



The range of subtle rise in serum progesterone levels following controlled ovarian hyperstimulation associated with lower in vitro fertilization pregnancy rates is determined by the source of manufacturer

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Abstract

Two previous studies found a correlation of higher pregnancy rates (PRs) with lower serum progesterone (P) levels at the time of human chorionic gonadotropin (hCG) injection in in vitro fertilization (IVF) cycles when luteal phase leuprolide acetate (LA)-human menopausal gonadotropin (hMG) was used for the controlled ovarian hyperstimulation (COH) regimen. In these two studies the radioimmunoassay (RIA) by Diagnostic Products Corporation (DPC) was used to measure P levels. This study attempted to corroborate these findings using a different RIA for P (Amersham) when the same COH regime was administered. The PR was significantly higher in the group where P was ≤ 1 ng/ml at the time of hCG (43.2%) versus the groups where the P level ranged from 1.1 to 2 ng/ml (15.8%). Viable PRs were also significantly higher in the lower P group. In contrast to the previous data with the DPC assay, no differences were seen with P < 0.5 ng/ml (36.4%) versus 0.5–1 ng/ml (44.6%). Nevertheless, using the Amersham RIA, the data does suggest decreasing PRs with higher serum P levels at time of hCG when using luteal phase LA-hMG COH regimen.

Key words: Human chorionic gonadotropin; IVF-ET; Pregnancy rates; Progesterone

1. Introduction

Several recent publications have suggested an association with lowered pregnancy rates (PRs)

with even subtle increases in serum progesterone (P) without overt premature luteinization occurring at the time human chorionic gonadotropin (hCG) is administered in in vitro fertilization (IVF) cycles, even when using gonadotropin releasing hormone agonist (GnRHa) down-regulation, followed by human menopausal gona-

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dotropin (hMG) stimulation for the controlled ovarian hyperstimulation (COH) regimen. Schoolcraft et al. found much higher PRs in patients whose serum P was <0.5 ng/ml at the time of hCG; these data were confirmed by Silverberg et al. [1,2].

Both of these studies used a commercial radioimmunoassay (RIA) kit for measuring serum P (Diagnostic Products Corporation, Los Angeles, CA); the inter- and intra-assay coefficients of variation (CV) were listed as 11.8% and 8.9%. We also use a commercial RIA kit for P measurement (Amersham-Amerlex, Arlington Heights, IL) in our laboratory and have found the inter- and intra-assay CV to be 9.1% and 11.9%, respectively.

The study presented herein evaluated whether a low serum P could also discern those patients with the highest PRs when the Amersham RIA was used to measure serum P. It is important to evaluate each RIA kit separately, because for some assays the CV may be larger and thus different conclusions may be reached. For example, Edelman et al. found no prognosticating value of the higher serum P level at the time of hCG on subsequent PRs, but their assay (Pantex, Santa Monica, CA) had a 34% CV.

2. Materials and methods

The subjects of the study were 119 consecutive patients having in vitro fertilization-embryo transfer (IVF-ET). Each patient was started on leuprolide acetate (LA) 1 mg/day beginning in mid-luteal phase. The dosage was decreased to 0.5 mg/day after 10 days if P was adequately suppressed to <1.5 ng/ml and estradiol (E_2) to <50 pg/ml. Human menopausal gonadotropins (hMG), 300 I.U./day (4 ampules) administered intramuscularly (IM) were started on day 11 and reduced to 225 I.U./day (3 ampules) after 4 days. Further changes were made as needed according to the results of serum E_2 and the number and size of the follicles, as determined by pelvic sonography using a transvaginal transducer. A single IM injection of 10 000 units of hCG was given when at least two follicles attained an average diameter of 18 mm and the serum E_2 was at least 800 pg/ml.

Patients were divided into five groups according

to the serum P concentration at the time of hCG administration: Group 1, P <0.5 ng/ml; Group 2, P in the range 0.5-1.0 ng/ml; Group 3, P in the range 1.1-1.5 ng/ml; Group 4, P in the range 1.6-2.0 ng/ml; Group 5, P \geq 2.1 ng/ml. Pregnancy rates were then calculated for each group.

3. Results

The PRs of the IVF-ET cycles according to P concentration in different groups are shown in Table 1. Pregnancy rates were considerably higher in P groups up to the level of 1.0 ng/ml, but began to decline when P exceeded this level. There were 29 pregnancies in 67 cycles (43.2%) in patients with sera P levels of <1.0 (Groups 1 and 2) versus only 6 pregnancies in 38 cycles (15.8%) in those patients with P level of 1.1-2.0 ng/ml (Groups 3 and 4) ($P = 0.007$, χ^2 analysis). The viable PR was 38.8% for Groups 1 and 2 (26/67) versus 13.1% for Groups 3 and 4 (5/38) ($P < 0.007$, Fisher's exact test). Comparing total and viable PRs of Groups 1 and 2 combined with Group 3 alone (5 of 30 or 16.6% for total and 4 of 30 or 13.3% for viable) found $P < 0.05$ for both categories.

