Changes in LH pulse frequency and amplitude in intact mares during the transition into the breeding season

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Summary. Two groups of mares were exposed to an abrupt, artificial increase or a natural increase in daylength. In both groups, mean LH pulse frequency increased with time of year and was accompanied by a reciprocal decrease in LH pulse amplitude. A non-pulsatile pattern of LH secretion was observed in some mares sampled close to the day of ovulation. Maximum mean LH pulse frequency and the onset of the breeding season occurred earlier in those mares exposed to an abrupt artificial increase in daylength. In blood samples collected frequently, mean serum LH concentrations increased in relation to time of year. However, during 60 days before ovulation, when LH pulse frequency increased, mean daily serum LH values only increased on Day –3 before ovulation. The magnitude of the periovulatory LH rise was greater before the second than the first ovulation of the breeding season. These results support the hypothesis that, in the mare, a photoperiod-induced seasonal alteration in LH pulse frequency and/or amplitude may play a role in the onset of the breeding season.

Introduction

In many mammalian species the secretion of luteinizing hormone (LH) is pulsatile (Dierschke *et al.*, 1970; Yen *et al.*, 1974; Baird, 1978; Rahe *et al.*, 1980; Gallo, 1981). In the mare, the secretion of LH is also pulsatile (Fitzgerald *et al.*, 1983, 1985) although the detection of pulsatile LH secretion in jugular venous blood appears limited to those experimental or physiological conditions when the frequency of LH pulses might be anticipated to be low. For example, in ovariectomized mares a pulsatile pattern of LH secretion can be identified during the spring months when serum LH concentrations are low or gradually rising in association with an increase in daylength. However, during the summer months, when serum LH values are maximally elevated in this long-day breeder, discrete LH pulses can no longer be demonstrated (Garcia *et al.*, 1979; Fitzgerald *et al.*, 1983). Similarly, in intact mares a pulsatile pattern of LH secretion is evident during the luteal phase of the oestrous cycle (Fitzgerald *et al.*, 1985) when progesterone exerts a negative feedback action on LH secretion (Ginther, 1979). However, before ovulation, when ovarian negative feedback effects on the hypothalamus–pituitary system are absent or minimal (Ginther, 1979), the pattern of LH secretion is non-pulsatile (Fitzgerald *et al.*, 1985).

In intact mares, the onset of the breeding season occurs in response to an increase in daylength and can be advanced by exposure of anoestrous mares to an artificial increase in daylength (Sharp & Ginther, 1975). The first ovulation of the breeding season is preceded by a period of prolonged oestrous behaviour accompanied by increased ovarian follicular development. Although LH has been implicated in both follicular development and ovulation of the mare (Pineda & Ginther, 1972; Licht *et al.*, 1979), serum LH remains at low levels in blood samples collected 2–3 times per week, until 6–7 days before the first ovulation of the breeding season (Freedman *et al.*, 1979; Turner *et al.*, 1979). During this 6–7-day period before ovulation, serum LH concentrations gradually increase to a maximum within 1 day after ovulation.

Based on the consideration that during the spring months the pattern of LH secretion in ovariectomized mares is pulsatile and that mean serum LH concentrations and LH pulse frequency both increase over a prolonged time period, the absence of a measurable increase in serum LH values in intact mares until 6–7 days before ovulation may reflect infrequent collection of blood samples. The purpose of the present study, therefore, was to investigate this possibility in two groups of intact anoestrous mares in which the onset of the breeding season occurred either in response to an abrupt, artificial increase in daylength or a gradual, natural increase in photoperiod. An additional and related objective was to characterize alterations in LH pulse frequency and amplitude in relation to the occurrence of the onset of oestrous cycles.

Materials and Methods

Animals and treatments. Two groups of anoestrous horse mares were maintained outdoors on pasture, except during sampling periods or experimental procedures, when they were housed in stalls. All mares received oats and hay daily and water was freely available.

Beginning 1 December, 4 mares (Group 1) were exposed to an abrupt artificial increase in daylength to advance the onset of the breeding season. Mares were kept in stalls between 15:00 and 07:00 h each day and exposed to artificial lighting between 16:00 and 23:00 h, thereby extending daylength to at least 16 h light per day. The remaining 5 mares (Group 2) were maintained in natural photoperiodic conditions for Lexington (38°2'N).

