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THE RELEASE OF LUTEINIZING HORMONE (LH) IN EWES DEPRIVED OF PROLACTIN DURING LACTATION

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OPSOMMING: VRYSTELLING VAN LUTEINISERENDE HORMOON (LH) BY OOIE WAARIN PROLAKTIEN SEKRESIE GEDURENDE LAKTASIE GE-INHIBEER IS

In 'n studie wat ten doel gehad het om vas te stel of prolaktien sekresie in lakterende ooie 'n nadelige effek uitoefen op die vrystelling van LH, en die reaksie van die eierstokke teenoor gonadotrofien stimulering, is 20 ooie wat oor 'n tydperk van agt dae gelam het, in twee vergelykbare groepe van 10 diere elk verdeel. Tussen dag 10 en 20 post-partum is die ooie in die een groep 12-uurliks ingespuit (onderhuids) met 1,2 mg ergocornine waterstof maleinaat (EC), 'n effektiewe onderdrukker van prolaktien sekresie. Gedurende dieselfde periode is die ooie in die tweede groep, wat gedien het as kontroles, met tussenposes van 12 uur ingespuit met 1,0 cm^3 van die EC oplosmiddel. Alle EC-behandelde en kontrole ooie is op dag 21 binnespiers ingespuit met 50 μg gonadotrofienvrystellings hormoon (Gn-VH). Vir die daaropvolgende ses uur is bloedmonsters met tussenposes van 15 minute verkry van elke ooi. Op dag 24 post-partum is die ooie onderwerp aan 'n buikoperasie, en die voorkoms van ovulasie aangeteken. Vanaf dag 24 tot op dag 60 post-partum is koggeiramme twee keer daagliks by die ooie geplaas om die voorkoms van estrus te bepaal. Al die ooie het 'n LH-piek getoon na inspuiting van die Gn-VH. Die grootte en duurte van die LH-pieke het aansienlik gevarieër tussen individuele diere. Die area onder die LH kurwe ('n aanduiding van die totale hoeveelheid LH vrygestel) na toediening van Gn-VH was 246,9 ±37,9 mm^2 in die EC-behandelde, en 208,5 ± 66,0 mm^2 in die kontrole ooie. Hierdie verskil was nie statisties betekenisvol nie. Drie EC-behandelde, en vier kontrole ooie het geovuleer nadat die Gn-VH toegedien is op dag 21 post-partum. Vanaf dag 21 tot en met dag 60 post-partum het geen ooi tekens van estrus getoon nie. Die resultate wat in die EC-behandelde en kontrole ooie verkry is, is saamgevoeg. Die area onder die LH kurwe in die diere wat ge-ovuleer het na Gn-VH toediening (303,8 ±90,9 mm^2) het nie betekenisvol verskil van die verkry in die ooie wat nie ge-ovuleer het nie (186,7 ±33,0 mm^2). Ses van die nege ooie (66,7%) wat toegeneem het in massa tussen dag 1 en 21 post-partum het ge-ovuleer nadat Gn-VH toegedien is, terwyl ovulasie plaasgevind het in slegs een van die agt ooie (12,5%) wat gedurende hierdie periode in massa afgeneem het. Weens die variasie in die LH responsie was dit nie moontlik om vas te stel of die onderdrukking van prolaktien sekresie in lakterende ooie lei tot 'n verhoogde vrystelling van LH, en 'n verbeterde reaksie van die eierstokke teenoor gonadotrofien stimulasie, al dan nie.

SUMMARY:

