

The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of Gn-RH

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Summary. Seasonally anoestrous ewes were injected i.v. with 250, 500 or 1000 ng Gn-RH at 2-h intervals for 8 days (2 sheep/treatment). Each injection of 250 or 500 ng Gn-RH resulted in a transient rise in plasma LH concentrations. Treatment with 1000 ng Gn-RH per injection resulted in a more sustained rise in plasma LH concentrations in 1 of 2 sheep during the early part of the treatment period. A preovulatory-type LH peak occurred 17–48 h after the start of treatment in all ewes, with a second preovulatory-type peak 106–133 h later in those ewes receiving 500 or 1000 ng Gn-RH per injection. Ovulation, with subsequent normal luteal function, occurred in all sheep. However, the rise in plasma progesterone concentrations appeared to be delayed in those ewes treated with 500 or 1000 ng Gn-RH compared to ewes treated with 250 ng Gn-RH. These data suggest that the absence of ovulation during seasonal anoestrus is due to an inadequate pattern of episodic LH secretion.

Introduction

Administration of a single injection of 150 or 300 µg synthetic Gn-RH induced a preovulatory-type LH surge and ovulation in the majority of seasonally anoestrous ewes, even though the LH surge was only about 25% of the magnitude of the natural peak at oestrus (Foster & Crighton, 1973, 1974; Crighton, Foster, Haresign & Scott, 1975). However, luteal function, as assessed by peripheral plasma progesterone concentrations, was completely absent in most animals (Crighton, Foster, Haresign, Haynes & Lamming, 1973; Haresign, Foster, Haynes, Crighton & Lamming, 1975). Administration of the same total dose of Gn-RH as a series of 5 injections (Crighton *et al.*, 1975) or following pre-treatment with oestradiol benzoate (Haresign & Lamming, 1978) resulted in a much greater induced preovulatory-type LH release, but did not increase the incidence of normal luteal function. Ovulation with subsequent luteal function was, however, produced by treating ewes with PMSG 24 h before injection of Gn-RH, prompting the suggestion that the lack of luteal function in ewes treated with Gn-RH alone was due to inadequate follicular development before the induction of ovulation (Haresign & Lamming, 1978).

The natural pattern of LH secretion in sheep is episodic in nature, basal levels of 0.1–2.0 ng/ml being interspersed with small short-lived episodes, each of 5–15 ng/ml and lasting about 30 min. During the luteal phase of the oestrous cycle and during seasonal anoestrus, these LH episodes occur irregularly at 3 to 12-h intervals (Baird, 1978; Baird, Swanston & Scaramuzzi, 1976; Scaramuzzi & Baird, 1977; Yuthasastrakosol, Palmer & Howland, 1977; B. R. Friman &

W. Haresign, unpublished data), but following regression of the corpus luteum their frequency increases until they occur at the rate of about one per 1–2 h immediately before the preovulatory LH peak (Yuthasastrakosol *et al.*, 1977; Baird, 1978). Each LH episode is followed by an increase in ovarian vein levels of oestradiol and this, together with the parallel increases in basal secretion of both LH and oestradiol during the follicular phase of the cycle, has led to the suggestion that the final stages of follicle development may be controlled by tonic LH secretion (Baird, 1978).

The purpose of the present study was to investigate whether small doses of Gn-RH, administered at 2-h intervals in an attempt to mimic the pattern of the LH secretion which occurs during the follicular phase of the oestrous cycle, were capable of inducing follicle development, ovulation and normal luteal function in seasonally anoestrous ewes. The doses were selected on the basis that they would induce small LH surges of 10–15 ng/ml plasma rather than the preovulatory-type LH peaks (60–200 ng/ml) which result from injections of 150 or 300 µg Gn-RH.

Materials and Methods

Animals and experimental design

Eight 5-year-old seasonally anoestrous Clun Forest ewes with an average liveweight of 50.5 kg were treated over an 8-day period in early August 1979. All ewes were housed in individual pens under conditions of natural daylength and temperature, and fed a diet of 'indoor' ewe concentrates and hay, with water always available. Injections of 250, 500 or 1000 ng synthetic Gn-RH (Lutal: Fabwerke Hoechst AG, Frankfurt, West Germany) in 2 ml sterile saline (9 g NaCl/l) were each given to 2 ewes via an indwelling jugular vein catheter at 2-h intervals for an 8-day treatment period. The 2 control ewes received saline alone.

