Prognostic value of beta-human chorionic gonadotropin is dependent on day of embryo transfer during in vitro fertilization

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Objective: To determine threshold β-hCG levels predictive of an ongoing pregnancy (OP), live birth (LB), and multiple gestation (MG) in IVF cycles resulting from day-3 (D3) vs. day-5 (D5) embryo transfers (ET), to compare IVF cycle characteristics and pregnancy outcomes in D3 vs. D5 ET groups, and to assess the degree to which maternal characteristics and cycle parameters were predictive of higher β-hCG levels.
Design: Retrospective analysis.
Setting: Infertility center.
Patient(s): Women who had ET performed for IVF cycles between July 2004 and January 2010.
Intervention(s): Embryo transfer performed on either D3 or D5 after oocyte fertilization.
Main Outcome Measure(s): Beta-hCG on day 15 after oocyte fertilization.
Result(s): Beta-hCG levels were significantly higher with D5 ET compared with D3 ETs (D3: 103.6 ± 4.4 IU/L vs. D5: 198.0 ± 10.6 IU/L), and a multivariate analysis demonstrated that D5 ET was a significant predictor of higher β-hCG levels. The β-hCG thresholds predictive of OP were 78 IU/L and 160 IU/L for D3 and D5 ET, which predicted OP in 96% and 91% of cases, respectively. Similarly, for LB, the β-hCG thresholds were 94 IU/L (79% positive predictive value [PPV]) and 160 IU/L (88% PPV), and for MG were 250 IU/L (18% PPV) and 316 IU/L (34% PPV), respectively.
Conclusion(s): Initial β-hCG levels are dependent on the day of ET and are a reliable and highly predictive tool for OP outcomes. (Fertil Steril® 2011;96:1362–6. ©2011 by American Society for Reproductive Medicine.)

Key Words: Day-3 embryo transfer, day-5 embryo transfer, beta-human chorionic gonadotropin, blastocyst-stage embryo, cleavage-stage embryo, predictive values

Human chorionic gonadotropin is secreted by syncytial trophoblast and appears in maternal circulation approximately 6–8 days after fertilization (1–3). It is well documented in the literature that levels of β-hCG in early pregnancy are predictive of pregnancy outcomes (4–14); however, the prognostic value of β-hCG thresholds has not been routinely integrated into clinical practice with IVF cycles. Many couples undergoing IVF endure a significant amount of stress and anxiety awaiting treatment outcomes, and a tool for early prediction of pregnancy outcomes based on cutoff β-hCG values would be beneficial to both the patient and clinician.

In IVF cycles, embryos can be transferred into the uterus either 2 or 3 days after fertilization (cleavage-stage embryo) or 5 to 6 days after fertilization (blastocyst-stage embryo). Compared with cleavage-stage embryos, blastocyst transfers offer the advantages of better viability and developmental potential, better synchronization between the stage of embryonic development and the endometrial environment, the opportunity to perform preimplantation genetic diagnosis when indicated, and higher implantation rates allowing transfer of fewer embryos and potentially decreasing the risk of higher-order multiple gestations (MG) (15). Embryos chosen for blastocyst transfer represent a select population of higher-quality embryos, whereas those embryos not selected for blastocyst transfer may be of lesser quality and at higher risk of aneuploidy. Given these inherent differences between cleavage-stage and blastocyst-stage embryos, embryo transfer (ET) at different developmental stages is likely to impact initial β-hCG levels, and thus differential patient counseling and recommendations dependent on day of ET would be appropriate. However, the majority of studies investigating the prognostic value of β-hCG thresholds did not separate their analyses according to day of ET. To establish reliable estimates of β-hCG cutoff values that may serve as clinically useful prognostic indicators of pregnancy outcomes, studies investigating specific β-hCG thresholds after day-3 (D3) and day-5 (D5) ETs are needed.

