Defining the rise of serum HCG in viable pregnancies achieved through use of IVF

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BACKGROUND: We aimed to characterize the rate of HCG rise associated with viable IVF pregnancies, and to evaluate the association between HCG rise and potentially influential factors. METHODS: We performed a retrospective cohort analysis of all viable pregnancies achieved through IVF at two centres between January 1999 and March 2004. RESULTS: Of the 455 pregnancies resulting in live births, 391 met inclusion criteria and contributed a total of 1052 HCG values. Using random effects models, the best pattern to describe the rise of log HCG was quadratic with the rate of increase slowing at 24 days post-oocyte retrieval. Limiting the analysis to measurements below the discriminatory zone, the linear model adequately characterized the profile. The average slope was 0.403, yielding a predicted increase of 1.50 (50% increase) in 1 day and 2.24 (124%) in 2 days. In the final model, absolute HCG values, but not rate of rise, were significantly higher for twins and triplets and significantly lower for patients with BMI >30 kg/m2. CONCLUSIONS: The HCG profile of viable pregnancies conceived with IVF is quadratic with an earlier plateau than has been reported for non-IVF pregnancies. The average rate of rise is comparable to previous estimates in symptomatic spontaneous conceptions.

Key words: body mass index/HCG rise/IVF/multiple pregnancies/viable pregnancies

Introduction

Serial measurements of serum HCG in early gestations represent an invaluable aid in the differentiation of normal and abnormal pregnancies. When ultrasonography is non-diagnostic, clinical practice relies upon established rates of HCG rise to estimate the likelihood of pregnancy viability and determine the need for intervention if an ectopic pregnancy is suspected. Initial studies by Kadar et al. (1981) described the pattern of HCG rise in normally developing pregnancies. These authors reported that an increase of <66% in 2 days was characteristic of non-viable gestations, suggesting that actions to distinguish an ectopic pregnancy from an abnormal intrauterine pregnancy were indicated. More recently, a study of a large cohort of women with spontaneously conceived pregnancies and symptoms of pain or bleeding found that the minimal rise in HCG [defined from a 99% confidence interval (CI)] for potentially normal gestations was slower than previously reported, i.e. 53% in 2 days (Barnhart et al., 2004). Based on these precise estimates, use of more conservative rules was recommended in order to reduce the risk of interrupting viable gestations.

Whether a similar pattern of HCG rise should be expected in pregnancies conceived through IVF has not been confirmed. Because there is an elevated risk of ectopic pregnancy among patients who conceive through assisted reproductive technologies such as IVF (Marcus and Brinsden, 1995), it is necessary to determine whether clinical rules derived from populations of spontaneously conceived pregnancies are applicable to this unique patient population. Previous studies attempting to characterize the HCG curve associated with IVF pregnancies are limited by cross-sectional study designs, limited sample size, and failure to analyse potentially influential variables (Confino et al., 1986; Zegers-Hochschild et al., 1994). Factors such as multiple embryo transfer and multiple gestations have been proposed to affect the rate of HCG rise (Speirs et al., 1983; Confino et al., 1986), thereby complicating the interpretation of serial values in early pregnancies resulting from IVF.

The objective of this study was to characterize the curve of serial HCG levels in a large cohort of patients who conceived through use of IVF and ultimately achieved live births. We aimed to determine the minimal rate of HCG rise and to evaluate the association between the rate of increase and potentially
influential factors such as multiple gestations. The curves defined by our data will facilitate the interpretation of serial HCG values in early IVF pregnancies and aid in the differentiation of normal and abnormal gestations in these patients at risk for ectopic pregnancy.

Materials and methods
This study was approved by the Institutional Review Board of the University of Pennsylvania. The study population was comprised of patients from two local infertility practices, one university-based and one community-based. All pregnancies which were conceived through use of IVF procedures performed between January 1999 and March 2004 were considered for inclusion. Those pregnancies which ultimately resulted in the live birth of one to three infants were eligible. For patients who contributed more than one pregnancy during the study period, only the first pregnancy that met inclusion criteria was used in the analysis. We excluded all spontaneous abortions and ectopic pregnancies, as well as gestations with more than three fetuses. Pregnancies resulting from alternative methods of assisted reproduction treatment, such as gamete or zygote intra-Fallopian transfer, cryopreservation and donor transfers, were also excluded.

