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Failure of oestrogen induced luteinizing hormone surge in women treated with mifepristone (RU 486) every day for 30 days

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It has been demonstrated previously that administration of the antiprogestin mifepristone (RU 486; 1–5 mg daily) inhibits or delays both the pre-ovulatory luteinizing hormone (LH) surge and ovulation. To investigate this mechanism, dynamic tests of pituitary ovarian function were performed in six healthy women before and during the administration of mifepristone (2 mg daily for 30 days). On day 9 of the control and treatment cycles, samples of blood were collected every 15 min over 12 h for measurement of LH concentration. After 10 h, the responsiveness of the pituitary was tested by the i.v. injection of 10 μg of gonadotrophin-releasing hormone (GnRH). On day 10 of the control and treatment cycles, two patches releasing 200 μg/day of oestradiol were applied to skin on the abdomen for 3 days. Blood was collected at 24, 48, 59, 72, 81 and 96 h after application of the oestrogen patches for the measurement of gonadotrophin and ovarian hormone concentrations. Follicular development continued in all women during their treatment with mifepristone, and ovulation was suppressed (four women) or delayed (two women). There was no significant difference in the basal concentration of LH between the control and treatment cycles (mean ± SE; 5.5 ± 0.4 versus 7.7 ± 0.4 IU/l respectively), or in the frequency (interpulse interval, 101 ± 12 versus 105 ± 13 min respectively) and the amplitude (2.1 ± 0.4 versus 2.6 ± 0.4 IU/l respectively) of LH pulses. The response to GnRH was similar. On day 10, the basal concentrations of LH, follicle-stimulating hormone (FSH), prolactin, oestradiol and progesterone and the diameter of the dominant follicle (15.7 ± 1.8 versus 13.3 ± 1.9 mm) were similar during control and treatment cycles. In control cycles, there were significant increases in the concentrations of LH and FSH within 72 h of application of the oestrogen patches. During treatment cycles, concentrations of FSH and LH remained low, and were significantly lower than the values observed during control cycles ($P < 0.006$). We conclude that the antiprogestin mifepristone disrupts ovulation by inhibiting the positive feedback effect of oestrogens and, hence, prevents or delays the generation of a pre-ovulatory LH surge.

Key words: luteinizing hormone pulses/mifepristone/oestrogen provocation

Introduction

Normal cyclical ovarian activity is dependent on a feedback system between the ovary and the hypothalamic–pituitary unit (Knobil, 1980). Follicular development is stimulated by luteinizing hormone (LH) and follicle stimulating hormone (FSH), the secretion of which is regulated by oestradiol, inhibin and progesterone. A key event in causing ovulation is the stimulation of a pre-ovulatory surge of LH that initiates a series of changes in the structure and function of the dominant follicle which result in rupture. While the principal cause of this discharge of LH is an increasing oestradiol concentration acting at the level of both the hypothalamus and the anterior pituitary, there is evidence in several species that progesterone may facilitate this action of oestradiol (Wildt *et al.*, 1981; Liu and Yen, 1983; Leyendecker *et al.*, 1990). In women, the progesterone concentration increases significantly around the onset of the midcycle LH surge due to increased secretion by the pre-ovulatory follicle (Hoff *et al.*, 1983; Djahanbakhch *et al.*, 1984). Progesterone facilitates the onset of an LH surge in women pretreated with oestradiol (Odell and Swerdloff, 1968; Chang and Jaffe, 1978), and it may be necessary for the concomitant release of FSH with LH (Aono *et al.*, 1976). The midcycle LH surge is delayed by the administration of a small dose of a progesterone receptor antagonist (mifepristone) in the late follicular phase of the cycle (Batista *et al.*, 1992a,b). The fact that this delay can be reversed by giving exogenous progesterone suggests strongly that endogenous progesterone may play a role in evoking the pre-ovulatory LH surge.

