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PLASMA LUTEINIZING HORMONE LEVELS IN EWES FAILING TO EXHIBIT OESTRUS DURING LACTATION AND IN EWES ISOLATED FROM RAMS

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OPSOMMING: LUTEINISERENDE HORMOON PEILE IN DIE PLASMA VAN OOIE ANESTRUS GEDURENDE LAKTASIE EN BY OOIE VAN RAMME AFGESONDER

Daar was geen betekenisvolle verskil in die peil van luteiniserende hormoon in die plasma van ooie wat geen oestrus gedurende laktasie getoon het nie en die tussen-estrus peil van ooie wat gereeld bronstig was. Die LH peil was beduidend laer by ooie wat van ramme afgesonder was as by soortgelyke, maar anestrus, ooie wat voortdurend by ramme geloop het. Die basale LH peil by ooie wat gereeld bronstig was, het relatief min gevarieer van dag tot dag, terwyl merkwaardigde skommelinge in die daaglikse monsters van ooie wat enstrus was, verkry is.

SUMMARY

The plasma LH level of ewes which did not exhibit oestrus during lactation was not significantly different from the inter-oestrus level of ewes showing regular oestrus. The LH level in the plasma of ewes kept isolated from rams was significantly lower than amongst similar, but anoestrous, ewes continuously associated with rams. In ewes which were cycling regularly the basal LH level remained relatively constant from day to day during inter-oestrus, whereas in anoestrous ewes the daily samples showed considerable fluctuation.

Cyclic oestrous activity in the ewe is not resumed immediately after parturition, and when breeding management dictates short intervals between successive lambings, the duration of the post-partum anoestrous period is important. The factors which affect the delay to first oestrus following parturition have been described by Hunter (1968, 1971), but little is known concerning the hormonal mechanisms controlling anoestrus.

It is probable that the breeding and non-breeding seasons in sheep are partly related to the rate of synthesis and/or release of luteinizing hormone (LH) from the adenohypophysis (Dutt, 1960; Robertson & Hutchinson, 1962). Accordingly, the level of LH in the circulation would differ between the anoestrous ewe and the ewe showing regular oestrus. Roche, Foster, Karsch, Cook & Dzuik (1970) drew no conclusions regarding the LH levels in such ewes, but noted that during the anoestrous period the LH levels were lowest during mid-anoestrus.

In this investigation of anoestrus, plasma levels of LH were measured in ewes which did not exhibit oestrus during lactation. Ewes which had been isolated from rams during anoestrus were also studied since such animals exhibit a lower incidence of oestrus (Lishman & Hunter, 1967) and ovulation (Hunter, 1969) than ewes which are associated with rams.

Procedure

Maiden and mature Merino ewes that had lambed in autumn were used in the experiment. The ewes were fed a ration of milled lucerne hay, maize silage and a concentrate mixture in such amounts that one group maintained body condition (high plane) while the other experienced an average loss of 20% in bodymass (low plane) during the 84-day lactation period. During the post-weaning period all ewes were fed the same amounts of feed. After lambing at the end of March 1970 twice-daily observations for oestrus were made using vasectomized rams.

At the conclusion of the lactation period blood samples were obtained from the ewes that had not exhibited oestrus during the suckling period. Samples obtained during the inter-oestrus period from ewes which were showing oestrus regularly at this time (late June), served as controls.

Just prior to the commencement of the new breeding season (mid-October) blood samples were taken from ewes which had been isolated from rams since weaning. Samples were drawn from at least five maiden and five mature ewes which had been randomly selected from amongst the animals on each of the two planes of nutrition. These were compared with ewes that had been continuously associated with rams, but which were anoestrus at the time of sampling. In all cases blood samples were collected by jugular venipuncture on five consecutive days and at the same time each day (14h00). Samples (10cm^3) were collected into heparinized test tubes, the plasma separated by centrifugation and the latter stored at −15°C until assayed.

LH was measured using the double-antibody radioimmunoassay developed by Niswender, Reichert, Midgley & Nalbandov (1969). Purified ovine LH (Papkoff preparation G3-206) was iodinated with ^{125}I (Radiochemical Centre, Amersham). NIH-LH-S16 was used as the standard hormone and all plasma values were expressed in terms of this preparation. The anti-ovine LH serum was used at an initial dilution of 1:100000 (Lishman, Stielau, Dreosti & Stewart, 1973). All determinations of plasma LH were performed in duplicate, using not more than 0,2cm^3 of plasma.

