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CLINICAL AND FUNCTIONAL CORRELATION OF PLASMA STEROIDS—DIAGNOSTIC USES

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SUMMARY

Plasma hormone quantification by saturation analysis can be employed to explain and interpret some aspects of ovarian physiology—I. Estradiol (E-2) and 17-hydroxyprogesterone (17 OH-P) have been evaluated in ovulatory and anovulatory cycles as indices to predict ovulation, the latter being more reliable—II. In another group, a single progesterone assay made 72 h after the elevation of the basal body temperature, of greater than 5·7 ng/ml, was sufficient for assessing ovulation, supported by endometrial histology and/or culdoscopy—III. In a group of patients with polycystic ovaries 17 OH-P peripheral levels were found to be 8–10 times higher than in women with normal ovaries; when both groups of women received glucocorticoids to suppress an adrenal source, 17 OH-P and FSH levels did not change significantly, though LH was considerably suppressed—IV. The E-2/testosterone (T) ratio was also evaluated in the women with polycystic ovaries who were given antiestrogens to induce ovulation; a mean E-2 plasma concentration of 0·048 ng/ml was necessary to produce an effect, but if testosterone concentration was >1·0 ng/ml, there could be a negative response—V. The peripheral estrogen levels in some diseases classified as "estrogen dependent" do not confirm the description "hyperestrogenemia" and, according to our results, they are better referred to as conditions with hyperestrogenic tissue concentrations.

Plasma hormone quantification by means of competitive protein binding or radioimmunoassay offers added sensitivity for explaining and interpreting some aspects of ovarian physiology [1–12]. Several endocrine studies have been carried out throughout the menstrual cycle using better and more precise methodology to measure daily blood steroid hormone concentration, providing an opportunity to understand some physiological and key events [10, 13–21]. Evaluation of the usefulness of these studies possible for diagnostic and therapeutic purposes is the intention of the present report. This report summarizes some of the results of studies concerned with ovarian steroids. These investigations have covered: I. Estradiol (E-2) and 17 hydroxyprogesterone (17 OH-P) plasma concentrations as indices in the prediction of ovulation. II. A single plasma progesterone (P) measurement to indirectly assess the occurrence of ovulation. III. The measurement of 17-hydroxyprogesterone in the diagnosis of women with polycystic ovaries (PCO). IV. The role of the estradiol/testosterone ratio (E/T) in PCO in the induction of ovulation using anti-estrogens. V. Peripheral estrogen levels vs estrogen tissue concentration.

The values expressed in the text, tables and figures represent mean ± S.D. Statistical analysis was performed by means of a two tails student "t" test.

I. *Estradiol and 17-hydroxyprogesterone plasma concentrations as indexes in the prediction of ovulation*

The pattern of E-2 and 17 OH-P in plasma during the human menstrual cycle has been reported by Strott and Abraham[4, 3]. These investigators found that the concentration of these hormones increased significantly prior to and coincident with the luteinizing hormone (LH) peak. The results of those studies indicate that E-2 levels provide a good index of follicular maturation and that 17 OH-P levels indicate luteinization of the follicle. However, there was a need to evaluate these two steroids in women with ovulatory and with anovulatory cycles, in order to compare the steroid production potential of the two different and opposite situations.

The values of plasma E-2 observed at the beginning of the menstrual anovulatory cycles (N = 10) were, $\bar{X} = 0{\cdot}047 \pm 0{\cdot}02$ ng/ml whereas those of the ovulatory ones (N = 10) were $X = 0{\cdot}083 \times 0{\cdot}02$ ng/ml ($P > 0{\cdot}05$) (see Table 1). The E-2 values were very uniform during the proliferative phase; 17 OH-P in the ovulatory cycles shows rhythmical concentration from the very beginning of the cycle. A correlation was made between E-2 and 17 OH-P values during the first part of the menstrual cycle in 10 ovulatory

Table 1. Indexes to predict ovulation (ng/ml) proliferative phase

PROLIFERATIVE PHASE		
OVULATORY (N=10)	v.s.	ANOVULATORY (N=10)
Estradiol		
Range: 0.007–0.525		0.021 – 0.152
*Mean: 0.083±0.023		0.047 ± 0.02
17 OH-P		
Range: 0.126–3.87		0.093 – 0.645
**Mean: 1.057±0.913		0.288 ± 0.075

