Urinary hCG patterns during the week following implantation

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BACKGROUND: Human chorionic gonadotrophin (hCG) is used to monitor pregnancy status. Yet the pattern of hCG excretion in the first week following implantation has not been adequately described.Therefore the aim of this study was to describe the average profile of hCG and its variability during the 7 days following estimated implantation in a population of naturally conceived pregnancies. METHODS: We measured daily hCG concentrations in first-morning urine for 142 clinical pregnancies from women with no known fertility problems. Mixed-effects regression models were used to estimate the hCG trajectory and its variability in relation to pregnancy outcomes. RESULTS: hCG rose 3-fold between the day of detection and the next day (95% CI = 2.7–3.4). The relative rate of rise decreased thereafter, reaching 1.6-fold (95% CI = 1.5–1.8) between days 6 and 7. HCG levels followed a log-quadratic trajectory, and the patterns of rise were unrelated to number of fetuses, risk of spontaneous abortion or sex of the baby. Later implantations (after 10 luteal days) produced slower rates of increase. CONCLUSIONS: Although mean hCG follows a log-quadratic trajectory during the first week of detectability, there is high variability across pregnancies. Later implantation may reflect characteristics of the uterus or conceptus that slow hCG production.

Keywords: pregnancy; hCG; spontaneous abortion; twins; fetal sex

Introduction

The growth and development of the human embryo are unobservable during the crucial early stages after implantation. The best information on early growth comes indirectly, through the measurement of hCG (human chorionic gonadotrophin) produced by the trophoblastic cells of the conceptus. HCG is a robust hormone, measurable in maternal serum and urine. Absolute hCG values and rates of rise in secretion and excretion have been reported to be associated with the embryo's genetic quality, site of implantation (intrauterine or ectopic) and developmental pace (Marshall et al., 1968; Kadar et al., 1981; Check et al., 1992; Ho et al., 1997; Urbancsek et al., 2002; Barnhart et al., 2004; Seeber et al., 2006). Consequently, there is an interest in hCG as a biomarker of embryonic health and a tool to evaluate the effects of endogenous and exogenous challenges on very early gestation (Lohstroh et al., 2007). First, however, it is necessary to describe the normal longitudinal daily excretion patterns of hCG and its natural variation among individuals.

HCG is an oligosaccharide glycoprotein hormone critical for pregnancy establishment and survival. It exerts a variety of intracrine, autocrine, paracrine and endocrine actions that play vital roles in the communication between conceptus and mother during early gestation (Yang et al., 2003). HCG's decisive early roles include the 'rescue' of the corpus luteum (Zeleznik and Pohl, 2006) and formation of the placental syncytium (Yang et al., 2003). Maintenance of the corpus luteum and its progesterone production (Liu et al., 1995; Baird et al., 2003) are crucial in supporting pregnancy until the placenta can produce adequate levels of progesterone (Csapo and Pulkkinen, 1978).

Though the mRNA for the hCG subunits has been detected as early as the 6–8-cell stage (Bonduelle et al., 1988), the intact protein first becomes detectable in the mother's blood and urine between 6 and 14 days after fertilization (Hay and Lopata, 1988; Wilcox et al., 1999; Lohstroh et al., 2006). Most research on longitudinal urinary excretion patterns of hCG has been conducted later in pregnancy [6 or more weeks after the last menstrual period (LMP)] (Braunstein et al., 1976; Gerhard and Runnebaum, 1984; Williams et al., 1995; Gol et al., 2005). Profiles for earlier gestational intervals have not been adequately characterized. This is particularly true for the first week post-implantation, which coincides with the onset of the placentation process (Malassine and Cronier, 2002).

Many past studies of very early hCG excretion have been based on clinical populations and involve women undergoing assisted fertility treatments (AFT), which limits their generalizability. Reproductive profiles of clinical patients may be inherently different from those of women in the general population, and could be affected by the exogenous drugs (or related stress) that accompany treatment (Batzer *et al.,* 1981; Lam *et al.,* 1999; Cwikel *et al.,* 2004). Additionally, many previous studies are based on cross-sectional designs, with each woman contributing only 1–3 data points (Lambers *et al.,* 2006; Seeber *et al.,* 2006). Such cross-sectional designs are inadequate for exploring the among-women variations in hCG excretion.