Blood samples (10 ml) for progesterone determination were collected daily by jugular venepuncture from 2 days before until 20 days after treatment. Blood samples (2 ml) for LH determination were collected via the indwelling catheter at 15-min intervals from 12 h before until 24 h after the first Gn-RH injection. A further 24-h period of 15-min blood sampling was carried out on Day 4 of the treatment. At other times during the treatment period blood samples for LH determination were taken at 2-h intervals immediately before the Gn-RH injection.

All ewes underwent laparoscopy to assess ovarian activity 2 days before and 4 days after the period of hormone treatment.

Progesterone assay

Plasma progesterone concentrations were determined by the radioimmunoassay method of Haresign *et al.* (1975). The assay showed negligible cross-reaction with other steroids and, within this study, the limit of sensitivity of the assay was 37 pg/tube (0.1 ng/ml), the inter- and intra-assay coefficients of variation were 9.6 and 4.6% respectively, and the mean extraction efficiency was $71.8 \pm 3.4\%$.

LH assay

Plasma LH concentrations were measured using the specific double-antibody radioimmunoassay technique described by Foster & Crighton (1974) with the following modifications. The volumes of plasma and diluent used were 100 µl and 150 µl respectively, and the first antibody was used at an initial working dilution of 1:105 000. Bovine serum albumin (1 g/l) was substituted for egg albumin in the assay buffer to overcome problems with precipitation, and incubation with the second antibody was increased from 24 and 48 h. These procedures changed

the limit of sensitivity of the assay to 0.3 ng NIH-LH-S17 equiv./ml. The inter- and intra-assay coefficients of variation within this study were 11.1% and 9.6% respectively.

Results

Ovarian activity

Laparoscopic observations 2 days before treatment showed that all ewes had reproductive tracts typical of those found during seasonal anoestrus, with little or no evidence of follicular development. At the second laparoscopy 4 days after the end of the treatment period, all Gn-RH-treated ewes had ovulated, and the resultant corpora lutea appeared macroscopically normal. The individual ovulation rates are shown in Table 1. Neither of the control ewes, receiving saline alone, showed any evidence of ovarian development.

Table 1. Time of preovulatory LH peaks and ovulation rates in seasonally anoestrous ewes treated with injections of Gn-RH every 2 h

Ewe	Gn-RH dose (ng/inj)	Interval from 1st injection to onset of LH peak (h)	Duration of LH peak (h)	Ovulation rate
31	250	22	14	2
32	250	32	14	2
33	500	32	12	3
		152	16	
34	500	40	8	3
		148	10	
35	1000	48	10	2
		154	12	
36	1000	17	10	2
		150	10	
37	0	—	—	—
38	0	—	—	—