*: P>0.001
**: P>0.05

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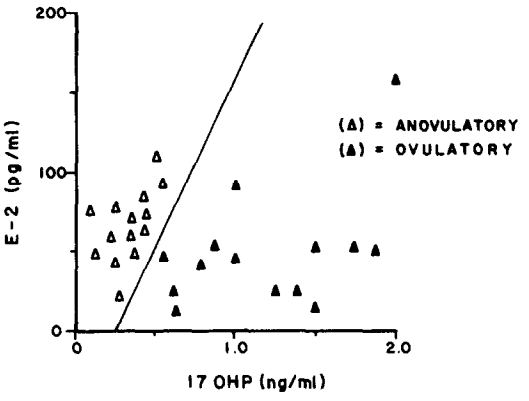


Fig. 1. Plasma E-2 and 17 OH-P correlation during the first part of the menstrual cycle. Each point represents a mean of at least 5 samples.

and anovulatory cycles, illustrated in Fig. 1. In the anovulatory group plasma values of E_2 were 0·047 ng/ml and 17 OH-P $<0·5$ ng/ml, while in the ovulatory group plasma E-2 values were 0·083 ng/ml and 17 OH-P $>0·5$ ng/ml.

In Fig. 2, progesterone (P) and 17 OH-P plasma levels in the first part of the ovulatory and anovulatory cycles are shown. Samples were obtained from the beginning of the menstrual bleeding, up to the initial rise in basal body temperature in the ovulatory cycles but since there is no rise in basal body temperature in the anovulatory group, the same number of samples were analyzed in the first part of the cycle for comparison. The values for P in the ovulatory cycles ranged from 0·099 to 2·015 ng/ml, while in the anovulatory cycles they were 0·095 and 0·313 ng/ml. The values for 17 OH-P in the ovulatory cycles were 0·126 to 3·874 ng/ml, whereas in the anovulatory cycles they ranged from 0·093 to 0·645 ng/ml.

Clinically the levels of 17 OH-P early in the first part of the menstrual cycle might be used as index to predict the occurrence of subsequent ovulation. Moreover, considering the sensitivity of current methods for measuring 17 OH-P and E-2, the determination of 17 OH-P is more reliable in the range of concentrations found in these studies [11].

II. *A single plasma progesterone measurement to indirectly assess the occurrence of ovulation*

Plasma P, endometrial histology, basal body temperature, and length of the menstrual cycle were evaluated simultaneously in 92 women studied randomly, in order to assess ovulation. Figure 3 is the composite of the four parameters studied. Among the population randomly studied, three groups were differentiated. In one group, P concentration above 5·7 ng/ml, secretory endometrium, and biphasic BBT were found; the second group had P levels below 1·43 ng/ml, proliferative endometrium, monophasic BBT and short or lengthy cycles. In a third group, progesterone ranged between 3·4 to 3·6 ng/ml, and the endometrium was proliferative; however, contrary to what was expected, basal body temperature was biphasic. It was concluded that consideration of length of the cycle, a definite rise in basal body temperature and a single progesterone determination were sufficient for assessing, indirectly, the occurrence of ovulation [17].

III. *Polycystic ovary (PCO): measurement of 17 OH-P in plasma in its diagnosis*

Experimentally, in 1969, the rat PCO was used to study the ability of nonluteal tissue to produce progestins when stimulated with an ovulatory dose of LH. In these studies the ovarian venous blood P, 17 OH-P and 20 OH-P concentration previous to the LH stimulation were analyzed; the 17 OH-P values were the highest found 137 ± 7 ng/ml/h, as compared with those of P: 11 ± 3 ng/ml/h, and 20 OH-P, 14 ± 5 ng/ml/h [21]. These results led us to undertake clinical studies in a group of 8 women with diagnosis of PCO by means of culdoscopy and ovarian biopsies. A minimum of 15 blood peripheral samples in each patient were drawn to analyse P and 17 OH-P. To evaluate the adrenal steroid source

