PHYSIOLOGY

Serum Progesterone Before and After Human Chorionic Gonadotropin Injection Depends on the Estradiol Response to Ovarian Hyperstimulation During In Vitro Fertilization–Embryo Transfer Cycles

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Purpose: Our purpose was to assess if periovulatory serum progesterone is reflective of ovarian responsiveness in controlled ovarian hyperstimulation (COH).

Methods: One-hundred forty-two in vitro fertilization–embryo transfer cycles in women using GnRH-a suppression and human menopausal gonadotropin (hMG) stimulation were evaluated. Responses were studied according to ovarian response to hMG and age. Outcome measures included peak serum estradiol, serum progesterone and estrogen/progesterone ratios on the day of hCG injection, number of harvested oocytes, fertilization rates, and delivered pregnancy rates.

Results: A periovulatory rise in serum progesterone (>0.9 ng/ml) occurred only among younger women (<40 years old) with a good response ($P<0.05$). Though the number of oocytes was greater in good responders, fertilization and pregnancy rates were similar among all women regardless of age and ovarian response.

Conclusions: Periovulatory levels of serum progesterone vary according to ovarian response to COH. Elevations in progesterone do not appear to be a manifestation of poor responders. Reduced periovulatory progesterone may reflect inadequate steroidogenesis.

KEY WORDS: controlled ovarian hyperstimulation; in vitro fertilization; premature luteinization.

INTRODUCTION

In spite of continued debate, periovulatory levels of progesterone (P) are often regarded as a marker for predicting successful outcome of in vitro fertilization (IVF)–embryo transfer (ET) cycles. Elevated serum P levels are felt to produce detrimental effects on either the oocyte or the endometrium, although adverse effects on oocyte and embryo quality have been excluded by many reports (1–5). P is believed to cause secretory changes in the endometrium and reduce implantation and pregnancy rates (PRs) (6,7) by advancing the “window of implantation.”

More recently, others have suggested that premature P secretion may be an early manifestation of the perimenopausal ovary (8). Fanchin *et al.* evaluated premature P elevation in IVF-ET cycles based on the quality of ovarian response to controlled ovarian hyperstimulation (COH) and found that women with a poor response to COH and premature luteinization were associated with lower PRs compared to patients exhibiting normal responses (9).

Additionally, others have reported that a three fold rise in serum P following human chorionic gonadotropin (hCG) administration is associated with significantly better pregnancy outcomes (10,11), although others have failed to note such an association (5). However, since estradiol (E_2) correlates well with the

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number of mature follicles, pre- and post-hCG P may also reflect the quality of response to COH. We, therefore, evaluated serum P prior to and following hCG to assess whether or not levels were reflective of the subsequent response to COH.

MATERIALS AND METHODS

Cycles ($n = 142$) of 97 patients undergoing IVF-ET were evaluated from January 1992 to December 1994. Patients with known male factor ($<20 \times 10^6$ and motility $<40\%$) and unexplained failed fertilization were excluded.

All patients began COH after pituitary desensitization with leuprolide acetate (LA; TAP Pharmaceuticals, Deerfield, IL), 1 mg/day. COH was initiated once serum E_2 was <30 pg/ml using 150 IU/day intramuscular (im) human menopausal gonadotropin (hMG), 150 IU/day im Metrodin (Serono Laboratories, Inc., Randolph, MA), or a combination of both. On initiation of menotropins, LA was decreased to 0.5 mg/day until the day of hCG. Monitoring was performed using serial vaginal sonography and serum E_2 levels. Dosing was individualized depending on the patient's response. When a minimum of three follicles ≥ 18 mm and a serum E_2 measuring at least 200 pg/ml per follicle were noted, the patient was given 10,000 IU of hCG (Profasi; Serono Laboratories, Inc.)