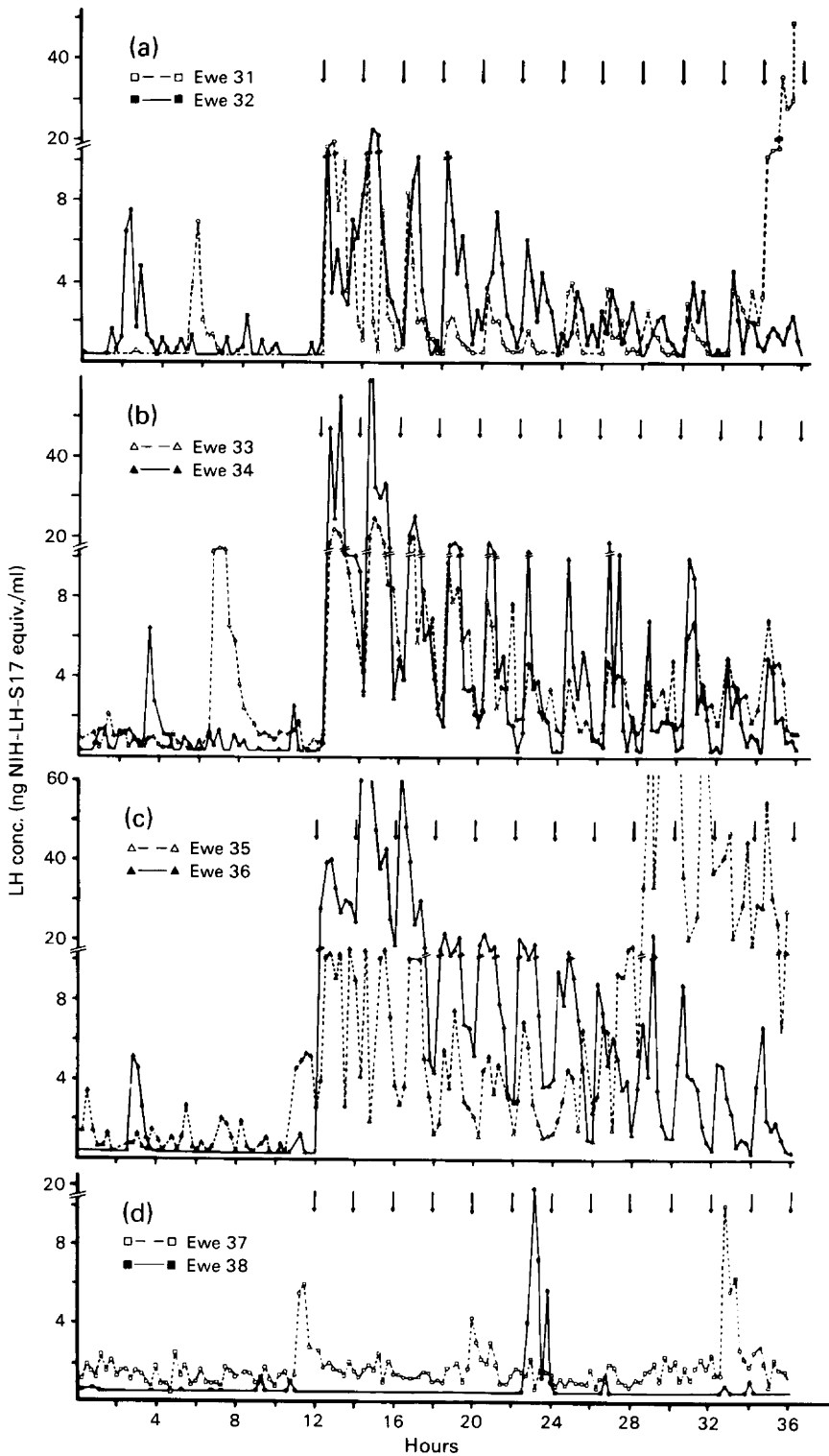
LH concentrations

A rise in LH concentrations was defined as an episode if (i) there was an increase of at least 50% above the preceding baseline value, (ii) there were at least two points between the peak value and the succeeding trough or baseline, and (iii) the rate of decline in concentrations after the peak was no greater than that allowed by the half-life of the hormone. As shown in Text-fig. 1, during the 12 h before the first Gn-RH injection, plasma LH concentrations were basal (0.5–2.0 ng/ml) for most of the time and included no more than a single, small LH episode (mean maximum concentration 6.8 ± 0.7 ng/ml, range 5.2–14.7 ng/ml). Each Gn-RH injection induced an immediate, but transient, rise in plasma LH concentrations and, in all Gn-RH-treated ewes, this response was greater to the initial 3 or 4 than to subsequent injections.

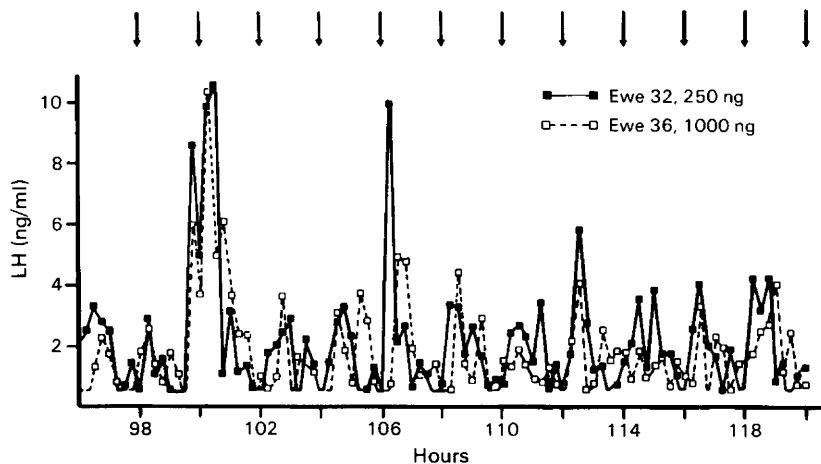
The 24-h period of 15-min blood collection carried out on Day 4 of the treatment period indicated a pattern of plasma LH concentrations in response to Gn-RH injections similar to that found during the first 24 h of treatment, except that, even at the 1000 ng dose level, LH concentrations returned to basal values between injections. Typical examples are shown in Text-fig. 2.

