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Calculation of plasma estradiol levels by analysis of number and size of follicles measured by ultrasound

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Abstract

OBJECTIVE: To determine the possibility of immediate estimation of plasma estradiol (E_2) levels in stimulated ovarian cycles using a multiple regression equation (MRE) based on the analysis of the number and size of ovarian follicles measured by pelvic ultrasound. METHOD: E_2 levels were measured by enzyme immunoassay (EIA) in 47 patients on the day of induction of ovulation with human chorionic gonadotropin (hCG). MRE was employed to calculate E_2 levels after analysis of the number of follicles present, divided into three groups according to their greatest ultrasonic diameters A (10–14 mm), B (15–16 mm) and C (≥17 mm). The follicular lots were analyzed retrospectively and a MRE was determined. In a later prospective study, plasma E_2 levels were measured by EIA and MRE in 36 patients on the day of induction of ovulation with hCG. RESULTS: The following MRE was obtained for plasma E_2 levels as a function of follicular diameter: MRE of E_2 (pg/ml) = 556 + 95 A + 98 B + 108 C. In the prospective study, the correlation coefficient for the measurements of E_2 levels by the two methods (EIA versus MRE) was 0.70. CONCLUSION: The use of MRE based on the analysis of number and size of ovarian follicles measured by ultrasound permits a relatively precise indirect estimate of plasma E_2 levels in stimulated ovarian cycles.

Keywords: Estradiol levels; Ultrasound; Follicle; Regression equation.

Introduction

Pelvic ultrasound and the determination of plasma estradiol levels are the fundamental methods used to monitor the process of follicular development [3]. In natural (unifollicular) cycles, there usually is a strong correlation (r = 0.968) between follicular growth and increased levels of plasma estradiol [1].

In stimulated (polyfollicular) cycles, estradiol measurement by radioimmunoassay (RIA) or by enzyme immunoassay (EIA) is usually performed on a daily basis to obtain information about the response of the ovarian follicles to the different therapeutic agents. However, these determinations are costly and require special equipment, and the results are not avaliable on the same day, especially in less developed countries.

In view of these considerations, the objective of the present study was to determine the possibility of immediate estimation of plasma estradiol levels in stimulated ovarian cycles using a multiple regression equation based on the analysis of the number and size of ovarian follicles measured by pelvic ultrasound.

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Materials and methods

A group of 47 patients aged 21–37 years was initially submitted to different protocols of ovarian stimulation at the Human Reproduction Center of the Sinhá Junqueira Maternity Foundation, as a preliminary phase in the use of invasive or noninvasive methods of assisted reproduction.

A blood sample was obtained from each patient at the time of human chorionic gonadotropin (hCG) administration for estradiol measurement by EIA (Serozyme, Serono; inter- and intra-assay variation, 6.2% and 8.7%, respectively). On the same occasion, the greatest diameter of the follicles were measured with a 5.0-MHz vaginal transducer. The human menopausal gonadotropin (hMG) therapy was continued until one or more follicles attained a size ≥ 17 mm diameter or two follicles ≥ 16 mm diameter, when a single intramuscular injection of 10 000 IU of hCG was given.

Then, follicles 10–14 mm in diameter were assigned to group A, follicles 15–16 mm in diameter were assigned to group B, and those ≥ 17 mm in diameter were assigned to group C. A multiple regression equation (MRE) was calculated for plasma estradiol values as a function of number of follicles in the different groups.

Plasma estradiol levels were then measured by EIA on the day of hCG administration in a prospective study carried out on another group of 36 patients submitted to ovarian stimulation with gonadotropins. The values obtained by the laboratory were then compared with those calculated by MRE. Data were analyzed statistically using the Statfast program coupled to a Macintosh Plus computer.

Results

The plasma estradiol values obtained by EIA and the distribution by number and size of follicles defined as A, B and C, for a total of 47 patients are shown in Table 1. The

Table 1. Determination of estradiol levels by enzyme immunoassay (EIA) and of follicle distribution by ultrasound on the day of hCG administration.