It has been reported that ovulation is inhibited when mifepristone is given to women for 30 days at a dose of 2–10 mg/day (Ledger *et al.*, 1992; Croxatto *et al.*, 1993). Follicular development continues, as indicated by the fluctuating quantities of oestrone excreted in the urine. An LH surge and ovulation occur within a few days of discontinuing the lowest dose (2 mg/day), suggesting that mifepristone inhibits the ability of oestradiol to evoke an LH surge. In the follicular phase, large doses (50–600 mg/day) of mifepristone suppress the secretion of LH and FSH, and follicular development is arrested (Liu *et al.*, 1987; Shoupe *et al.*, 1987; Luukkainen *et al.*, 1988; Permezel *et al.*, 1989). In this study we have investigated the pituitary–ovarian activity in women who were given 2 mg/day of mifepristone for 30 days. The pattern of pulsatile secretion of LH and FSH in the mid-follicular phase and the response to exogenous oestrogen (oestrogen provocation) were compared during both a control and a treatment cycle.

Materials and methods

Subjects

Six healthy female volunteers aged 25–35 years and of normal height (162–172 cm), weight (53.9–71.6 kg) and mean ± SE body mass

index (23.4 ± 1.3 kg/m^2) were recruited into this study. Informed consent was obtained for the study, which was approved by the local Ethical Committee. Barrier contraception was advised for women who had not been sterilized. None had used hormonal contraception within 3 months of entry to the study. All the women had a history of regular menstrual cycles (25–35 days). After a general physical examination, blood was collected for haematology and biochemical screening to exclude anaemia or any systemic disorder. Subjects were issued with a menstrual record card and were asked to record details of episodes of bleeding and any untoward symptoms throughout the study.

Study design

This study was conducted over three consecutive cycles. Each subject took two placebo capsules per day during the control and follow-up cycles. During the treatment cycle, they took two 1 mg mifepristone capsules per day for 30 days commencing on the day of onset of menses following the control cycle. Throughout the study the women, who were unaware of the order of placebo and treatment cycles, collected a sample of urine passed on rising (early morning urine), which was stored at –20°C until analysis.

On the morning of day 9 of the control and treatment cycles, women were admitted to the Clinical Research Unit of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh, UK. An indwelling catheter was inserted into a vein on the forearm and blood samples (3 ml) were collected every 15 min for 10 h. Between samples, the catheter was kept patent by filling it with 0.9% saline containing 1000 IU of heparin per 100 ml. After 10 h, 10 μg of gonadotrophin-releasing hormone (GnRH; Gonaderelin; Hoechst, Hounslow, UK) were injected as a bolus over ~15 s, and blood samples were collected every 15 min for a further 105 min.

On day 10 (next day) at 9 a.m., two (100 μg) estraderm patches (Ciba Laboratories, Horsham, UK) were applied to skin on the abdomen after collecting a blood sample. Further blood samples were collected for the measurement of gonadotrophin and ovarian hormone concentrations at 24, 48, 59, 72, 81 and 96 h after application of the estraderm patches. Ovarian ultrasound was performed on days 9 and 10 using an abdominal probe (3.5 mHz; Diasonics DRF 250, BM (Scotland) Ltd, Strathclyde, UK), and the number and size of follicles were recorded. The estraderm patches were removed from all patients after 72 h.

Hormone analysis

FSH, LH, oestradiol, progesterone and prolactin were measured by radioimmunoassays, using methods which have been published previously by our centre (Yong *et al.*, 1992). The intra- and interassay coefficients of variation were 8.4, 6.9, 8.0, 8.0 and 4.2 versus 9.7, 8.8, 11.0, 10.0 and 6.5% respectively.

Throughout the study, subjects collected a daily sample of early morning urine. Aliquots were stored frozen at –20°C until assayed for urinary LH, oestrone glucuronide and pregnanediol glucuronide (Yong *et al.*, 1992). The intra- and interassay coefficients of variation were 6.9, 6.0 and 10.0 versus 9.7, 9.0 and 13.0% respectively. Concentrations of urinary steroids were expressed as a ratio of the creatinine concentration measured colorimetrically (Jaffe reaction).

