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Response of seasonally anoestrous ewes to small-dose multiple injections of Gn-RH with and without progesterone pretreatment

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Summary. Four groups, each of 5 seasonally anoestrous ewes, were treated i.v with small doses (75, 125, 250 or 500 ng) of Gn-RH at 2-h intervals for 48 h. A further 15 ewes received 14 days pretreatment with progesterone and then the 250 ng Gn-RH treatment. Gn-RH injections induced an episodic pattern of LH secretion which differed significantly for the doses of Gn-RH used. A preovulatory LH surge occurred in all but 1 of the ewes during the period of Gn-RH treatment. Ovulation occurred in all 15 ewes pretreated with progesterone and in 19/20 ewes treated with Gn-RH alone. Although normal luteal function occurred in all ewes pretreated with progesterone, it was present in only 5 of the 20 ewes treated with Gn-RH alone. Oestrus, as shown by mating, occurred at a mean time of $34{\cdot}7 \pm 2{\cdot}6$ h after the start of Gn-RH treatment only in those ewes receiving progesterone pretreatment. These results indicate that progesterone pretreatment has a marked effect on the ability of small doses of Gn-RH to induce ovulation and normal luteal function in seasonally anoestrous ewes.

Introduction

A single injection of 150 or 300 μg Gn-RH into seasonally anoestrous ewes results in an immediate preovulatory-type LH surge and ovulation, but normal luteal function is completely absent in most ewes (Crighton, Foster, Haresign, Haynes & Lamming, 1973; Haresign, Foster, Haynes, Crighton & Lamming, 1975). The lack of normal luteal function in such animals has been attributed to inadequate follicle development before the induction of ovulation (Haresign & Lamming, 1978).

Evidence from the patterns of gonadotrophin secretion during the follicular phase of the oestrous cycle in the ewe has suggested that the final phases of follicle growth and development may well be under the control of episodic LH secretion (Yuthasastrakosol, Palmer & Howland, 1977; Baird, 1978), and this is supported by the fact that multiple injections of small doses of either LH or Gn-RH can induce ovulation and luteal function in prepubertal lambs (Ryan & Foster, 1980) and seasonally anoestrous ewes (McNeilly, O'Connell & Baird, 1980; McLeod, Haresign & Lamming, 1982). The present experiment was designed to extend the findings of McLeod *et al.* (1982) and to assess whether pretreatment with progesterone would result in a fertile oestrus following Gn-RH treatment.

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Materials and Methods

Animals and management

The 37 seasonally anoestrous Clun Forest ewes (mean liveweight 63·8 ± 1·5 kg), all of which had given birth to lambs in the spring, were used between mid-June and early July 1980. All ewes were housed under conditions of natural daylength and temperature in individual pens and fed a diet consisting of 'indoor' ewe concentrates and hay, with water always available.

Treatments and blood sampling

Ewes were injected with 75 ng (Group 1, Ewes 1–5), 125 ng (Group 2, Ewes 6–10), 250 ng (Group 3, Ewes 11–15), or 500 ng (Group 4, Ewes 16–20) of Gn-RH (Lutal: Fabwerke Hoechst AG, Frankfurt, West Germany) in 2 ml sterile saline (9 g NaCl/l). The injections were given into an indwelling jugular vein catheter (inserted 2 days before treatment) at 2-h intervals for 48 h. A further 5 ewes (Group 5, Ewes 21–25) received the 250 ng Gn-RH treatment following a 14-day period of pretreatment with progesterone. Each ewe received 3 progesterone implants (Sil-Estrus: Abbott Labs, Athens, Greece) containing 375 mg progesterone in a silicone-elastomer matrix; the implants were inserted subcutaneously in the axilla region and removed immediately after the second Gn-RH injection.

Blood samples (2 ml) for LH determination were collected via the catheter from all ewes at 15-min intervals from 12 h before until 12 h after the period of Gn-RH administration (i.e. a total period of 72 h). A further 10 ewes (Group 6, Ewes 26–35) received the same treatment schedule as that used for ewes in Group 5, except that blood samples for LH determination were collected at 2-h intervals around the time of Gn-RH treatment. Two control ewes (Group 7, Ewes 36 and 37) received saline alone, and blood samples for LH determination in these animals were collected at 15-min intervals as for ewes in Groups 1–5.

Blood samples (10 ml) for progesterone determination were collected daily by jugular venepuncture from 3 days before until 24 days after the Gn-RH treatment. In addition those ewes pretreated with progesterone implants had daily blood samples for progesterone analysis collected over the period while the implants were *in situ*.