4. Discussion

Most centers monitor IVF-ET cycles with sera E_2 levels and sonographic studies of follicle di-

Table 1
Pregnancy rate according to progesterone concentration at the time of hCG administration

Group	Serum P (ng/ml)	No.	% total	Preg-nancies (total)	Preg. rate (sac on ultra-sound) (%)	Viable pregnancy (%)
1	<0.5	11	9.2	4	36.4	3 (27.3)
2	0.5-1.0	56	47	25	44.6	23 (41.1)
3	1.1-1.5	30	25.2	5	16.7	4 (13.3)
4	1.6-2.0	8	6.7	1	12.5	1 (12.5)
5	\geq 2.1	14	11.7	1	7.1	0 (0)
Total		119	100	36	30.2	31 (26.01)

ameter and number [3-5]. Some centers advocate the monitoring of endometrial thickness or sonographic echo patterns as well, to determine the optimal time to administer hCG [6-8]. The importance of the adverse effect of premature luteinizing hormone (LH) surge in monitoring hMG, especially when trying to superovulate, is well known, and has even been shown to be an adverse factor in natural unstimulated cycles [9,10]. Thus, many centers will monitor IVF cycles with rapid LH measurements also.

The incidence of premature luteinization with COH for IVF-ET is considerably reduced, but not eliminated, through the use of GnRHa [11-16]. Recently Mio et al. have provided data demonstrating that following clomiphene citrate (CC)-hMG COH for IVF, subtle P changes (measured by an RIA by Nippon Diagnostic Products) can occur during the follicular phase, without the rise in LH, and may be associated with a decreased fertility rate [17].

Thus, our data corroborate these other studies on the importance of measurement of sera P during the follicular phase when preparing for IVF-ET, even when a GnRHa is used. Furthermore, we show that the Amersham RIA can also be used effectively to demonstrate the adverse effect of higher sera P levels. However, with this assay, no adverse effect of P levels between 0.6 and 1.0 compared with ≤ 0.5 ng/ml could be determined. Each laboratory will have to evaluate its own results according to the assay kit employed. There are many centers that no longer measure serum P levels during COH for IVF-ET when GnRH agonists are used, because of the very low incidence of premature rise of LH. The importance of these data is to make the clinician aware that subtle rises in serum P may occur.

The mechanism of subtle P rise despite GnRHa is not known. One hypothesis is that the rising sera follicle stimulating hormone (FSH) and E_2 levels may induce an increased amount of LH receptors and thus the granulosa cells responds with a rise in P, even with suppressed LH [18]. Other explanations are also possible. Several growth factors have been evaluated as possible regulators of granulosa cell progesterone biosynthesis; partially purified platelet derived growth factor was found to aug-

ment FSH stimulated progesterone biosynthesis in the rat [19]. Highly purified somatomedin-C (Sm-C)/insulin-like growth factor (Sm-C/IGF-1) had a limited effect on granulosa cell progesterone biosynthesis, but concurrent treatment with this peptide produced substantial increments in the FSH stimulated accumulation of progesterone [20,21]. Sm-C/IGF-1 has been shown to induce substantial progesterone biosynthesis on its own [22], rather than in synergism with FSH [20,21,23]. Other studies have shown that porcine IGF-II was not able to stimulate progesterone biosynthesis on its own, but was capable of synergizing with FSH to produce progesterone [23]. Also, studies of the role of insulin in the regulation of granulosa cell progesterone biosynthesis revealed a stimulatory effect exerted at micromolar concentrations in the rat [24,25], porcine [26-33] and possibly monkey [34] granulosa/luteal cells. Though most of these studies involved animals, IGF-I receptors were recently demonstrated in human corpora lutea and were found not to correlate with either serum or cytosol P and 17-OHP in the corpus luteum and were found to increase after clomiphene stimulation [35].

Confirmation of the importance of ultra low levels of P are needed by other centers whether using RIA or non-isotopic immunoassay. It is not clear whether the subtle P rise in itself decreases the PR, e.g. by advancing the secretory effect on endometrium, or is in some other way associated with reduction in fecundity. In addition, further work in the area of local ovarian factors may provide further insight to this phenomenon.

Schoolcraft et al. demonstrated that pre-hCG elevation in serum P did not result in lower oocyte fertilization and cleavage rates but yet lowered the PR, suggesting that the adverse effect of the subtle rise of P might be on the endometrium rather than the embryo [1]. The subtle rise in P may advance secretory changes of the endometrium, thus closing the window of receptivity. Further data supporting this concept was provided by Check et al. using a shared donor oocyte program and the same Amersham RIA assay as this study, where the viable PR in donor and recipients where serum P was ≤ 1 ng/ml and was equal at 14.4% per cycle, and the PR of recipients from donors where P was

>1 ng/ml was similar (12.7% per cycle), but the PR in donors where P > 1 ng/ml was reduced at 7.2% [36].

A recent manuscript by Fanchin et al. corroborated the association of a lower PR with IVF-ET when the P level has had a subtle rise before hCG is given [37]. They also used an RIA method (kit from Coatria-Biomerieux Laboratories, Marcy-L'Etoile, France) with intra and inter-assay CVs of $\leq 5\%$ and $\leq 7\%$, respectively. Fanchin et al. did not evaluate whether an even lower cut-off of 0.5 ng/ml would better distinguish the high from low pregnancy groups. Thus, the only paper to date refuting that the pre-hCG serum P can help predict a higher from a lower pregnancy group was that of Eldestein et al. [38]. However, the CV for the Pantex (Santa Monica, Calif.) RIA for P used in this study was >30%.

Thus, these data not only corroborate several other studies suggesting that a subtle rise of P before hCG, despite GnRH agonists may lower PRs and refutes the study by Eldestein, but emphasizes the importance of using an RIA assay for P with a low CV in the low range of P. One has to determine what cut-off for each specific assay delineates the high from low PR group. This may be especially true for non-isotopic methods for measuring P since many of these assays have never evaluated the CV of P in these low ranges.

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