Follicular development, oestrus and ovulation. Follicular development was assessed by palpation of the ovaries *per rectum* at least every 3 days, or daily after identification of a follicle 25 mm in diameter or larger. The occurrence of ovulation was indicated by the collapse of a follicle. Oestrus was monitored by teasing daily with a stallion.

Collection of blood samples. Blood samples (5 ml) were collected daily at 09:00 h by jugular venepuncture beginning on 30 December (Group 1) or 2 February (Group 2), until each mare had completed an oestrous cycle. Changes in pulsatile LH secretion were determined in additional samples collected via an intravenous catheter. For Group 1 mares, blood samples (5 ml) were collected every 15 min for 24-h periods before (15 November), on the day of (1 December) and 2 weeks after (16 December) exposure to an abrupt increase in daylength. Thereafter, it was anticipated that LH pulse frequency might increase and therefore the duration of frequent sample collection (every 15 min) was reduced to 12 h and performed every 2 weeks until ovulation. Frequent samples were also collected for 24 h from mares of Group 2 on 12 December and 5 January; thereafter, samples were collected for 12 h every 2 weeks until ovulation. All frequent sample collection periods began at 12:00 h. During the hours of darkness, blood samples were collected with the aid of a dim red light. The cannula was inserted 30 min before sample collection and maintained patent by flushing with saline (9 g NaCl/l). At each sample withdrawal, the first 1 ml was discarded and a further 5 ml collected. All blood samples were kept overnight at 4°C. Serum was collected and stored frozen until assayed.

Radioimmunoassay of LH. Luteinizing hormone concentrations were measured in duplicate 10–200 µl samples of serum using a radioimmunoassay procedure described previously (Loy *et al.*, 1982). A horse pituitary LH preparation (E98A), kindly supplied by Dr H. Papkoff, was used as standard. This preparation had 2.97 times the activity of NIH-LH-S1 according to the ovarian ascorbic acid depletion assay (Licht *et al.*, 1979). The sensitivity of the radioimmunoassay was 0.014 ng/tube. Intra-assay and interassay coefficients of variation were 10 and 13% respectively for a serum pool that produced a 50% inhibition of binding of radiolabelled LH to antibody (30 assays).

Analysis of data. Changes in mean serum LH concentrations before ovulation, in samples collected daily, were analysed using a repeated measures analysis of variance. Multiple comparisons using the least-squares means (SAS User's Guide, 1985) were used to identify the timing of the onset of the periovulatory increase in serum LH values, relative to ovulation. Additionally, in both groups of mares, orthogonal contrasts were used to test for linear, quadratic and cubic effects of the periovulatory rise in serum LH concentrations during a 10-day period before the first and second ovulations of the breeding season.

The occurrence of an LH pulse was identified as described previously (Fitzgerald *et al.*, 1985). Changes in LH pulse frequency, with respect to time of year and occurrence of ovulation, were tested by χ^2 analyses. In the case of days before ovulation, data were blocked into sampling intervals of 10 days. This procedure resulted in 7 time periods (blocks) ranging from 10–0 days to > 60 days before ovulation. Appropriate contrasts were used to test for differences between blocks. Since the duration of frequent sample collection was not constant, changes in LH pulse frequency were statistically analysed for blood samples collected between 12:00 and 24:00 h.

Comparison of mean serum LH concentrations determined in blood samples collected daily or in periods of frequent sample collection during 60 days before ovulation was made by analysis of variance. The relationship between LH pulse frequency and amplitude was determined by regression analysis. Values are presented as mean $\pm$ s.e.m.

Results

Oestrus, follicular development and occurrence of ovulation

Ovulation occurred earlier ($P < 0.01$) in Group 1 than Group 2 mares (65.7 ± 6.8 vs 135.6 ± 3.7 days after 1 December), corresponding to 4 February and 14 April, respectively.

In all mares, ovulation was preceded by prolonged oestrous behaviour averaging 25 and 50 days in Groups 1 and 2, respectively. In both groups, the period of prolonged oestrus included days when mares were unresponsive or passive towards the teaser stallion. Therefore, this apparent difference in duration of oestrus between the respective groups was not statistically evaluated.