During the frequent blood sampling period ewes were exposed to a colour-marked entire ram at least once every 4 h and allowed to mate if in oestrus. Laparoscopy was performed under barbiturate anaesthesia 2 days before and 4 days after the end of the frequent blood sampling period to assess ovarian activity.

Progesterone assay

Progesterone concentrations were measured by the radioimmunoassay method of Haresign *et al.* (1975). The assay showed negligible cross-reaction with other steroids and the limit of sensitivity was 37 pg/tube (0·1 ng/ml plasma). Within this study the inter- and intra-assay coefficients of variation were both $< 10\%$ and the mean extraction efficiency was 67·3 ± 3·2%.

LH assay

Plasma LH concentrations were determined by the specific double-antibody radioimmunoassay of Foster & Crighton (1974) as modified by McLeod *et al.* (1982). The limit of sensitivity of the assay was 0·3 ng NIH-LH-S17 equiv./ml plasma and the inter- and intra-assay coefficients of variation were both $< 10\%$. An LH episode was defined by the criteria of McLeod *et al.* (1982)

Statistical analysis was by Student's t test or χ^2 analysis as appropriate.

Results

Ovarian activity and oestrus

Laparoscopic examination 2 days before Gn-RH injections showed that all ewes had regressed reproductive tracts typical of the seasonally anoestrous ewe, with little or no evidence of follicular development. At the second laparoscopy 4 days after the end of Gn-RH treatment all but one of the ewes (Ewe 14, Group 3) treated with Gn-RH had ovulated and the resultant corpora lutea looked macroscopically normal. Neither of the two control ewes had ovulated. The mean (±s.e.m.) ovulation rates for the different Gn-RH treatments are given in Table 1. These were not significantly different from each other.

Table 1. Ovulation rate, characteristics of LH release and the incidence of luteal function in seasonally anoestrous ewes (5/group, Groups 1–5; 10 in Group 6; 2 in Group 7) treated with small-dose multiple injections of Gn-RH at 2-h intervals for 48 h

			Preovulatory LH surge			Incidence of luteal function	
Group	Ovulation rate	Mean maximum conc. of the first 8 Gn-RH-induced LH episodes (ng/ml)	Time from 1st Gn-RH injection to onset of surge (h)	Duration of surge (h)	Maximum conc. (ng/ml)	Normal	Delayed (or abnormal*)
1	1·20 ± 0·20	4·77 ± 0·44^a	21·80 ± 6·99^c	16·20 ± 0·88	146·5 ± 15·4	1	1 (+1*)
2	1·40 ± 0·24	4·93 ± 0·38^a	22·90 ± 5·59^c	16·50 ± 0·76	176·0 ± 22·7	2	1
3	1·80 ± 0·37	7·74 ± 0·74^b	17·70 ± 0·70^c	14·50 ± 0·80	213·6 ± 18·3	1	
4	1·50 ± 0·29†	7·40 ± 0·55^b	20·40 ± 1·48^c	13·75 ± 1·06	174·8 ± 13·5	1	
5	1·73 ± 0·15	6·21 ± 0·47^b	33·89 ± 1·75‡^{d}	13·77 ± 0·70‡	166·0 ± 19·3‡	5	
6		—			—	10	
7	—	—	—		—		

* One ewe showed only a transient rise in progesterone; see text.

† Excluding one ewe which failed to ovulate.

‡ Excluding one ewe in which no LH peak was observed during the frequent sampling period; see text.

