Early Hormonal Markers of Pregnancy Outcome After In Vitro Fertilization and Embryo Transfer

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Purpose: To investigate the relative power of HCG, estradiol, and progesterone determinations in the prediction of pregnancy outcome after IVF. These prognostic hormonal factors were studied as single and combined predictors.

Methods: Serum concentrations of β -HCG, progesterone, and estradiol were measured 12–13 days after embryo transfer (study point 1) and 7 days later (study point 2) in a series of 20 consecutive infertile patients having a first-trimester spontaneous clinical abortion after an IVF-embryo transfer cycle. As a control group ($n = 60$), the next three IVF-embryo transfer cycles resulting in an ongoing pregnancy after each miscarried IVF cycle in our assisted reproduction program was used. The discrimination attained between the two study groups (ongoing pregnancies and miscarriages) was evaluated by logistic regression and receiver operating characteristic (ROC) curve analysis.

Results: Mean hormone concentrations at study points 1 and 2 were higher in the ongoing pregnancy than in the abortion group. Regarding pregnancy outcome the percentage increment of HCG serum levels ($\geq 1321\%$), with an accuracy (predictive value of pregnancy outcome) of 81.2% (sensitivity 98%, specificity 50%), had the best prognostic reliability but no significant differences were found when this parameter was compared with the predictive value of HCG concentration (≥ 72 IU/l) at study point 1 (diagnostic accuracy 80.5%; sensitivity 70%; specificity 80%). When ROC analysis was used, the best predictor of ongoing pregnancy according to the AUC^{ROC} was HCG concentration at study point 2 but again no significant differences were found when this parameter was compared with the predictive value of HCG serum levels at study point 1. A multiple marker strategy did not help distinguish viable from nonviable pregnancies.

Conclusion: A single, early (days 12–13 after embryo transfer) HCG quantitative serum measurement in IVF cycles not only is diagnostic but also has good predictive value for pregnancy outcome.

KEY WORDS: Abortion; HCG; IVF; pregnancy outcome; progesterone.

INTRODUCTION

Human embryos secrete human chorionic gonadotropin (HCG) into the culture medium 5–

8 days after fertilization and 1 week after conception pregnancy-specific HCG (as β -HCG) is detectable in peripheral maternal blood. β -HCG can be detected in 5% of cycles 8 days after the preovulatory luteinizing hormone surge, in 16% 9 days afterwards, in 53%

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10 days afterwards, and in 100% of all cycles from day 11 (1). Early and rapidly increasing HCG production by the embryo stimulates the corpus luteum so that estradiol and progesterone production can be maintained. Therefore, endocrinological parameters in the early gestation period have been used to predict an abnormal pregnancy (2,3).

From a medical standpoint, there are increased risks of adverse outcomes in *in vitro* fertilization (IVF) pregnancies compared with natural conceptions, including mainly spontaneous abortions (4). Thus, in pregnancies achieved with assisted reproduction, the early determination of pregnancy prognosis is of critical importance and from a patient's emotional perspective, an early prognostic indicator of the pregnancy status would be helpful in counseling as well as alleviating the anxiety invariably associated with the IVF.

Various studies have addressed the question about early hormone values and their relationship to pregnancy outcome after IVF. However, while several studies conclude that a single hormone measurement in early pregnancy can reliably predict pregnancy outcome (5–14), other reports suggest that serial hormone determinations for differential diagnosis of early pregnancy are superior to single measurements with regard to diagnostic power (15–18). On the other hand, while most of those previous reports relied on the use of HCG as a single marker in early pregnancy (5–10,12,14,15,17,18), others investigated also the prognostic value of serum progesterone and estradiol as predictors of early gestational normalcy (11,16,19).

Therefore, this study was undertaken to investigate the relative power of HCG, estradiol, and progesterone determinations in the prediction of pregnancy outcome after IVF. These prognostic hormonal factors were studied as single and combined predictors.