Our objective is to provide a description of daily urinary excretion of hCG during the first week after the estimated onset of implantation, using a sample of naturally conceived pregnancies in women with no known fertility problems. We have previously focused on pregnancies resulting in early spontaneous abortion (before 6 weeks LMP) (Wilcox *et al.,* 1999; Baird *et al.,* 2003). Here, we evaluate daily concentrations in first-morning urinary hCG from clinical pregnancies (i.e. those that survived at least 6 weeks LMP), and investigate the extent to which fetal number, fetal viability or fetal sex might affect the pattern of hCG rise.

Methods

Study population

Analyses were based on data collected in the context of the North Carolina Early Pregnancy Study (NCEPS), which had as its original objective to determine the incidence of early, sub-clinical, spontaneous abortion (before 6 weeks LMP). The field portion of the study was carried out between 1982 and 1986. A total of 221 women, who were planning to conceive, were enrolled at the onset of their attempt. Eligibility for participation required that women had no known chronic health or fertility problems, and were not under any hormone treatment. Participants' ages ranged from 21 to 42 years (mean = 30); most were white and college-educated (Wilcox *et al.,* 1988). All participants provided informed consent. This research was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences.

Study design

Participants kept daily dairies in which they recorded menstrual bleeding and symptoms of pregnancy. Women collected first morning urine samples daily in 30 ml wide-mouth polypropylene jars with screw tops. The collection of samples continued 8 weeks into the pregnancy or for up to 6 months if no clinical pregnancy was identified. Samples were stored in the participants' home freezers (without preservatives), with weekly retrieval and transport to a central storage unit where they were kept at $-20°C$. Urine specimens were then packed in dry ice and shipped by overnight freight to Columbia University for hCG assay (Wilcox *et al.,* 1985, 1988).

Hormonal assays and reproductive parameters

HCG assays were conducted from 1983 to 1987, within 6–24 months after the end of each woman's participation. HCG assays were carried out in triplicate, using an immunoradiometric assay (Sepharose-IRMA B101-R525) (Wilcox *et al.,* 1988). Laboratory personnel were blind to the outcomes of the clinical pregnancy. The specific primary target of the IRMA was intact hCG, with cross-reactivity to βhCG (∼70%)

(McChesney *et al.,* 2005). The detection limit was 0.01 ng/ml (Armstrong *et al.,* 1984). Converting to bioassay units of purified reference preparations, 1 ng in the IRMA is approximately equivalent to 0.013 IU. Within-assay variability in pooled samples was 21%; with this removed, the variability among assays was 15% (Wilcox *et al.,* 1988). All specimens for a given woman were measured in the same assay batch, except that additional dilutions were carried out if necessary for high concentrations of hCG. The stability of intact hCG was assessed by subjecting purified analytes in urine to 40 cycles of freezing and thawing and to storage at 4°C for 4 weeks. No loss of immunodetection for intact hCG analytes was evident during the freeze–thaw process or at any time during that storage period (McChesney *et al.,* 2005).

Specimens were also analysed for steroid metabolites in duplicate, using radioimmunoassay to determine urinary concentrations of estrone-3-glucuronide (E_1G) and pregnanediol-3-glucuronide (PdG) (Wright *et al.,* 1978; Samarajeewa *et al.,* 1979).

Concentrations of intact hCG are similar in urine and serum (Wehmann and Nisula 1981; Norman *et al.,* 1987; McChesney *et al.,* 2005). Urine collection is non-invasive, which is an advantage for studies requiring frequent specimen collection and non-clinical settings. Creatinine concentrations were measured for a subset of urine samples. Variations in urine concentration of creatinine were trivial in relation to the steep daily rise of hCG (McChesney *et al.,* 2005). Accordingly, no adjustments for creatinine were performed in the present analysis.

Definitions and reproductive end-points

Hormonal patterns were used to estimate ovulation and the onset of implantation. The day of ovulation was inferred from the rapid decline of the ratio of E_1G to PdG (Baird *et al.,* 1995; Baird *et al.,* 1991), which marks the luteinization of the ovarian follicle. This estimator of ovulation has been validated by Ecochard *et al.* (2001), who used ultrasound of the rupturing follicle to demonstrate that the urinary steroid hormone ratio is as good a marker of ovulation as the LH surge, and better than the LH peak.