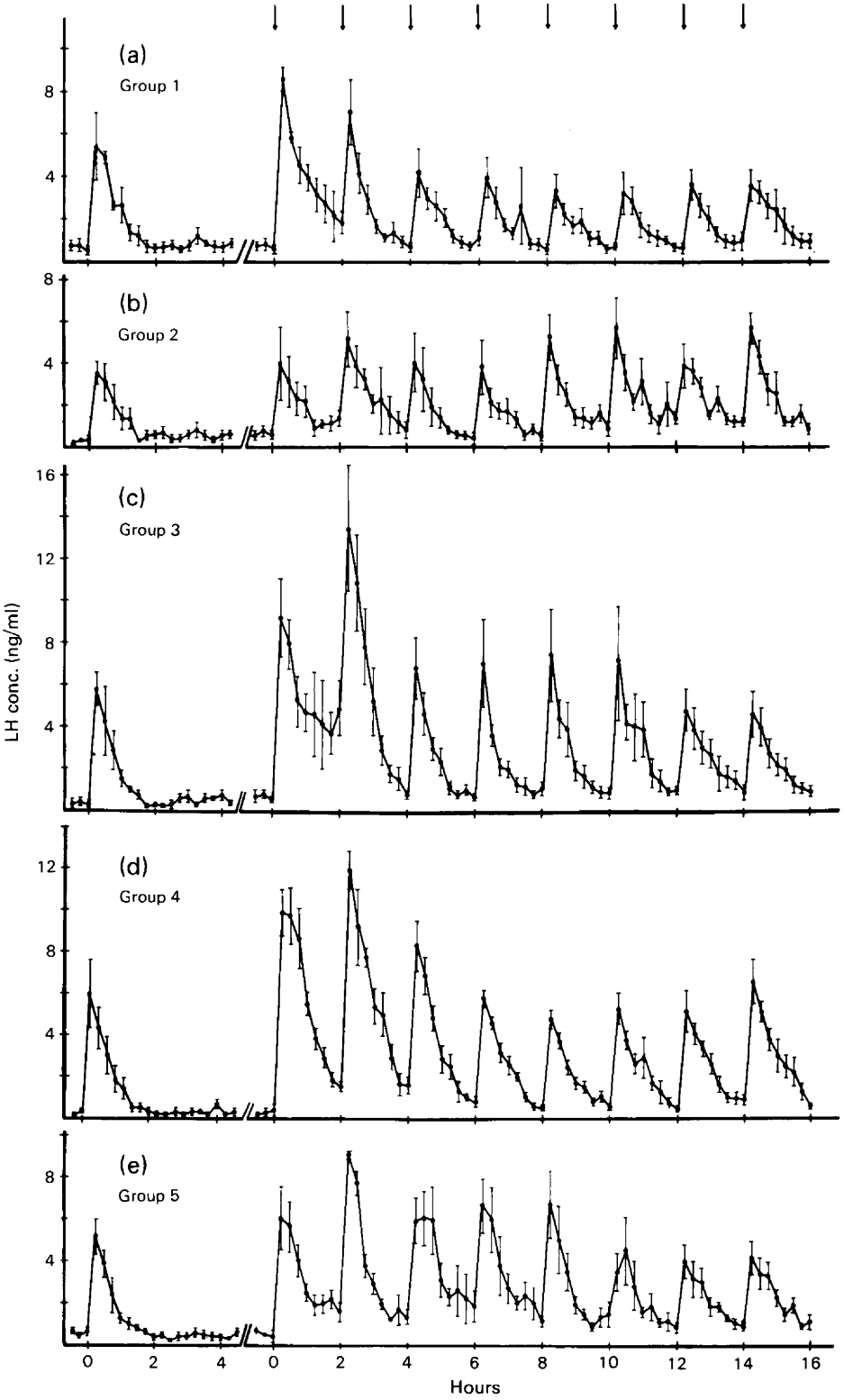
Within columns, means with different superscripts are significantly different: *a* versus *b*, $P < 0{\cdot}005$; *c* versus *d*, $P < 0{\cdot}001$.

None of the ewes treated with saline (Group 7) or Gn-RH alone (Groups 1–4) showed oestrus, whereas 14 of the 15 ewes pretreated with progesterone (Groups 5 and 6) came into oestrus at a mean time of 34·7 ± 2·6 h (range 24–46 h) after the start of treatment. In the remaining ewe (Ewe 21, Group 5) oestrus occurred between 60 and 72 h after the start of treatment and was not accurately timed.

LH concentrations

The mean LH profiles before and over the first 16 h of the Gn-RH treatment period for ewes in Groups 1–5 are shown in Text-fig. 1.

Over the 12 h pretreatment period plasma LH concentrations remained basal for most of the time, and included a mean of 1·5 ± 0·2 LH episodes, with no significant differences between treatment groups. Each Gn-RH injection induced an immediate but transient rise in plasma LH concentrations. The response to the initial 2 or 3 injections tended to be greater than that to subsequent injections, particularly at the higher Gn-RH dose levels. In addition, the mean pituitary response (when measured in terms of the mean maximum LH concentration attained in the Gn-RH-induced episodes) to the first 8 injections of Gn-RH was significantly ($P < 0{\cdot}05$) greater at the higher dose levels (Table 1). Progesterone pretreatment (Group 5) had no significant effect on pituitary response to Gn-RH simulation. Blood samples for LH analysis from ewes in Group 6 were not collected frequently enough to monitor the pattern of LH concentrations in response to Gn-RH treatment.



Text-fig. 1. Mean (±s.e.m.) plasma LH concentrations before and for the first 16 h after the start of i.v. injections of Gn-RH into seasonally anoestrous ewes (Groups 1–5) at 2-h intervals for 48 h. The progesterone implants were removed immediately after the second Gn-RH injection from ewes in Group 5. The arrows indicate the times of Gn-RH injections. The data from the preinjection period have been normalized about a natural episode.