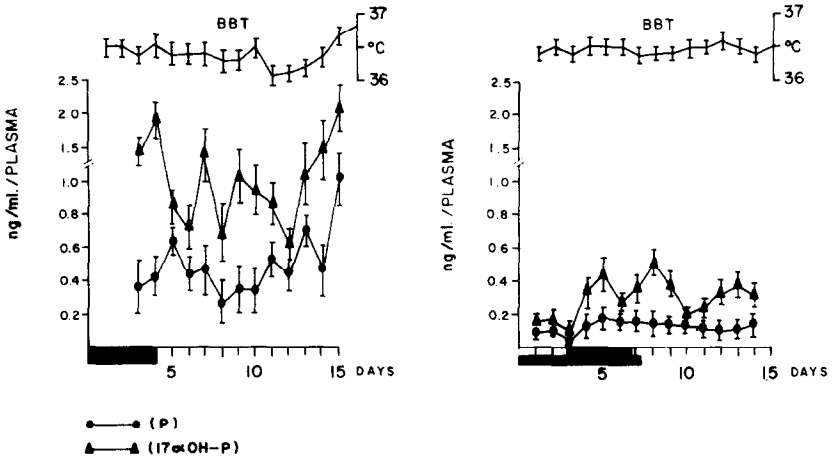


Fig. 2. Plasma levels of progesterone (P) and 17 hydroxyprogesterone (17-OH-P) in the first part of the menstrual cycle. Each point represents a mean of at least five samples.

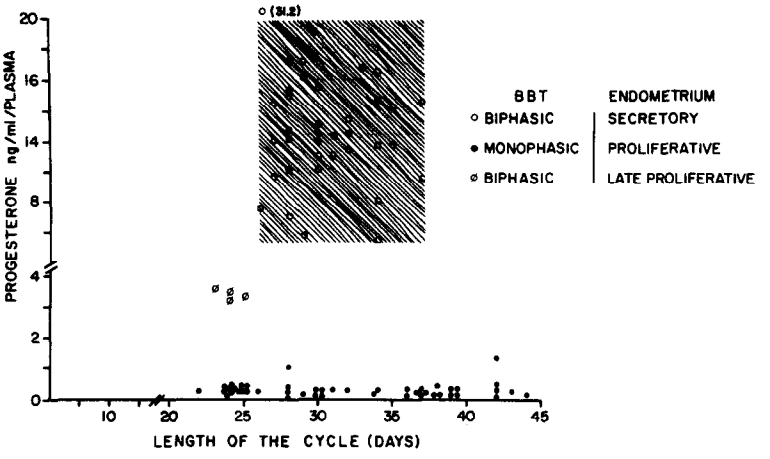


Fig. 3. Relationship among plasma P, length of the cycle, BBT and endometrium histology in ovulatory and anovulatory cycles.

the patients received parametasone acetate (PA) 6 mg/day for five consecutive days (Fig. 4). P values were: 0·35 ± 0·21 ng/ml and during PA treatment 0·25 ± 0·15 ng/ml, while 17 OH-P values were: 3·6 ± 2·4 ng/ml and during PA treatment: 5·5 ± 3·2 ng/ml. The levels of 17 OH-P did not change significantly with the use of glucocorticoids and were 8–10 times higher than those found in the proliferative phase before the midcycle peak (0·65 ± 0·31 ng/ml) [18].

It might well be that this high 17 OH-P concentration is related to the multiple cystic follicles of the PCO. These results are in keeping with Weidenfeld's findings who reported high pregnantriol urinary excretion in the same type of patients [22].

Corticosteroid therapy has been used in PCO based on the assumption that those compounds could re-establish the cyclic variations of gonadotropins by blocking ACTH secretion. The effect of PA on ovarian steroids and gonadotropins during the normal menstrual cycle has been observed [23]. As can be seen in Table 2, neither 17 OH-P nor FSH changed significantly, although LH was considerably suppressed. This study suggests the possibility that the effect of corticosteroids on the ovulatory mechanism is the regulation of LH release at the hypothalamic level, regardless of their effect on ACTH.

IV. *The role of the estradiol/testosterone ratio in the PCO in the induction of ovulation using anti-estrogens*

Ovulation can be induced in the polycystic ovary (PCO) in several ways: corticosteroid therapy [24], gonadotropins [25], anti-estrogen [26–27], combination of gonadotropins and anti-estrogens [28], and the combination of corticosteroids and anti-estrogens [25]; the wedge cuneiform ovarian resection has also been a surgical procedure used to solve the ovulatory failure. Under all these treatments, the PCO has the steroidogenic potential to produce a similar hormone pattern as the normal ovary [29]. However, none of those treatments allows one to analyse the dynamics involved in the normalization of the ovarian function. The present study was undertaken to evaluate the estradiol and testosterone plasma concentration in the PCO as one of the factors involved in the anti-estrogenic response to Clomiphene Citrate.

