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# Acute changes in pulsatile LH and FSH secretion after ovariectomy in rats: treatment with oestradiol for 24 h suppresses LH, but not FSH, for at least 48 h

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Concentrations of LH in female rats do not increase to concentrations seen in the male at 24 h after castration until up to one week after ovariectomy, while FSH concentrations increase rapidly in both sexes. We hypothesized that the lag in LH rise is due to the imposition of a prolonged suppression by the high concentrations of oestradiol seen during each cycle at pro-oestrus, which prevents a rapid response to the removal of negative feedback. This hypothesis was tested by studying the effect of exposure to pro-oestrus oestradiol concentrations, administered in Silastic capsules, for 24 h on the pulsatile release of LH and FSH. In the first experiment, treatment with oestradiol for 24 h began on day 3 after ovariectomy and resulted in a significant suppression of LH pulse frequency, which appeared to persist for up to 4 days after removal of the implant. In the second experiment, exposure for 24 h to high, pro-oestrous oestradiol concentrations beginning on day 3 or 7 after ovariectomy significantly suppressed mean LH concentrations and LH pulse amplitude compared with vehicle-treated controls, while FSH secretion did not differ between the two treatments. These results suggest that the acute lag in the rate of LH rise after ovariectomy depends on a prolonged suppressive effect of oestradiol and that the rise in oestradiol during the cycle, which exerts a positive feedback to trigger the preovulatory gonadotrophin surges, also exerts a negative feedback for 3–4 days, and this contributes to the relative stability of cycle duration in female rats.

## Introduction

Gay and Midgley (1969) first characterized a sex difference in the rate of rise of gonadotrophin concentrations following gonadectomy in rats. They and others (Blackwell and Amoss, 1971; Tapper *et al.*, 1972; Brown-Grant and Greig, 1975) found that, upon removal of gonadal negative feedback, LH concentrations in males increase to five to ten times control concentrations within 8 to 12 h after castration, while in females the LH response to ovariectomy at any stage of the oestrous cycle is slower. Serum LH doubles by 24–48 h and does not increase further until day 4 or 5 after ovariectomy (Gay and Midgley, 1969; Blackwell and Amoss, 1971; Tapper *et al.*, 1972). FSH, in contrast, rises quickly within the first 24–48 h in both sexes, reaching two to four times the precastration values (Brown-Grant and Greig, 1975; Spitzbarth *et al.*, 1988).

The rise in mean LH concentrations after ovariectomy results from increases in both LH pulse amplitude and pulse frequency. Ovariectomy at various stages of the cycle results in significant increases in LH pulse amplitude and frequency by 24 h (Leipheimer *et al.*, 1984, 1985; Gallo and Bona-Gallo,

1985). Despite these initial increases, pulsatile LH release does not increase further until between days 4 and 10 after ovariectomy (Leipheimer and Gallo, 1983; Higuchi and Kawakami, 1982). Pulsatile FSH secretion has not been characterized as extensively as pulsatile LH secretion. We reported that by 24 h after metoestrous ovariectomy, FSH pulse frequency does not change significantly and FSH pulse amplitude approximately doubles (Luderer and Schwartz, 1991). Other workers have found that by four weeks after ovariectomy FSH pulses occur at about half the frequency of LH pulses (Lumpkin *et al.*, 1984; De Paolo, 1985).

Oestradiol replacement can completely reverse the increases in concentrations of serum LH after ovariectomy, and partially suppress serum FSH toward concentrations in intact animals and can reinstate afternoon surges of both gonadotrophins (Savoy-Moore and Schwartz, 1980). Oestradiol continues to exert stimulatory and suppressive effects on LH secretion in ovariectomized rats, even after the source of oestradiol has been removed and circulating concentrations of oestradiol have fallen to values observed in ovariectomized animals. In one study, exposure of ovariectomized rats to supraphysiological oestradiol concentrations for 24 h resulted in a 50% suppression of morning concentrations of LH and robust afternoon LH surges for 4 days after removal of the implant (Legan *et al.*, 1975).

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Together the data from post-castration and oestradiol replacement studies suggest that removal of oestradiol alone may be responsible for the pattern of LH, but not FSH, increase following ovariectomy. We hypothesized that oestradiol exerts its suppressive effects by setting in motion a cascade of events, such as the synthesis of a hypothalamic protein(s) with a long half-life, for which the continued presence of oestradiol is not required. To test this hypothesis, we treated ovariectomized rats with oestradiol and compared the changes in LH and FSH pulsatility after removal of oestradiol with changes after ovariectomy. Thus, the aims of the following studies were (i) to test the hypothesis that oestradiol exerts a prolonged suppressive effect on pulsatile LH, but not FSH, secretion in the adult female rat, and (ii) to characterize the pulsatile nature of FSH secretion in metoestrous rats and at various times after ovariectomy and to further characterize the LH lag after ovariectomy. An abstract of this work was presented at the 71st Annual Meeting of the Endocrine Society, 1989.

## Materials and Methods

### Animals

Adult female Charles River (Portage, MI) Sprague-Dawley rats (200–300 g; 75–125 days of age) were housed three or four to a cage in a temperature-controlled room with lights on from 05:00 to 19:00 h. Animals had free access to tap water and standard laboratory rat chow. Only animals that displayed at least two consecutive 4-day oestrous cycles were used for the experiments.

### Surgery

Surgery was performed under ketamine hydrochloride (Bristol Laboratories, Syracuse, NY)/xylazine (Mobray Corporation, Shawnee, KS) anaesthesia (10 mg and 1 mg, respectively, per 100 g body weight). Bilateral ovariectomies were performed at 09:00 h on metoestrus. Thereafter, between 09:00 and 12:00 h, the animals were fitted with right atrial catheters via the external jugular vein. The tubing was tunnelled under the skin and exteriorized at the nape of the neck. The catheters were made of 2.5–3.0 cm lengths of Silastic tubing (0.025 i.d.  $\times$  0.047 o.d.; Dow Corning, Midland, MI) connected to longer lengths of polyethylene tubing (Clay-Addams, Parsippany, NJ). Catheters were inserted on the day of ovariectomy or of oestrus for the intact controls in Expt 1 and 24 h before collection of serial blood samples in Expt 2.