Additional inclusion criteria required that patients had one or more serum HCG level documented in the medical record and subsequently validated in each centre’s computerized database. Values which were measured at intervals of ≥24 h and ≤7 days were included in the analysis. Serum HCG concentrations were determined using the Abbott AxSYM total β immunoassay (Abbott Laboratories, Abbott Park, IL, USA) at the university-based centre and the DPC Immulite assay (Diagnostic Products Corporation, Los Angeles, CA, USA) at the community-based centre. These two automated immunometric assays have been shown to yield comparable results and few errors when compared to radioimmunoassay (Cole et al., 2001). Inter- and intra-assay variations were <10% at both centres. Results are expressed as mIU/ml, using the third International Reference Preparation.

Data regarding patient demographics and details of the IVF cycle including use of ICSI or assisted hatching (AH) and number of embryos replaced at the time of transfer, were extracted from patient medical records. At each centre, data recorded for the purpose of mandated reporting to the Centers for Disease Control were reviewed to confirm the number of gestational sacs and live-born infants for each patient.

The curves created by HCG determinations up to 7 weeks gestational age [35 days post- oocyte retrieval or 49 days from last menstrual period (LMP)] were evaluated. For all analyses, time was measured by gestational age (days from LMP) and all HCG values were transformed to the natural log scale in order to better approximate a normal distribution and reduce the influence of large values. Cross-sectional analyses of the initial HCG level for each subject were performed. We employed multivariable linear regression to determine which factors influence HCG concentrations.

To characterize the shape of the HCG curves, longitudinal analyses were conducted using random effects techniques. Random effects models estimate a population average curve by aggregating estimated HCG profiles from each individual subject. Application of these models accounts for repeated measures of HCG contributed by each subject and allows for variation in the number and timing of observations (Laird and Ware, 1982). In our analysis, linear and quadratic models accounting for random slopes for the association between log(HCG) and time (in days), as well as random intercepts were explored. For variables found to be associated with absolute HCG concentrations, interactions with the slope were assessed to determine whether there was an effect on the rate of rise.

To optimize the clinical utility of these data, we then limited the analysis to HCG measurements obtained prior to 39 days gestation (25 days post-oocyte retrieval). This sub-group of early gestational ages was selected because we were interested in describing the pattern of rise that occurs during the period when monitoring of serial HCG levels is clinically most appropriate, i.e. when ultrasound is non-diagnostic. Previous studies have shown that if gestational age is known and multiple pregnancy is a possibility, the discriminatory zone at which a diagnosis can be achieved by transvaginal ultrasound is best defined by gestational age ≥24 days post-conception (≥38 days from LMP) rather than by HCG concentration (Kadar et al., 1994).

Population average values for slope, standard errors, and upper and lower confidence bounds on the rate of increase in log(HCG) were estimated from the multivariable linear regression model which assumed that log(HCG) was normally distributed. The final model was used to calculate expected values at clinically pertinent points in time. Primary data management and analyses were conducted using STATA version 8 (StatCorp, College Station, TX, USA). Random effects models were analysed using SAS statistical software (SAS Institute, Cary, NC, USA).

Results
Of the 455 pregnancies which ultimately achieved live births during the study period, 409 had HCG determinations confirmed in the appropriate centre’s computerized database. Values that were documented in the medical record, but could not be confirmed in the computerized database, were assumed to be performed at outside laboratories and were therefore not included in the analysis. Of the 409 remaining pregnancies, 18 were identified as having more than one pregnancy which met inclusion criteria. For these patients, only data from the first eligible pregnancy were included in the analysis, yielding a final study sample of 391 subjects who contributed a total of 1052 HCG values. The following descriptive data are presented as mean ± SD. On average, the initial HCG assessment was obtained 16.3 ± 1.8 (range 8–26) days after oocyte retrieval, representing a mean gestational age of 30.3 ± 1.8 (range 22–40) days from LMP. The average number of observations per subject was 2.02 ± 1.66 (range 1–6). The mean interval between HCG determinations was 3.32 ± 1.66 (range 1–7) days.