Conception was inferred when the urinary concentration of hCG exceeded 0.025 ng/ml for 3 consecutive days (Wilcox *et al.,* 1988). For each conception, the onset of implantation was approximated as the first day of sustained production of urinary hCG above 0.015 ng/ml (Wilcox *et al.,* 1999). Although hCG is reliably detected in maternal urine only 6 or more days after fertilization (Wilcox *et al.,* 1999; Lohstroh *et al.,* 2006), the conceptus probably begins secreting hCG earlier (Bonduelle *et al.,* 1988; Hay and Lopata, 1988). The specific phase of the implantation process in which hCG reaches maternal circulation and is excreted in urine is unknown (Chang *et al.,* 1998).

Sample size

Of the 199 conceptions chemically detected in our study, 151 survived at least 6 weeks after LMP ('clinical pregnancy'). The initiation of implantation was identified for 142 of the 151 pregnancies. In one of the 142 pregnancies, a urine sample was missing near the onset of hCG rise. We inferred that this was the likely day of implantation, based on the fact that there was no detectable hCG on the previous day, and by the subsequent day hCG was already more than 2-fold higher than any confirmed day of first detection in our sample. Critical hCG information was missing for the nine remaining pregnancies, and these were excluded from further analysis. Of the 142 pregnancies in which implantation could be identified, 121 ended in singleton births, 6 in twin births, 13 in spontaneous abortions, 1 in an ectopic pregnancy and 1 in a molar pregnancy (all identified by participants' self-report).

Statistical analyses

Hormonal values were log-transformed for analysis in order to normalize distributions and reduce the influence of outliers. Urinary hCG excretion trajectories were evaluated using mixed model analyses (Proc Mixed in SAS 9.1; SAS Institute, Cary, NC, USA) with a random-effect term to account for correlations among observations within women. Comparisons of hCG trajectories among pregnancies according to their outcomes, sex of the fetus and time to implantation were performed by including the characteristic in question as a predictor in the mixed model. The fit of competing models was assessed by comparing the models' restricted log-likelihoods using chi-squared tests.

Results

hCG trajectory during the first week following detection

During the first week following detection, average maternal urinary concentration of hCG increased rapidly and continuously (Tables I and II and Fig. 1). The overall pattern of hCG excretion (Fig. 1) was well described by the following regression equation:

$$\ln \text{hCG} = \beta_0 + \beta_1 \times \text{time} + \beta_2 \times \text{time}^2$$

in which time is day in relation to hCG detection (day of hCG detection = 0), $\beta_0 = -2.837$ (SE = 0.065), $\beta_1 = 1.070$ (SE = 0.031), $\beta_2 = -0.046$ (SE = 0.005); P-values <0.0001 for all terms. The rate of increase in hCG excretion was steepest between the first day of hCG detection and the following
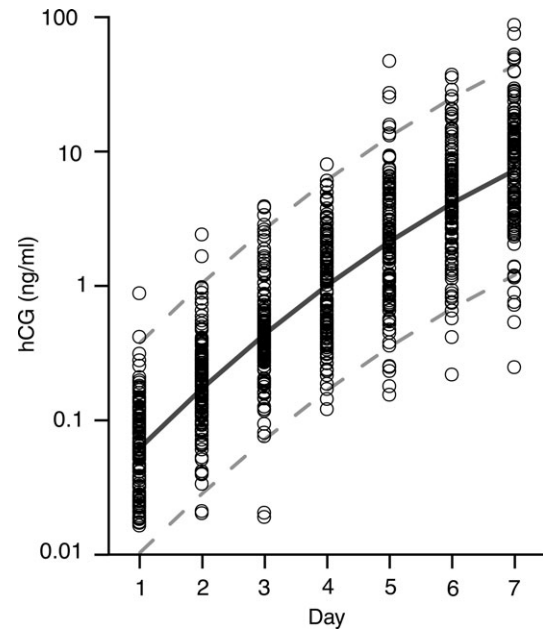

Figure 1: Predicted hCG excretion pattern and observed values during the first week of detection for 142 clinical pregnancies
Circles represent individual data points, the central solid line represents the hCG trajectory predicted by the regression equation and the broken lines represent the 95% probability band for the model. Day 1 = day of detection (hCG > 0.015 ng/ml)

day. The rate of increase progressively slowed in subsequent days (Table II).