### Oestradiol implants

Silastic tubing (0.062 i.d.  $\times$  0.125 o.d.) was plugged at one end with Silastic polymer; segments were filled and the other end was also plugged with polymer. In Expt 1 crystalline oestradiol (Sigma, St Louis, MO) was dissolved in peanut oil by warming to 65°C at a concentration of 150  $\mu\text{g ml}^{-1}$ . Lengths of 10 mm per 100 g body weight were used to produce serum concentrations of  $23.3 \pm 2.2 \text{ pg ml}^{-1}$ . In Expt 2 crystalline oestradiol was packed into 5 mm lengths of tubing. These implants produced serum concentrations of  $77.6 \pm 1.5 \text{ pg ml}^{-1}$ . For comparison, oestradiol concentrations in the control ovariectomized animals fell to  $8.0 \pm 1.3 \text{ pg ml}^{-1}$  within 8 h after

ovariectomy and did not change significantly thereafter, and oestradiol concentrations in intact pro-oestrous rats in our colony range from 20 to 80  $\text{pg ml}^{-1}$ . The implants were incubated overnight in 0.1% Type A gelatin (Grayslake Gelatin Co., Grayslake, IL), 0.9% saline to prevent an initial 'surge' of oestradiol after implantation.

### Collection of blood samples

Blood samples, 0.5 ml, were withdrawn through the sampling catheter every 6 min for 3 h beginning at 09:00 h. After every second sample, 1.0 ml of a blood replacement mixture (Ellis and Desjardins, 1982), consisting of erythrocytes from donor females reconstituted with human plasma protein fraction (Plasmanate, Cutter Laboratories), was injected. This mixture contains no immunoassayable LH, FSH or GnRH, and contains concentrations of oestradiol comparable to those of ovariectomized rats. The samples were then allowed to clot overnight at  $-4^\circ\text{C}$ , were centrifuged at 6000  $g$  for 20 min and the serum was stored frozen at  $-70^\circ\text{C}$  until radioimmunoassay for LH and FSH. In addition, 0.5 ml of blood for oestradiol radioimmunoassay was withdrawn from each rat in Expt 1 at 12:00 and 17:00 h on every day from day 0 to day 11 and replaced with an equal volume of heparinized saline.

### Autopsies

The animals were decapitated and trunk blood was collected. The uteri were dissected out; wet and dry uterine weights and uterine fluid weights were obtained. Autopsies occurred on metoestrus or day 11 at 17:00 h in Expt 1 and on the day of collection of serial blood samples at 16:00 h in Expt 2.

### Radioimmunoassays

Plasma fractions were analysed using a rat:rat or an ovine:rat system for LH (NIDDK RP-2 in Expt 1 or NIH LH S25 in Expt 2 as standard and anti-rat LH antibody S-10 from the NIDDK kit). Results are expressed in terms of RP-2 (S25 is equivalent to RP-2). A rat:rat system was used to measure FSH (NIDDK rFSH RP-2 as standard and NIDDK anti-rat FSH antibody S-11). The intra-assay coefficients of variation (CV) of the LH and FSH assays were 19% and 26%, respectively for the first assay for Expt 1, 8% and 12%, respectively for the second assay for Expt 1, 6% and 6% for Expt 2. The interassay CVs were 32% for LH and 17% for FSH. Serum concentrations of oestradiol were also measured by radioimmunoassay after ether extractions, using [ $^3\text{H}$ ]oestradiol (Amersham, Arlington Heights, IL) and 0.1 ml oestradiol antiserum (GDN 244, provided by G. D. Niswender, Colorado State University, Fort Collins, CO) at a working dilution of 1:50 000. The basic procedure for this assay has been validated by Nequin *et al.* (1975). The intra-assay CVs were 3% and 2% for the two assays for Expt 1 and 12% for Expt 2. The interassay CV was 17%.

### Statistical analysis

The ULTRA pulse detection program (Van Caeter, 1988) was used to determine significant LH and FSH pulses. This program

takes as input the data values, the coefficients of variation (CV) of the concentration ranges of the assay and a threshold expressed in terms of number of assay CVs. For these analyses a two CV threshold was used. Values for mean pulse amplitude and mean pulse frequency for each animal were obtained from the ULTRA analysis. Mean hormone concentrations, pulse amplitude and pulse frequency were analysed by two-way analysis of variance (ANOVA), with day post-ovariectomy and treatment as between variables, using the CRUNCH interactive statistical package (Crunch Software, Oakland, CA). Differences among treatment groups were assessed by post-hoc *t* test and were considered significant if  $P < 0.05$ .

#### *Experiment 1: pulsatile FSH and LH secretion in control rats and ovariectomized rats treated with oestradiol for 24 h*

Female rats were allocated to three treatment groups. Group 1 consisted of intact controls. The animals in groups 2 and 3 were ovariectomized on metoestrus (day 0). Atrial catheters were placed on the day of ovariectomy for groups 2 and 3 and on oestrus for group 1. Group 2 received vehicle implants and group 3 received oestradiol implants. The implants were inserted at 09:00 h on day 3 after ovariectomy and removed at 09:00 h on day 4. Serial blood samples were withdrawn on metoestrus for the intact controls. For treatment groups 2 and 3, sampling occurred on 1 day between day 1 and day 11 after ovariectomy (samples were collected from a total of 21 groups,  $n = 2-4$  per group).

#### *Experiment 2: effect of treatment with oestradiol for 24 h beginning on day 3 or 7 on pulsatile LH and FSH release on day 5 or 9 after ovariectomy*

Rats were ovariectomized at 09:00 h on metoestrus (day 0). There were two control and two oestradiol treated groups: one control group ( $n = 4$ ) received empty implants from 09:00 h day 3 to 09:00 h day 4; the other control group ( $n = 4$ ) received empty implants for 24 h beginning at 09:00 h day 7. The treated groups ( $n = 4$  per group) received implants filled with crystalline oestradiol during the same periods. Serial blood samples were collected either on day 5 for those animals that received implants on day 3 or on day 9 for those that received implants on day 7.