In the final sample, there were 224 singletons, 135 twins, and 32 triplets. Baseline HCG concentrations were significantly higher for twins and triplets compared to singletons ($P < 0.0001$) and for triplets compared to twins ($P < 0.0001$). The patients were predominantly Caucasian and nulliparous, and had an average of 3.01 ± 0.86 (range 1–6) embryos replaced at the time of transfer. Linear regression analysis of the initial values of log(HCG) were significantly influenced by the number of gestational sacs ($P < 0.0001$) and maternal body mass index (BMI) ($P = 0.01$). HCG levels were higher among twins and triplets compared to singletons, and lower among women with greater BMI. In the multivariable regression, there was no independent effect of number of embryos transferred, use of ICSI, or use of AH on initial log(HCG) values. Infertility centre was also analysed as an independent variable and was found to have no significant effect.

Using random effects models, the best pattern to characterize the rise of log(HCG) was quadratic with the rate of increase

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slowing after 24 days post-oocyte retrieval (38 days from LMP; Figure 1). Multivariable analyses revealed that absolute values of log(HCG) were significantly influenced by number of gestational sacs and maternal BMI. Interactions between the slope and number of gestational sacs and between the slope and BMI were not significant, indicating that these factors did not affect the rate of rise over time. In the final model, BMI was considered as a categorical variable to facilitate clinical interpretation. Groups were defined as BMI <20 (bmigrp1, n = 70), BMI 20–25 (reference group, n = 346), BMI 25–30 (bmigrp2, n = 117), and BMI >30 (bmigrp3, n = 91).

Overall, the optimal model included a fixed quadratic effect and a random linear effect for gestational age (number of days from LMP) allowing for random slopes and random intercepts, as well as fixed effects for number of gestational sacs and groups of BMI. The population average profile for the rise in log(HCG) per days of gestational age (days) was described by the following equation:

\[
\text{log(HCG)} = 0.842(\text{days}) - 0.007(\text{days}^2) + 0.406(\text{twin}) + 0.825(\text{triplet}) + 0.130(\text{bmigrp1}) - 0.049(\text{bmigrp2}) - 0.266(\text{bmigrp3}) - 13.74.
\]

A graphic representation of predicted values from the model is shown in Figure 2. Predicted absolute HCG values by number of gestational sacs and BMI are listed in Table I. Data presented in the Table I are meant to provide a reference for patients who conceive through IVF and ultimately achieve live births.

When the analysis was limited to measurements obtained prior to 39 days gestation, the linear model adequately characterized the profile of log(HCG). The equation which defines this line is as follows:

\[
\text{log(HCG)} = 0.403(\text{days}) + 0.414(\text{twin}) + 0.812(\text{triplet}) + 0.119(\text{bmigrp1}) - 0.07(\text{bmigrp2}) - 0.289(\text{bmigrp3}) - 6.76.
\]

In the final model, absolute HCG values, but not rate of rise, were significantly higher for twins (P < 0.0001) and triplets (P < 0.0001). Patients with BMI > 30 had significantly lower HCG values (P = 0.009) than patients with normal BMI, but rates of rise were similar.

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**Figure 1.** Curve generated from serial log(HCG) concentrations of women who conceived through IVF and ultimately achieved live births (391 patients, 1052 observations). CI = confidence interval.

**Figure 2.** Generated curve of serial log(HCG) values in women who achieved singleton, twin and triplet live births through use of IVF.

**Table I.** Predicted mean HCG values (mIU/ml) over time by number of gestational sacs and maternal body mass index

<table>
<thead>
<tr>
<th>Body mass index (kg/m²)</th>
<th>Day 14 post-ET (30 days from LMP)</th>
<th>Day 16 post-ET (32 days from LMP)</th>
<th>Day 21 post-ET (37 days from LMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleton</td>
<td>(30 days from LMP)</td>
<td>(32 days from LMP)</td>
<td>(37 days from LMP)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>210.61</td>
<td>476.28</td>
<td>2866.94</td>
</tr>
<tr>
<td>20–25</td>
<td>184.93</td>
<td>418.22</td>
<td>2517.45</td>
</tr>
<tr>
<td>25–30</td>
<td>176.09</td>
<td>398.22</td>
<td>2397.06</td>
</tr>
<tr>
<td>&gt;30</td>
<td>141.74</td>
<td>320.54</td>
<td>1929.47</td>
</tr>
<tr>
<td>Twin</td>
<td>(30 days from LMP)</td>
<td>(32 days from LMP)</td>
<td>(37 days from LMP)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>316.08</td>
<td>714.80</td>
<td>4302.71</td>
</tr>
<tr>
<td>20–25</td>
<td>277.55</td>
<td>627.66</td>
<td>3778.19</td>
</tr>
<tr>
<td>25–30</td>
<td>264.28</td>
<td>597.65</td>
<td>3597.52</td>
</tr>
<tr>
<td>&gt;30</td>
<td>212.72</td>
<td>481.06</td>
<td>2895.75</td>
</tr>
<tr>
<td>Triplet</td>
<td>(30 days from LMP)</td>
<td>(32 days from LMP)</td>
<td>(37 days from LMP)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>480.58</td>
<td>1086.81</td>
<td>6542.01</td>
</tr>
<tr>
<td>20–25</td>
<td>421.99</td>
<td>954.32</td>
<td>5744.51</td>
</tr>
<tr>
<td>25–30</td>
<td>401.82</td>
<td>908.69</td>
<td>5469.81</td>
</tr>
<tr>
<td>&gt;30</td>
<td>323.44</td>
<td>731.43</td>
<td>4402.82</td>
</tr>
</tbody>
</table>