Case	EIA pg/ml	Number of follicles		
		10–14 mm	15–16 mm	≥17 mm
1	2446	11	2	2
2	1066	2	1	2
3	1690	3	2	4
4	436	3	1	1
5	1013	4	2	4
6	2297	8	1	1
7	339	5	2	3
8	1151	2	1	2
9	1239	6	3	6
10	1613	7	5	1
11	2824	1	0	6
12	196	8	1	1
13	1100	3	1	5
14	1300	2	1	2
15	1340	3	1	6
16	2312	5	1	4
17	2989	3	4	6
18	1397	2	0	3
19	1171	4	4	2
20	1816	5	3	1
21	2624	7	3	3
22	1790	8	1	4
23	1944	2	4	1
24	4595	11	4	1
25	724	6	1	3
26	780	5	1	3
27	315	2	0	3
28	2166	10	4	2
29	2038	4	1	5
30	4446	9	3	3
31	804	4	0	1
32	1494	14	3	4
33	1148	5	1	2
34	1733	6	2	2
35	1605	3	0	5
36	3923	18	15	8
37	1953	4	5	6
38	1137	2	2	3
39	804	2	3	0
40	950	3	1	2
41	1007	5	2	1
42	964	6	1	1
43	1371	2	0	6
44	1319	1	0	6
45	1183	2	4	2
46	860	4	1	1
47	964	6	1	1

Table 2. Prospective analysis of estradiol levels measured by EIA and by a multiple regression equation.

Case	EIA pg/ml	Equation pg/ml	Number of follicles		
			10–14 mm	15–16 mm	≥17 mm
1	1302	1381	3	1	4
2	1019	1741	7	3	2
3	2288	1879	7	0	6
4	373	864	2	0	1
5	676	965	1	2	1
6	1147	1266	2	3	2
7	1158	2552	2	1	4
8	1583	2444	4	4	5
9	929	1073	1	2	2
10	1839	2199	11	6	0
11	2066	1394	2	1	5
12	719	1253	3	3	1
13	2198	1796	2	2	6
14	1852	1338	4	4	0
15	1655	1342	6	1	1
16	2645	2353	9	4	5
17	1298	1555	7	0	3
18	2036	1561	5	2	3
19	1247	1718	2	1	8
20	1055	1080	2	0	3
21	2272	1407	1	1	6
22	1547	1840	10	0	3
23	1293	985	1	0	3
24	3001	2605	13	6	2
25	2989	2025	5	2	7
26	3077	3071	21	3	2
27	1571	1358	4	2	2
28	5882	3246	6	5	15
29	3884	2267	5	7	5
30	1605	1728	8	2	2
31	1376	1283	3	0	4
32	2825	1774	6	1	5
33	700	1260	4	1	2
34	2054	1745	9	0	3
35	1423	1590	2	3	5
36	2108	1839	7	4	2

following MRE was obtained for plasma estradiol levels as a function of follicular diameter:

$$\text{MRE of estradiol (pg/ml)} = 566 + 95\,A + 98\,B + 108\,C.$$

Table 2 presents the plasma estradiol levels measured by EIA and those inferred by application of the MRE to a total of 36 patients submitted to cycle stimulation. Statistical analysis did not show a significant difference between the estradiol values determined by EIA and those determined by MRE (Student's t-test, $P = 0.26$). The correlation coefficient for the measurements of estradiol levels was 0.70 between the two methods.

Discussion

There is an excellent correlation between maximal diameter of the dominant follicle (natural cycle) and serum estradiol level [1]. The total volume of all developing follicles also correlates with the estradiol concentration [3]. One mature preovulatory follicle will be detected in association with a serum estradiol level of approximately 250–300 pg/ml. Since ultrasonography has been used to monitor hMG therapy (polyfollicular cycles), it will be important to measure estradiol during stimulated ovarian cycles. In this situation, there is little information about correlation between estradiol levels and follicular development determined by echography.

Lee et al. [2] detected variability among 5 radioimmunoassay methods for the measurement of estradiol concentration in the serum of 9 patients submitted to ovulatory stimulation for intratubal gamete transfer. Significant differences were detected among estradiol values measured in the same sample by the 5 techniques. The imprecision of estradiol measurement has been reported in the literature but is not fully recognized in the area of gynecology [4]. Antibodies for use in these assays have been produced for substances such as steroids, which are not normally antigenic (haptens). This has been accomplished by chemically coupling the haptens with antigenic proteins. With this principle of competitive binding, estradiol can be measured by an antibody (produced against an estradiol-bovine serum albumin complex, etc.). Unfortunately, to date neither the specificity nor the

avidity of such antibodies has been as high as anticipated.

The present data demonstrate that, within certain limits, it is possible to use a multiple regression equation (based only on the ultrasound method) to measure estradiol levels in stimulated ovarian cycles, as shown by the absence of significant differences between the population of estradiol values measured by EIA and those measured by a multiple regression equation. Similarly, the correlation coefficient was good (0.70) between the two methods. However, it is advisable for each service to develop its own multiple regression equation since important variations can occur when different methods are used for follicle measurement by ultrasound (mean follicular diameter or greatest diameter) and for estradiol calculation with different commercial kits.

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