Variability among pregnancies

Individual hCG profiles varied markedly. We illustrate this variability in five selected individual profiles of surviving pregnancies (Fig. 2). Some individual profiles follow a pattern similar to the mean model (e.g. Fig. 2(A)), whereas others diverge from it in various ways (e.g. Fig. 2(B–D)). There are pregnancies, e.g. in which the rate of increase remains relatively constant (e.g. Fig. 2(E)). In other pregnancies, hCG rates of increase slow down for a few days (e.g. Fig. 2(B–D)) and then increase abruptly and, in some cases, decrease again afterwards (e.g. Fig. 2(B) and (D)). Abrupt increases appeared to be more common towards the beginning of the week and decelerations were more common towards the end.

hCG excretion and pregnancy survivability

The trajectory of hCG rise in the first week was not different for spontaneous abortions and live births ($P = 0.80$). Pregnancies ending in spontaneous abortion presented as much variability in their hCG trajectories as did surviving singleton pregnancies (Levene's test for homogeneity, $P = 0.61$) (Fig. 3). Furthermore, among these clinical pregnancies, the mean time to implantation was identical (9.15 days after estimated ovulation) between surviving pregnancies and those resulting in spontaneous abortion.

hCG excretion in twin pregnancies

Our sample included six sets of twins, all monozygotic. Zygosity was assessed several years after birth using a validated set of
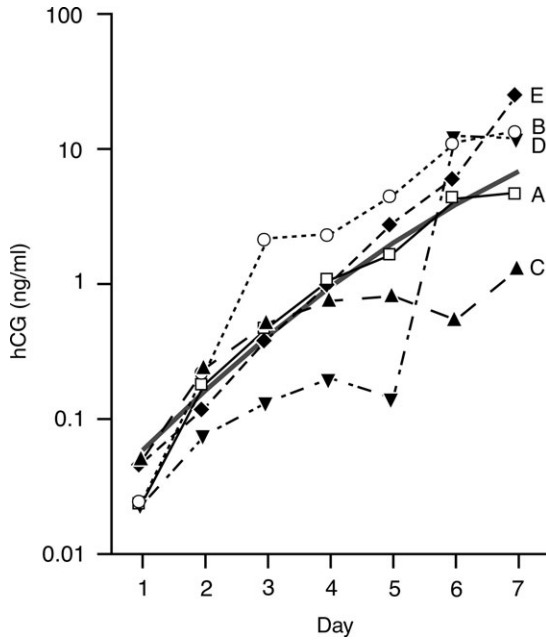
Table I. Daily geometric mean hCG concentrations in first morning urine during the first week following detection calculated for 142 clinical pregnancies.

Days[a]	n	Geometric mean (ng/ml)	95% CI
1	141	0.05	0.05–0.06
2	142	0.17	0.15–0.20
3	140	0.40	0.35–0.47
4	137	0.91	0.78–1.07
5	137	1.94	1.63–2.31
6	136	3.99	3.40–4.69
7	133	6.76	5.66–8.07

[a]Day 1 = day of detection (hCG > 0.015 ng/ml).

Table II. Daily rates of increase in urinary excretion of hCG during the first week following detection for 142 clinical pregnancies.

Days[a]	n	Rates of increase[b]	95% CI	10 percentile	Median	90 percentile
1–2	141	3.0	2.7–3.4	1.3	2.9	7.1
2–3	140	2.3	2.0–2.5	1.1	2.2	5.3
3–4	137	2.3	2.1–2.5	1.0	2.0	6.2
4–5	137	2.1	1.8–2.4	0.9	2.0	5.9
5–6	136	2.0	1.7–2.3	0.9	2.0	5.3
6–7	133	1.6	1.4–1.8	0.8	1.7	3.6

[a]Day 1 = day of detection (hCG > 0.015 ng/ml).
[b]The rate of increase was calculated by exponentiating the means of daily differences [ln hCG $_{\text{day2}}$ − ln hCG$_{\text{day1}}$]. A value of 3.0 reflects a 3-fold increase and a value of <1 reflects a decline in hCG levels from one day to the next.