Serum was obtained and assayed on the day of hCG and approximately 10 to 12 hr following hCG. Serum P concentrations were determined prospectively during the time of the patient's cycle. However, the data collected were not used to influence cycle management. Oocyte retrieval was performed 34 to 36 hr after hCG under transvaginal ultrasound guidance. Oocytes were cultured and embryo(s) transferred 48 hr later. The luteal phase was supported with 200 mg of micronized P administered vaginally on the day of embryo transfer. Pregnancy was confirmed with serial β -hCG titers commencing 12 days following embryo transfer. Clinical pregnancies were detected with transvaginal ultrasound. Excluded from evaluation were chemical pregnancies.

Serum samples were assayed for follicle stimulating hormone (FSH) and E_2 using direct I-125 RIA kits (Pantex, Santa Monica, CA) and P using coated-tube radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), respectively. Intra- and inter-assay coefficients of variation (CVs) were 3.4% and 4.2% for FSH, 5.0% and 8.4% for E_2 , and 3.2% and 5.6%

for P, respectively. The sensitivity of the P assay was 0.09 ng/ml.

Premature luteinization was arbitrarily defined as serum P ≥ 0.9 ng/ml on the day of hCG administration as reported by others (1–7). All cycles were analyzed according to the response to COH. Categorical descriptives represent a slight modification of groups studied by Fanchin *et al.* (8). A poor ovarian response was defined as a serum $E_2 \leq 500$ pg/ml on the day of hCG administration and five or fewer mature oocytes (MII) obtained at oocyte retrieval. Women were further subgrouped into patients <40 and ≥ 40 years old (Groups I and IV). A good ovarian response comprised women <40 and ≥ 40 years old with a serum $E_2 \geq 1500$ pg/ml and ≥ 10 MII oocytes obtained at retrieval (Groups III and VI). An intermediate response comprised women <40 and ≥ 40 years old with a serum E_2 between 500 and 1500 pg/ml and 5 to 10 MII oocytes obtained at aspiration (Groups II and V). Outcome measures included peak serum E_2 , P, E_2/P ratios on the day of and the day following hCG, number of oocytes (total and MII) obtained at aspiration, fertilization rates, embryos transferred (ET), and delivered pregnancy rates.

Statistical analysis was performed using chi-square for categorical comparisons and ANOVA and Bonferroni t test to compare the diagnostic significance of each parameter. Statistical significance was defined as $P < 0.05$. Results are expressed as mean $\pm$ SE.

Groups I, II, and III were significantly younger (34.7 ± 1.1 , 34.2 ± 0.4 , and 33.7 ± 0.6 years) than Groups IV, V, and VI (40.5 ± 0.6 years, 41.1 ± 0.2 years, and 43.0 ± 0.7 years) ($P < 0.05$). Day 3 FSH profiles were not significantly different among the groups (Group I, 11.6 ± 1.5 mIU/ml; Group II, 10.7 ± 0.5 mIU/ml; Group III, 10.3 ± 0.6 mIU/ml; Group IV, 10.3 ± 0.9 mIU/ml; Group V, 11.2 ± 1.1 mIU/ml; Group VI, 11.0 ± 1.9 mIU/ml; P , NS).

RESULTS

Premature luteinization was noted in 28 cycles (19.7%) and occurred only in women <40 years old. This event was witnessed a greater number of times in Group III (51.3%; 19/37) compared to all other groups (Group I, 0%, 0/10; Group II, 14%, 9/64; Group IV, 0%, 0/8; Group V, 0%, 0/17; and Group VI, 0%, 0/6; $P < 0.05$). The PR was not different among groups [Group I, 20% (2/10); Group II, 22% (14/64); Group III, 16% (6/37); Group IV, 0% (0/8); Group V, 12% (2/17); Group VI, 17% (1/6); P , NS] (Table I). Good