Results

Ewes anoestrus throughout lactation

The mean plasma LH levels during five consecutive days, in the various groups, is shown in Table 1. Although

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Table 1

Plasma LH levels in ewes not exhibiting oestrus during lactation (anoestrus) compared to inter-oestrous samples from cycling ewes (oestrus)

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 ± S.E.	No. of ewes in which a single daily sample exceeded 5,0 ngLH/cm^3
High	Oestrus	Maiden	5	2,95 ± 0,67	0
		Mature	6	3,75 ± 0,61	0
	Anoestrus	Maiden	3	4,32 ± 0,87	1
		Mature	6	4,19 ± 0,61	2
Low	Oestrus	Maiden	3	3,13 ± 0,87	0
		Mature	5	3,53 ± 0,67	0
	Anoestrus	Maiden	13	3,96 ± 0,42	5
		Mature	10	3,27 ± 0,48	1

Table 2

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 ± S.E.	No. of ewes in which at least one daily sample exceeded 5,0 ng LH/cm^3
High	Continuous	Maiden	5	3,35 ± 0,49	1
		Mature	5	4,09 ± 0,49	2
	Isolated	Maiden	7	2,76 ± 0,42	1
		Mature	8	2,95 ± 0,39	2
Low	Continuous	Maiden	5	3,85 ± 0,49	1
		Mature	7	4,66 ± 0,42	4
	Isolated	Maiden	7	2,94 ± 0,42	1
		Mature	9	3,19 ± 0,39	1