In a study aimed to establish whether prolactin secretion in lactating ewes influences the release of LH, and the response of the ovary to gonadotropin stimulation, 20 Merino ewes which lambed over an eight-day period were divided into two comparable groups of 10 animals. Between day 10 and 20 post-partum the lactating ewes in the one group received 12-hourly injections (subcutaneous) of 1,2 mg ergocornine hydrogen maleinate (EC), an effective inhibitor of prolactin secretion. Using the same schedule the ewes in the second group were injected with 1,0 cm^3 of the suspension vehicle, and served as controls. On day 21 post-partum the EC-treated and control ewes were each injected (intramuscularly) with 50 μg of gonadotropin-releasing hormone (Gn-RH). During the ensuing six hours blood samples were obtained from each ewe at intervals of 15 minutes. The ewes were laparotomized on day 24, and the occurrence of ovulations noted. Thereafter, and until day 60 post-partum, vasectomized rams were joined with the ewes twice daily to detect overt oestrus. All the experimental ewes exhibited an LH surge in response to Gn-RH administration. The magnitude and duration of the surges varied considerably between individual animals. The area under the LH curve (an indication of the total quantity of LH released) in the EC-treated ewes (246,9 ±37,9 mm^2) did not differ significantly from that measured in the controls (208,5 ±66,0 mm^2). Although three EC-treated, and four control ewes ovulated, no signs of oestrus were observed in any ewes between day 21 and 60 post-partum. After pooling the results obtained in the EC-treated and control ewes, it was evident that the area under the LH curve in the animals which ovulated in response to Gn-RH (303,8 ±90,9 mm^2) did not differ significantly from that measured in the ewes which failed to ovulate (186,7 ±33,0 mm^2). Six of the nine ewes (66,7%) which gained in mass between day 1 and 21 ovulated in response to Gn-RH, whereas ovulation occurred in only one of the eight ewes (12,5%) which lost mass during this period. In view of the variation encountered in the LH response of the ewes it was not possible to conclude whether or not the suppression of prolactin secretion in lactating ewes results in an enhanced release of LH, and an improved ovulatory response to gonadotropin stimulation.

The stimulus of suckling results in a rapid release of prolactin into the bloodstream of ewes (Fell, Beck, Brown, Catt, Cumming & Goding, 1972; Lamming, Moseley & McNeilly, 1972) and cows (Karg & Schams, 1974). Evidence obtained by Pelletier & Thimonier (1973) indicates that the process of lactation exerts an inhibitory influence on the release of LH in ewes. Minaguchi & Meites (1967) found that suckling acts on the hypothalamus of rats to depress the release of LH-releasing hormone and of prolactin inhibiting factor (PIF), the former resulting in suppression of LH release and the latter in increased prolactin secretion. Symington (1969) subsequently proposed that in farm animals an antagonistic relation exists between the secretion of prolactin and LH during lactation, and this may contribute to the phenomenon of lactation anoestrus. Keller (1968) and Tyson, Friesen & Anderson (1972) are of the opinion that prolactin released in response to suckling in humans decreases the ovarian response to circulating gonadotropins.

Ergocornine, an ergot-derivate, effectively suppresses prolactin secretion in sheep (Louw, Lishman, Botha & Baumgartner, 1974; Niswender, 1974). This report describes the release of LH, and subsequent ovarian activity in lactating ewes in which ergocornine was used to

suppress prolactin secretion during the early post-partum period.

Procedure

Following synchronization of oestrus, 20 mature Merino ewes were mated to lamb over an eight-day period commencing 16th October, 1974. In South Africa this period of the year represents the early phase of the breeding season in Merino ewes.

Immediately after lambing (day 0 post-partum) each ewe was placed on a ration consisting of maize silage *(ad lib.)*, 0,9 kg lucerne hay and 0,7 kg maize meal per day. A creep feed (80 parts maize meal and 20 parts lucerne meal) was made available to the lambs three weeks after parturition. At intervals of seven days throughout the experiment the ewes and lambs were separated, and feed and water withheld for six hours prior to weighing of animals.

The 20 lactating ewes were divided into two groups of 10 animals each, such that the mass of the ewes, and the age and mass of the lambs in each group were comparable. At intervals of 12 hours between days 10 and 20 post-partum (on average) the ewes in the one group were injected subcutaneously with 1,2 mg ergocornine hydrogen maleinate (EC). A single injection of EC (1,2 mg) effectively suppresses prolactin secretion for at least 14 hours (Louw, 1974). The remaining 10 ewes served as controls and received 1,0 cm^3 of the suspension vehicle (6,0% ethanol in 0,9% saline) at intervals of 12 hours. On day 15 post-partum blood samples were obtained from the ewes and the plasma subsequently assayed for prolactin content to check whether the EC effectively suppressed prolactin release.

