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THE EFFECTS OF FEED RESTRICTION, OESTROGEN PRIMING AND STAGE OF THE OESTROUS CYCLE ON GN-RH-INDUCED RELEASE OF LH IN EWES

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OPSOMMING: DIE INVLOED VAN VOERBEPERKING, VOORBEHANDELING MET ESTROGEN EN STADIUM VAN DIE ESTRUSSIKLUS OP DIE AFSKEIDING VAN LH NA GnVH TOEDIENING BY OOIE

Die invloed van ondervoeding gedurende 'n herfs laktasie, voorbehandeling met estrogeen en stadium van die estrussiklus op die afskeiding van LH na stimulering met GnVH is bestudeer by Merino-ooie. Die basale LH-konsentrasie voor GnVH-inspuiting is nie deur die behandelings beïnvloed nie. Voorbehandeling met estrogeen het die piek LH-konsentrasie betekenisvol verhoog beide op die derde ($P < 0,001$) en vyftiende dag ($P < 0,05$) van die estrussiklus. In vergelyking met piek LH-konsentrasie het die totale afskeiding van LH 'n minder konsekwente reaksie op voorbehandeling met estrogeen getoon. Ooie wat behandel is toe die reserwes van die hipofise hoog was (15de dag), het meer LH afgeskei (piek LH en totale LH) as wanneer die ooie vroeg in die siklus (3de dag) behandel is. Ondervoeding het die gevoeligheid van die ooie tot GnVH verminder, aangesien 'n laer piek LH-peil waargeneem is by hierdie ooie in vergelyking met ooie wat hul liggaamsmassa gedurende laktasie gehandhaaf het (15de dag; $P < 0,001$).

SUMMARY:

The effect of underfeeding during autumn lactation, oestrogen priming and stage of the oestrous cycle on the release of LH in response to GnRH was studied in Merino ewes. The basal LH level prior to GnRH administration was not influenced by the treatments applied. Oestrogen priming significantly increased the peak LH level, both on days 3 ($P < 0,001$) and 15 ($P < 0,05$) of the oestrous cycle. In comparison, the total LH release showed a less consistent response to oestrogen priming. Ewes treated when pituitary stores of LH were expected to be high (day 15) released more LH (peak LH and total LH) than when treated early in the cycle (day 3). Underfeeding reduced the sensitivity of the ewes to GnRH since a lower peak LH level was observed in such animals when compared to ewes which maintained their bodymass during lactation day 15; $P < 0,001$).

The decreased reproductive rate of underfed female mammals is considered to be due, at least in part, to changes in the synthesis and/or release of the hormones involved in reproduction (Mulinos & Pomerantz, 1940; Lamming 1960; Leathem, 1966; Howland 1972). When ewes were underfed during autumn lactation their oestrous activity declined to a minimum shortly before the onset of the new breeding season in spring (Lishman, Stielau & Botha, 1974). In an attempt to cast some light on the mechanism by which underfeeding reduces the incidence of oestrus, the Gn-RH-induced release of LH was measured during late September.

Procedure

The experimental ewes were from a flock of 120, two to seven-year old Merino ewes which had lambed between 14 March and 14 April 1974. Three days after parturition ewes, with single lambs, were randomly allocated to either the adequate or restricted levels of feeding. The former ration maintained the weight of the ewes during the 12-week lactation, while the latter resulted in a 23% loss during this time. After weaning of the lambs, all the ewes received a ration which maintained body condition in those ewes not fed a restricted diet during lactation. The composition of the rations fed has been described by Lishman *et al.* (1974).

The original intention was to utilize only ewes that were anoestrus during September and therefore to increase the proportion that became anoestrus the flock was kept isolated from rams. However, during August ovarian examination of 10 ewes, selected at random, revealed that 50% possessed an active corpus luteum. Consequently, the experiment was modified and the release of LH measured shortly after and before oestrus, when pituitary reserves were likely to be low and high, respectively (Roche, Foster, Karsch, Cook & Dzuik, 1970).