Table I. Summary of Women <40 and ≥40 Years Old Based on the Response to COH

Group	E ₂ , d0 ^a (pg/ml)	E ₂ , d1 ^b (pg/ml)	P, d0 (ng/ml)	P, d1 (ng/ml)	E2/P, d0	E2/P, d1	No. oocytes	MII	FR ^c (%)	ET	PR (%)
I (n = 10)	368 ± 32	526 ± 56	0.39 ± 0.06	1.28 ± 0.15	11.1 ± 3.4 [‡]	2.4 ± 0.3	7.9 ± 0.4	3.0 ± 0.6	70 ± 8	2.3 ± 0.4	20
II (n = 64)	1019 ± 30	1352 ± 53	0.55 ± 0.03 [♦]	2.3 ± 0.13	5.8 ± 0.4	1.8 ± 0.1	7.0 ± 0.5	5.2 ± 0.4	65 ± 4	2.6 ± 0.2	22
III (n = 37)	1996 ± 123 [*]	2423 ± 146 [*]	0.84 ± 0.07 [♦]	4.3 ± 0.35 [†]	4.7 ± 0.4	1.9 ± 0.2	11.4 ± 0.8 [■]	8.7 ± 0.1 [■]	56 ± 5	2.4 ± 0.3	16
IV (n = 8)	363 ± 45	460 ± 71	0.32 ± 0.04	1.46 ± 0.23	8.7 ± 1.6	1.6 ± 0.3	2.5 ± 0.2	1.0 ± 0.2	50 ± 7	1.0 ± 0.3	0
V (n = 17)	711 ± 61	925 ± 74	0.39 ± 0.03	1.5 ± 0.15	5.7 ± 0.5	1.6 ± 0.1	4.8 ± 0.5	3.4 ± 0.4	60 ± 6	2.2 ± 0.5	12
VI (n = 6)	2193 ± 292 [*]	2150 ± 145 [*]	0.61 ± 0.07 [♦]	1.96 ± 0.57	2.9 ± 0.3	0.6 ± 0.4	14.3 ± 3.5 [•]	14.3 ± 3.5 [◻]	61 ± 14	4.6 ± 0.6 [◻]	17

^a Day of hCG.^b Day following hCG.^c Fertilization rate.^{*} P < 0.05 compared to Groups I, II, IV, and V.[♦] P < 0.05 compared to Groups I, IV, and V.[†] P < 0.05 compared to Groups I, II, IV, V, and VI.[‡] P < 0.05 compared to Groups II, III, IV, V, and VI.[■] P < 0.05 compared to Group II.[•] P < 0.05 compared to Groups IV and V.[◻] P < 0.05 compared to Groups I and II.[◻] P < 0.05 compared to Groups IV and V.[◻] P < 0.05 compared to Group IV.

responders in Groups III and VI had a significantly higher serum E_2 on the day of and the day following hCG compared to poor and intermediate responders, regardless of age (Group III, 1996 ± 123 and 2423 ± 146 pg/ml; and Group VI, 2173 ± 292 and 2150 ± 145 pg/ml; compared to Group I, 368 ± 32 and 526 ± 56 pg/ml; Group II, 1019 ± 30 and 1352 ± 53 pg/ml; Group IV, 363 ± 45 and 460 ± 71 pg/ml; and Group V, 711 ± 61 and 925 ± 74 pg/ml; $P < 0.05$). The day of hCG, serum P was significantly higher in Group II (0.55 ± 0.03 ng/ml), Group III (0.87 ± 0.70 ng/ml), and Group VI (0.61 ± 0.70 ng/ml) compared to Group I (0.39 ± 0.06 ng/ml), Group IV (0.32 ± 0.04 ng/ml), and Group V (0.39 ± 0.03 ng/ml) ($P < 0.05$). The day following hCG, serum P was significantly higher in Group III (4.3 ± 0.4 ng/ml) compared to all other groups (Group I, 1.3 ± 0.5 ng/ml; Group II, 2.3 ± 0.1 ng/ml; Group IV, 1.4 ± 0.6 ng/ml; Group V, 1.5 ± 0.2 ng/ml; Group VI, 1.9 ± 0.6 ng/ml; $P < 0.05$). E_2/P ratios on the day of hCG were higher in Group I (11.1 ± 3.4) compared to all other groups (Group II, 5.8 ± 0.4 , $P < 0.05$; Group III, 4.7 ± 0.4 , $P < 0.05$; Group IV, 8.7 ± 1.6 , P NS; Group V, 5.7 ± 0.5 , $P < 0.05$; Group VI, 2.9 ± 0.3 , $P < 0.05$). However, no differences in E_2/P ratios were noted the day following hCG among all groups (Group I, 2.4 ± 0.3 ; Group II, 1.8 ± 0.1 ; Group III, 1.9 ± 0.2 ; Group IV, 1.6 ± 0.3 ; Group V, 1.6 ± 0.1 ; Group VI, 1.6 ± 0.4 ; P , NS).