Fifteen hours after the last EC or saline-ethanol injection, on day 21 post-partum, each of the 20 ewes was injected (intramuscularly) with 50 μg of synthetic gonadotropin-releasing hormone (Gn-RH). At intervals of 15 minutes, for the following six hours, blood samples were obtained from each ewe via a jugular cannula. The blood was collected into heparinized syringes, centrifuged and the plasma stored at – 15°C until assayed for LH by the double-antibody radio immunoassay of Niswender, Reichert, Midgley & Nalbandov (1969). Validation of the assay used in this laboratory has been described by Lishman (1972).

On day 24 post-partum the ewes were laparotomized, and the occurrence of fresh ovulations noted. Thereafter, and until day 60 post-partum observations for oestrus were made by bringing vasectomized rams to the ewes twice daily.

The significance of differences between the quantity of LH released in the EC-treated and control ewes was analysed by the "Student" t-test.

Results

The prolactin concentration of the plasma samples obtained on day 15 post-partum varied from 3,3 to 9,6 ng/cm^3 in the EC-treated ewes, and from 199,0 to 620,4 ng/cm^3 in the controls, indicating that the EC effectively suppressed prolactin release. From the growth of the lambs it appeared that the lack of circulating prolactin did not adversely influence milk production in the ewes.

A surge in the level of LH (> 10 ng/cm^3 plasma) following treatment with Gn-RH was observed in all animals. Three criteria of hormone secretion were used to characterize the individual LH surges measured viz., maximum LH level, duration of the surge, and area under the LH curve, the latter acting as an indication of the total quantity of LH released. The response of the individual EC-treated and control ewes varied greatly and the results are summarized in Table 1. The area under the LH curve in the EC-treated ewes (mean = 246,9 ±37,9 mm^2) tended to be larger, but did not differ significantly from that measured in the controls (208,5 ±66,0 mm^2). Similarly, differences between maximum LH levels, and the duration of the LH surge in the EC-treated and control ewes were not significant. At least one corpus luteum was observed in three of the EC-treated, and four of the control ewes at laparotomy. None of the experimental ewes had exhibited oestrus when observations for oestrus ceased.

Since the quantity of LH released, and the number of corpora lutea observed in the EC-treated ewes did not differ significantly from that measured in the controls, all results obtained in the experiment were pooled. The distribution of the maximum LH levels around their median was then subjected to Fisher's exact test and it was found that a significantly larger ($P < 0,05$) number of EC-treated ewes (8) exhibited maximum LH levels in excess of the median than the number of controls (2).

The age of the ewes, and the exact interval between lambing and Gn-RH administration (varied from 17 to 24 days) did not influence the quantity of LH released in the ewes, or the response of the ovaries to LH stimulation. The mean area under the LH curve in the seven ewes which ovulated following Gn-RH administration (303,8 ± 90,9 mm^2) did not differ significantly from the mean observed in the 13 ewes which failed to ovulate (186,7 ±32,0 mm^2). Noteable differences in the response of the ovaries to LH stimulation were also ob-

Table 1

Changes in mass, LH secretion and ovarian activity in experimental ewes

Treatment	Ewe No.	Ewe mass at lambing (kg)	Mass gain/ loss between day 0 and 21 post-partum	LH secretion in response to Gn-RH			Number of corpora lutea observed on Day 24 post-partum
				Maximum LH level (ng/cm^3)	Duration of LH surge (min)	Area under LH curve (mm^2)	
	2	36,8	0	43,2	218	47,6	0
Control	5	45,4	0,9	85,6	291	175,2	1
	6	39,5	+1,4	180,8	273	327,4	0
(Saline-							
ethanol	8	52,2	−5,0	97,2	248	150,5	0
injections,	11	37,2	−0,9	50,0	136	48,3	0
Day 10–20	13	55,4	−1,8	412,8	340	755,4	1
post-partum)	16	46,3	+1,8	120,8	213	100,0	2
	17	32,7	+0,4	66,4	234	107,5	0
	18	47,2	+0,5	121,2	266	184,6	1
	20	50,8	−0,4	117,2	266	188,2	0
	Mean:	44,4		129,5	248,5	208,5	
	S E	±2,4		±34,0	±17,1	66,0	
Prolactin	3	48,6	−1,4	58,4	218	88,5	0
suppression	4	43,1	−3,6	124,0	270	198,3	0
	7	42,2	+1,4	170,8	265	257,6	1
(EC injections	9	41,8	−0,9	76,8	229	108,5	0
Day 10–20	10	31,8	+0,4	198,4	282	232,5	1
post-partum)	12	30,0	0	189,2	271	312,7	0
	14	52,2	−1,4	154,4	266	242,9	0
	15	42,7	+2,2	193,6	318	421,3	2
	19	48,1	+1,1	213,6	283	447,2	0
	21	32,7	0	132,0	254	159,2	0
	Mean:	41,3		151,1	265,6	246,9	
	S E	±2,4		± 16,7	±8,9	±37,9	