## Results

#### *Experiment 1: pulsatile FSH and LH secretion in control rats and ovariectomized rats treated with oestradiol for 24 h*

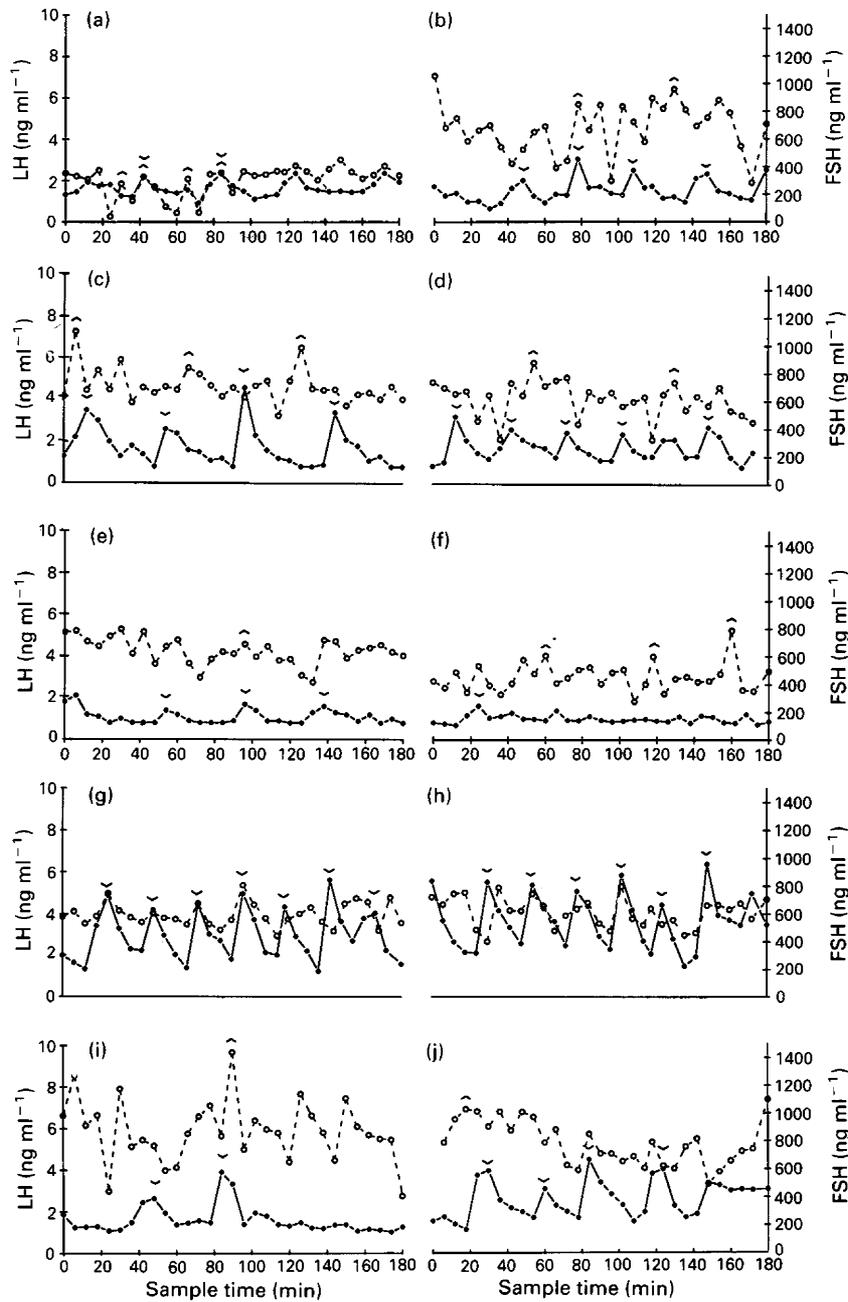
By 3 h after ovariectomy circulating oestradiol had fallen to about  $7-8 \text{ pg ml}^{-1}$  and did not decrease further until autopsy on day 11 in the oil-treated controls (data not shown). Treatment with oestradiol implants resulted in an almost threefold increase in serum concentrations of oestradiol on day 3 ( $7.8 \pm 0.5 \text{ pg ml}^{-1}$  in the oil-treated animals versus  $23.3 \pm 2.2 \text{ pg ml}^{-1}$  in the oestradiol treated animals). Individual profiles of pulsatile LH and FSH release for representative animals from several treatment groups are shown (Fig. 1).

LH secretion was clearly pulsatile in metoestrous and ovariectomized animals. FSH pulses occurred less frequently and with more variable amplitudes (Fig. 2). Mean concentrations of LH, LH pulse amplitude and pulse frequency in the oil-treated animals increased significantly with day after ovariectomy ( $P < 0.001$ ;  $P < 0.002$ ;  $P < 0.04$ , respectively). Post-hoc *t* test showed that mean concentrations of LH and LH pulse amplitude were significantly higher in serum of animals on days 4, 6, 7, 9, 10, 11 after ovariectomy than in metoestrous animals. LH pulse frequency was significantly increased compared with metoestrous concentrations by day 3 (metoestrus < days 3, 6, 7, 9, 10, 11 by post-hoc *t* test). The post-hoc tests show that all three parameters of LH secretion were not consistently increased until day 9 after ovariectomy. In the oil-treated animals mean FSH concentrations also increased with day after ovariectomy ( $P < 0.001$ ; metoestrus < days 2-11 by post hoc *t* test), while FSH pulse frequency decreased with day ( $P < 0.05$ ; metoestrus > days 2, 4, 6, 7, 9, 10 by post-hoc *t* test) and pulse amplitude did not change significantly. Oestradiol treatment suppressed LH pulse frequency ( $P < 0.05$ ), which appeared to persist for 4 days after capsule removal (pulse frequency in the oestradiol-treated animals was lower than that in the oil-treated animals on days 3, 5, 6, 7, 8 and 9). The effect of oestradiol on mean concentrations of LH and the interaction between treatment and day on LH pulse amplitude approached significance ( $P = 0.075$ ;  $P = 0.071$ ). Treatment with oestradiol did not affect FSH secretion, although the interaction between treatment and day after ovariectomy on FSH mean concentrations approached significance ( $P = 0.066$ ).