Data analysis

The length of the follicular phase of a particular cycle was defined as the number of days from the first day of menses to the day of the LH peak in urine inclusive. The luteal phase was calculated as the interval between the LH peak and the day before the onset of menses. Two separate methods were used to identify LH pulses in blood samples collected during the period of frequent sampling on day 9. In our original method, a pulse was defined as occurring when the two consecutive samples exceeded the mean of the two previous samples (basal), and the value of at least one (peak) exceeded the basal value by at least twice the coefficient of variation of the assay (Baird *et al.*, 1977). The second method involved the use of a Munro Pulsar program in which the G parameters were set at $G_1 = 4.0$, $G_2 = 3.0$, $G_3 = 2.5$, $G_4 = 2.0$ and $G_5 = 1.5$ (Merriam and Wachter, 1982). There were no significant differences detected by either method between the number of pulses, the height of pulses or the interpulse interval. The sensitivity of the anterior pituitary to GnRH was calculated as the difference in LH and FSH between the basal and peak values following GnRH injection. Statistical analyses were performed using a paired t-test, an analysis of variance (ANOVA), a Newman–Keuls test or a Wilcoxon sign rank test as appropriate.

Results

In the control cycle, all women showed a normal ovulatory cycle with a midcycle urinary LH peak (mean ± SE; 5.0 ± 1.1 IU/l) and a luteal phase of normal length (14.5 ± 0.8 days). There was no significant difference in the diameter of the dominant follicle on day 10 between control and treatment cycles (15.7 ± 1.8 versus 13.3 ± 1.9 mm). However, ovulation was inhibited in four of the six women during the treatment cycle, as indicated by low concentrations of pregnanediol in urine (Figure 1). In these four patients, the oestrone excretion showed a biphasic pattern, with the concentration decreasing after a peak on day 12 before increasing again during the last 10 days of treatment. An LH surge occurred within 4 days of discontinuing mifepristone administration and ovulation occurred, as indicated by the rise in pregnanediol excretion. The length of the luteal phase in the follow-up cycle was similar to that in the control cycle in these four women (12.7 ± 1.3 versus 13.8 ± 0.7 days; not significant).

Two women showed endocrine changes indicative of ovulation during the treatment cycle (Figure 2). The LH surge was delayed by 6 and 2 days respectively, and the luteal phases were shorter compared with their control cycles (12 and 11 versus 14 and 18 days). Normal ovulatory cycles occurred in both these patients on discontinuing the treatment (luteal phase length 14 and 16 days respectively).

Pulsatile luteinizing hormone secretion and response to gonadotrophin-releasing hormone

In control and treatment cycles, LH pulses occurred at intervals of ~100 min (101 ± 12 and 105 ± 13 min respectively; Figure 3). Both LH pulse amplitude (2.1 ± 0.4 and 2.6 ± 0.4 IU/l) and mean basal concentration (5.5 ± 0.4 and 7.7 ± 0.4 IU/l) were slightly higher in the treatment cycle ($P > 0.05$; not significant). The mean area of the pulses was increased after treatment with mifepristone (556 ± 86 versus 890 ± 148 arbitrary units; $P < 0.04$; Wilcoxon sign rank test). There was no significant difference in the response to GnRH, although there was a considerable variation in response between individuals (Figure 4). The response to GnRH was greater in those mifepristone cycles where the concentration of oestradiol was lower (Figure 3, subject 6).