All Gn-RH-treated ewes showed a preovulatory-type LH peak (maximum concentration 68–145 ng/ml and of 8–16 h duration) 17–48 h after the start of treatment, with a further preovulatory-type LH peak 106–133 h later in those ewes receiving 500 or 1000 ng Gn-RH per injection (Table 1).

With the exception of occasional episodes, the LH concentrations in the control ewes remained basal throughout the entire treatment period (Text-fig. 1d).



Text-fig. 1. Plasma LH concentrations in ewes in response to injections every 2 h of (a) 250 ng, (b) 500 ng or (c) 1000 ng Gn-RH per injection or (d) saline only. Blood samples were collected at 15-min intervals from 12 h before until 24 h after the start of treatment. The time of each Gn-RH injection is indicated by arrows.

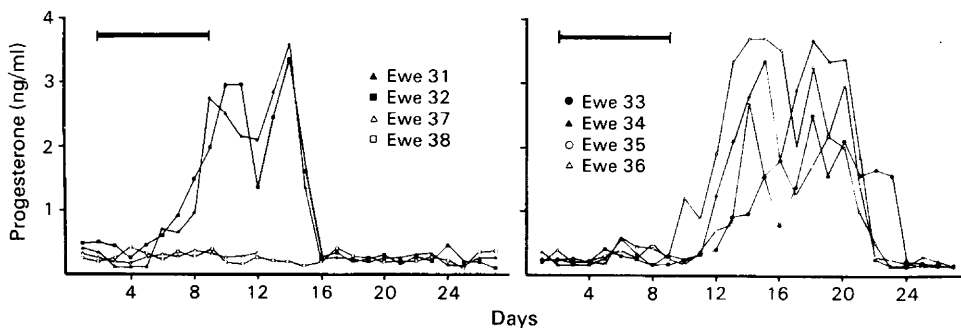


Text-fig. 2. Plasma LH concentrations in response to injections of Gn-RH every 2 h on Day 4 of the treatment period. Blood samples were collected at 15-min intervals and the arrows indicate the time of each Gn-RH injection.

Progesterone concentrations

In all Gn-RH-treated ewes the post-ovulatory pattern of plasma progesterone concentrations was similar to that found during the normal oestrous cycle. Values rose from basal (<0.5 ng/ml) to >2.5 ng/ml (maximum concentrations 3.3 ± 0.2 ng/ml), and remained elevated for 10–12 days (Text-fig. 3). However, this rise started 3–4 days later in ewes treated with 500 or 1000 ng Gn-RH per injection than it did in ewes treated with 250 ng per injection.

Plasma progesterone concentrations remained basal throughout the sampling period in ewes treated with saline alone (Text-fig. 3).



Text-fig. 3. Peripheral plasma progesterone concentrations in saline-treated control ewes (Ewes 37 and 38) or ewes treated with 250 ng (Ewes 31 and 32), 500 ng (Ewes 33 and 34) or 1000 ng (Ewes 35 and 36) of Gn-RH at 2-h intervals during seasonal anoestrus. The treatment period is indicated by the horizontal bar.

Discussion

The ability of single i.v. injections of 150 or 300 μ g Gn-RH to induce ovulation in seasonally anoestrous ewes has been widely reported. However, following this type of treatment the induced ovulation rate never exceeded one, even in breeds that consistently produce multiple ovulations at natural oestrus (Crighton *et al.*, 1973; Haresign *et al.*, 1975). In contrast, repeated injections

of low doses of Gn-RH as used in this present study induced multiple ovulations and normal luteal function in all 6 treated ewes.

