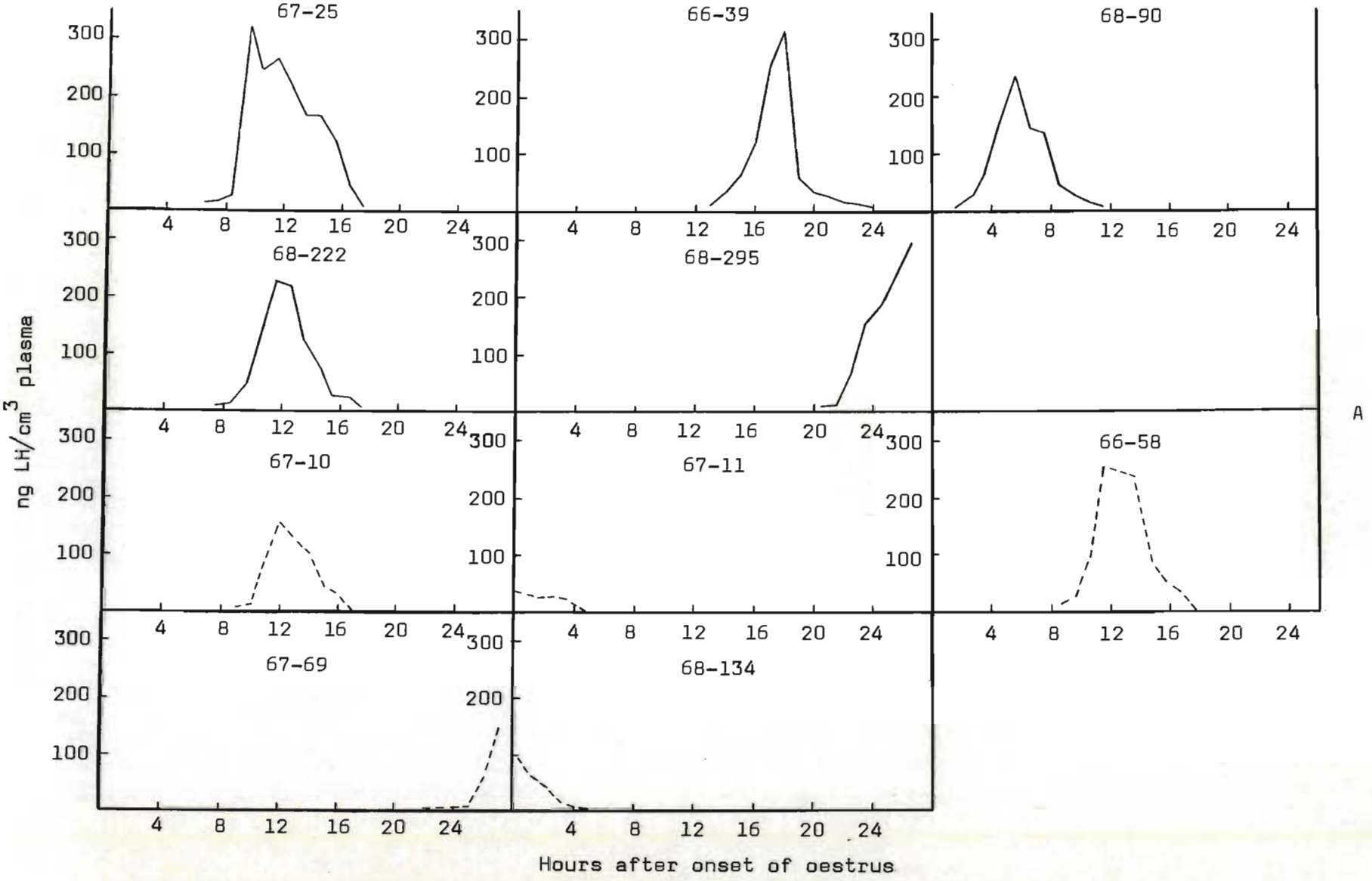

441
67-76
141
495
300
200
100
4
8
12
16
20
24
ng LH/cm3 plasma
Hours after onset of oestrus

B


64
66-104
68-284
306
441
67-2
66-56
68-132
67-76
152
300
200
100
4
8
12
16
20
24
ng LH/cm3 plasma
Hours after onset of oestrus