tained in this experiment. Ewe 5 (bodymass 46,3 kg), which ovulated in response to the Gn-RH injection, exhibited a maximum LH level of 85,6 ng/cm^3, and the area measured under the LH curve was 175,2 mm^2. On the other hand, a ewe of similar mass (49, 2 kg) failed to ovulate in response to a maximum LH level of 213,6 ng/cm^3, and a measured area under the curve of 447,2 mm^2.

Variation in the quantity of LH released was too great to allow correlation between ewe mass (day 21 post-partum) and the quantity of LH secreted in response to Gn-RH. However, six of the nine ewes (66,7%) which gained in mass between lambing and day 21 ovulated in response to LH stimulation, whereas ovulation occurred in only one of the eight ewes (12,5%) which lost mass during this period (Table 1).

Discussion

The present study was conducted to determine whether prolactin exerted antigonadal effects during lactation in ewes. An evaluation of the findings is complicated by the marked variation between animals in the quantity of LH released and by the use of a relatively insensitive measure of ovarian responsiveness. Notwithstanding these limitations the finding that the EC-treated ewes tended to exhibit higher peak LH levels than the controls suggests that the inhibition of prolactin secretion, as a means of enhancing LH release in lactating animals, warrants further investigation.

The results obtained in this experiment indicate that the pituitary gland of the lactating ewe is capable of releasing LH in response to the stimulus of Gn-RH as

early as day 17 post-partum. This finding is in contrast to that of Chamley, Findlay, Cumming, Buckmaster & Goding (1974) and Jenkin & Heap (1974), who noted that the pituitary is insensitive to Gn-RH stimulation during the 40 days which follow lambing. On the other hand, Restall & Radford (1974) observed a release of LH on day 12 post-partum in lactating ewes treated with a progestagen prior to administration of releasing hormone, and on day 26 when progestagen priming did not precede the Gn-RH injection. The cause of the variable results obtained in studying pituitary sensitivity to Gn-RH stimulation is not clear. In this context it is interesting to note that beef heifers are capable of releasing LH in response to Gn-RH on day 1 post-partum (Cummins, Cumming, Knight & Lawson, 1975).

Crighton, Scott & Foster (1974) found that the height and duration of the LH peaks induced by Gn-RH in anoestrous ewes are of lower magnitude than those which occur during normal oestrus. Maximum LH levels in the present study varied from 43,2 to 412,8 ng/cm^3, and are considerably higher than the level of 72,5 ng/cm^3 which Restall & Radford (1974) measured in lactating Border Leicester x Merino ewes injected with 50 μg of Gn-RH.

The results presented in Table 1 indicated that factors other than the quantity of LH which probably reaches the ovary, determine the ability of the ewe to ovulate during the early post-partum period. The finding that ovulation occurred more readily in ewes which gained in mass after lambing than in animals which lost mass (Table 1) should be viewed with caution, since individual mass gains were relatively small, and did not exceed 2,2 kg.

The seven ewes which ovulated in response to Gn-RH stimulation on day 21 (Table 1) had not exhibited overt oestrus by day 60 post-partum, indicating that normal cyclicity did not follow the formation of the induced corpus luteum. This phenomenon can be explained on the basis of the finding by Haresign, Foster, Haynes, Crighton & Lamming (1975) that, following administration of Gn-RH to anoestrous ewes, plasma progesterone concentrations either remained basal, or rose to levels lower than those found during the luteal phase of the cycle. This problem clearly requires solution before Gn-RH can be successfully used to induce early reproductive activity in lactating ewes.

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