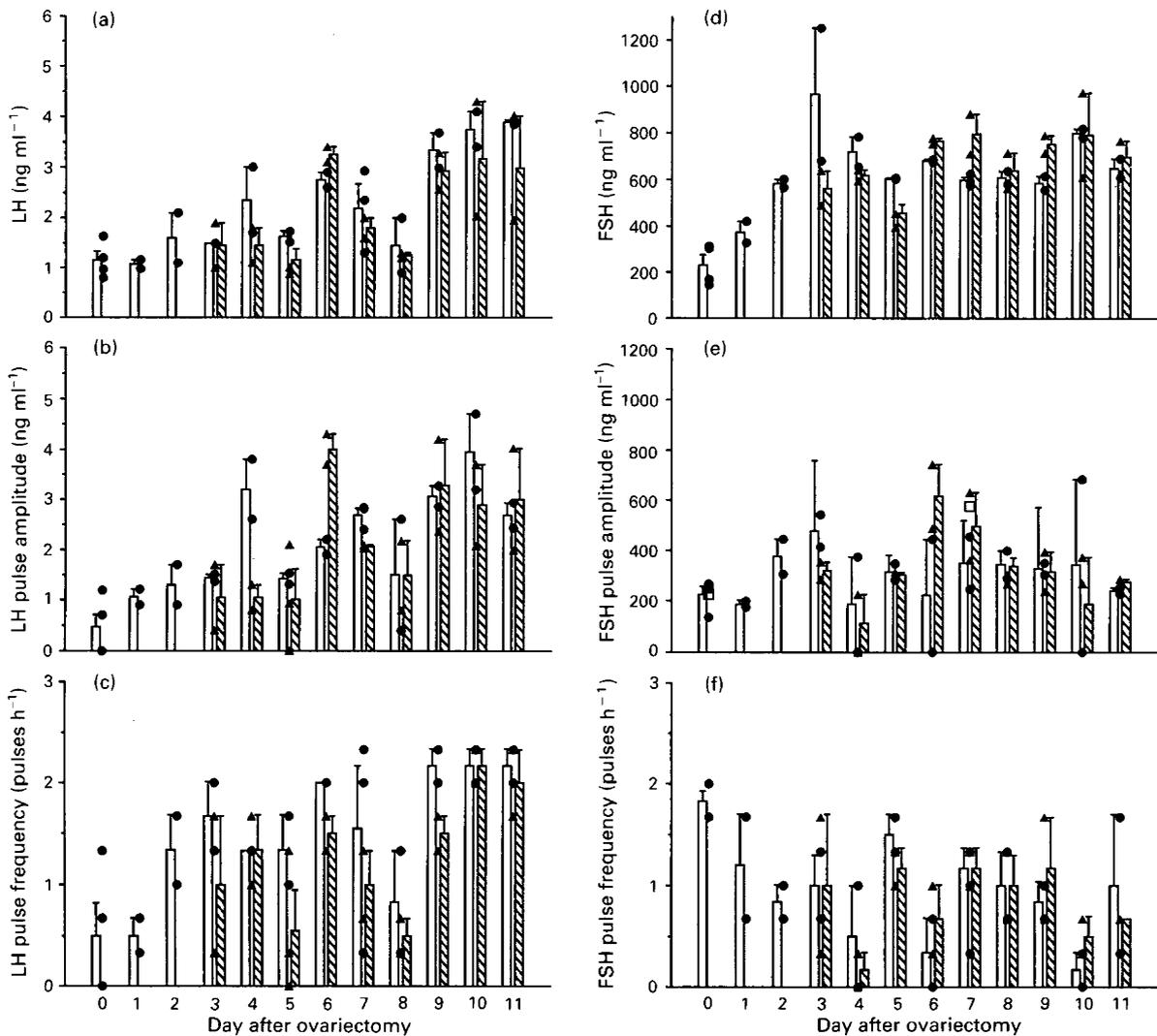
#### *Experiment 2: effect of treatment with oestradiol for 24 h on day 3 or day 7 on pulsatile LH and FSH release on day 5 or 9 after ovariectomy*

The near significance of oestradiol administration on pulsatile LH secretion in Expt 1 led us to speculate that increasing the dose of oestradiol to high physiological concentrations might enhance its suppressive effect. Alternatively, the suppressive effect might become more evident if the implantation of oestradiol capsules was delayed until a day when LH concentrations were reliably increased in all animals. Thus, all the animals in this experiment received a higher dose of oestradiol, and capsule implantation was delayed in half the animals.

The LH and FSH release profiles from representative animals in each group and the summarized data are shown (Figs 3 and 4). Mean concentrations of LH in the controls were significantly higher ( $P < 0.003$ ) on day 9 than on day 5 after ovariectomy, because of a non-significant increase in pulse amplitude and a significant increase in frequency ( $P < 0.02$ ; Fig. 4a-c). LH pulse amplitude ( $P < 0.02$ ) and mean concentrations ( $P < 0.004$ ) were significantly suppressed on days 5 and 9 after oestradiol treatment beginning on days 3 and 7, and LH pulse frequency suppression approached significance ( $P = 0.076$ ). FSH pulsatility did not differ between the 2 days in the control animals, and FSH was unaffected by treatment with oestradiol (Fig. 4d-f). The uterine wet and dry weights were significantly increased in the groups treated with oestradiol for 24 h compared with the control groups ( $P < 0.003$ ,  $P < 0.04$ , respectively; data not shown).



**Fig. 1.** Representative LH (●—●) and FSH (○---○) release profiles obtained from intact metoestrous or ovariectomized rats treated with oil- or oestradiol-filled implants (Expt 1). The animals were ovariectomized and received atrial catheters on metoestrous morning (day 0). Each animal underwent frequent blood sampling (every 6 min) from 09:00 to 12:00 h on a single day, as indicated below. Rats that were serially bled after day 2 received s.c. implants containing  $150 \mu\text{g}$  oestradiol  $\text{ml}^{-1}$  oil or control oil-filled capsules (length of  $10 \text{ mm}$  ( $100 \text{ g}$ ) $^{-1}$  body weight) from 09:00 h on day 3 until 09:00 h on day 4. Significant LH and FSH pulses as determined by the ULTRA program are indicated by the downward and upward-pointing arrowheads, respectively. (a) Intact metoestrous, (b) oil-treated, sampled day 3, (c) oil-treated, sampled day 4, (d) oil-treated, sampled day 5, (e) oestradiol-treated, sampled day 4, (f) oestradiol-treated, sampled day 5, (g) oil-treated, sampled day 7, (h) oil-treated, sampled day 9, (i) oestradiol-treated, sampled day 7, (j) oestradiol-treated, sampled day 9.