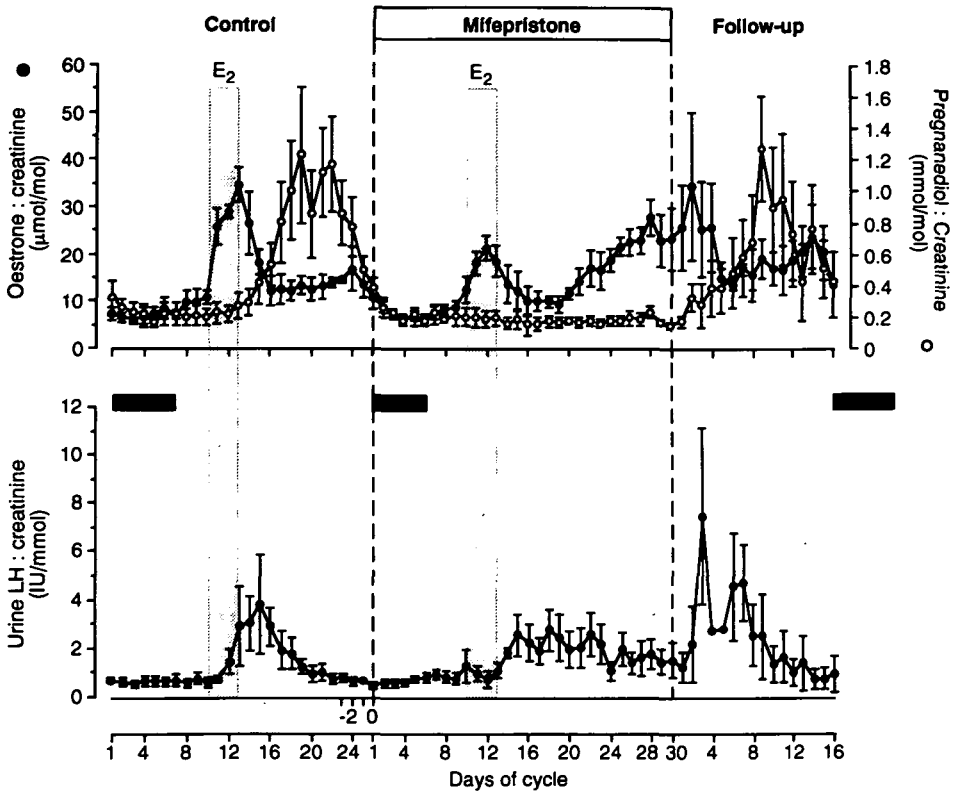

Figure 1. Urinary excretion of oestrone, pregnanediol glucuronide and luteinizing hormone (LH; mean ± SE) in four women before (control) and after (follow-up) the oral administration of mifepristone (2 mg/day). Two oestradiol (E_2) patches releasing a total of 200 µg oestradiol/day were applied to the skin on days 10, 11 and 12, as indicated by the stippled bars. The black bars indicate menstrual bleeding.