Daily venous blood samples were obtained in women with PCO for fifteen days regardless of the

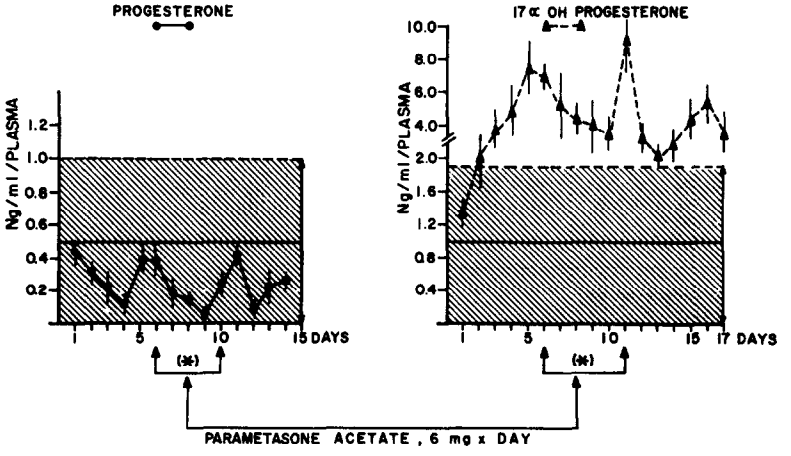


Fig. 4. Progestin concentration in the PCO before, during and after glucocorticoid suppression.

Table 2. Ovarian steroids and gonadotropins (ng/ml) (mid-cycle peak)

	CONTROL	PARAMETASONE
17 OH-P	4.08 ± 0.6 (*)	3.4 ± 0.7 (P>0.05)
E-2	0.296 ± 0.082 (*)	0.126 ± 0.03 (P<0.01)
LH	1111.1 ± 102.8 (**)	260.5 ± 83.7 (P<0.001)
FSH	579.0 ± 57.0 (**)	467.0 ± 96.0 -----

(*) $\bar{X}$ ± S.D., N = 5 (**) $\bar{X}$ ± S.E., N = 15

day of the cycle. After the last blood sample was obtained, Clomiphene Citrate was administered (100 mg/day for five consecutive days) on three occasions. A minimum of 25–30 days was allowed to elapse between each occasion in order to evaluate whether or not ovulation had occurred. The criteria for ovulation were as follows: I. Definite rise in basal body temperature, II. secretory endometrial changes, and, III. plasma progesterone above 5·7 ng/ml.

The groups were separated according to the mean estradiol and testosterone concentration found in PCO, as well as the ratio of those hormones which are shown in Table 3. Induction of ovulation was achieved with Clomiphene Citrate in all the cases where the E/T ratio was 0·07 or higher. As can be seen, when the estradiol/testosterone ratio was 0·06 or lower (because of higher testosterone concentrations) ovulation did not take place after the anti-estrogen therapy.

The results showed (see Fig. 5) that a minimum mean estradiol concentration of 50 pg/ml is necessary to obtain a Clomiphene Citrate effect. They also suggest that testosterone could act as an anti-estrogenic substance competing at the hypothalamic level for the cyclic release of gonadotropins.

V. *Estrogen peripheral levels vs estrogen tissue concentrations*

With the availability of new techniques developed initially for the determination of plasma steroid concentration, we decided to measure estradiol simultaneously in peripheral blood and in some tissues of the human female reproductive tract. A preliminary communication of the direct measurement of the estrogen content of human breast tissue was recently reported [31]. The general outline of the extraction of steroids from tissue is shown in Table 4.

Figure 6 shows the results in plasma, endometrium and myomas. In all of the following patients, daily peripheral blood samples were obtained for five consecutive days, before during and after the surgical procedures. Group A, disfunctional uterine bleeding, had E-2 plasma levels of 0·040 ± 0·026 ng/ml which were lower than those previously mentioned for normal menstruating women of the same age. Nonetheless, in this group A, the E-2 endometrial concentration was 0·437 ± 0·175 ng/g. In a group of women younger than 35 years with diagnosis of hyperplasia of the endometrium, the peripheral levels of E-2 were 0·041 ± 0·021 ng/ml, but their E-2 endometrial concentration was higher (0·530 ± 0·120 ng/g). In the group of uterine myomas four simultaneous parameters were evaluated: the plasma E-2 levels were the lowest obtained (0·017 ± 0·006 ng/ml); these were followed by the endometrial concentration of 0·437 ± 0·053 ng/g; the highest values were found in the myoma tissue (0·693 ± 0·252 ng/g). The hormone concentration of the myometrium was similar to that found in the myomas (0·519 ± 0·178 ng/ml).