Figure 2: Selected set of five individual hCG urinary excretion trajectories (labelled A–E) to illustrate between-pregnancy variability
The central solid line represents the hCG trajectory predicted by the regression equation for 142 clinical pregnancies. Icons and associated lines represent the five individual trajectories. Day 1 = day of detection (hCG > 0.015 ng/ml)

questions answered by the mother. A visual inspection of hCG trajectories for the twin pregnancies shows considerable variation, with an average trajectory very similar to that for surviving singletons (Fig. 4).
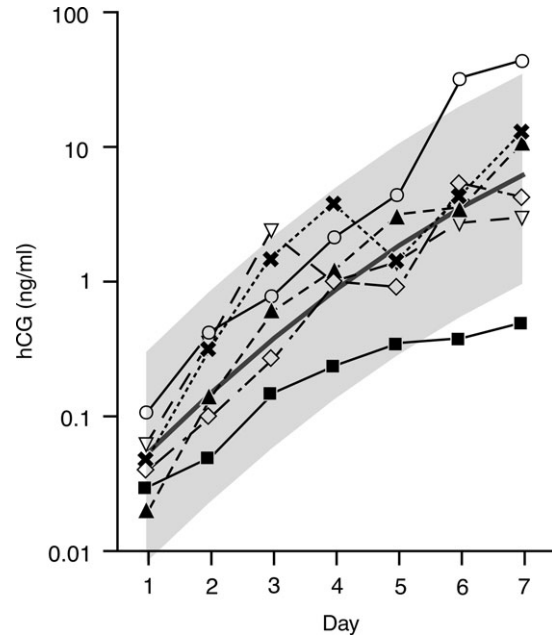

Figure 3: Urinary hCG excretion profiles for 13 clinical spontaneous abortions during the first week following implantation. Numbers represent the length of gestation (from ovulation to bleeding)
The smooth central solid line represents the hCG trajectory predicted by the regression equation for 121 surviving singletons pregnancies and the gray area represents the 95% confidence interval. Day 1 = day of detection (hCG > 0.015 ng/ml)

Figure 4: Urinary hCG excretion profiles for six sets of monozygotic twins during the first week of detection
The central solid line represents the hCG trajectory predicted by the regression equation for 121 surviving singleton pregnancies and the gray area represents the 95% confidence interval. Icons and associated lines represent the 6 individual trajectories. Day 1 = day of detection (hCG > 0.015 ng/ml)

hCG excretion and sex of the fetus

Sex of the fetus (65 boys and 56 girls) was not a significant predictor of urinary hCG levels ($P = 0.09$) among pregnancies resulting in surviving singletons (Fig. 5).

Time to implantation and hCG excretion trajectories

As we previously reported for these data (Wilcox *et al.,*1999), the time between estimated day of ovulation and estimated day of implantation ranged from 6 to 12 days (mean = 9.1, SD = 1.12, median = 9). The time it takes for a conceptus to implant may reflect quality of the conceptus or the uterus. In our sample of clinical pregnancies, time to implantation emerged as a significant predictor of the pattern of hCG rise ($P < .0001$). As shown in Fig. 6, conceptuses that implanted early (7 days after ovulation or earlier) tended to have lower hCG levels on the day of implantation but higher rates of increase during the first week, compared with conceptuses that implanted late (11 days or later). By the end of the first week following hCG detection, late implanters showed lower mean levels of hCG.