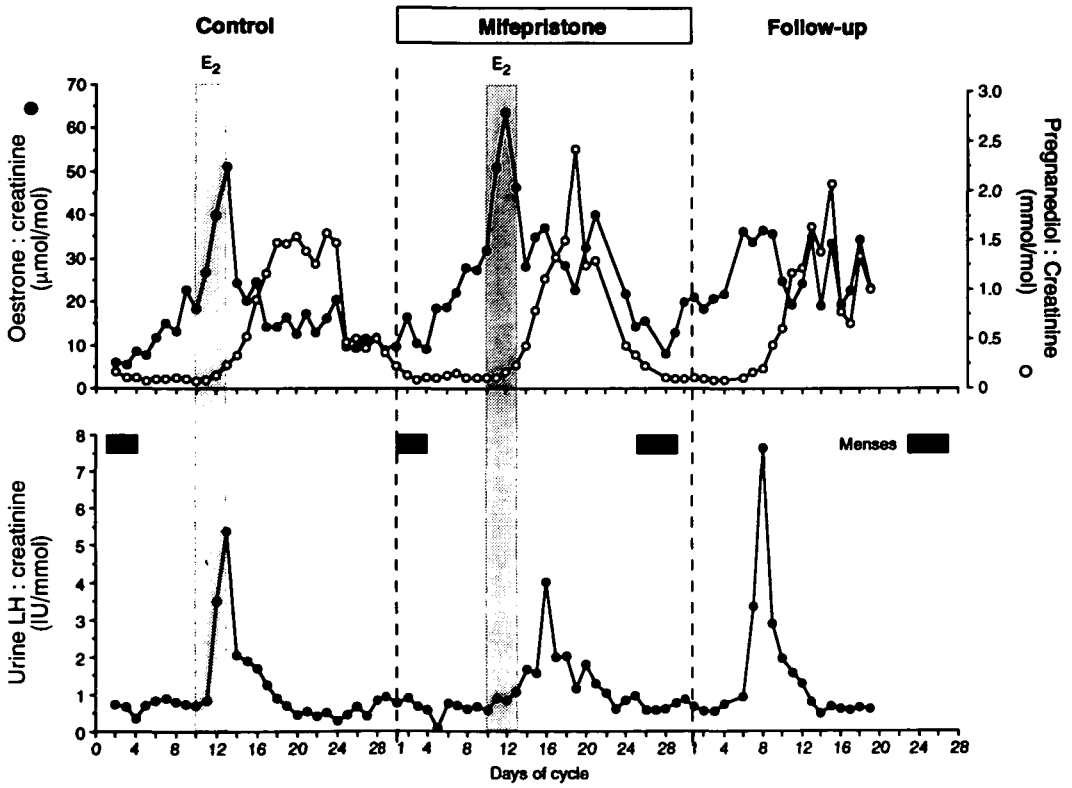

Figure 2. Urinary excretion of oestrone, pregnanediol glucuronide and luteinizing hormone (LH) in a woman who ovulated during the administration of mifepristone. Two oestradiol (E_2) patches releasing a total of 200 µg oestradiol/day were applied to the skin on days 10, 11 and 12, as indicated by the stippled bars. The black bars indicate menstrual bleeding.

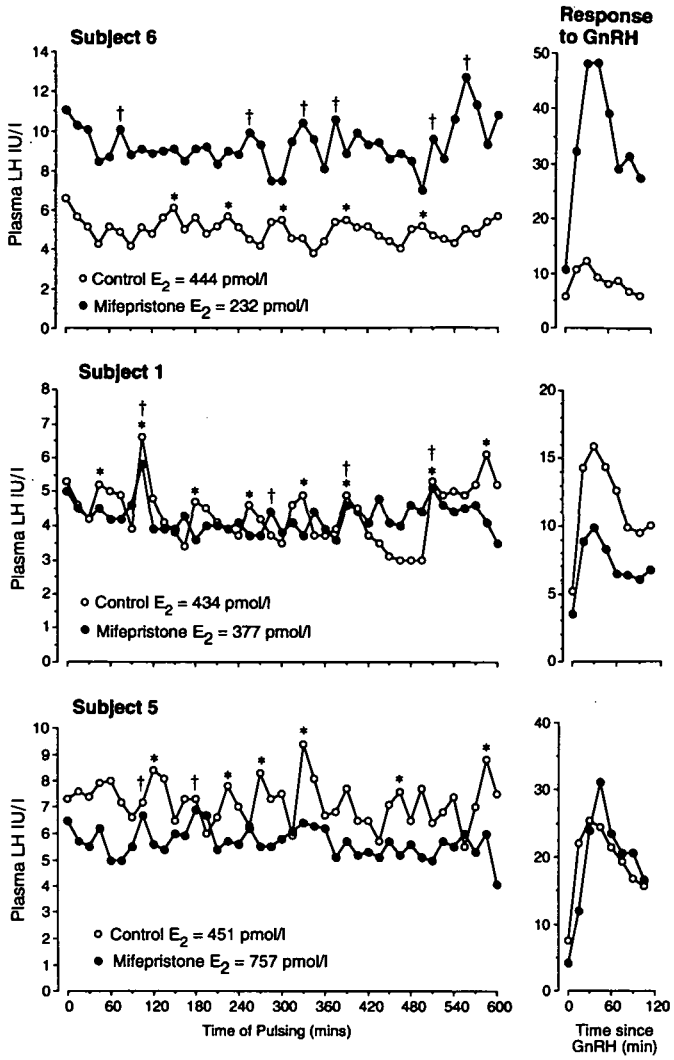

Figure 3. Concentration of luteinizing hormone (LH) in samples of plasma collected every 15 min from three women on day 9 of control (○) and treatment (●) cycles. The concentration of oestradiol (E_2) at the start of the collection is indicated. The response to i.v. injection of a bolus of 10 μg of gonadotrophin-releasing hormone (GnRH) after 10 h is illustrated on the right side of the figure. Significant pulses of LH during control (*) and treatment (†) cycles are indicated by the symbols.