*Derived from final equation: log(HCG) = 0.842(\text{days}) - 0.007(\text{days}^2) + 0.406(\text{twin}) + 0.825(\text{triplet}) + 0.130(\text{bmigrp1}) - 0.049(\text{bmigrp2}) - 0.266(\text{bmigrp3}) - 13.74.

LMP = last menstrual period; ET = embryo transfer.
Using this model, expected rates of increase in HCG values were derived and are shown in Table II. On average, patients experienced a relative increase of 1.50 (50% increase, 99% CI 1.50–1.52) in 1 day and 2.24 (124%, 99% CI 2.19–2.30) in 2 days. Due to the large sample size, this estimated rate of rise is quite precise as is reflected in the 99% confidence intervals. Of the actual values observed, the slowest confirmed rise was 1.14 (14%) in 1 day and 1.30 (30%) in 2 days. Relative rates of increase observed in the 1st percentile were 1.17 (17%) in 1 day and 1.38 (38%) in 2 days, and in the 5th percentile were 1.30 (30%) in 1 day and 1.70 (70%) in 2 days. These data can be used by clinicians for comparison when monitoring serial HCG levels before a definitive diagnosis can be achieved with ultrasound.

Discussion

Using sophisticated modelling and a large sample size, we precisely describe the HCG profiles of pregnancies which were conceived through IVF and ultimately achieved live births. Overall, the curve was quadratic with an earlier plateau than has previously been reported for pregnancies conceived spontaneously or through other methods of infertility treatment. Multiple investigators have evaluated the rise of HCG in various populations of fertile, infertile, symptomatic and asymptomatic women for the purposes of estimating curves to distinguish normal from abnormal pregnancies (Pittaway and Wentz, 1985; Fritz and Guo, 1987; Kadar and Romero, 1987; Kadar et al., 1990; Check et al., 1992). In past years, there was much controversy about whether the pattern that best described the rise in HCG was log-linear or quadratic. Categorization of the existing literature based on the presence or absence of symptoms of pain or bleeding in the first trimester suggests that indeed, the quadratic curve represents HCG rise when the population is asymptomatic and the gestational age is certain (Table III). Previous studies have estimated rates of increase among infertile patients treated with various methods of ovulation induction, but have not specifically investigated pregnancies resulting from IVF. Additionally, these earlier studies were limited by their use of doubling time, a measure which is sensitive to variations in the interval of testing (DT = [(log 2)*(time interval in days)]/[log(HCG2)/(HCG1)]) (Pittaway and Wentz, 1985).

In our study, we restricted the sample to pregnancies achieved through IVF and analysed our data using random effects methods which are flexible and adjust for varying numbers of evaluations and intervals of testing. While fixed effects models account for variability or error associated with individual observations, they assume that the error terms have independent and identical distributions. Because repeated measurements within subjects over time cannot be considered independent, such models are not appropriate. Random effects models are ideal for evaluating repeated measurements because they account for variability that occurs with each observation at each time interval within a subject as well as the between-subject variability (Laird and Ware, 1982).