The present study used data collected at a large infertility facility to establish β-hCG cutoff values predictive of ongoing pregnancy (OP), live birth (LB), and MG for IVF cycles involving D3 and D5 ETs. To determine these thresholds, we evaluated β-hCG values drawn 15 days after oocyte fertilization (day 12 after a D3 ET and day 10 after a D5 ET) for D3 and D5 ETs using receiver operating characteristic (ROC) curves. Secondary aims of the present investigation were to compare the IVF cycle characteristics and pregnancy outcomes in D3 vs. D5 ET groups and to assess the degree to which

Received June 15, 2011; revised September 22, 2011; accepted September 23, 2011; published online November 1, 2011.
A.S.Q.K. has nothing to disclose. Y.C.-A. has nothing to disclose. M.J.B. has nothing to disclose. W.S.M. has nothing to disclose. D.I.H. has nothing to disclose. V.N.W. has nothing to disclose. D.R.C. has nothing to disclose. G.F.M. has nothing to disclose. S.J.O. has nothing to disclose.
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0015-0282/$36.00
doi:10.1016/j.fertnstert.2011.09.042

maternal characteristics and cycle parameters were predictive of higher β-hCG levels.

MATERIALS AND METHODS

We retrospectively reviewed the electronic medical records of patients who underwent IVF between July 2004 and January 2010 from an infertility center in Margate, Florida. This study was exempt from institutional review board review because of its retrospective noninterventional nature. No patients were contacted, and no identifying patient information was used for the purposes of this study. Inclusion criteria for this study included [1] nondonor cycles with fresh embryos that were transferred on either D3 or D5 and [2] β-hCG drawn on day 15 (D15) after oocyte fertilization. Because cycles involving D15 β-hCG levels represented the largest group available for analysis, the study was restricted to only these particular cycles.

Treatment Protocol

Patients underwent IVF according to standard stimulation protocols. Protocols involved pituitary down-regulation with either GnRH agonist administered in the midluteal phase of the prior cycle (long protocol) or diluted GnRH agonist on day 2–4 of the cycle (microdose protocol). Alternatively, GnRH antagonist short protocols started when the leading follicle reached 14 mm. Controlled ovarian stimulation was achieved with hMG and/or recombinant FSH. The response to stimulation was monitored with serum E_2 and transvaginal ultrasound. Human chorionic gonadotropin was administered to stimulate the final stages of follicular development when follicles reached maturity, defined by two to four leading follicles reaching >18 mm. Oocyte aspiration was performed 34–36 hours after hCG administration under transvaginal ultrasound guidance, and oocytes were then placed in Quinn's advantage cleavage media (SAGE In Vitro Fertilization). Fertilization occurred via conventional IVF, with sperm added to oocyte culture 4–6 hours after oocyte retrieval, or intracytoplasmic sperm injection when indicated on the basis of low number or poor-quality oocytes or for male factor infertility if sperm parameters were suboptimal according to World Health Organization or strict Kruger's criteria. If at least four embryos at the four-cell stage with minimal or no fragmentation were present, embryos were moved to Quinn's advantage blastocyst media (SAGE In Vitro Fertilization), and ET was delayed until D5. Using this stringent criterion, a smaller group of embryos were available for blastocyst transfer. Number of embryos transferred was dependent on maternal age, embryo quality, and the availability of surplus high-quality embryos. Patients were started on daily IM injections of P for luteal-phase support after oocyte retrieval.

All patients underwent β-hCG testing D15 after oocyte fertilization, regardless of the day of ET. Serum β-hCG concentrations were measured using a chemiluminescent enzyme immunometric assay (Immulite hormone analyzer; DPC Corporation). The assay has a detection ranging from 1.1 to 5,000 IU/L. The intra-assay and interassay coefficients of variation varied from 3.6% to 5.2% and from 7.8% to 9.9%, respectively. All patients underwent transvaginal ultrasound at 5 to 6 weeks' gestation or when β-hCG exceeded 2,000 IU/L to determine the location and number of pregnancies. Ongoing pregnancy was defined as pregnancies that progressed beyond 20 weeks' gestation.