Discussion

We evaluated maternal hCG urinary excretion patterns during the first week following detection in 142 naturally conceived clinical pregnancies. Daily mean urinary hCG concentrations rose rapidly, with daily increases ranging from an initial 3-fold rise to a 1.6-fold rise by the end of the week. In contrast with the smooth increase in the overall mean, individual hCG trajectories were highly variable. Sharp increases were
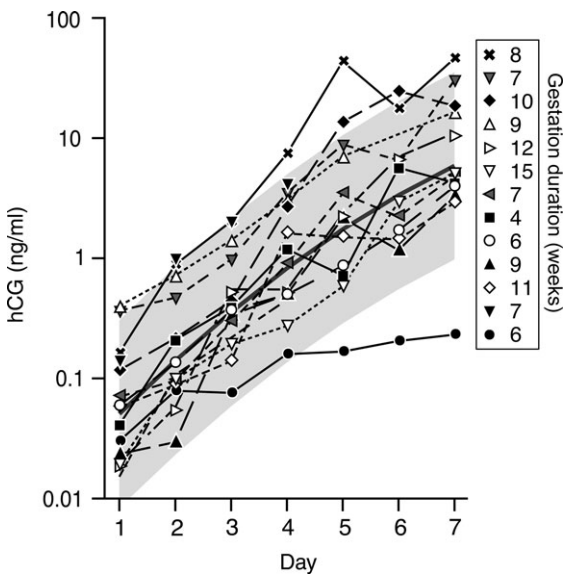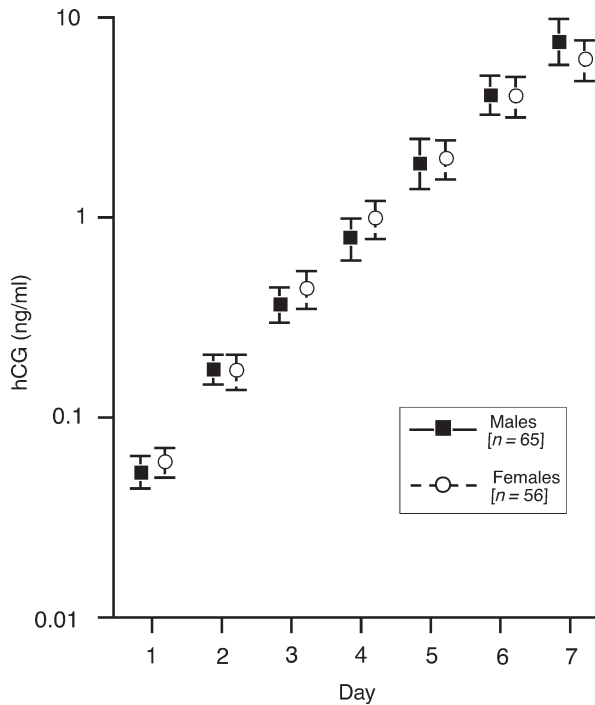
Figure 5: Daily hCG geometric means (GM) for surviving male and female fetuses during the first week of hCG detection
Icons represent daily GMs and bars are drawn to plus/minus one standard error. Day 1 = day of detection (hCG > 0.015 ng/ml)

sometimes interspersed with relative plateaus (Fig. 2). Some of this may be measurement error, although the use of triplicate assays reduces such error. The observed variation may also
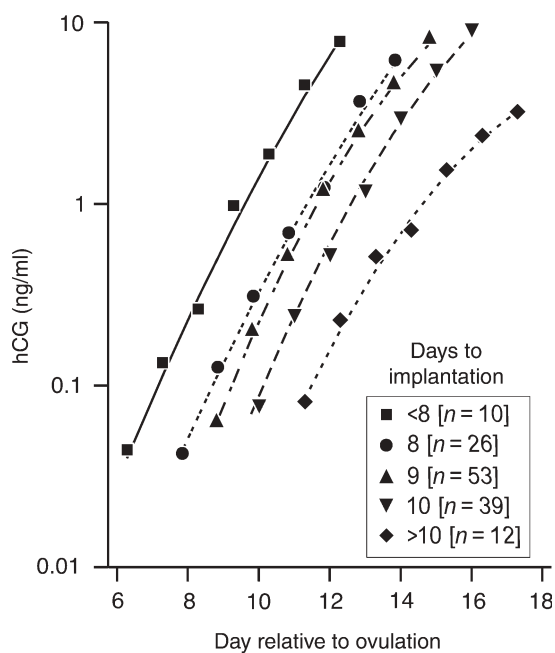

Figure 6: Daily hCG trajectories by time elapsed between ovulation and first hCG detection (i.e. time to implantation) for 142 clinical pregnancies during the first week of detection
Lines represent the trajectories predicted by the regression model for each day for each time to implantation category. The icons associated with each line represent the observed daily geometric means for each category. Day 0 = estimated day of ovulation

reflect day-to-day changes in the trophoblastic invasion process, as hCG-producing cells are brought in proximity to the maternal blood supply. Alternatively, there could be variable sequestering of hCG in maternal tissues prior to excretion. Our sample was ethnically and socioeconomically homogeneous; a more diverse sample could possibly show even higher heterogeneity in early hCG profiles.

Comparing our findings with those of previous studies is complicated by differences in design, sensitivity of the hCG assay used and data analysis methods. A study based on 25 pregnancies achieved via artificial insemination reports higher average hCG values for the first 5 days following detection than the values we observed for the same period (Ho *et al.,* 1997). The differences in results can be at least partially explained by the higher sensitivity of our assay which allowed earlier pregnancy detections. Two prior studies that examined longitudinal hCG rise patterns at the very earliest time of pregnancy for naturally conceived pregnancies were based on very small samples (Lenton and Woodward, 1988; Lohstroh *et al.,* 2006). Lohstroh *et al.* (2006) reported hCG data from 13 naturally conceived clinical pregnancies for days 10–14 from the FSH peak. The daily absolute increases they observed are similar to ours. Lenton *et al.* (1982, 1988) report data from 18 spontaneously conceived pregnancies. They observed a deceleration, in the day-to-day relative rate of increase during the first 12 days following implantation, i.e. consistent with the pattern we describe. A recent review of nine studies by Chung *et al.* (2006) provides evidence suggesting that the deceleration in the relative rate of increase of hCG levels continues after the first week post-implantation.