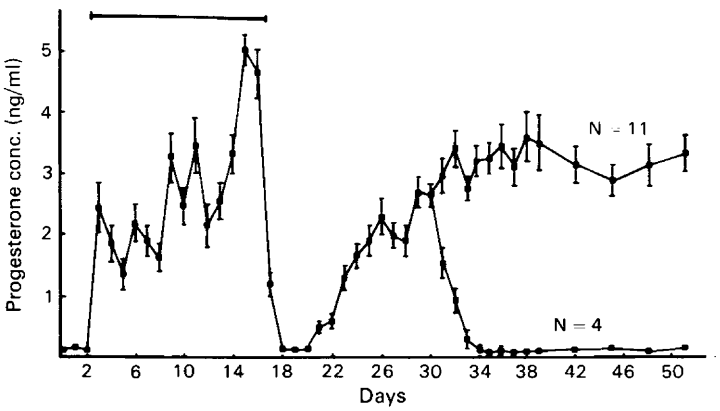
A preovulatory LH peak occurred in all but one of the ewes (No. 21) treated with Gn-RH during the frequent blood sampling period, and it was this ewe which was particularly late showing oestrus. In terms of the preovulatory LH surge the only significant difference between groups (Table 1) was in the interval from the start of Gn-RH injections to the onset of the preovulatory LH surge, this being significantly longer ($P < 0{\cdot}001$) for those ewes pretreated with progesterone.

In the two control ewes injected with saline alone, natural LH episodes occurred at irregular intervals with a mean rate of $1{\cdot}75 \pm 0{\cdot}08$ per 12 h throughout the entire frequent blood sampling period.

Progesterone concentrations

The numbers of ewes in each treatment group showing normal luteal function are shown in Table 1. Normal luteal function was defined as an elevation in plasma progesterone concentrations for at least 9 days, starting within 4 days of the preovulatory LH surge and reaching a maximum concentration of >1·5 ng/ml. Only 5 of the 20 ewes treated with Gn-RH alone showed normal luteal function compared to all 15 ewes pretreated with progesterone ($P < 0{\cdot}001$). One ewe in Group 1 and one ewe in Group 2 showed a significant rise in progesterone concentrations which remained elevated for 9 and 13 days respectively, but this rise did not start until 8 and 10 days respectively after the preovulatory LH surge. In another ewe in Group 1 there was a small rise in plasma progesterone concentrations at the expected time but this was only of 4 days duration and did not exceed 0·8 ng/ml. The mean plasma progesterone concentrations for the 15 ewes pretreated with progesterone implants (Groups 5 and 6) are shown in Text-fig. 2. Progesterone concentrations rose approximately 3 days after treatment with Gn-RH. In 4 of the ewes it fell about 10–12 days later, a pattern typical of the normally cyclic ewe. In the remaining 11 ewes the concentrations remained elevated until the end of the sampling period, indicative of pregnancy.

When all treatment groups were considered collectively, the mean interval from the start of Gn-RH treatment until the onset of the preovulatory LH peak was $35{\cdot}89 \pm 1{\cdot}45$ h (range 26–40 h, N = 19) in those ewes which had normal luteal function, but only $22{\cdot}00 \pm 1{\cdot}07$ h (range 18–30 h, N = 12) for those ewes which had no luteal function ($P < 0{\cdot}001$). In the two ewes with delayed luteal function the preovulatory LH surge began within the first 6 h of Gn-RH treatment, and in the ewe showing only a transient rise in progesterone concentrations the LH surge commenced 40 h after the first Gn-RH injection.



Text-fig. 2. Mean (±s.e.m.) plasma progesterone concentrations in 15 seasonally anoestrous ewes receiving 250 ng Gn-RH/injection at 2-h intervals for 48 h after a 14-day pretreatment period with progesterone implants (——) which were removed immediately after the second Gn-RH injection.

Discussion

The pattern of episodic LH secretion in control ewes and in treated ewes before the first Gn-RH injection is consistent with that previously reported to occur during seasonal anoestrus (Scaramuzzi & Baird, 1977; McLeod *et al.*, 1982). Treatment with the doses of Gn-RH used in this study immediately increased the episode frequency to 1 per 2 h, and this rate continued until the time of the preovulatory LH surge. The fact that the induced LH episodes continued to show a similar amplitude up to the onset of the preovulatory LH surge indicates that, with low doses of Gn-RH, the pituitary does not become refractory to repeated stimulation as it does with much higher doses (Crighton, Scott & Foster, 1974; Crighton, Foster, Haresign & Scott, 1975), which is consistent with earlier observations (McLeod *et al.*, 1982). Moreover, these data suggest that the phenomenon of "down-regulation" reported by others (see Knobil, 1980) may well be the result of using too high a dose level of Gn-RH or one of its agonists.