Statistical Analysis

Statistical analyses were performed with SPSS version 18 and SAS 9.2 (SAS Institute). To assess β-hCG cutoff values predictive of OP, LB, and MG for each ET group, ROC curves were performed using a nonparametric distribution method. Receiver operating characteristic curves are a graphic representation of sensitivity (or true-positive rate) vs. 1 minus specificity (or false-positive rate). Discrimination thresholds were chosen on the basis of optimal sensitivity and specificity. The percentages for area under the curve and 95% confidence intervals were generated for each ROC curve. In addition, β-hCG values between 78 IU/L and 400 IU/L were used as reference points to demonstrate the likelihood of OP and LB at different β-hCG values.

To determine differences between D3 and D5 ET groups, parametric and nonparametric analyses were conducted after determining whether the variables met the normality and homoscedasticity assumptions. Student *t* tests and Mann-Whitney *U* test were conducted to analyze continuous and discrete ordinal variables, and nominal data were analyzed with χ^2 tests. All *t* tests were two-tailed, and Bonferroni's correction was used to adjust for multiple comparisons (16) unless otherwise stated. A *P* value of $<.05$ was considered statistically significant.

A hierarchical linear regression analysis was also conducted to determine the variables that significantly predicted higher serum β-hCG levels using a generalized mixed linear model approach in a SAS system. This system provides a flexible framework to build hierarchical models correlating measurements made on the same level of a random factor, including subject-specific regression models, while a variety of covariance and correlation structures can be specified for residuals. A restricted/residual maximum likelihood estimation with a compound symmetry covariance structure was used to generate various models that analyzed individual characteristics and cycle parameters, while accounting for multiple IVF cycles performed within an individual. Several successive models were performed with R^2 and R^2 changes used to ascertain the overall variance explained, *F* tests to assess the statistical significance of each individual model, and *t* tests to evaluate the significance of each predictor to each model. The final regression analysis excluded the following variables that violated multicollinearity assumptions (with correlation coefficients >0.70): body mass index, peak E_2 levels, number of follicles >14 mm, number of oocytes retrieved, inseminated, and fertilized, and the presence of fetal cardiac activity.

RESULTS

The medical records of 2,953 patients who underwent 5,263 IVF cycles were reviewed. Data excluded from analyses included cycles involving donor oocytes, frozen embryo transfers, and ETs performed on days other than D3 or D5. Of these remaining 2,621 cycles, 1,729 cycles had β-hCG drawn D15 after oocyte fertilization and met the inclusion criteria for this study. Mean female age in our study group overall was 35.0 ± 0.1 (SEM) years (range, 20–44 years), with the predominant ethnicity being Caucasian (56.4%) and the most common infertility diagnosis being male factor infertility (20.1%). When categorized into D3 and D5 ET groups, women who underwent D5 ET were significantly younger and had significantly lower basal FSH, higher baseline antral follicle count (AFC), higher E_2 on day of hCG administration, more oocytes retrieved and fertilized, and fewer embryos transferred ($P<.001$). Numbers of cycles resulting in pregnancy, LB, singletons, and twins were significantly higher in the D5 ET group ($P<.001$) (Table 1). Though not significantly different between groups, D5 ET tended to have fewer triplet gestations.

Similar comparisons were done when D3 and D5 ETs were subdivided into singleton and twin gestations. Female age ($P<.001$, $P<.001$), basal FSH ($P<.001$, $P=.017$), and numbers of embryos transferred ($P<.001$, $P<.001$) were significantly lower in blastocyst transfers, and E_2 on day of hCG and number of oocytes retrieved and fertilized were significantly higher in D5 ETs in both singleton and twin gestations ($P<.001$, $P<.001$). Overall, β-hCG was significantly higher in D5 ETs compared with D3 ETs (Table 1, $P<.001$), and this trend remained significant when data were grouped into singleton and twin gestations ($P<.001$, $P<.001$).

The final hierarchical linear regression model predicting serum β-hCG levels was highly significant ($P<.001$) and accounted for more than 70% of the explained variance. Higher serum β-hCG levels were significantly predicted by a greater number of sacs seen on ultrasound ($P<.001$) and a D5 ET ($P=.003$) (Table 2). Because number of sacs was the strongest predictor of initial β-hCG levels, two separate regressions were performed for singleton and multiple gestations. Higher β-hCG levels were still significantly predicted by a D5 ET in both groups ($P=.001$, $P<.001$).