Response to oestrogen provocation

There was no significant difference in the mean basal concentrations of LH, FSH, prolactin, oestradiol and progesterone on days 9 or 10 between control and treatment cycles (Figure 5). Following application of the oestradiol patches, there was an increase in the oestradiol concentration in both control and treatment cycles ($P < 0.0004$). After 48 h, values in control cycles were significantly higher than during treatment with mifepristone ($P < 0.003$). In control cycles, this was followed by an increase in LH and FSH concentrations, which peaked 48–72 h later. In contrast, during the mifepristone cycle, LH concentrations remained low until the patches were removed 72 h later. FSH concentrations were significantly lower when compared with the values on day 10 ($P < 0.01$; Newman–Keuls). Compared with the control cycle, LH and FSH concentrations were significantly lower in the mifepristone cycle over

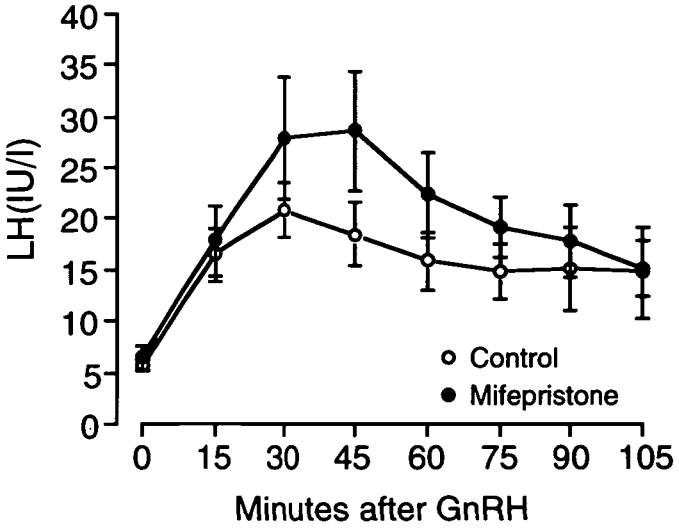

Figure 4. The mean ± SE concentration of luteinizing hormone (LH) in six women after i.v. injection of a bolus of 10 μg of gonadotrophin-releasing hormone (GnRH) on day 9 of the control cycle and during treatment with mifepristone (2 mg/day).

the time of oestradiol patch application ($P = 0.006$; ANOVA). Two women (subjects 1 and 4) complained of hot flushes and sweats for up to 5 days after removing the patches.

The excretion of oestrone, pregnanediol and LH in urine showed similar responses to plasma hormones (Figure 6). The LH excretion was significantly lower during the 72 h of oestradiol patch application in treatment compared with control cycles ($P = 0.004$; ANOVA). The peak LH concentration in urine was delayed by 3 days compared with the control cycle (13.8 versus 16.8 days; $P < 0.02$; paired t-test), although there was no significant difference in the magnitude of the peak (5.01 versus 4.32 IU/mmol creatinine).

In control but not treatment cycles, progesterone concentration increased significantly 72–96 h after applying the oestradiol patch. There were no significant changes in the prolactin concentration in either control or treatment cycles (Figure 5).

Discussion

A normal ovulatory cycle occurred in all six women during their control cycle when an oestradiol patch which delivered 200 μg oestradiol/day for 3 days was administered. The concentration of oestradiol on day 12 was >1000 pmol/l, which is higher than the mean concentration expected in the late proliferative phase of a normal cycle (Figure 5). In all women, a surge of LH and FSH occurred within 48 h of applying the oestradiol patches, the magnitudes being comparable with those of a normal midcycle. The subsequent increases in progesterone concentration and pregnanediol excretion suggest strongly that ovulation occurred, although direct confirmation using ultrasound measurements was not available. It is likely that although administration of the oestradiol patch advanced the onset of the LH surge by ~48 h compared with a spontaneous cycle, by day 10 the dominant follicle was capable of ovulating in response to the LH surge.