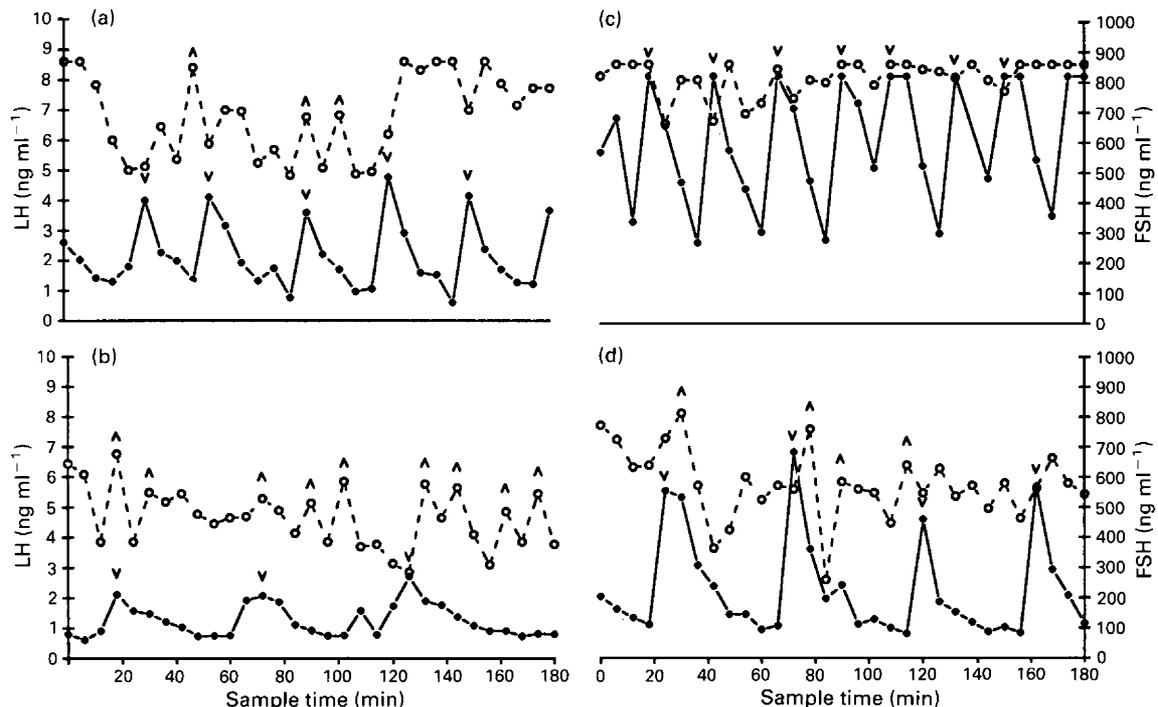


**Fig. 2.** Summary of (a–c) pulsatile LH and (d–f) FSH release in animals from Expt 1. The animals were ovariectomized and received atrial catheters on metoestrous morning (day 0). Each animal underwent frequent blood sampling (every 6 min) from 09:00 h to 12:00 h on a single day. Rats that were serially bled after day 2 received s.c. implants containing 150  $\mu\text{g}$  oestradiol  $\text{ml}^{-1}$  oil or control oil-filled capsules (length of 10 mm ( $100 \text{ g}^{-1}$ ) body weight) from 09:00 h on day 3 until 09:00 h on day 4. The filled dots and triangles represent individuals, and the open and striped bars represent the mean  $\pm$  SEM for each sampling group. In the oil-treated animals ( $\square$ ,  $\bullet$ ) (a) mean concentrations of LH, (b) LH pulse amplitude and (c) LH pulse frequency and (d) mean concentrations of FSH increased significantly with day after ovariectomy ( $P < 0.001$ ;  $P < 0.002$ ;  $P < 0.05$ ;  $P < 0.001$ , respectively); (e) FSH pulse amplitude did not change and (f) FSH pulse frequency decreased with day ( $P < 0.05$ ). There was a significant suppressive effect of oestradiol treatment ( $\square$ ,  $\blacktriangle$ ) on LH pulse frequency ( $P < 0.05$ ). The effect of oestradiol on mean concentrations of LH approached significance ( $P = 0.075$ ).

## Discussion

These studies demonstrate that the increases in mean FSH secretion after ovariectomy are not due to increases in pulse amplitude or in pulse frequency in rats. In contrast, Levine and Duffy (1988) found that in male rats, in addition to high baseline values, FSH pulse frequency and amplitude, which both doubled by 24 h after castration, also played a role in the increase in mean FSH concentrations. The experiments reported here also demonstrate that the delay in the rate of

serum LH rise following ovariectomy lasts for up to a week following an initial small increase. Significant increases in LH pulsatility occur within 24–48 h after ovariectomy (Leipheimer and Gallo, 1983; Luderer and Schwartz, 1991; present study); however, the rate of increase then decreases considerably compared with LH secretion in castrated male rats (Ellis and Desjardins, 1984; Spitzbarth *et al.*, 1988) or to FSH secretion in castrated rats of either sex (Spitzbarth *et al.*, 1988, present study). Mean concentrations of LH and LH pulse amplitude in the present study increased threefold between day 5 and day 9,



**Fig. 3.** Representative LH (●—●) and FSH (○—○) release profiles obtained from Expt 2. (a, b) Day 3 and (c, d) day 7 ovariectomized rats received either (a, c) blank or (b, d) crystalline oestradiol-filled implants ( $n = 4$  per group) for 24 h beginning at 09:00 h. Blood samples were withdrawn every 6 min from 09:00 to 12:00 h one day after the implants were removed (a and b, day 5, or c and d, day 9). Significant LH and FSH pulses as determined by the ULTRA program are indicated by downward and upward pointing arrowheads, respectively.