No information was available with regard to the actual content of estrogen at the tissue level with a simultaneous determination at peripheral levels, in diseases classified as of "estrogen dependent". The

Table 3. Functional evaluation of the PCO and the use of anti-estrogens

I. Induction of Ovulation		
Estradiol:	$\bar{X}$ = 0.074 ± 0.018*	(N = 122)
Testosterone:	$\bar{X}$ = 0.796 ± 0.223	(N = 122)
E/T Ratio:	= 0.092	
II. Without induction of Ovulation		
Estradiol:	$\bar{X}$ = 0.058 ± 0.036*	(N = 131)
Testosterone:	$\bar{X}$ = 3.788 ± 2.452	(N = 131)
E/T Ratio:	= 0.015	

– P>0.001: Comparing E/T ratios of group I and II
*: Mean ± standard deviation (ng/ml)
N: Number of samples.

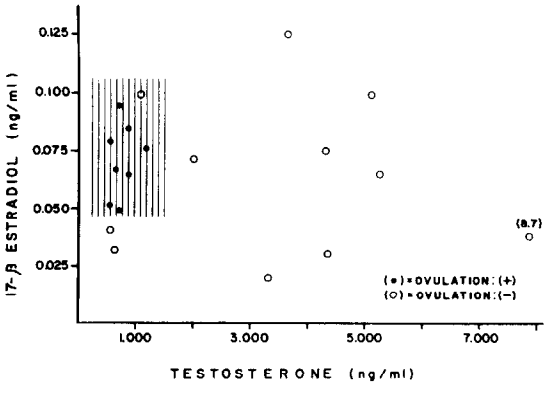
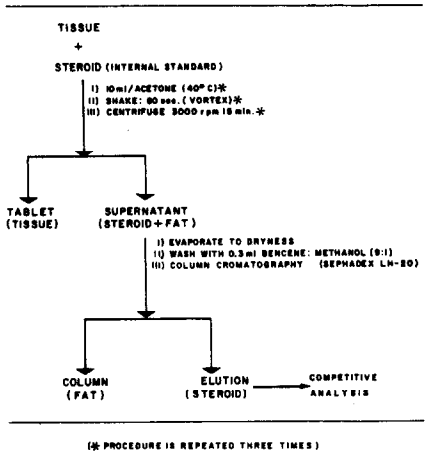


Fig. 5. Relationship between estradiol and testosterone in the PCO and the effect of Clomiphene Citrate in the induction of ovulation.

Table 4. Steroid tissue extraction



results indicate that tissue estradiol concentrations were higher than plasma peripheral estradiol concentrations in the female reproductive tract tissues studied. The expression "relative excess of estrogen" used to explain certain abnormalities of the breast [32] and also the term "hyperestrogenemia" used in some cases of endocrine diseases [33] should be better referred to as hyperestrogenic tissue concentration. These results help to explain some hormonal changes occurring at the tissue level. The measurement of tissue estrogen content could be used as a better test of the anti-estrogenic effect of some compounds [34].

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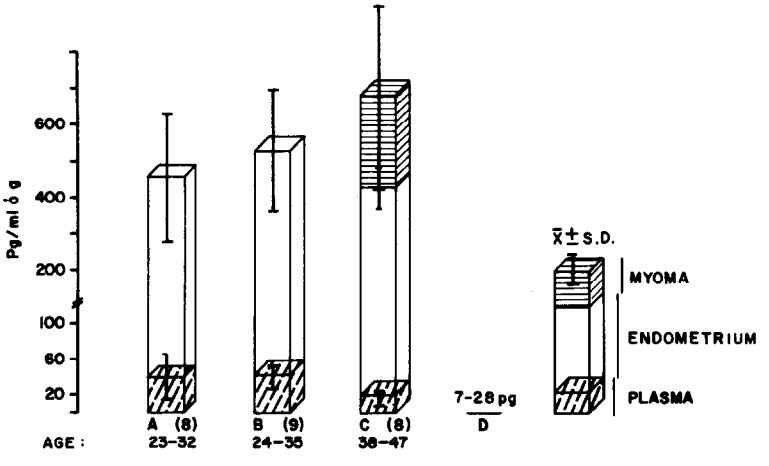

Fig. 6. Peripheral estrogen levels v.s. estrogen tissue concentrations in the human female reproductive tract. (A = dysfunctional uterine bleeding, B = hyperplasia of the endometrium, C = uterine myomas and D = non-endocrine tissues).

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