MATERIAL AND METHODS

A total of 80 women was included in the present investigation. For this study we have collated the data from a cohort of women having a clinical pregnancy after IVF-embryo transfer and where we have frozen blood samples still stored for measurement of estradiol and progesterone as reported below. Embryo transfer was performed 3 days after oocyte recovery and in our IVF program β -HCG serum concentrations are routinely measured 12–13 days after embryo transfer (study point 1) and 7 days later (study

point 2). As it is the policy of our laboratory to store blood samples from IVF cycles carried out during the previous months, we were able to gather data on a series of 20 consecutive infertile patients having a first-trimester spontaneous clinical abortion after an IVF-embryo transfer cycle. As a control group ($n = 60$), the next three IVF-embryo transfer cycles resulting in an ongoing pregnancy after each miscarried IVF cycle in our assisted reproduction program was used. Clinical pregnancy was diagnosed by increasing serum concentrations of β -HCG after embryo transfer and the subsequent demonstration of an intrauterine gestational sac by ultrasonography. Only singleton pregnancies were included for the specific purpose of this study. Ongoing pregnancy was defined by the presence of fetal heart activity beyond the 12th week of gestation. Miscarriage was defined as cessation of development of intrauterine pregnancy seen in transvaginal sonography during the first trimester.

The overall mean age of the women was 34.8 ± 0.5 years (mean $\pm$ SEM) (range 27–41). All of them were regularly menstruating premenopausal women and none of them had occult ovarian failure on the basis of their cycle day 3 FSH concentrations of <12 IU/l (standard International Reference Preparation [IRP] 78/549). All women underwent the same previously described IVF treatment protocol (20). As intracytoplasmic sperm injection (ICSI) may result in lower than expected HCG levels (21), only patients undergoing standard IVF were included.

Hormones in serum were measured using commercially available kits. Estradiol was measured by a competitive immunoassay (Immuno 1, Bayer, Tarrytown, NY, USA). The sensitivity of the assay was 10 pg/mL and the interassay coefficients of variation 5%. Progesterone was determined by a competitive chemiluminiscent immunoassay (Immulate, DPC, Los Angeles, CA, USA). The sensitivity of the method was 0.2 ng/mL and the interassay coefficient of variation was 6.7%. Total β -HCG was measured by a solid-phase, two site chemiluminiscent enzyme immunoassay standardized against the Third International Standard 75/537 (Immulate, Diagnostic Products Co., Los Angeles, CA, USA), with a detection limit of 2 IU/l. The interassay coefficient of variation was 5.8%. Ultrasonic scans were performed using a 5 MHz vaginal transducer attached to an Aloka sector scanner (Model SSD-620; Aloka Co. LTD, Tokyo, Japan).

For statistical analysis the Mann–Whitney U-test was used. Arithmetic means $\pm$ SEM and 95% CI

constituted the descriptive analysis. Logistic regression was used to predict the chance of having an ongoing pregnancy after IVF and embryo transfer. Hormone concentrations at study points 1 and 2 as well as percentage increments for hormones measured were introduced as continuous variables (assuming proportional hazards) in the univariate and multivariate logistic regression analysis (stepwise procedure; model criteria: $P_{in} = 0.05$, $P_{out} = 0.10$). Logarithmic transformation was performed on all variables because of their nonnormal distribution. A logistic regression model was performed to assess the joint effect of variables and to estimate the sensitivity and specificity values for all possible combinations of the study variables. The discrimination attained between the two study groups (ongoing pregnancies and miscarriages) was evaluated by receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, and area under the ROC curve (AUC^{ROC}) were obtained for each model. Ninety-five percent CIs were calculated for each of the estimates. The models' area under the ROC curve were compared using the method of Hanley and McNeil (22). Areas of 1.0 and 0.5 denote no overlapping and no discrimination, respectively, between groups. Data were analyzed by SPSS (SPSS Inc., Chicago, IL) statistical software.

RESULTS

Table I shows main demographic patient characteristics and mean hormonal values of study groups at the two study points. Mean age and body mass index were similar in both groups of patients. At study

point 1 HCG and progesterone serum levels were significantly higher in the ongoing pregnancy than in the abortion group. Estradiol levels tended to be higher in the ongoing pregnancy group, though this was not statistically significant ($P = 0.07$). The three hormonal parameters investigated were significantly higher among ongoing pregnancies at study point 2. However, when abortion and ongoing pregnancy groups were compared with respect to the percentage increment of hormonal values from study point 1 to study point 2, results were statistically significant for HCG but not progesterone and estradiol (Table I).