A single injection of 150 or 300 µg Gn-RH to seasonally anoestrous ewes results in an immediate preovulatory-type LH surge which is similar to, but significantly smaller than, that at a natural oestrus (Crighton, Scott & Foster, 1974). In contrast, the levels of Gn-RH used in the present study were insufficient to induce such a surge at the two lower dose levels used, but rather resulted in an extended period of episodic LH secretion. The preovulatory-type LH peaks which did occur 17–48 h after the start of treatment did not appear to be directly related to the injections of Gn-RH but, more likely, were the result of endogenous endocrine responses to this period of elevated gonadotrophin output. Although plasma oestrogen concentrations were not measured in this experiment it is probable that the preovulatory LH peaks resulted from the positive feedback effects of high levels of oestrogen on the hypothalamo-pituitary axis (Karsch, Legan, Ryan & Foster, 1977), particularly since similar treatments result in oestrus in seasonally anoestrous ewes which are pretreated with progesterone (B. J. McLeod & W. Haresign, unpublished data).

The presence of a second preovulatory-type LH surge in 4 of the ewes in this trial suggests that, in the absence of a rise in progesterone concentrations resulting from the first preovulatory LH surge in these ewes, the ovarian–hypothalamo–pituitary axis continued to respond with a periodicity of 106–133 h (Table 1). The negative feedback effects of the earlier elevation in plasma progesterone in response to the first LH peak in Ewes 31 and 32 would account for the absence of a second preovulatory peak in these animals as shown by Karsch *et al.* (1977).

It has been reported that plasma progesterone concentrations rise approximately 4 days after the preovulatory LH peak in cyclic ewes (Thorburn, Bassett & Smith, 1969; Yuthasastrakosol *et al.*, 1973). This would suggest that the increased progesterone concentrations noted in this experiment arose from corpora lutea resulting from the first preovulatory LH peak in Ewes 31 and 32, and from the second pre-ovulatory peak in Ewes 33–36. The reasons for this are not clear although it is possibly related to the different dose-dependent patterns of episodic LH secretion before the first preovulatory-type LH surge. It is notable that the induced LH episodes on Day 4 of treatment were very similar and not apparently related to dose. It is not clear whether ovulation occurred in response to the first preovulatory LH peak in Ewes 33–36 because no laparoscopic examination was made until 4 days after the end of the treatment period, and at that time the corpora lutea of all 6 treated ewes were very similar in appearance.

The high incidence of multiple ovulation and the endogenous initiation of preovulatory LH peaks in treated ewes support the suggestion of a possible direct role for tonic LH secretion in stimulating the final stages of follicle development and oestrogen synthesis in the ewe. A similar close relationship between episodic LH secretion and reproductive activity has been shown by the ability of repeated injections of small doses of LH to induce ovulation in prepubertal ewe lambs (Ryan & Foster, 1980). The role of changes in FSH concentrations in the responses noted cannot be ignored. Unfortunately, FSH was not measured in this experiment but similar work in the ram has shown that FSH is very slow to respond to multiple injections of low doses of Gn-RH (Lincoln, 1979). This would suggest that the induction of ovulation and luteal function in at least the 2 ewes treated with 250 ng Gn-RH per injection was primarily due to induced changes in LH rather than FSH secretion.

This hormone treatment regimen has also been shown to induce reproductive activity during post-partum anoestrus in beef cows (Riley, Peters & Lamming, 1981), to induce menstrual cycles in prepubertal monkey (Wildt, Marshall & Knobil, 1980) and hypogonadotrophic women (Crowley & McArthur, 1980; Leyendecker, Wildt & Hansmann, 1980) and to restore sexual activity to seasonally anoestrous rams (Lincoln, 1979). In addition, these data suggest that the absence of ovulation in seasonally anoestrous ewes is due to an inadequate pattern of episodic LH secretion. Correction of this inadequacy with small-dose multiple injections of Gn-RH resulted in ovulation and normal luteal function in all ewes.

We thank Hoechst Pharmaceuticals for financial support and supplies of synthetic Gn-RH; The National Institutes of Health for standard LH; and Dr B. J. A. Furr for the progesterone antiserum.