The occurrence of oestrus was generally accompanied by an increase in the size of the ovaries although, initially, follicles could not be palpated. Subsequently, palpable follicles were recorded and in 3 and 4 mares in Groups 1 and 2, respectively 1 or 2 follicles reached preovulatory dimensions (≥ 35 mm diameter) but regressed before development of a similar large follicle that ovulated. In the remaining 2 mares, the development of a smaller follicle (≤ 25 mm diameter) was recorded before identification of the preovulatory follicle.

Daily serum LH profile before the first ovulation of the breeding season

In 4 mares (Group 1) exposed to an abrupt artificial increase in daylength, beginning 1 December, mean serum LH concentrations in samples collected daily for 24 days before ovulation remained < 1 ng/ml until Day -3 . Thereafter, serum LH increased ($P < 0.001$) to a maximum level of 3.59 ± 0.70 ng/ml on Day -1 before ovulation (Fig. 1, upper panel). Similarly, in 5 mares (Group 2) exposed to natural photoperiodic conditions and from which samples were collected for 60 days before ovulation, mean serum LH rose ($P < 0.001$) on Day -3 to attain 3.55 ± 0.98 ng/ml on the day of ovulation. Although during a 24-day period before ovulation serum LH concentrations were not different ($P > 0.05$) between groups and in both the first increase in serum LH levels occurred 3 days before ovulation, the periovulatory LH rise during a 10-day period was linear ($P < 0.01$) for Group 2 whereas in Group 1 serum LH increased abruptly on Day -3 before ovulation and therefore the periovulatory LH rise showed a significant ($P < 0.01$) quadratic effect.

Changes in mean serum LH concentrations and the pattern of LH secretion established from frequent collection of blood samples are presented below for each group both in relation to the time of year and in combination after normalization of the data with respect to the time of occurrence of ovulation.

LH pattern in relation to time of year

In both groups, a minor but significant ($P < 0.05$) increase in mean serum LH values was observed in relation to time of year. With the exception of one mare in late April (Group 2), in which the day of frequent sample collection coincided with ovulation, mean serum LH concentrations remained < 1 ng/ml (Table 1).

Mean LH pulse frequency also increased in relation to time of year and maximum pulse frequency occurred earlier ($P < 0.001$) in Group 1 than Group 2 (2 February and 10 April, respectively; Table 1). Within each group, the increase in LH pulse frequency was associated with an increase in mean serum LH values although this trend was not apparent on all occasions studied. In this regard, mean LH pulse frequency in Group 1 increased between 1 December and 19 January in association with an increase in mean serum LH concentrations. However, between 15 December and 2 February mean LH pulse frequency increased approximately 5-fold whereas mean serum LH concentrations remained unchanged ($P > 0.05$). In Group 2, mean LH pulse frequency increased ($P < 0.001$) between 16 January and 10 April and was associated with an increase ($P < 0.05$) in mean serum

Fig. 1. Mean ($\pm$ s.e.m.) serum LH concentrations from samples collected daily for 24 days before ovulation in 4 mares (Group 1) exposed to abrupt increase in daylength, beginning 1 December (upper panel) and for 60 days in 5 mares (Group 2) exposed to natural photoperiodic conditions (lower panel).

Table 1. Changes in mean ($\pm$ s.e.m.) serum LH concentrations, LH pulse frequency and amplitude in mares in relation to time of year

Group	Time of year	N*	Serum LH (ng/ml)	No. of LH pulses per 12 h (12:00–24:00 h)	LH pulse amplitude (ng/ml)
1	21 Nov.	0/4	0.23 $\pm$ 0.05	0.00	
	1 Dec.	1/4	0.19 $\pm$ 0.09	0.00	1.51†
	15 Dec.	2/4	0.35 $\pm$ 0.11	0.50 $\pm$ 0.28	2.49 $\pm$ 1.24†
	5 Jan.	2/4	0.36 $\pm$ 0.12	1.00 $\pm$ 0.70	0.66 $\pm$ 0.23
	19 Jan.	3/4	0.73 $\pm$ 0.27	1.25 $\pm$ 0.63	0.70 $\pm$ 0.06
	2 Feb.	2/2	0.47 $\pm$ 0.07	2.50 $\pm$ 0.49	0.56 $\pm$ 0.10
	17 Feb.	0/1	0.87	0.00	
2	12 Dec.	2/5	0.49 $\pm$ 0.20	0.60 $\pm$ 0.39	1.43 $\pm$ 0.28†
	16 Jan.	1/5	0.17 $\pm$ 0.05	0.20 $\pm$ 0.20	1.11†
	14 Feb.	3/5	0.55 $\pm$ 0.16	0.60 $\pm$ 0.24	2.39 $\pm$ 0.32
	28 Feb.	3/5	0.83 $\pm$ 0.40	0.80 $\pm$ 0.37	1.98 $\pm$ 0.71
	13 Mar.	4/5	0.46 $\pm$ 0.06	1.00 $\pm$ 0.31	1.39 $\pm$ 0.54
	27 Mar.	5/5	0.81 $\pm$ 0.27	1.80 $\pm$ 0.37	1.09 $\pm$ 0.16
	10 Apr.	3/3	0.92 $\pm$ 0.25	3.33 $\pm$ 0.66	0.70 $\pm$ 0.08
	24 Apr.	0/1	3.00	0.00	

