

## RESPONSE OF PLASMA ESTRADIOL-17 $\beta$ TO SYNTHETIC FSH-LH/RH: A PRELIMINARY STUDY§

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### SUMMARY

A group of progesterone positive secondary amenorrhic patients was compared to 2 oligomenorrhic patients concerning the response to a single i.v. injection of 100  $\mu$ g of synthetic FSH-LH/RH. It was found that in all of them, plasma estradiol-17 $\beta$  (E<sub>2</sub>) fell immediately following the injection of FSH-LH/RH and frequently showed a rebound. In the oligomenorrhic patients E<sub>2</sub> rose, with a peak at 75 min. In some amenorrhic patients the fall in E<sub>2</sub> preceded the rise in LH suggesting a direct effect of the hypothalamic hormone on the ovary.

### INTRODUCTION

The effect of synthetic follicle stimulating hormone-luteinizing hormone/releasing hormone (FSH-LH/RH) upon pituitary function has been extensively documented in the literature. However, only a very limited number of reports have included data on concomitant steroid assays which allow the study of gonadal response to the artificially induced release of endogenous gonadotropins. A simultaneous evaluation of the pituitary-gonadal axis, as a whole, in response to synthetic FSH-LH/RH may offer interesting information both on physiologic and pathogenic grounds. The purpose of this presentation is a preliminary report of such an investigation in women.

### MATERIALS AND METHODS

Six patients with well established amenorrhea were selected for this study in view of their steady hormonal state that would allow a better interpretation of the results. They all had a withdrawal bleeding after medication with progesterone (progesterone positive) indicating that they all shared the same common denominator, i.e. they were not hypoestrogenic. Moreover, since these patients had no other detect-

able relevant source of estrogens it could be assumed that their ovaries were not inactive. In fact, despite variations in the etiology of amenorrhea among these patients, the only important feature that they all had in common in order to be included in this study was a responsive ovary, since the last target organ to be investigated was precisely the gonad. Two patients with oligomenorrhea characterized by delayed ovulations were studied for comparison, in the hope that longer preovulatory phases of the cycle might offer a convenient model to interpret the response of follicles that were destined to ovulate. Plasma FSH, LH and estradiol-17 $\beta$  (E<sub>2</sub>) were used as parameters.

#### *Assay of plasma estradiol*

Plasma E<sub>2</sub> was measured by a known radioimmunoassay [1]. A highly specific antiserum was used [2, 3].

#### *Collection of blood samples*

After a resting period of 30 min, starting at 10 a.m., two blood samples were collected 15 min apart with the patient in a lying position. A second sample was withdrawn immediately before i.v. injection of 100  $\mu$ g of synthetic FSH-LH/RH (Serono) over a period of 30 s. Additional blood samples were collected every 15 min up to 3 h. In some patients additional samples were collected every 3 min from 15 min before to 15 min after the injection (from time -15 to +15 min). Whenever possible, blood plasma from several patients was frozen and assays run in duplicate at the same time.

#### *Assay of FSH and LH*

Radioimmunoassay of FSH and LH was done according to the methods described by Midgley [4, 5] using kits available from Serono Laboratories, Rome, Italy.

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§ Most of these observations were first reported during the "Serono Symposium on Hypothalamic Hormones", Milan, Italy, Oct. 14-16, 1974 (Book of Abstracts) and the "First Intern. Congress on Human Reproduction", Rio de Janeiro, Brazil, Nov. 11-14, 1974 (Excerpta Medica I.C.S. No. 370-in press).

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## RESULTS

Although this is a small group of amenorrheic patients compared to an even smaller group of controls, there are differences between these two groups in what concerns the response of  $E_2$  to the rise of LH and FSH stimulated by synthetic FSH-LH/RH.

Whereas in the patients shown in Figs. 1, 2a, 2b, 3 and 6 the fall in  $E_2$  was concomitant with the elevation of LH in all four and of FSH in three (Figs. 1, 3, and 6), the same was not observed in those illustrated in Figs. 4 and 5 in whom the drop in  $E_2$  preceded the rise in gonadotropins. Except for the case represented in Fig. 1, in whom only 4 samples were collected for  $E_2$  assay, the drop of  $E_2$  was followed by a rebound phenomenon in five tests (Figs. 2a, 2b, 3, 5 and 6) and by a steady low level in one test (Fig. 4). The lowest level of  $E_2$  was generally reached between 45 and 60 min after the injection FSH-LH/RH.