To simplify collection of blood samples the oestrous cycles of the ewes were synchronised by insertion of progesterone pessaries (G.D. Searle), followed on removal after 15 days by a single i.m. injection of 500 µg oestradiol benzoate (ODB). Observations for oestrus were then initiated using vasectomized rams twice daily. On day 3 of the second cycle following synchronisation, i.e. approximately 23 days after removal of the progestagen pessaries, 58 ewes were divided into 6 treatment groups (Table 1). Six hours prior to the i.m. injection of Gn–RH, (Abbott) on 24 September, 3 of these groups received a priming dose of 100 µg ODB. Directly thereafter, indwelling silastic jugular catheters (Portex) were inserted into all ewes and at two-hour intervals blood samples (5 ml) were withdrawn into heparinized syringes. At 12h00 the ewes were injected with the allocated dose of Gn-RH in saline, (Table 1) and for the next 8 hours, blood samples were obtained every 30 minutes. On day 15 of the cycle subsequent to that when Gn-RH was first administered 54 ewes from the flock of 120 were re-allocated, at random, to the treatments applied on day 3 of the previous cycle. However, in an attempt to improve the priming effect of oestrogen the dose of ODB was divided into 3 injections each

of 30 μ g. These were administered at 8h intervals, commencing 22h before Gn–RH treatment. Blood samples were drawn as before, but at 15 minute intervals after Gn–RH injection. Following centrifugation the plasma samples were stored at $-15^{o}C$ until assayed for LH by the double-antibody radioimmunoassay of Niswender, Rechert, Midgley & Nalbandov (1969). This assay has been validated by Lishman (1972). NIH-LH-S16 was used as standard.

Results and Discussion

The data from the 2x2x2x3 factorial treatment arrangement were analyzed by least squares procedures appropriate for unequal subclass numbers. A model which accounted for the effects of day of the cycle, oestrogen priming, level of feeding and dose of Gn–RH used. Three characteristics of the LH release were measured viz., the basal level prior to Gn–RH, the highest level to which the hormone rose in the plasma following Gn–RH (peak LH) and the total release of LH (estimated from area under the LH release curve). The basal LH level (4,47 ± 1,16 ng/ml) was not significantly influenced by oestrogen priming or any of the treatments applied, whereas the least squares means in Table 1 indicate that oestrogen priming significantly increased the peak LH level both on day 3 (increase = 5,6 ng/ml; $P < 0,001$) and day 15 (increase = 10,6 ng/m; $P < 0,05$). This suggested that the divided priming regime applied on day 15 was more effective than the single dose given on day 3, but since the priming procedures varied the conclusion remains only tentative. The response to oestrogen priming, measured in terms of the total LH release (Table 1) was more variable than the peak LH levels and a significant positive response ($P < 0,05$), was obtained only in the restricted ewes on day 3 and the unrestricted animals on day 15. These results support the findings which indicate that oestrogen plays an important role in modifying the pituitary response to Gn-RH in the ewe, (Reeves, Arimura & Schally, 1971 a & b; Jackson, 1975; Coppings & Malven 1976), cow (Convey, 1973) and rat (Libertun, Cooper, Fawcett & McCann, 1974). In view of the observation that ODB elicits an LH surge within approximately 15 hours (Reeves, Beck & Nett, 1974; Jackson, 1975) and the present finding that 100 μg ODB does not elevate LH levels by six hours after administration, but does sensitize the pituitary to exogenous Gn-RH within this time, supports the hypothesis favouring a dual action of oestrogen viz., a quick action to sensitize the pituitary and a slower action on the hypothalamus to elevate the levels of Gn-RH. The conclusions of Nett, Akbar & Niswender (1974), Cumming (1975) and Jackson (1975) provide support for this hypothesis. However, Coppings & Malven (1976) proposed that the pituitary is sensitized, briefly, only 15 h after administration of oestradiol – 17β and that facilitated release of endogenous Gn–RH occurs 12 to 20h after administration of this oestrogen.