*Proportion of total number of mares exhibiting pulsatile LH release during 24- or 12-h sample collection period.

†Mean LH pulse amplitude includes all LH pulses observed during 24-h sample collection period.

LH values (Table 1). However, as noted for Group 1 mares, a 5-fold rise in LH pulse frequency between 14 February and 10 April was unaccompanied by an increase ($P > 0.05$) in mean serum LH concentrations (Table 1).

As shown in Table 1, there was in both groups a trend for a decrease in LH pulse amplitude in relation to time of year; however, insufficient data precluded further analysis of this observation.

LH pattern in relation to time of ovulation

Changes in mean serum LH concentrations and the pattern of LH secretion were assessed by blocking days before ovulation (Day = 0) into sampling intervals of 10 days. This procedure resulted in 7 time periods ranging from 10–0 days (Period 1) to >60 days (Period 7) before ovulation; composite data for all 9 mares are depicted in Fig. 2.

Fig. 2. Changes in mean serum LH concentrations (open bars), LH pulse frequency (●) and amplitude (▲) during the transition into the breeding season. Numbers at the bottom of the open bars indicate the number of mares. Data were normalized to the day of ovulation by using an arbitrary sampling interval of 10 days.

Mean serum LH concentrations increased ($P < 0.001$) between Periods 7 and 1, from 0.29 ± 0.05 (N = 9 mares) to 1.55 ± 0.31 ng/ml (N = 6 mares). During this period, mean LH pulse frequency also increased ($P < 0.001$) from 0.19 pulses/12 h in Period 7 (N = 9, range 0–2 pulses) to 2.37 pulses/12 h in Period 2 (N = 8, range 1–4 pulses). However, between Periods 6 and 2 mean LH pulse frequency increased 6-fold but mean serum LH concentrations remained low and increased ($P < 0.05$) only during the final 10 days before ovulation (Period 1). Within Period 1, mean LH pulse frequency apparently decreased but the observed frequency was not different from that in Period 2 before ovulation and may reflect the absence of identifiable LH pulses in 3 of 6 mares examined during this period. Finally, mean serum LH concentrations established from periods of frequent sample collection during 60 days before ovulation were similar ($P > 0.05$) to values determined in samples collected daily for 24 (Group 1) or 60 days (Group 2) before ovulation.

A significant ($P < 0.02$) reciprocal relationship was observed between LH pulse frequency and amplitude during the transition into the breeding season (Fig. 2). In general, LH pulse amplitude was > 1 ng/ml between Days >60 and 30–20 before ovulation whereas 21 of 29 LH pulses (72%) observed during the final 20 days before ovulation were < 1 ng/ml.

Comparison of the periovulatory increase in serum LH concentrations before the first and second ovulations of the breeding season

The interval between the first and second ovulations of the breeding season was not different ($P > 0.05$) between groups and averaged 22.5 ± 0.8 days ($N = 9$; range 17–26 days). During a 10-day period preceding the respective ovulations, mean daily serum LH concentrations were not different between groups ($P > 0.05$) and, therefore, results were pooled for comparisons between the first and second ovulation. As depicted in Fig. 3, the periovulatory rise in serum LH before the first ovulation was manifest as a linear increase ($P < 0.001$) with respect to time. In contrast, that preceding the second ovulation fitted a quadratic function ($P < 0.001$), indicating that mean serum LH concentrations increased more rapidly between Days 10 to 3 before the second than first ovulation and increased thereafter to a greater level ($P < 0.05$) on the day of ovulation (9.50 ± 1.71 vs 3.56 ± 1.78 ng/ml, $N = 9$).