Patients G.P. (Fig. 2b) was reinvestigated in more detail two months later in order to clarify the relationships between  $E_2$ , FSH and LH. It was noticed that before the injection of FSH-LH/RH the fluctuations of  $E_2$  and LH were parallel, whereas between +15 and +30 (Fig. 2c) the fluctuations were the reverse of the depressive effects that were previously observed.

The response found in the oligomenorrheic patients (Fig. 7) showed a rise in  $E_2$  following a rise in LH as expected from stimulation with FSH-LH/RH.

## DISCUSSION

As a general interpretation of these results, it might be assumed that the amenorrheic patients had follicles which were much less developed than those of oligomenorrheic patients, with delayed ovulations. Thus, the effect of FSH-LH/RH (directly, or through LH) might consist of, first, inhibition of estrogen biosynthesis in follicles starting to undergo atresia or in im-

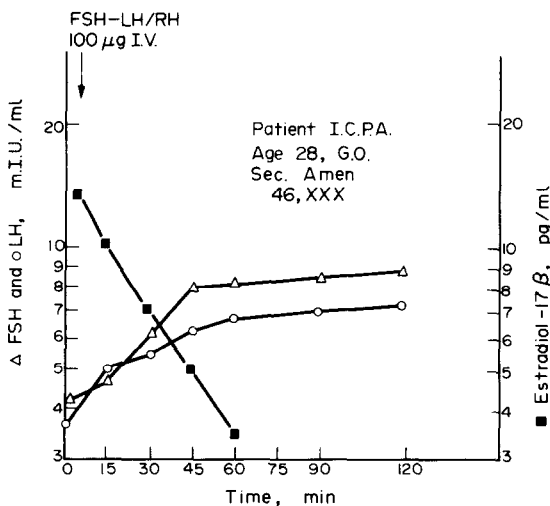


Fig. 1.

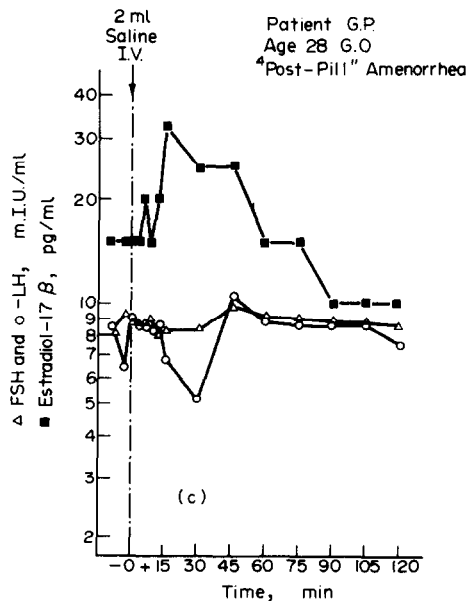
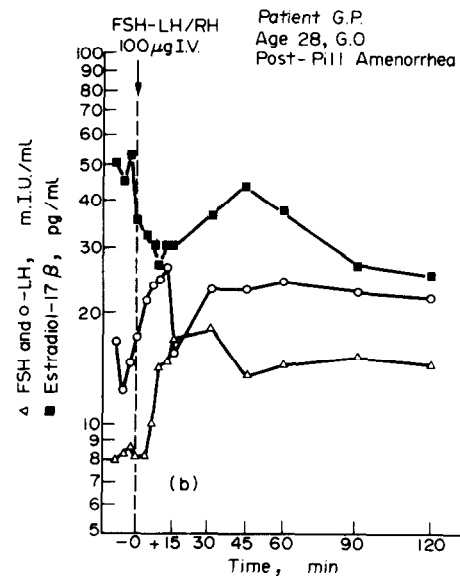
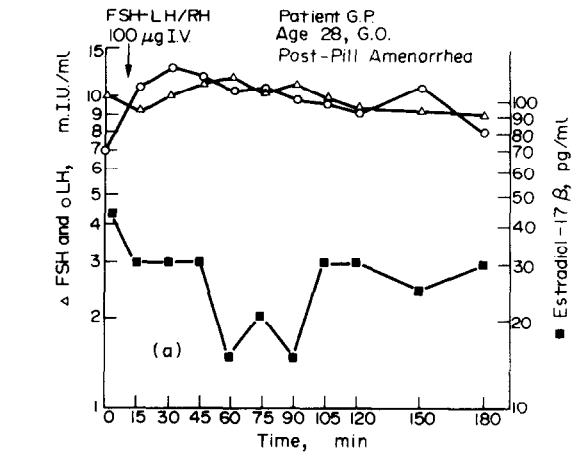


Fig. 2.