The quadratic curve described by these data indicates that the rate of increase slows at ~24 days post-ovocyte retrieval, corresponding to a gestational age of 38 days (5 weeks and 3 days) and an approximate HCG concentration of 3000 mIU/ml. This relatively early plateau in HCG production may reflect differential rates of implantation and resorption of non-viable embryos in pregnancies resulting from IVF. Alternatively, it is possible that the administration of exogenous progesterone, which is common practice in IVF treatment, leads to a diminished need for the HCG-induced steroidogenesis of the corpora lutea (Kohen et al., 2003).

Within the clinically pertinent range of gestational ages at which ultrasound is not likely to be diagnostic, we determined that a log-linear pattern can be used to describe the increase in serum HCG. Our data indicate that the population of IVF pregnancies which achieved a live birth, on average, demonstrated a relative HCG increase of 50% in 1 day and 124% in 2 days (slope = 0.403). This rate of HCG rise is comparable to previous estimates for spontaneous intrauterine pregnancies (IUP) (Table III) and is not different for multiple gestations.

While the lower bound of the 99% CI suggests that the slowest rise in pregnancies resulting from IVF may be faster than that reported for symptomatic spontaneous pregnancies, this finding should not be interpreted as a clinical rule. Due to our large sample size and the exact nature of estimated gestational age in our study population, we have achieved a great degree of precision, thus narrowing the confidence interval around the mean. The 99% confidence interval represents the degree of assurance that the true mean slope of HCG rise in our population lies between 0.391 and 0.415. However, the more clinically relevant data are presented in the range and percentiles of observed rates of rise. The 1st, 5th and 95th percentiles represent the distribution of our study population, indicating that the actual rates of rise can be notably slower or faster than the average. Indeed, the slowest rate of rise in our population of pregnancies known to result in a live birth was 14% in 1 day and 30% in 2 days. To avoid the inadvertent interruption of

<table>
<thead>
<tr>
<th>Slope of HCG rise</th>
<th>1 days later</th>
<th>2 days later</th>
<th>7 days later</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st percentile</td>
<td>0.161</td>
<td>1.17</td>
<td>1.38</td>
</tr>
<tr>
<td>5th percentile</td>
<td>0.265</td>
<td>1.30</td>
<td>1.70</td>
</tr>
<tr>
<td>Mean</td>
<td>0.403 (0.391–0.415)</td>
<td>1.50 (1.50–1.52)</td>
<td>2.24 (2.19–2.30)</td>
</tr>
<tr>
<td>95th percentile</td>
<td>0.510</td>
<td>1.67</td>
<td>2.78</td>
</tr>
</tbody>
</table>

Values in parentheses are 99% confidence intervals.
viable pregnancies in an attempt to diagnose or treat abnormal pregnancies, conservative threshold values are essential. Prospective evaluation of critical values is needed to establish and validate a clinical rule. Moreover, it is important to acknowledge that observation of a ‘normal’ rise in HCG does not eliminate the possibility of a miscarriage or ectopic pregnancy. Such diagnoses should be sought definitively using methods such as ultrasonography or uterine evacuation.

With respect to HCG rise among multiple gestations, previous studies have provided conflicting evidence. Some authors have reported faster rates of rise and higher absolute concentrations among multiple gestations compared to singletons (Confino et al., 1986), while others have found no difference (Kelly et al., 1991; Check et al., 1992). One study of 48 singletons and 50 multiples conceived through IVF found that there was no difference in doubling times but absolute HCG values were higher among multiple gestations (Zegers-Hochschild et al., 1994). Modelling HCG levels longitudinally over time in a large cohort of patients, our study confirms that absolute values but not rate of rise are higher in twins and triplets compared to singletons. We also observed that HCG concentrations were significantly lower among obese women (BMI > 30 kg/m²) compared to normal-weight women, but rates of increase were similar. Physiological mechanisms underlying this finding are speculative, but may be related to the fat tissue’s capacity to act as a steroid hormone reservoir and site of hormone metabolism (Deslypere et al., 1985).

Diagnostic algorithms using serial HCG levels to differentiate normal and abnormal early gestations are based on log-linear curves derived from a population of spontaneous IUP. We determined that, while the optimal curve to describe the rise of HCG in viable pregnancies conceived through IVF is quadratic, a similar average rate of rise can be expected. Absolute values of HCG are higher in multiple gestations and lower in obese women, but these factors do not influence the rise over time. Our data should aid in the interpretation of serial HCG values in early IVF pregnancies, allow for extrapolation of expected HCG levels, and facilitate the counselling of patients being monitored.

References