Fig. 3. Mean ($\pm$ s.e.m.) serum LH concentrations in 9 mares sampled daily during 10 days before the first (●) and second (▲) ovulations of the breeding season.

Discussion

The foregoing results demonstrate that, in the mare transition from seasonal anoestrus to the breeding season is associated with a photoperiod-induced, seasonal increase in the frequency of pulsatile LH secretion. A causal relationship between daylength and the observed increase in LH pulse frequency is suggested since an abrupt, artificial increase in daylength, applied during the winter months, advanced the timing of the increase in LH pulse frequency and the initiation of the breeding season, compared with that observed in mares maintained under natural photoperiodic conditions. The present results therefore suggest that a daylength-mediated seasonal increase in LH pulse frequency may play a role in the mechanisms governing the onset of the breeding season of this species.

In Group 2 mares, from which blood samples were collected daily for 60 days before ovulation, mean daily serum LH values between 60 and 11 days before ovulation were similar to mean concentrations in frequent sample collection periods. This observation suggests that, during a 60-day period before ovulation, blood samples collected daily appear to provide a reliable estimate of changes in mean serum LH concentrations. The present results therefore support earlier observations that in the mare the onset of the breeding season is not associated with a measurable increase in mean daily serum LH values until a few days before ovulation (Freedman *et al.*, 1979; Turner *et al.*, 1979). In addition, the present results extend these observations by providing evidence that the absence of a measurable increase in mean daily serum LH concentrations, until immediately before ovulation, is not a reflection of infrequent blood sample collection. However, in the present study daily blood samples were collected only from Group 2 mares for 60 days before ovulation. Furthermore, mean serum LH values determined in periods of frequent sample collection at > 60 days were lower than values observed during the final 60 days before ovulation. It is possible, therefore, that a longer duration of daily blood sample collection might also yield evidence of a seasonal increase in serum LH concentrations.

Between 60 and 11 days before ovulation mean LH pulse frequency increased approximately 5-fold. That mean serum LH concentrations in both daily and frequently collected blood samples did not reflect this increase in LH pulse frequency was unexpected, since this finding contrasts with our previous observations for ovariectomized mares, at a comparable time of year (Fitzgerald *et al.*, 1983). In ovariectomized mares a 4-fold increase in LH pulse frequency during the spring months was associated with a 9-fold increase in mean serum LH concentrations. One explanation to account for this discrepancy might be related to the amplitude of LH pulses. The present study demonstrates that in intact mares LH pulse amplitude is reciprocally related to LH pulse frequency whereas in ovariectomized mares the seasonal increase in LH pulse frequency during the spring months was not accompanied by a detectable change in LH pulse amplitude (Fitzgerald *et al.*, 1983).

A reciprocal relationship between LH pulse frequency and amplitude has been observed in various species under different experimental conditions (Yen *et al.*, 1974; Goodman & Karsch, 1980; Rahe *et al.*, 1980). Heretofore, such a relationship has not been reported for the mare and the mechanisms underlying this phenomenon remain to be elucidated. Studies with ovariectomized ewes, in which communication between the hypothalamus and pituitary gland has been interrupted (Clarke *et al.*, 1984), or in rhesus monkeys bearing lesions of the arcuate nucleus (Wildt *et al.*, 1981), indicate that high-frequency exogenous GnRH stimulation is associated with low LH pulse amplitude and *vice versa*. Furthermore, there is an upper limit to the ability of the pituitary gland to respond to pulsatile GnRH administration, at least in the rhesus monkey (Wildt *et al.*, 1981). The observed reduction in LH pulse amplitude in mares entering the breeding season may therefore be caused by a decrease in pituitary responsiveness resulting from increased high-frequency GnRH stimulation. Additionally, the absence of LH pulses in some mares sampled a few days before ovulation may indicate that in the mare there may be an upper limit to the ability of the pituitary gland to respond to high-frequency GnRH stimulation. During the breeding season the frequency of hypothalamic GnRH release may increase to the extent that the pituitary gland is unable to respond by secreting discrete LH pulses. In support of this proposal, a non-pulsatile pattern of LH secretion is evident during the breeding season in ovariectomized mares (Fitzgerald *et al.*, 1983) and in intact mares during the follicular phase of the oestrous cycle (Fitzgerald *et al.*, 1985) when ovarian negative feedback effects on the hypothalamus-pituitary system are absent or minimal (Ginther, 1979). In ovariectomized ewes, a non-pulsatile pattern of LH secretion has been reported in a small number of animals when LH pulse frequency is maximal (Robinson *et al.*, 1985).