References

- Baird, D.T.** (1978) Pulsatile secretion of LH and ovarian estradiol during the follicular phase of the sheep oestrous cycle. *Biol. Reprod.* **18**, 359–364.
- Baird, D.T., Swanston, I. & Scaramuzzi, R.J.** (1976) Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the oestrous cycle. *Endocrinology* **98**, 1490–1496.
- Crighton, D.B., Foster, J.P., Haresign, W., Haynes, N.B. & Lamming, G.E.** (1973) The effects of a synthetic preparation of gonadotrophin releasing factor on pituitary and ovarian function in anoestrous ewes. *J. Physiol., Lond.* **231**, 98P–99P, Abstr.
- Crighton, D.B., Scott, S.A. & Foster, J.P.** (1974) An attempt to simulate by injection of luteinizing hormone releasing hormone in the anoestrous sheep, the pattern of release observed at oestrus and the effects of this on luteinizing hormone release. *J. Endocr.* **61**, lxiii–lxiv, Abstr.
- Crighton, D.B., Foster, J.P., Haresign, W. & Scott, S.A.** (1975) Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle. *J. Reprod. Fert.* **44**, 121–124.
- Crowley, W.F. & McArthur, J.W.** (1980) Simulation of the normal menstrual cycle in Kallman's Syndrome by pulsatile administration of luteinizing hormone-releasing hormone (LH-RH). *J. clin. Endocr. Metab.* **51**, 173–175.
- Foster, J.P. & Crighton, D.B.** (1973) Preliminary observations on the administration of a synthetic preparation of gonadotrophin-releasing factor to cyclic and anoestrous ewes. *J. Endocr.* **57**, xxv, Abstr.
- Foster, J.P. & Crighton, D.B.** (1974) Luteinizing hormone (LH) release after single injections of synthetic LH-releasing hormone (LH-RH) in the ewe at three different reproductive stages and comparison with natural LH release at oestrus. *Theriogenology* **2**, 87–100.
- Haresign, W. & Lamming, G.E.** (1978) Comparison of LH release and luteal function in cyclic and LH-RH treated anoestrous ewes pre-treated with PMSG or oestrogen. *J. Reprod. Fert.* **52**, 349–353.
- Haresign, W., Foster, J.P., Haynes, N.B., Crighton, D.B. & Lamming, G.E.** (1975) Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod. Fert.* **43**, 269–279.
- Karsch, F.J., Legan, S.J., Ryan, K.D. & Foster, D.L.** (1977) The feedback effects of ovarian steroids on gonadotrophin secretion. In *Control of Ovulation*, pp. 29–48. Eds D. B. Crighton, G. R. Foxcroft, N. B. Haynes & G. E. Lamming. Butterworths, London.
- Leyendecker, G., Wildt, L. & Hansmann, M.** (1980) Pregnancies following chronic intermittent (pulsatile) administration of Gn-RH by means of a portable pump ("Zyklomat")—a new approach to the treatment of infertility in hypothalamic amenorrhea. *J. clin. Endocr. Metab.* **51**, 1214–1216.
- Lincoln, G.A.** (1979) Use of a pulsed infusion of luteinizing hormone-releasing hormone to mimic seasonally induced endocrine changes in the ram. *J. Endocr.* **83**, 251–260.
- Riley, G.M., Peters, A.R. & Lamming, G.E.** (1981) Induction of pulsatile LH release and ovulation in post-partum acyclic beef cows by repeated small doses of Gn-RH. *J. Reprod. Fert.* **63**, 559–565.
- Ryan, K.D. & Foster, D.L.** (1980) Neuroendocrine mechanisms involved in the onset of puberty in the female: concepts derived from the lamb. *Fedn Proc. Fedn Am. Socs exp. Biol.* **39**, 2372–2377.
- Scaramuzzi, R.J. & Baird, D.T.** (1977) Pulsatile release of luteinizing hormone and the secretion of ovarian steroids in sheep during anestrus. *Endocrinology* **101**, 1801–1806.
- Thorburn, G.D., Bassett, J.M. & Smith, I.D.** (1969) Progesterone concentration in plasma of the sheep during the oestrous cycle. *J. Endocr.* **45**, 459–469.
- Wildt, L., Marshall, G. & Knobil, E.** (1980) Experimental induction of puberty in the infantile female Rhesus monkey. *Science, N.Y.* **207**, 1373–1375.
- Yuthasastrakosol, P., Palmer, W.M. & Howland, B.E.** (1973) Hormone levels in the anoestrous and cycling ewe. *J. Anim. Sci.* **37**, 334.
- Yuthasastrakosol, P., Palmer, W.M. & Howland, B.E.** (1977) Release of LH in anoestrous and cyclic ewes. *J. Reprod. Fert.* **50**, 319–321.

Received 17 April 1981