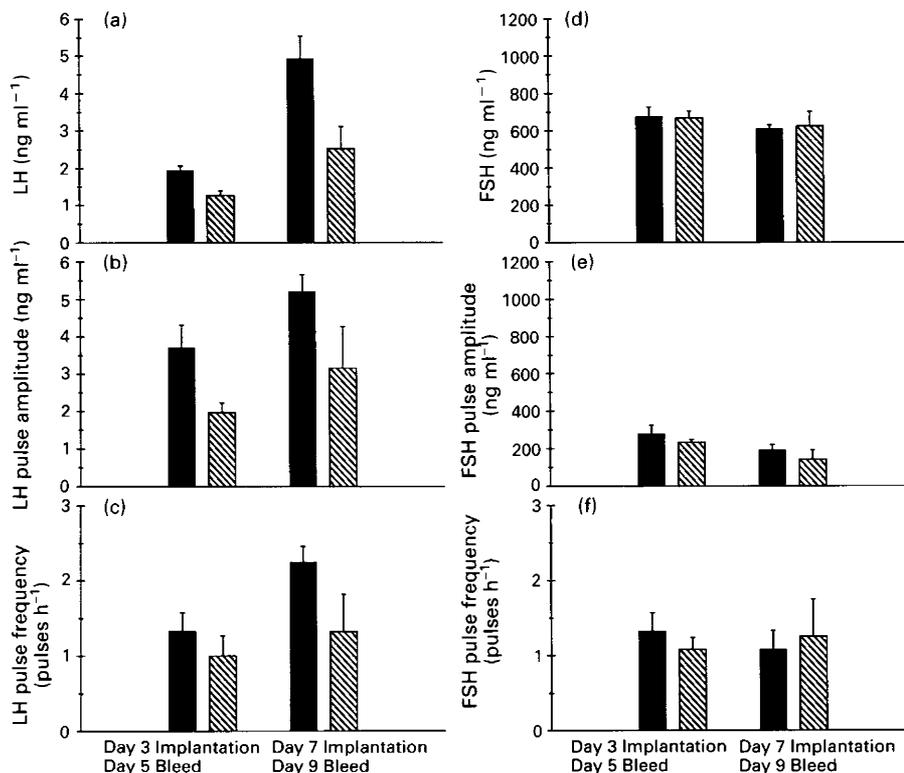
while mean concentrations of FSH increased threefold by day 1–2. Taken together, the present and previous studies suggest that LH secretion in female rats responds immediately, but only partially, to the removal of negative feedback, and is then restrained from further increases for up to a week.

The finding of decreased FSH pulse frequency after ovariectomy is consistent with previous studies in which FSH pulse frequency at one month after ovariectomy (De Paolo, 1985) was half that of LH pulse frequency. These results suggest that pulse-like increments of FSH release are not driven by pulses of GnRH. Administration of GnRH antisera or antagonists to gonadectomized rats results in a rapid suppression of serum LH to concentrations in intact animals, but FSH secretion is suppressed neither as rapidly nor as profoundly (Blake and Kelch, 1981; Charlesworth *et al.*, 1984; Condon *et al.*, 1986; Kartun and Schwartz, 1987). Moreover, FSH pulse amplitude and frequency remain unchanged after GnRH antagonist treatment (Culler and Negro-Vilar, 1986). The excitatory neurotransmitter *N*-methyl-D,L-aspartate, which acts by stimulating GnRH release (Bourguignon *et al.*, 1989), elicits pulse-like increments in LH secretion without increasing FSH secretion (Luderer *et al.*, 1993; Strobl *et al.*, 1993). While GnRH stimulates both LH secretion (Wildt *et al.*, 1981) and synthesis (Gharib *et al.*, 1990) *in vivo*, GnRH may influence FSH secretion primarily by stimulating its synthesis (Dalkin *et al.*, 1989; Rodin *et al.*, 1989). Alternatively, the apparent lack of minute-to-minute regulation of FSH by GnRH may be due to the much less rapid metabolic clearance of FSH than

of LH (Gay and Bogdanove, 1968; Bogdanove and Gay, 1969; Schwartz, 1983).

The results of the experiments reported here demonstrate that administration of oestradiol for 24 h suppresses LH pulse amplitude and mean concentrations significantly and tends to decrease pulse frequency for at least 24 h after removal of the implant. In contrast, FSH secretion was not suppressed at this time. These results expand on previous studies, which showed that mean LH concentrations in ovariectomized rats remain suppressed for several days after exposure to oestradiol (Legan *et al.*, 1975) and that FSH secretion is less susceptible to suppression by oestradiol than is LH (Campbell and Schwartz, 1979; Savoy-Moore and Schwartz, 1980). Thus, divergence in LH and FSH secretion after ovariectomy may be due to a prolonged suppressive effect of oestradiol on LH, but not on FSH. The lag in the rate of LH rise during the first week after ovariectomy was shown not to be merely caused by a suppressive effect of any remaining circulating oestradiol of adrenal or gonadal origin by previous work: adrenalectomy and oestrogen antagonist treatment superimposed on ovariectomy do not change the pattern of LH rise (Savoy-Moore and Schwartz, 1980).

The fact that pulsatile LH secretion remains suppressed 24 h after oestradiol concentrations are no longer increased (present study) and that LH surges are seen for 4 days after exposure to oestradiol for 29 h (Legan *et al.*, 1975) suggests that in cyclic rats the high dioestrous and pro-oestrous oestradiol concentrations are necessary not only as a signal for the preovulatory



**Fig. 4.** Summary of (a–c) pulsatile LH and (d–f) FSH release from Expt 2. Day 3 and day 7 ovariectomized rats received either blank (■) or crystalline oestradiol-filled (▨) implants ( $n = 4$  per group) for 24 h beginning at 09:00 h. Blood samples were withdrawn every 6 min from 09:00 to 12:00 h one day after the implants were removed day 5, or day 9. (a) and (d) represent the mean  $\pm$  SEM plasma concentrations for each of the four experimental groups during the 3 h bleeding period; (b) and (e) show the mean  $\pm$  SEM pulse amplitude for the 3 h period, and (e) and (f) the mean  $\pm$  SEM pulse frequency for the 3 h period. (a) Mean concentrations of LH and (c) LH pulse frequency were significantly higher on day 9 than on day 5 after ovariectomy ( $P < 0.003$ ;  $P < 0.02$ ). Mean concentrations of LH and (b) LH pulse amplitude were significantly decreased in the oestradiol treated animals compared with the controls ( $P < 0.004$ ;  $P < 0.02$ ). Suppression of LH pulse frequency approached significance ( $P = 0.076$ ). There was no significant effect of day or treatment on FSH.

gonadotrophin surges, but also for the maintenance of low LH concentrations during the remainder of the cycle. If the concentrations of oestradiol responsible for positive feedback did not exert a prolonged suppressive effect on LH, LH concentrations might remain high or rise quickly after oestradiol concentrations fall on the afternoon of pro-oestrus. Undoubtedly, progesterone also plays a crucial role in negative feedback during the oestrous cycle. The increase in progesterone after oestrus has been shown to prevent LH surges from recurring on subsequent days (Freeman *et al.*, 1976), and treatment with progesterone superimposed on treatment with oestradiol enhances the suppression of LH by oestradiol (Savoy-Moore and Schwartz, 1980). Although administration of dioestrous concentrations of progesterone alone at the time of ovariectomy prevents the small increase in LH pulse amplitude that occurs 24 h later (Leipheimer *et al.*, 1984), even pro-oestrous concentrations of progesterone inhibit the increase in mean LH concentrations observed at 4 days after ovariectomy only slightly in the absence of oestradiol (Goodman, 1978). Thus, it may be that the prolonged suppressive effect of oestradiol on LH pulse amplitude observed here

would be enhanced in the presence of progesterone; however, it is unlikely that the LH lag could be due to the effects of progesterone alone as we have shown using the progesterone antagonist RU 486 (Luderer and Schwartz, 1991).