The time at which the relative rate of hCG rise starts to slow down as pregnancy advances has long been a topic of debate (Fritz and Guo, 1987). Studies discussing this issue focus on different intervals within the first trimester and data from the earliest stages of pregnancy are rarely well represented. Some of these previous studies show an early deceleration pattern (Lenton *et al.,* 1982; Pittaway and Wentz, 1985; Daya, 1987; Fritz and Guo, 1987; Check *et al.,* 1992), whereas others report a constant exponential rise (Kadar *et al.,* 1981; Zegers-Hochschild *et al.,* 1994; Barnhart *et al.,* 2004).

Early hCG levels are regarded as a predictor of pregnancy outcome (Check *et al.,* 1992; France *et al.,* 1996; Ho *et al.,* 1997; Yaron *et al.,* 2002; Hauzman *et al.,* 2004; Lohstroh *et al.,* 2007). While this is clearly true for very early losses (before 6 weeks) (Baird *et al.,* 2003; Lohstroh *et al.,* 2006; Wilcox *et al.,* 1999), early hCG levels are a poor predictor of clinical spontaneous abortions. In our data, conditional on survival to 6 weeks, the urinary hCG trajectory during the first week had no association with spontaneous abortion. This observation is consistent with results reported by Lohstroh *et al.* (2005), who found no association between hCG levels on day of detection and pregnancy outcome either in 62 fertile women undergoing artificial insemination or in 10 naturally conceived pregnancies (Lohstroh *et al.,* 2006). There is some evidence, however, that differences in hCG slopes may emerge during the second week, but findings are based on relatively small samples or IVF patients (Lohstroh *et al.,* 2006,

2007; Porat *et al.,* 2007). Also, variations in hCG bioactivity and hyperglycosylated hCG levels, which we did not measure, may prove to be useful in assessing pregnancy viability during the peri-implantation period (Ho *et al.,* 1997; Lohstroh *et al.,* 2006; Kovalevskaya *et al.,* 2007).

The number of developing embryos has been proposed to affect the trajectory of hCG (Zegers-Hochschild *et al.,* 1994; Hauzman *et al.,* 2001; Urbancsek *et al.,* 2002; Chung *et al.,* 2006). Some studies which focused on the earliest gestational stages have, however, failed to observe an effect (Kelly *et al.,* 1991; Check *et al.,* 1992). During the week following the estimated day of implantation we found no differences in either absolute hCG values or rates of rise between the twins and singleton pregnancies.

By the second trimester, women bearing female fetuses have higher hCG levels on average than those carrying males (Brody and Carlstroem, 1965; Santolaya-Forgas *et al.,* 1997; Spencer 2000). Yaron *et al.* (2001, 2002) report sex differences in hCG levels by the third week post-fertilization, but others have failed to observe differences that early in gestation (Braunstein *et al.,* 1980; Kletzky *et al.,* 1985; Gol *et al.,* 2005). We found no sex-related differences in hCG levels among surviving pregnancies during the first week of detection, suggesting that sex differences arise later.

Delay in implantation is a strong predictor of early pregnancy loss (before 6 weeks) (Wilcox *et al.,* 1999). Here, we report that, among pregnancies surviving at least 6 weeks, those that implanted after luteal day 10 had a slower hCG rise. The finding of a slower hCG rise might seem to support the hypothesis that time to implantation is related to embryo quality (Bolton *et al.,* 1989; Woodward and Lenton 1992; Rogers 1995). Both late implantation and a slower hCG rise may reflect a slower growing conceptus (Liu *et al.,* 1995; Wilcox *et al.,* 1999). However, conditional on survival to 6 weeks, neither late implantation nor a slower rate of hCG rise during the first week were associated with survival in our data.

In summary, maternal urinary hCG levels are quite variable during the first week following implantation. During this gestational stage, hCG patterns rise very rapidly but the relative rate of rise decelerates as the week advances. Among the factors evaluated, the only significant predictor of hCG trajectories during the first week was time to implantation.

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