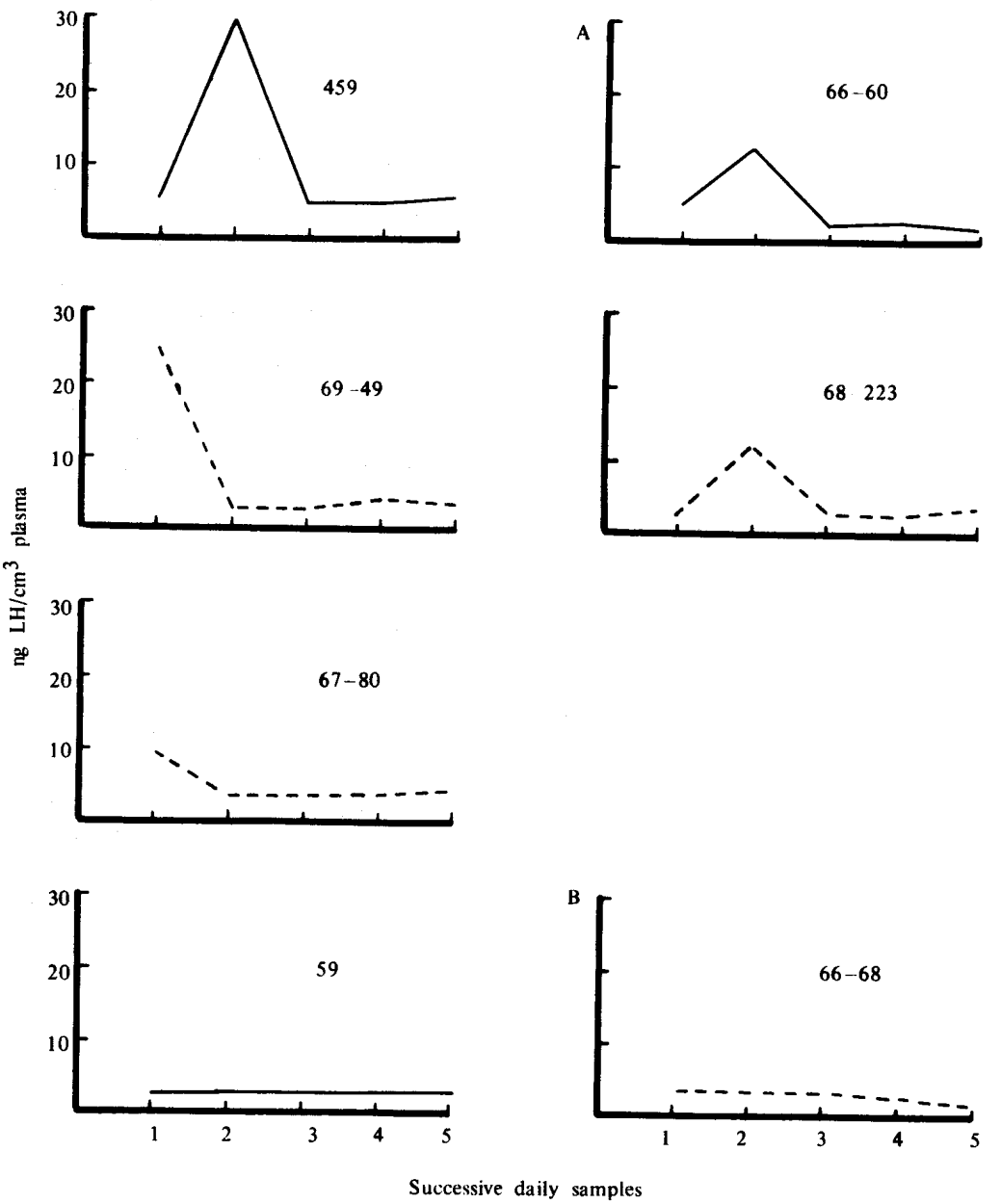

Fig. 1. *Plasma LH levels illustrating marked daily variation in ewes not exhibiting oestrus during lactation (A) compared to dioestrous levels of two randomly selected cycling ewes (B).*

——— *High plane of nutrition during lactation.*

– – – – *Low plane of nutrition during lactation.*

mean plasma LH levels in maiden ewes tended to differ from those in mature ewes the differences were not statistically significant (least squares analysis). Similarly, neither the reproductive state nor the plane of nutrition had a significant effect on the mean plasma LH level (Table 1). In certain individuals one of the daily samples showed a marked deviation from the remaining values (Table 1) and the more striking examples are illustrated in Fig. 1. This phenomenon was not observed amongst the ewes exhibiting regular oestrus.

Ewes isolated from rams

During late anoestrus (October, 1970) plasma LH levels were lower in ewes isolated from rams than in ewes in continuous contact with rams (least square analysis, $P = 0{,}01$; Table 2). The occurrence of elevated levels of LH ($>5{,}0$ ng/cm^3) in anoestrous ewes was again evident.

Discussion

The results presented here do not support the hypothesis that some ewes fail to ovulate and remain anoestrus throughout lactation because of an inadequate, tonic release of LH from the pituitary gland. This is in accordance with the finding that pituitary stores of gonadotrophin are not reduced during anoestrus (Roche *et al.*, 1970). However, the findings of Roche *et al.* (1970) and Pelletier & Thimonier (1973) suggest that the ability of the pituitary to rapidly synthesise and release gonadotrophin, in amounts sufficient to induce ovulation, is impaired during lactation.

It has recently been demonstrated that in sheep (Cumming, Brown, Blockey & Goding, 1971) and in man (Nankin & Troen, 1972; Root, De-Cherney, Russ, Duckett, Garcia & Wallach, 1972; Murray & Corker, 1973) there is a pulsatile release of LH from the pituitary gland. However, the suppressive action of gonadal steroids maintains the tonic release of LH at a relatively low level (Brown, Cumming, Goding & Hearnshaw, 1972).

The minor surges in plasma LH reported here and also observed in anoestrous (Butler, Bolt & Malvern, 1971) and ovariectomized ewes (Roche *et al.*, 1970; Reeves, O'Donell & Denorscia, 1972) probably reflects an interruption of the negative feed back system (Reeves *et al.*, 1972). Under such conditions there is a cyclic release of LH (Reeves *et al.*, 1972) rather than repetitive abrupt discharges.

Such fluctuations in hormone levels can have an important influence on the results obtained, depending on the sampling procedure followed. Findings based on samplings repeated several times each day and continued over a number of days would be preferable.

Evidence currently available suggests that various factors can influence the level of LH in the circulation. This is indicated by the finding that both in underfed (Howland, 1971a, 1972; Howland & Skinner, 1973) and young (Howland, 1971b) laboratory animals the serum LH level is reduced. Our results do not demonstrate a consistent effect of age or nutritional level, but suggest that a further factor viz., association with rams can influence the basal level of LH in the blood. The latter is in conformity with the results of Lishman & Hunter (1966, 1967), Hunter (1969) and Lishman (1969) where it was demonstrated that ewes which are isolated from rams tend to enter a state of anoestrus.

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