The prolonged suppression of LH by oestradiol presumably occurs via a genomic mechanism of action (Evans, 1988). Treatment of ovariectomized rats with oestradiol *in vivo* suppresses  $\alpha$ , LH $\beta$  and FSH $\beta$  mRNA concentrations (Gharib *et al.*, 1990) and transcription rates (Shupnik *et al.*, 1988) in the pituitary. Systemic treatment with oestradiol also induces or inhibits the synthesis of proteins in the ventromedial nucleus and in the preoptic area of rats (Jones *et al.*, 1988). Whether the effects of oestradiol on the gonadotrophin subunit or hypothalamic protein synthesis persist after exposure to the steroid has ceased has not been tested. In *Xenopus*, however, a single injection of oestradiol has been shown to induce serum retinol-binding protein mRNA for at least 125 days, even though oestradiol concentrations return to control values within 24 h (McKearin and Shapiro, 1988).

The prolonged LH response to negative feedback of oestradiol, which this study demonstrates, is clearly unique to the

sexually differentiated female reproductive axis. Gay and Hauger (1977) showed that after the removal of implants of the sex-opposite steroid from gonadectomized adult rats, LH concentrations in males increased rapidly and LH concentrations in females showed the typical lag, disproving the hypothesis that the last steroid to which the hypothalamic-pituitary axis is exposed determines the LH response to gonadectomy. Prepubertal rats do not show the same sex difference as do adults: LH concentration increases rapidly in females and is delayed in males (Rabii and Ganong, 1976; Lorenzen and Ramaley, 1981), suggesting that the ability to respond to high oestradiol concentrations with a LH surge is not compatible with a rapid LH response to the removal of negative feedback and that the events at puberty that enable the females to respond to high oestradiol concentrations with a gonadotrophin surge are also responsible for the LH lag after ovariectomy.

In summary, these experiments have confirmed that there is a lag in the rate of rise in LH pulse amplitude and frequency as well as mean concentrations after ovariectomy which may last for as long as 9 days, and demonstrated that the increase in mean FSH concentrations that occurs during the first 4 days after ovariectomy is not due to increasing amplitude or frequency of FSH pulses, and that exposure to high circulating oestradiol concentrations for 24 h can suppress LH pulsatility, but not FSH pulsatility, for at least 24 h after removal of oestradiol. This finding suggests that the lag in the rate of LH rise during the first week after ovariectomy is probably caused by prolonged suppressive effects of oestradiol. Moreover, these results indicate that the high dioestrous and pro-oestrous oestradiol concentrations may be important not only for triggering the preovulatory gonadotrophin surges, but also for maintaining low concentrations of LH secretion during the remainder of the oestrous cycle.