Although the results of several studies suggest that pituitary gland responsiveness is reduced by high-frequency GnRH stimulation (Wildt *et al.*, 1981; Clarke *et al.*, 1984), in the mare we have observed that pituitary responsiveness to a single injection of GnRH is sometimes increased when serum LH concentrations are elevated and when, therefore, endogenous pulsatile GnRH stimulation

might be anticipated to be frequent. Injection of ovariectomized mares with GnRH (200 µg) during the spring months induces an elevation in serum LH concentrations comparable to the amplitude of endogenous LH pulses occurring at this time of year. In contrast, during the summer months, when serum LH values are maximally elevated but LH pulses cannot be identified, pituitary responsiveness to this GnRH dose is increased (B. P. Fitzgerald, unpublished). Whilst these results must be interpreted cautiously, since a single bolus injection may not be viewed as a physiological stimulus for LH release, it seems feasible in the light of these findings that the observed reduction in LH pulse amplitude during the transition period may reflect a decrease in GnRH pulse amplitude.

The present results confirm previous observations that the magnitude of the periovulatory LH rise preceding the first ovulation is lower than for subsequent ovulations during the breeding season (Oxender *et al.*, 1977; Freedman *et al.*, 1979). The mechanisms underlying this modification of the amplitude of the periovulatory LH rise are unknown although it is unlikely, as suggested earlier (Freedman *et al.*, 1979), that this seasonal alteration is directly modulated by daylength. The present results demonstrate that serum LH concentrations associated with the first and second ovulations of the breeding season were similar in mares exposed to an abrupt artificial increase in daylength or maintained under natural photoperiodic conditions. An alternative mechanism might be an alteration in pituitary responsiveness during the breeding season, perhaps reflecting an increase in pituitary LH content as reported for mares during the transition from anoestrus to the breeding season (Hart *et al.*, 1984).

Irrespective of the magnitude of the periovulatory LH rise preceding the first and subsequent ovulations of the breeding season, the duration of the first oestrous cycle was comparable to the known duration of oestrous cycles in the middle of the breeding season (i.e. about 22 days). This observation contrasts the sequence of events associated with the onset of oestrous cycles in the ewe. In a high proportion of ewes, the start of the breeding season may be characterized by one or more short luteal phases, as indicated by transient elevations in serum progesterone concentrations for 2–3-day periods before the occurrence of a luteal phase characteristic of the breeding season (Walton *et al.*, 1977). In the ewe such transient elevations in progesterone, which also occur in other species at puberty or at the initiation of ovulatory cycles after a prolonged period of anovulation (Gonzalez-Padilla *et al.*, 1975; Foster, 1977; Nass *et al.*, 1979), may be caused by immaturity of the preovulatory follicle at the time of the preovulatory LH surge (Legan *et al.*, 1985). Furthermore, it has been proposed that such brief elevations in serum progesterone, which are preceded by a pre-ovulatory LH surge (l'Anson, 1985), may play an important role in the initiation of the breeding season by synchronizing the random anoestrous pattern of follicular development with an ensuing LH surge, thereby ensuring onset of a normal oestrous cycle (Legan *et al.*, 1985). That this sequence of events does not occur in the mare, since serum progesterone concentrations increase only after the first ovulation of the breeding season (Oxender *et al.*, 1977), suggests that in this species the follicle destined to ovulate is mature at the time of the first periovulatory LH rise, thereby ensuring an oestrous cycle of normal length. Furthermore, the observation that in a high proportion of mares large preovulatory size follicles develop and regress before the development of the follicle destined to ovulate suggests that this pattern of follicular development may function as a fail-safe mechanism to ensure complete follicular maturation at the time of the first periovulatory LH rise. Finally, the stimulus for the first periovulatory LH rise is unknown but the abrupt nature of this increase suggests a positive feedback stimulus provided by the ovaries.

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