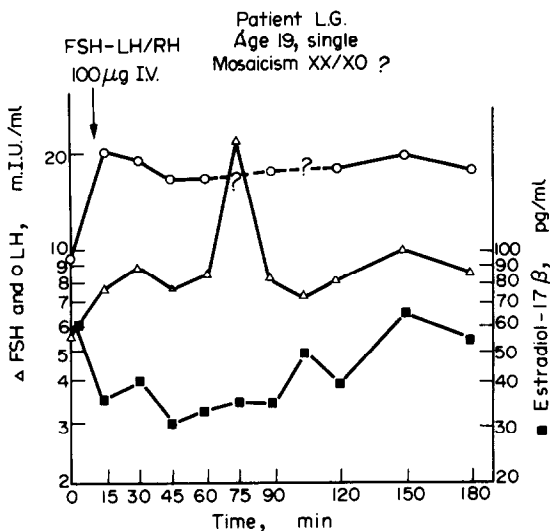


Fig. 3.

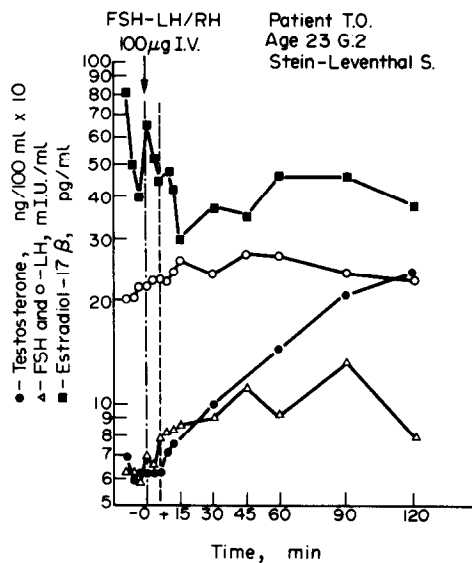


Fig. 6.

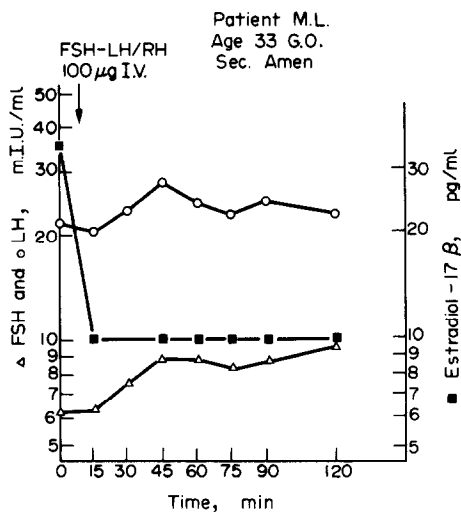


Fig. 4.

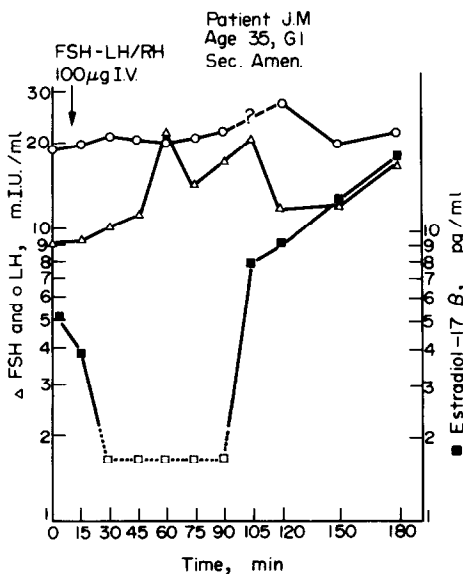
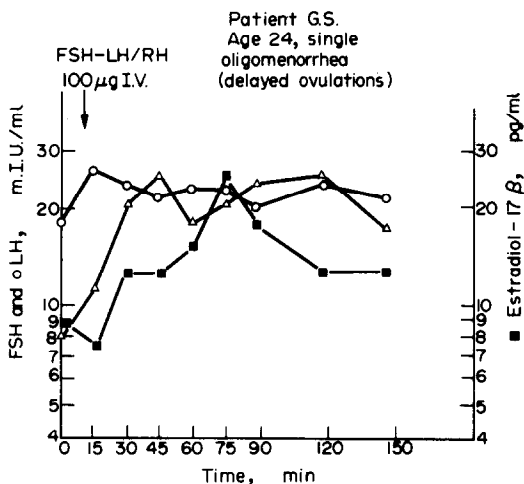


Fig. 5.