In the four women who failed to ovulate during treatment

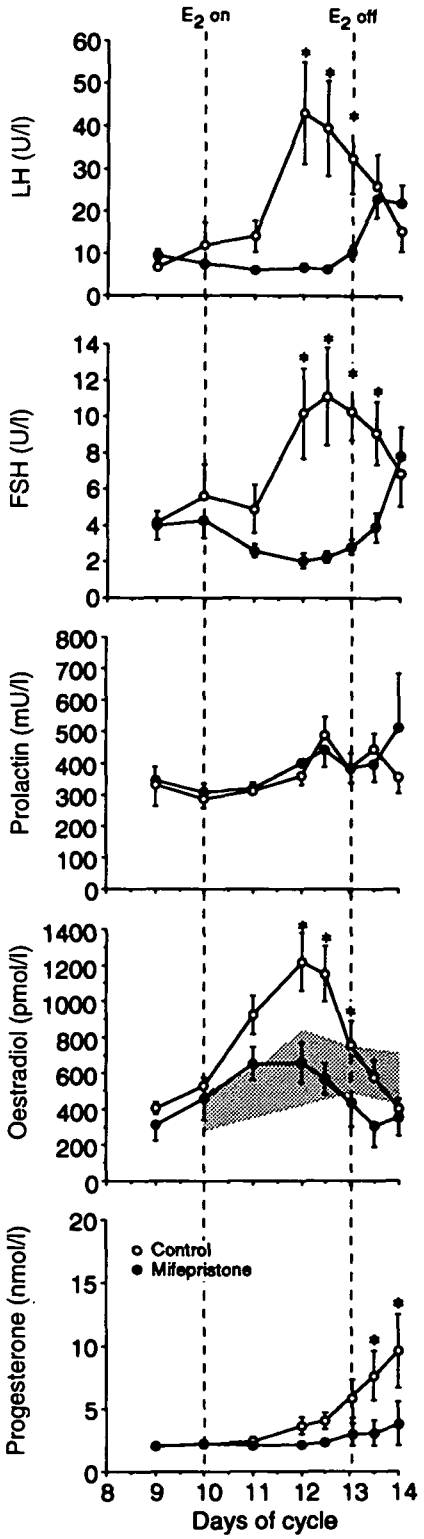

Figure 5. Concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, oestradiol and progesterone (mean ± SE) in the plasma of six women during an oestrogen provocation test. Two oestradiol (E_2) patches releasing a total of 200 μg oestradiol/day were applied to the skin between the points indicated by E_2. The shaded area indicates two SE from the mean of oestradiol concentration in the plasma of six women during normal cycles. * Significant difference between control and mifepristone cycles ($P < 0.05$).

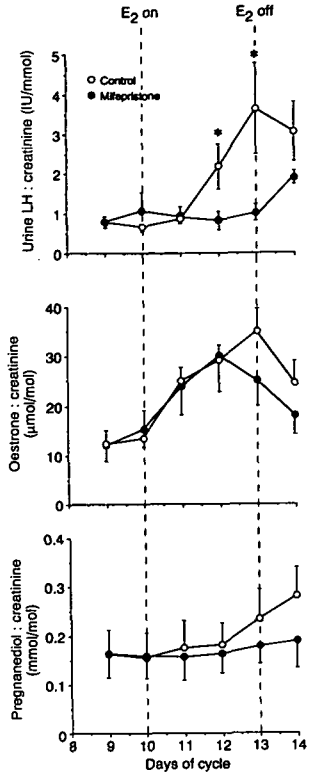

Figure 6. Excretion of oestrone, pregnanediol glucuronide and luteinizing hormone (LH; mean ± SE) in the urine of six women during an oestrogen provocation test. Two oestradiol (E_2) patches releasing a total of 200 μg oestradiol/day were applied to the skin between the points indicated by E_2. * Significant differences between control and mifepristone cycles ($P < 0.05$).

with mifepristone, increases in the excretion of oestrone in urine and the concentration of oestradiol in plasma during oestradiol patch application were less than in the control cycle (Figures 1, 5 and 6). As the quantity of oestradiol released by the patch (200 μg/day) was identical in each cycle, this indicates that there was a suppression in the secretion of oestradiol by the ovary. The most likely explanation is that after the administration of exogenous oestradiol during the treatment cycle, development of the dominant follicle was inhibited because of a suppression of FSH. The decline in excretion of oestrone in urine after day 12 reflects the collapse of large follicles, and the progressive increase in oestrone excretion after day 20 reflects the growth of a new large follicle (Figure 1). In the two women who ovulated during the treatment cycle, concentrations of oestrone were similar to those observed during the control cycle (Figure 2).