The ewes treated on day 15 of the oestrous cycle exhibited a significantly ($P < 0,01$) greater peak and total release of LH after 25 μg and 100 μg Gn–RH than those treated on day three (Table 2). This trend is in agreement with that reported by Hooley, Baxter, Chamley, Cumming & Findlay (1974) and Rippel, Johnson, Mauer & Webel (1974). If the quantity of LH released is related to pituitary reserves (Jenkin, Heape & Symons, 1977) then the 4-fold difference in pituitary content of LH between days 3 and 15 (Roche *et al.*, 1970) would be expected to result in a difference in peak LH values greater than the 24,6 ng/ml obtained in the present study. The difference, as a percentage of the value on day 3, was greatest at the lowest level of Gn–RH, hence the significant "day of cycle X dose of Gn–RH" interaction, when comparing days 3 and 15. This interaction was also reflected in the lower total LH release on day 15 than on day 3 when 50 μg GnRH was administered (Table 2).

Zolman, Gonvey & Britt (1974) proposed that the interaction between the dose of Gn–RH and the day of the oestrous cycle on which the releasing hormone was injected, was due to variations in the level of oestrogen. Our results are somewhat contradictory in that it could be expected that administration of a priming dose of 100 μg ODB would nullify any effect of differences in the level of endogenous oestrogen. However, a stage-of-cycle effect was still evident and if the circulating levels of progesterone and oestrogen levels had been measured, the results may have become clearer. In support of the present results is the finding that in rats the increased responsiveness to Gn–RH could not be correlated with the oestrogen levels at that time (Araki, Ferin, Zimmerman & Vande Wiele, 1975). Furthermore, Castro-Vazques & McCann (1975) demonstrated that ovariectomy did not block the increased responsiveness near the time of oestrus. Both Castro-Vazquez & McCann (1975) and Zeballos & McCann (1975) noted that priming with Gn–RH increased the responsiveness to subsequent releasing hormone.

Although the animals which received the maintenance diet (unrestricted) during lactation exhibited a higher peak LH value than those which were restricted at this time (Table 1) the differences were significant ($P < 0,001$) only on day 15. Beal, Kaltenbach & Dunn (1975) recorded a similar response regarding the total LH response, but not the peak LH level, in heifers fed 61% of their energy requirements. In an earlier study by Dunn, Rone, Kaltenbach, van der Walt, Riley & Akbar (1974) the peak LH was in effect higher in underfed beef cows. In the study reported here, on day 3 the total LH response was lower ($P < 0,001$) in the restricted ewes only, in those not primed with oestrogen. On day 15 this response was noted only in the primed ewes and those not primed exhibited the reverse trend.

The reduced LH release in the restricted ewes, as measured by peak LH levels, does not necessarily imply a deficiency of pituitary stores, since Rippel, Johnson & White (1974) demonstrated that the pituitary concentration of LH in anoestrous ewes was not influenced

Table 1

Least squares estimated release of LH (mean ± S.E.M.) after injection of Gn-RH in ewes as influenced by level of feeding, oestrogen priming and stage of the oestrous cycle

Level of feeding	Dose Gn–RH (µg)	Character-istic of LH release	Day 3 of oestrous cycle				Day 15 of oestrous cycle			
			n	Primed (100 µg ODB)	n	Unprimed	n	Primed (3 x 30 µg ODB)	n	Unprimed
Unre-stricted	25	Peak[1]	4	82,1 ± 4,5	5	60,9 ± 4,5	4	113,8 ± 4,3	5	102,7 ± 4,3
		Total[2]		121,7 ± 10,3		118,0 ± 10,3		191,7 ± 13,2		114,6 ± 11,5
	50	Peak	5	156,9 ± 5,6	5	135,6 ± 5,6	5	179,6 ± 3,9	4	168,4 ± 4,4
		Total		284,6 ± 8,8		280,9 ± 8,8		277,9 ± 12,7		200,9 ± 11,7
	100	Peak	5	251,1 ± 3,8	5	239,8 ± 3,8	5	288,2 ± 4,5	4	277,1 ± 4,1
		Total		417,4 ± 8,8		413,7 ± 8,8		494,7 ± 11,5		417,6 ± 12,6
Restricted	25	Peak	5	75,8 ± 3,8	5	54,5 ± 3,8	5	92,0 ± 4,5	4	80,8 ± 4,0
		Total		142,6 ± 8,8		79,3 ± 8,8		130,8 ± 11,4		145,2 ± 13,3
	50	Peak	5	150,5 ± 3,8	5	129,3 ± 3,8	4	157,7 ± 4,5	4	146,6 ± 4,6
		Total		305,5 ± 9,7		242,2 ± 9,7		217,0 ± 13,5		231,4 ± 13,5
	100	Peak	5	244,7 ± 3,8	5	223,4 ± 3,8	5	266,4 ± 4,1	5	255,2 ± 4,0
		Total		438,3 ± 8,8		375,1 ± 8,8		433,8 ± 11,3		448,1 ± 11,3