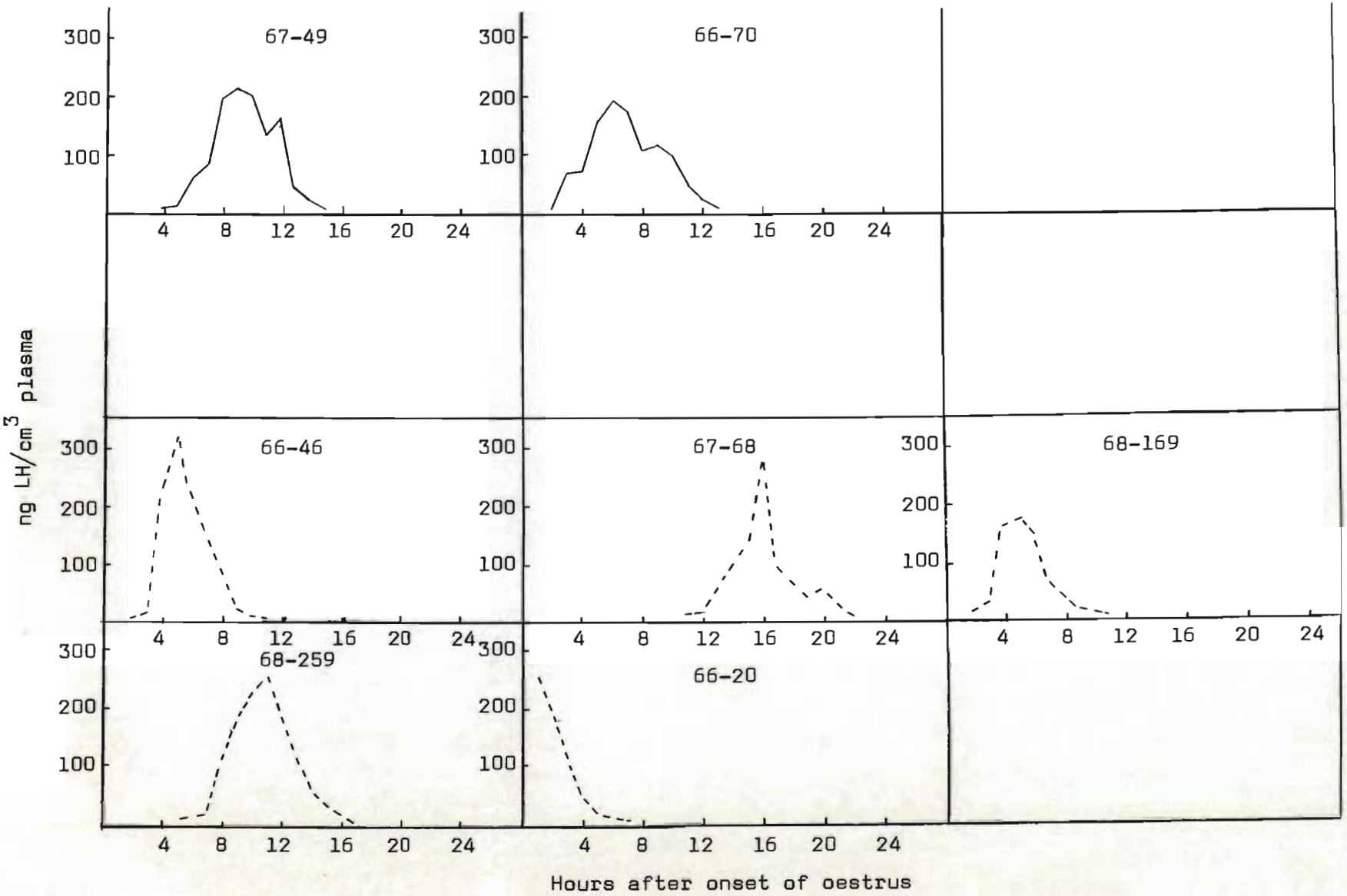

67-68
67-71
288
66-6
66-7
66-134
157
300
200
100
4
8
12
16
20
24
ng LH/cm3 plasma
Hours after onset of oestrus