Although the midcycle LH surge was delayed or suppressed in all subjects, there was no significant effect on the basal concentrations of LH and FSH on days 9 or 10 during treatment with mifepristone. Larger doses of mifepristone (25–600 mg) administered during the follicular phase of the cycle consistently cause suppression of both FSH and LH, and follicular growth is terminated (Liu *et al.*, 1987; Permezel *et al.*, 1989). However, previous studies using daily doses of 1–10 mg of mifepristone have demonstrated no significant effect on the basal concentrations of LH or FSH, although only single daily

blood samples were analysed (Croxatto *et al.*, 1993). In our study, a detailed analysis of the pattern of pulsatile LH secretion did not reveal any differences between control and mifepristone cycles in the frequency or amplitude of LH pulses. It is unlikely, therefore, that impairment of the oestrogen-induced LH surge is due to an effect of mifepristone on the hypothalamus.

In this study, the magnitude of the LH increase in response to GnRH on day 9 of the mifepristone cycle was highly variable between subjects (Figure 4). In some women, in whom the increase in oestradiol concentration during the follicular phase was delayed (e.g. subject 6), the basal concentration of LH was raised and the response to GnRH exaggerated. Thus, interpretation of the effect of mifepristone on the pituitary responsiveness to GnRH is difficult because of the variation in the endocrine environment between individuals. The fact that there was no significant change in the amount of LH released by exogenous GnRH demonstrates that inhibition of the oestrogen-induced LH surge by mifepristone is not caused by decreased responsiveness of the pituitary to GnRH.

In control cycles, there was an increase in the concentrations of LH and FSH within 48 h of application of the oestradiol patches. The magnitude of this surge was similar to that which occurs in spontaneous ovarian cycles and was sufficient to induce ovulation as indicated by the progressive increases in progesterone concentration in plasma and excretion of pregnanediol in urine. In the mifepristone cycle, lack of an LH surge, despite oestradiol concentrations within the range found during normal spontaneous cycles (Figure 5), indicates a suppression of the positive feedback mechanism by which oestrogen induces a release of LH from the anterior pituitary. The mechanism of this positive feedback effect of oestrogen is not entirely clear, but evidence from a number of species suggests that it is caused by both an increased discharge of GnRH from the hypothalamus and a facilitation of the LH discharge from the pituitary (Knobil, 1980; Fink, 1988). Progesterone receptors are present in both hypothalamus and anterior pituitary, and hence mifepristone could act at either of these sites. The failure to observe a significant change in the frequency or amplitude of LH pulses during the treatment cycle makes it unlikely that mifepristone at this dose has a significant effect on the hypothalamus. Rather, mifepristone may inhibit or delay the LH surge by reducing directly the sensitivity of pituitary gonadotroph to the positive feedback effects of oestrogen. In keeping with this observation is the fact that progesterone can facilitate an LH discharge in oestrogen-treated post-menopausal women (Odell and Swerdloff, 1968; Aono *et al.*, 1976) and overcome the inhibitory effect of mifepristone (Batista *et al.*, 1992a,b). The fact that mifepristone delays the onset of the LH surge in women with hypothalamic hypogonadism, in whom follicular development and ovulation are induced by exogenous GnRH, suggests that the pituitary is the main site of action (Batista *et al.*, 1994). This inhibitory effect of mifepristone cannot be due solely to the decreased sensitivity of the gonadotroph to GnRH because the response to exogenous GnRH was increased slightly (not significantly). These studies illustrate the complex nature of the positive feedback mechanism which involves a coordinated simultaneous discharge of LH by thousands of individual gonadotrophs (Fink, 1988).

In our study, an impairment of the ability of oestrogen to provoke an LH surge was demonstrated in all women during treatment with mifepristone. However, follicular development continued and ovulation occurred eventually in two patients after a delay of ~7 days. Thus, if used as a contraceptive, these women could potentially be at risk of pregnancy, although it is unknown whether the follicle would ovulate a healthy oocyte capable of being fertilized. It is likely that even at low doses, mifepristone may prevent implantation due to a direct inhibitory effect on the formation of a normal secretory endometrium (Batista *et al.*, 1992a,b; Cameron *et al.*, 1994). However, it seems likely that a dose of $\geq$5 mg of mifepristone may be necessary if the inhibition of follicular growth and the suppression of ovulation are to be achieved reliably (Baird, 1993).

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