[1] Highest concentration in plasma following Gn–RH (ng/ml)

[2] Derived from area under LH release curve (arbitrary units)

Table 2

Least square estimated release of LH (mean ± S.E.M.) after injection of Gn–RH in ewes as influenced by stage of the oestrous cycle in addition to level of feeding and oestrogen priming

Level of feeding	Dose GN–RH (µg)	Character-istic of LH release	Oestrogen primed				No oestrogen			
			n	Day 3	n	Day 15	n	Day 3	n	Day 15
Unre-stricted	25	Peak[1]	4	89,0 ± 3,5	4	113,5 ± 3,5	4	72,6 ± 3,4	5	97,1 ± 3,1
		Total[2]		138,4 ± 9,7		165,9 ± 9,6		105,5 ± 9,8		133,0 ± 8,6
	50	Peak	5	159,4 ± 3,0	5	184,0 ± 3,0	5	143,0 ± 2,9	4	167,6 ± 3,4
		Total		301,1 ± 8,3		256,0 ± 8,7		268,2 ± 8,3		223,1 ± 9,9
	100	Peak	5	260,3 ± 3,1	5	284,9 ± 3,0	5	243,9 ± 3,0	4	268,5 ± 3,4
		Total		434,0 ± 8,3		472,5 ± 8,3		401,7 ± 8,3		439,5 ± 8,3
Restricted	25	Peak	5	71,9 ± 3,4	5	96,5 ± 3,1	5	55,5 ± 3,3	4	80,1 ± 3,4
		Total		125,7 ± 8,5		153,2 ± 8,5		92,7 ± 8,5		120,2 ± 9,6
	50	Peak	5	142,4 ± 3,0	4	166,9 ± 3,4	5	126,0 ± 3,3	4	150,5 ± 3,5
		Total		288,4 ± 8,3		243,3 ± 8,2		255,5 ± 9,3		210,4 ± 9,3
	100	Peak	5	243,3 ± 3,0	5	267,8 ± 2,9	5	226,9 ± 3,2	5	251,5 ± 3,1
		Total		421,3 ± 8,3		459,7 ± 8,3		388,3 ± 8,3		426,8 ± 8,3

[1] Highest concentration in plasma following Gn–RH (ng/ml)

[2] Derived from area under LH release curve (arbitrary units)

by successive injections of Gn-RH. Furthermore, Memon, Antoniewicz, Benevenga, Pope & Casida (1969) noted a decline in plasma LH concentrations without pituitary concentrations being affected in underfed ewes. Howland (1976) is of the opinion that synthesis of Gn-RH and the sensitivity of its target tissue are normal in the underfed rat. The similarity between the peak LH level in unprimed, well-fed ewes and primed, restricted ewes, obtained in the present study, suggests that anoestrus in underfed ewes (Allen & Lamming, 1961; Hunter 1962, Smith, 1962, Lamond, Gaddy & Kennedy, 1972, Lishman *et al.*, 1974) could be the result of an inhibited oestrogen secretion, and consequently inadequate steroid priming (Howland, 1976). Rawlings, Kennedy, Chang, Hill & Henricks (1977) have proposed a similar mechanism for the onset of seasonal anoestrus. Clearly, a deficiency in basal LH could be reflected in inadequate steroid production (Howland, 1976), but the present results do not suggest such a deficiency. An insensitivity to LH on the part of the ovary (Gombe & Hansel, 1973) is possibly one of the reasons why underfed females go into anoestrus. An aspect which requires testing, in the malnourished female, is the ability of the hypothalamo-hypophysial system to rapidly synthesise gonadotrophin just prior to oestrus (Roche *et al.*, 1970).

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