D

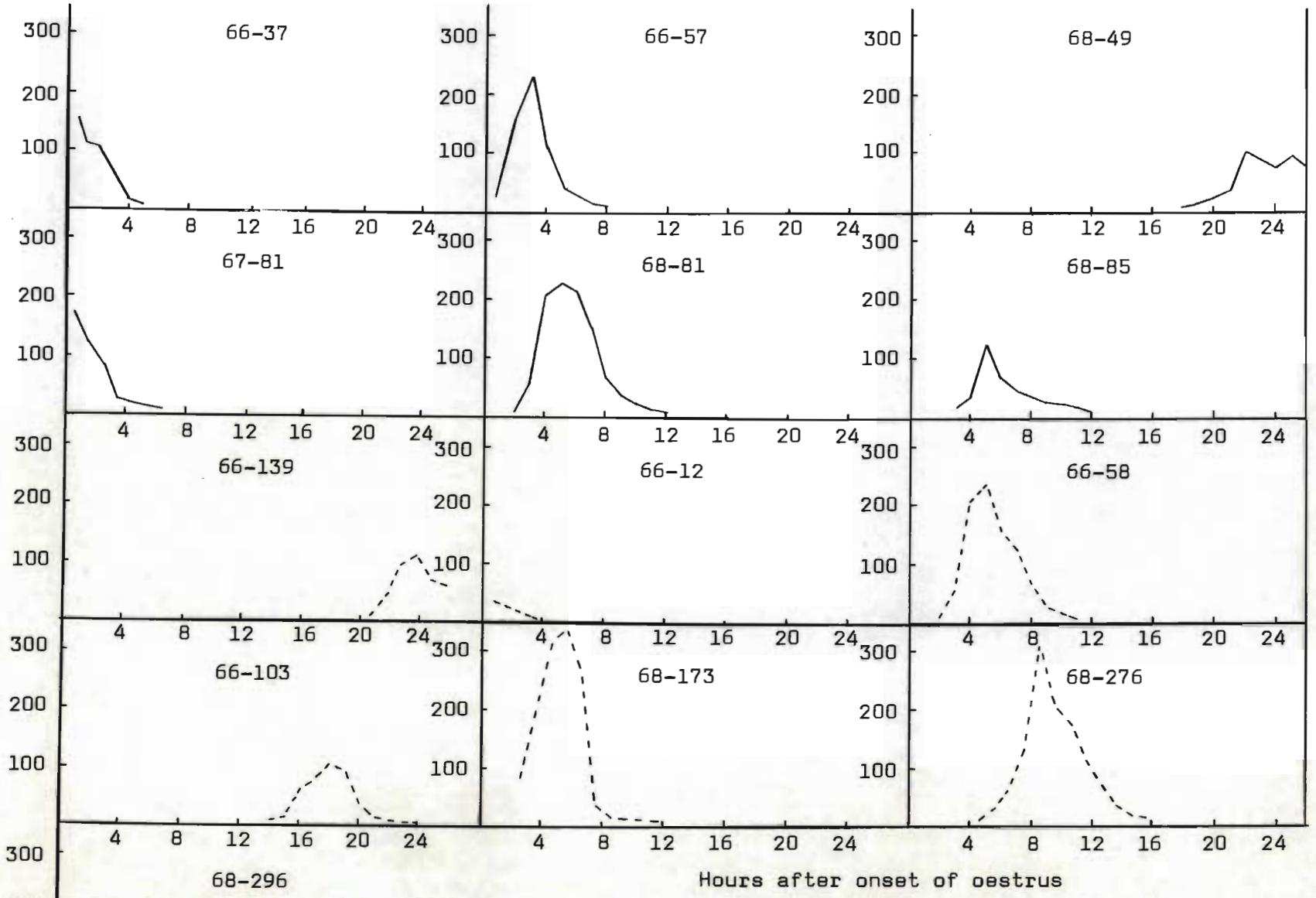


Appendix Fig. 2. LH concentration of jugular plasma measured at oestrus during 1971 in ewes maintained on high (——) or low (----) planes of nutrition during lactation.

A


67-49
66-70
66-46
67-68
68-169
68-259
66-20
300
200
100
4
8
12
16
20
24
ng LH/cm3 plasma
Hours after onset of oestrus

B


66-37
66-57
68-49
67-81
68-81
68-85
66-139
66-12
66-58
66-103
68-173
68-276
68-296
300
200
100
4
8
12
16
20
24
ng LH / cm3 plasma
Hours after onset of oestrus

C

178