Table II shows logistic regression analysis probability estimates for HCG, progesterone and estradiol at study points 1 and 2 as well as their percentage increments, and the best combinations of all variables considered regarding outcome of pregnancy. On the basis of this study, the percentage increment of HCG serum levels ($\geq 1321\%$), with an accuracy (predictive value of pregnancy outcome) of 81.2% (sensitivity 98%, specificity 50%), had the best prognostic reliability but no significant differences were found when this parameter was compared with the predictive value of HCG concentration (≥ 72 IU/l) at study point 1 (diagnostic accuracy 80.5%; sensitivity 70%; specificity 80%). A multiple biomarker strategy did not help distinguish viable from nonviable pregnancies in early gestation.

To analyze further the diagnostic accuracy of hormonal parameters to discriminate between abortion and ongoing pregnancies the AUC^{ROC} values determined with ROC analysis for each hormone and the best combination of them are also shown (Table II). The best single predictor of ongoing pregnancy

Table I. Demographic Characteristics and Mean Hormonal Values of Study Groups at the Two Study Points^a

Study point/parameter	Abortion ($n = 20$)	Ongoing pregnancy ($n = 60$)	p
Age (years)	35 ± 0.4	34.6 ± 0.5	ns ^b
BMI (kg/m^2)	23.9 ± 0.5	24.1 ± 0.8	ns
Study point 1			
HCG-1(IU/l)	54.1 ± 22	141.5 ± 37.1	<0.01
Progesterone-1(ng/mL)	191.7 ± 42	392.8 ± 34.4	<0.01
Estradiol-1(pg/mL)	2154 ± 575	3147 ± 439	0.07
Study point 2			
HCG-2(IU/l)	747.15 ± 349	2597 ± 356.1	<0.001
Progesterone-2(ng/mL)	215.2 ± 54.3	413.1 ± 42	<0.01
Estradiol-2(pg/mL)	2702 ± 760	4110 ± 415.3	<0.05
Percentage increment			
HCG- Δ (IU/l)	1283.7 ± 952	1741.6 ± 64.1	<0.001
Progesterone- Δ (ng/mL)	12.5 ± 6.7	5.3 ± 3.8	ns
Estradiol- Δ (pg/mL)	25.4 ± 14.5	30.6 ± 19.2	ns

^a Values are means $\pm$ SEM.

Table II. Diagnostic Accuracy of Different Hormonal Parameters Alone or in Combination to Discriminate Between Abortion and Ongoing Pregnancy

Study point/parameter	Sensitivity (%)	Specificity (%)	Diagnostic accuracy ^a	AUC ^{ROC}	95% CI	Cut-off value
Study point 1						
HCG-1(IU/l)	70	80	80.5	0.76	0.64–0.85	72
Progesterone-1(ng/mL)	63	80	73.2	0.72	0.61–0.82	299
Estradiol-1(pg/mL)	77	50	60.1	0.60	0.48–0.73	2910
Study point 2						
HCG-2(IU/l)	94	60	77.8	0.77	0.66–0.86	1448
Progesterone-2(ng/mL)	81	60	76.4	0.75	0.64–0.85	311
Estradiol-2(pg/mL)	94	50	66.3	0.68	0.56–0.78	3052
Percentage increment						
HCG-Δ(IU/l)	98	50	81.2	0.75	0.64–0.84	1321
Progesterone-Δ (ng/mL)	73	60	61.2	0.58	0.46–0.70	9.7
Estradiol-Δ (pg/mL)	62	70	53.4	0.56	0.44–0.64	10.9
Parameters combined						
HCG-2+Progesterone-2	68	78	73.4	0.75	0.65–0.83	
HCG-Δ+Progesterone-2	72	75	79.2	0.77	0.66–0.88	
HCG-1+Progesterone-2	81	65	76.4	0.70	0.58–0.79	

^aPercentage of correctly predicted pregnancies outcome.

according to the AUC^{ROC} was HCG concentration at study point 2 but again no significant differences were found when this parameter was compared with the predictive value of HCG serum levels at study point 1. Also, a dual or triple-marker strategy did not show substantial improvement over the single-marker strategy. Overall, these results indicate that a single, early HCG quantitative serum measurement in IVF cycles not only is diagnostic but also has good predictive value for pregnancy outcome.