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## References

- Blackwell RE and Amoss MS (1971) A sex difference in the rate of rise of plasma LH in rats following gonadectomy *Proceedings of the Society for Experimental Biology and Medicine* **136** 11–14
- Blake CA and Kelch RP (1981) Administration of anti-LHRH serum to rats: effects on periovulatory secretion of LH and FSH *Endocrinology* **109** 2175–2179
- Bogdanove EM and Gay VL (1969) Studies on the disappearance of LH and FSH in the rat: a quantitative approach to adeno-hypophysial secretory kinetics *Endocrinology* **84** 1118–1131
- Bourguignon JP, Gerard A and Franchimont P (1989) Direct activation of gonadotropin-releasing hormone secretion through different receptors to neuro-excitatory amino acids *Neuroendocrinology* **49** 402–408
- Brown-Grant K and Greig F (1975) A comparison of changes in the peripheral plasma concentrations of luteinizing hormone and follicle-stimulating hormone in the rat *Journal of Endocrinology* **65** 389–397
- Campbell CS and Schwartz NB (1979) Time course of serum FSH suppression in ovariectomized rats injected with porcine follicular fluid (folliculostatin): effect of estradiol treatment *Biology of Reproduction* **20** 1093–1098
- Charlesworth MC, Grady RR, Shin L, Vale WW, Rivier C, Rivier J and Schwartz NB (1984) Differential suppression of FSH and LH secretion by follicular fluid in the presence or absence of GnRH *Neuroendocrinology* **38** 199–205
- Condon TP, Sawyer CH and Whitmayer DI (1986) Episodic patterns of luteinizing hormone and follicle-stimulating hormone release: differential secretory dynamics and adrenergic control in ovariectomized rats *Endocrinology* **118** 2525–2533
- Culler MD and Negro-Vilar A (1986) Evidence that pulsatile follicle-stimulating hormone secretion is independent of endogenous luteinizing hormone-releasing hormone *Endocrinology* **118** 609–612
- Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR and Marshall JC (1989) The frequency of gonadotropin-releasing hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression *Endocrinology* **117** 917–924
- De Paolo LV (1985) Differential regulation of pulsatile luteinizing hormone (LH) and follicle-stimulating hormone secretion in ovariectomized rats disclosed by treatment with an LHRH antagonist and phenobarbital *Endocrinology* **117** 1826–1833
- Ellis GB and Desjardins C (1982) Male rats secrete luteinizing hormone and testosterone episodically *Endocrinology* **110** 1618–1627
- Ellis GB and Desjardins C (1984) Orchidectomy unleashes pulsatile luteinizing hormone secretion in the rat *Biology of Reproduction* **30** 619–627
- Evans RM (1988) The steroid and thyroid hormone receptor superfamily *Science* **240** 889–895
- Freeman MC, Dupke KC and Croteau CM (1976) Extinction of the estrogen-induced daily signal for LH release in the rat: a role for the proestrous surge of progesterone *Endocrinology* **99** 223–228
- Gallo RV and Bona-Gallo A (1985) Lack of ovarian steroid negative feedback on pulsatile luteinizing hormone release between estrus and diestrus day 1 in the rat estrous cycle *Endocrinology* **116** 1525–1528
- Gay VL and Bogdanove EM (1968) Disappearance of endogenous and exogenous luteinizing hormone activity from the plasma of previously castrated, acutely hypophysectomized rats: an indirect assessment of synthesis and release rates *Endocrinology* **82** 359–368
- Gay VL and Hauger RL (1977) A sex-related pattern of gonadotropin secretion in the castrated rat: effects of changing the inhibitory steroid or pituitary LH content *Biology of Reproduction* **16** 527–535
- Gay VL and Midgley AR (1969) Response of the adult rat to orchidectomy and ovariectomy as determined by LH radioimmunoassay *Endocrinology* **84** 1359–1364
- Gharib SD, Wierman ME, Shupnik MA and Chin WW (1990) Molecular biology of the pituitary gonadotropins *Endocrine Reviews* **11** 177–199
- Goodman RL (1978) A quantitative analysis of the physiological role of estradiol and progesterone in the control of tonic and surge secretion of luteinizing hormone in the rat *Endocrinology* **102** 142–150
- Higuchi T and Kawakami M (1982) Changes in the characteristics of pulsatile luteinizing hormone secretion during the oestrous cycle and after ovariectomy and oestrogen treatment in female rats *Journal of Endocrinology* **94** 177–182
- Jones KJ, McEwen BS and Pfaff DW (1988) Quantitative assessment of early and discontinuous estradiol-induced effects on ventromedial hypothalamic and preoptic area proteins in female rat brain *Neuroendocrinology* **48** 561–568
- Kartun K and Schwartz NB (1987) Effects of a potent antagonist to gonadotropin-releasing hormone on male rats: luteinizing hormone is suppressed more than follicle-stimulating hormone *Biology of Reproduction* **36** 103–108
- Legan SJ, Coon GA and Karsch FJ (1975) Role of estrogen as initiator of daily LH surges in the ovariectomized rat *Endocrinology* **96** 50–56
- Leipheimer RE and Gallo RV (1983) Acute and long-term changes in central and pituitary mechanisms regulating pulsatile luteinizing hormone secretion after ovariectomy in the rat *Neuroendocrinology* **37** 421–426
- Leipheimer RE, Bona-Gallo A and Gallo RV (1984) The influence of progesterone and estradiol on the acute changes in pulsatile luteinizing hormone release induced by ovariectomy on diestrus day 1 in the rat *Endocrinology* **114** 1605–1612
- Leipheimer RE, Bona-Gallo A and Gallo RV (1985) Ovarian steroid regulation of pulsatile luteinizing hormone release during the interval between the mornings of diestrus 2 and proestrus in the rat *Neuroendocrinology* **41** 252–257
- Levine JE and Duffy MT (1988) Simultaneous measurement of luteinizing hormone (LH)-releasing hormone, LH, and follicle-stimulating hormone release in intact and short-term castrate rats *Endocrinology* **122** 2211–2221
- Lorenzen JR and Ramaley JA (1981) Ontogeny of sex differences in LH and FSH levels 48 h after castration in the rat *American Journal of Physiology* **E241** 460–464

- Luderer U and Schwartz NB (1991) Sex differences in acute LH response to gonadectomy remain after progesterone antagonist and dopamine agonist treatment *Biology of Reproduction* **45** 918–926
- Luderer U, Strobl FJ, Levine JE and Schwartz NB (1993) Differential gonadotropin responses to *N*-methyl-D,L-aspartate in metestrus, proestrus and ovariectomized rats *Biology of Reproduction* **48** 857–866
- Lumpkin MD, DePaolo LV and Negro-Vilar A (1984) Pulsatile release of follicle-stimulating hormone in ovariectomized rats is inhibited by porcine follicular fluid (inhibin) *Endocrinology* **114** 201–206
- McKearin DM and Shapiro DJ (1988) Persistent estrogen induction of hepatic *Xenopus laevis* serum retinol binding protein mRNA *Journal of Biological Chemistry* **263** 3261–3265
- Nequin LG, Alvarez JA and Campbell CS (1975) Alterations in steroid and gonadotropin release resulting from surgical stress during the morning of proestrus in 5-day cyclic rats *Endocrinology* **97** 718–724
- Rabii J and Ganong WF (1976) Responses of plasma 'estradiol' and plasma LH to ovariectomy, ovariectomy plus adrenalectomy, and estrogen injection at various ages *Neuroendocrinology* **20** 270–281
- Rodin DA, Lalloz MRA and Clayton RN (1989) Gonadotropin-releasing hormone regulates follicle-stimulating hormone  $\beta$ -subunit gene expression in the male rat *Endocrinology* **125** 1282–1289
- Savoy-Moore RT and Schwartz NB (1980) Differential control of FSH and LH secretion. In *International Review of Physiology, Vol. 22. Reproductive Physiology III*, pp 203–248 Ed. RO Greep. University Park Press, Baltimore
- Schwartz NB (1983) Selective control of FSH secretion. In *Role of Peptides and Proteins in Control of Reproduction*, pp 193–213 Eds SM McCann and D Dhindsa. Elsevier Science Publishers, Amsterdam
- Shupnik MA, Gharib SD and Chin WW (1988) Estrogen suppresses rat gonadotropin gene transcription *in vivo* *Endocrinology* **122** 1842–1846
- Spitzbarth TL, Horton TH, Lifka J and Schwartz NB (1988) Pituitary gonadotropin content in gonadectomized rats: immunoassay measurements influenced by extraction solvent and testosterone replacement *Journal of Andrology* **9** 294–305
- Strobl FJ, Luderer U, Schwartz NB and Levine JE (1993) Differential gonadotropin responses to *N*-methyl-D,L-aspartate in intact and castrate male rats *Biology of Reproduction* **48** 867–873
- Tapper CM, Naftolin F and Brown-Grant K (1972) Influence of the reproductive state at the time of operation on the early response to ovariectomy on the rat *Journal of Endocrinology* **53** 47–57
- Van Cauter E (1988) Estimating false-positive and false-negative errors in analyses of hormonal pulsatility *American Journal of Physiology* **254** 786–794
- Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE and Knobil E (1981) Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey *Endocrinology* **109** 376–385