In women ≥ 40 years old, the total numbers of oocytes and MII oocytes in good responders (Group VI, 14 ± 4 and 12 ± 3.5) were significantly higher than in intermediate and poor responders (Group IV, 2.5 ± 0.2 and 2.5 ± 0.2 ; and Group V, 4.8 ± 0.5 and 3.4 ± 0.4) ($P < 0.05$). In women < 40 years old, the total number of oocytes was significantly higher only in good responders (Group III, 11.4 ± 0.8) compared to intermediate responders (Group II, 7.0 ± 0.5 ; $P < 0.05$), while Group III had significantly more MII oocytes (8.7 ± 0.1) than both Group I (3 ± 0.6) and Group II (5 ± 0.4). Fertilization rates were similar among all groups (Group I, $70 \pm 8\%$; Group II, $65 \pm 4\%$; Group III, $56 \pm 5\%$; Group IV, $72 \pm 7\%$; Group V, $60 \pm 6\%$; Group VI, $61 \pm 14\%$; $P < 0.05$).

DISCUSSION

As expected, our results indicate that, regardless of age, poor responders have a significantly reduced peak serum E_2 and number of oocytes obtained at retrieval. However, unlike other reports (7,8), premature luteinization was not seen in women ≥ 40 years old and

poor responders < 40 years old, but only in good and intermediate responders to COH. Thus, deleterious effects, if any, could not be ascertained. In contrast, our study revealed significantly lower serum P and E_2/P ratios on the day of hCG in poor responders compared to superior responders. This may reflect reduced or abnormal folliculogenesis in poor responders. This is consistent with the reported positive correlation of E_2 levels with the number of mature follicles noted by others (4).

While serum P on the day of hCG was reflective of the response to COH, it was not associated with differences in pregnancy outcomes. This may be secondary to a type II sampling error (small numbers) or be a result of the use of gonadotropin releasing hormone agonist down-regulation, which was not used in other studies (7). Compared to the findings of Fanchin *et al.* (8), where premature luteinization occurred in all types of responders to COH, in our experience it occurred only in good or intermediate responders < 40 years old and was not associated with any differences in PRs [21.1% (4/19) and 11.1% (1/9) vs 11.1% (2/18) and 23.6% (13/55); P , NS].

Serum P following hCG has also been used as an outcome predictor, with threefold increases resulting in significantly higher PRs (9,10), although the physiologic significance of this observation remains unclear. Good responders to COH in women < 40 years old have significantly higher serum P following hCG compared to other groups, again suggesting that a larger cohort of mature follicles will produce more E_2 and P not only prior to hCG but also following its administration. Pregnant cycles had similar post-hCG P compared to nonpregnant cycles (3.0 ± 0.5 vs 2.5 ± 0.2 ng/ml; P , NS) and was not predictive of outcome.

In conclusion, we showed that periovulatory elevations of serum P reflect the response to COH, but premature elevations do not appear to be a manifestation of a diminished ovarian reserve in down-regulated cycles. The definition of premature luteinization as described by others seems to be a normal physiologic response to COH. Poor responders are more likely to have reduced serum P levels prior to and following hCG, which reflects compromised steroidogenesis and may be a manifestation of a diminished ovarian reserve.

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