DISCUSSION

Early prediction of outcome in pregnancies following assisted reproduction treatment is important for both patients and clinical staff. Hormonal markers have been sought to distinguish between viable and nonviable pregnancies before verification of live intrauterine pregnancy by transvaginal sonography is possible. Several placental hormones have been proposed as useful diagnostic markers for early pregnancy but because HCG is the earliest detectable marker, it is the mainstay of modern pregnancy diagnosis. HCG can be detected as early as days 8–11 following ovulation, shortly after implantation (1). Exogenous HCG is used in stimulated IVF cycles but as clearance studies have shown, the HCG measured in serum on days 12–13 after embryo transfer, as did in this study, is of trophoblastic origin (23).

HCG is a glycoprotein consisting of an unspecific α -subunit, which is identical to LH and FSH, and a specific β -subunit. While the levels of the α -subunit

rise until the 36th week of pregnancy, the peaks for the β -subunit and for the whole HCG molecule are found between 8th and 10th weeks of gestation (1). The consistent nature of this pattern has made quantitative determinations of HCG an invaluable tool in the clinical assessment of early gestational normality. Implicit in the use of a single or serial HCG testing is the assumption that the serum HCG levels increase exponentially during early gestation. Thus, it has been proposed that the absolute HCG value may reflect the quality of implantation while the rate of HCG increase would reflect the activity of trophoblasts. Accordingly, the absolute HCG value should be used as a marker of implantation success and the HCG increase rate should be used in the clinical evaluation of suspected abnormal pregnancy (10,17). In keeping with this postulate some studies have suggested that paired measurements of serum concentrations of HCG have a higher diagnostic accuracy for prediction of pregnancy outcome than single measurements (15–17). This is not supported by results in the present study showing that HCG measurement of day 12–13 after embryo transfer combined with HCG measurement 7 days later did not improve significantly the predictive accuracy for pregnancy outcome. Therefore, our report adds further evidence favouring the use of early, single quantitative measurement of HCG after embryo transfer in IVF cycles as an excellent method of predicting pregnancy outcome (13).

The above is further supported by the fact that possible combinations of HCG determination on days 12–13 after embryo transfer with progesterone and estradiol measured at that time and 7 days later did not

improve the predictive value of HCG alone. This was true irrespective of absolute values or percentage changes being considered. This in contrast with a previous report concluding that determination of serum HCG, estradiol, and progesterone concentrations between 2 and 4 weeks after oocyte collection provides clinically useful information not only in the prediction of ectopic IVF pregnancy, but also, conversely, in the identification of IVF pregnancies that are destined to be ongoing (16). However, in that study, the predictive value of a positive result was only 18% (16). In contrast, a later report analyzing 282 pregnancies resulting from IVF, was unable to demonstrate that serum progesterone and estradiol values could predict pregnancy outcome using receiver-operator characteristic curves (19).

Testing of multiple serum samples for routine screening is neither practical nor cost-effective. A single, inexpensive test that could reliably predict pregnancy outcome would be a great boon to IVF patients as well as to their physicians. Although transvaginal ultrasonography offers a valuable tool in the prognosis of early pregnancy, it does not become useful until at least two weeks later than routine HCG testing. In addition to being more sensitive than transvaginal ultrasonography, HCG testing impinges no additional cost on the patient and is obtained routinely in IVF cycles (7). Thus, in conclusion, this report and previous studies in the literature suggest that a single, early (days 12–13 after embryo transfer) quantitative serum measurement of HCG in IVF cycles not only is diagnostic but also has good predictive value for pregnancy outcome. Neither the use of paired measurements of serum concentrations of HCG 7 days later nor the simultaneous determination of serum progesterone and estradiol were shown to improve significantly the predictive value of early HCG determination alone.

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