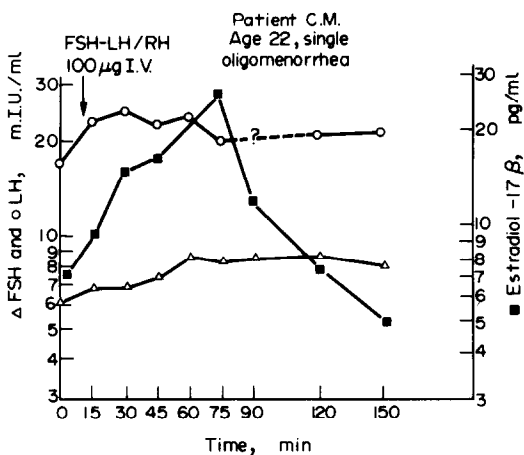


Fig. 7.

mature follicles [6] and, second stimulation of follicles responding positively to gonadotropins because of a higher local concentration of estrogens, according to the findings of Harman and Ross[13], and Sanyal and Taymor[14].

In the group of amenorrheic patients, there was a drop in  $E_2$  following the injection of FSH-LH/RH which in two out of six tests did not accompany the rise in FSH and LH (Figs. 4 and 5). This observation may suggest a direct effect of the hypothalamic hormone on the ovary [6]. Such a possibility of a rapid direct effect of FSH-LH/RH on the ovary would be in accordance with its short half-life of 2.7 min (by bioassay) and its complete disappearance from the circulation after 20 min (by bioassay) [7, 8]. However, if one considers the remaining four tests, the drop in  $E_2$  was simultaneous with the rise in LH (and FSH) (Fig. 2b). This could be explained by the findings of Hay and Moor[9, 10] that LH inhibits estrogen secretion from sheep graafian follicles, both *in vitro* and *in vivo*, and stimulates the production of cAMP and progesterone. It is relevant to emphasize that LH exerts its effect on the ovary *via* cAMP [11]. Therefore it would appear that FSH-LH/RH could influence ovarian steroid biosynthesis either directly or through the release of pituitary gonadotropins.

The observations of Katz and Carr[10] who found a rise in plasma levels of  $E_2$ , in a similar group of patients under the same experimental conditions, do not invalidate these results since the authors only published the concentrations of  $E_2$  found at times +240 and +360 min. They were probably measuring during the rebound period also found by us (Fig. 5) and may have missed the initial stages of depression. The case illustrated in Fig. 6, a patient with a typical Stein-Leventhal syndrome (with hirsutism), was interesting since it showed that plasma testosterone levels rose uniformly after time +6 min when the fall in  $E_2$  was apparent in association with the rise in LH. The values of  $E_2$  from time -9 to +6 min should be considered as the expression of a fluctuating secretory pattern. It is interesting that in patient G.P. (Fig. 2b), there seemed to be a biphasic secretory LH pattern in forming a mirror image of what was seen for  $E_2$ . It was observed that FSH did not fluctuate whereas LH fell between times +15 and +30, while  $E_2$  rose.

When this patient was taken as its own control (Fig. 2c) and injected with saline only it is evident that the results obtained in her two previous stimulations (Figs. 2a and 2b) are significant since the fluctuations of  $E_2$  and LH were divergent. Thus, at times +3, +6 and +9 min there was a drop in  $E_2$  accompanying a rise in LH. A drop in LH at time +15 min was concomitant with a rise in  $E_2$ . The secretion of LH after the stimulation followed a biphasic pattern.

The oligomenorrheic patients (Fig. 7) responded differently, both showing an  $E_2$  peak at 75 min. They

both had a menstrual bleeding two weeks later probably because they were either at the time of ovulation or because they ovulated in response to FSH-LH/RH.

## CONCLUSIONS

The results show that FSH and LH can either stimulate the secretion of  $E_2$  or depress it (followed or not by a rebound phenomenon) depending on the functional status of the ovary.

The presented results may offer an explanation for the physiological fall in  $E_2$  prior to the mid-cycle LH peak [15].

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