The fact that oestrus occurred concomitantly with the preovulatory LH surge in all of the ewes pretreated with progesterone supports the suggestion of McLeod *et al.* (1982), that the preovulatory LH surge after multiple injections of low doses of Gn-RH is due to the positive feedback action of oestradiol rather than being a direct result of the Gn-RH injections. These results suggest that the final stages of follicular development in the ewe are under the direct control of episodic LH secretion, supporting the data of McNeilly *et al.* (1980).

The low incidence of luteal function in ewes treated with Gn-RH alone (5 out of 20) would at first sight seem to be at variance with the results of McLeod *et al.* (1982). However, the previous animals received Gn-RH injections for 8 days and 4/6 of these had two preovulatory LH surges, the second occurring on Day 6 of treatment some 4–5 days after the first. In these particular animals the rise in progesterone concentrations was similarly delayed. Blood samples were not taken over a sufficiently long period of time to establish whether the 2 ewes in the current trial with delayed luteal function produced a second LH peak. Although they had ovulated when examined at laparoscopy 4 days after Gn-RH treatment, both also had at least two large follicles of near preovulatory size at that time, and may have ovulated again. Both of these ewes produced an LH surge within the first 6 h of treatment with Gn-RH, whereas none of the other ewes showed a preovulatory peak <18 h after the start of Gn-RH injections, suggesting that there was inadequate follicular development in these 2 animals before the follicle was exposed to preovulatory concentrations of LH.

Although there was a significant dose-response of the pituitary to Gn-RH stimulation in those ewes treated with Gn-RH alone this did not appear to be related to the presence or absence of luteal function in these animals. However, there was a marked difference in the incidence of normal luteal function between the ewes treated with Gn-RH alone and those pretreated with progesterone. The reasons for this are not immediately clear, but may well be associated with the significantly later preovulatory LH surge in these animals. Indeed, when compared over all treatments, those ewes showing normal luteal function had a very much later preovulatory LH surge, and therefore the ovarian follicles of these animals were exposed to episodic LH secretions for longer than those not producing a functionally normal corpus luteum. Hammond (1944), Robinson (1950) and Matton, Bherer & Dufour (1977) have all reported continual phases of growth and atresia of follicles during seasonal anoestrus, suggesting that the responses obtained may reflect differences in the stage of follicular development when Gn-RH treatment began. It would appear from these data that the follicle requires a prolonged period of exposure to episodic LH secretion if it is to develop into a functionally normal corpus luteum after ovulation. Moreover, it seems that the ability to respond to episodic LH secretion by developing the capacity to secrete oestrogen can occur asynchronously with the development of the capacity to luteinize properly.

It is not clear whether the effect of progesterone in this respect is a direct one at the ovarian level or an indirect one acting via gonadotrophin secretion from the hypothalamic-pituitary unit.

The insertion of progesterone implants into seasonally anoestrous ewes reduces the frequency of endogenous LH episodes (B. R. Friman & W. Haresign, unpublished data) and this may have been sufficient to arrest follicular development to such an extent that, when progesterone is removed from the system, the follicles are all at an appropriate stage of development to respond fully to the Gn-RH-induced LH episodes.

Although no control ewes treated with progesterone implants alone were included in this trial, previous work has shown that the removal of such implants from anoestrous ewes does not result in the induction of oestrus, ovulation or the pattern of LH secretion observed in the Gn-RH-treated ewes primed with progesterone in the current experiments (B. J. McLeod & W. Haresign, unpublished data).

These data clearly demonstrate that seasonal anoestrus in the ewe is characterized by an inadequate pattern of episodic LH secretion, and that correction of this by repeated injections of Gn-RH in progesterone-primed ewes can result in the induction of a fertile oestrus. Similar treatment regimens have also been shown to induce ovulation and normal luteal function during post-partum anoestrus in beef cows (Riley, Peters & Lamming, 1981), in prepubertal monkeys (Wildt, Marshall & Knobil, 1980) and hypogonadotrophic women (Crowley & McArthur, 1980; Leyendecker, Wildt & Hansmann, 1980). In addition, these data lend support to the suggestion of Haresign & Lamming (1978) that the absence of normal luteal function in seasonally anoestrous ewes treated with a large amount of Gn-RH alone is due to inadequate follicular development before the induction of ovulation.

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