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# A decrease in serum estradiol levels after human chorionic gonadotrophin administration predicts significantly lower clinical pregnancy and live birth rates in *in vitro* fertilization cycles†

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**BACKGROUND:** Although close observation of serum estradiol (E2) levels remains a mainstay of assessing clinical response to controlled ovarian stimulation, the prognostic value of any change in E2 levels after administration of hCG remains unclear. The objective of this study is to evaluate the relationship between serum E2 response after hCG administration and the clinical pregnancy and live birth rates in fresh IVF cycles.

**METHODS:** We conducted a retrospective cohort study of women aged 21–45 years undergoing their first IVF cycle from 1999 to 2008 at a single practice. We compared the post-hCG serum E2 level with values on the day of hCG trigger. IVF cycles were stratified by post-hCG E2 response and appropriate parametric and non-parametric statistics were performed. Clinical intrauterine pregnancy and live births were the primary outcomes of interest. Multivariable logistic regression models were created to identify predictive factors associated with outcomes while adjusting for potential confounders.

**RESULTS:** Among the 1712 IVF cycles, 1065 exhibited a >10% increase (Group A), 525 had a plateau (±10%, Group B) and 122 showed a >10% decrease (Group C) in post-hCG E2 levels. While the E2 levels on the day of hCG were similar across groups, Group C had more patients with diminished ovarian reserve, required higher gonadotrophin doses and had the lowest implantation rates. After adjusting for age, total gonadotrophin dose, infertility diagnosis, number of oocytes and number of transferred embryos, the associations between post-hCG E2 decline (Group C) and clinical pregnancy [adjusted odds ratio (aOR): 0.53; 95% confidence interval (CI): 0.33–0.84, $P = 0.007$] and live birth (aOR: 0.40; 95% CI: 0.22–0.71, $P = 0.002$) were significant. We also found significant associations between E2 plateau (Group B) and clinical pregnancy (aOR: 0.73; 95% CI: 0.57–0.94, $P = 0.013$) and live birth (aOR: 0.74; 95% CI: 0.56–0.97, $P = 0.032$) when adjusting for the same factors.

**CONCLUSIONS:** In our study, >10% decrease in E2 levels after hCG administration was associated with 40–50% reduction in clinical pregnancy and live birth rates. Similarly, post-hCG E2 plateau (±10%) lowered the clinical pregnancy and live birth rates by >25%. Our study suggests that the change in the post-hCG E2 level is another parameter that can be used by clinicians to counsel patients regarding their likelihood of success with assisted reproductive technologies prior to oocyte retrieval.

**Key words:** IVF/ICSI outcomes / estrogen / hCG / implantation / pregnancy

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# Introduction

The birth of Louise Brown in 1978 signified a landmark breakthrough in reproductive medicine, as she was the first successful live birth conceived by IVF (Steptoe and Edwards, 1978). Since then, there have been significant advances in stimulation protocols, laboratory techniques and culture conditions aimed to improve clinical outcomes from IVF. However, IVF success rates are still modest, necessitating clinicians to use various parameters to counsel patients regarding their likelihood of conception. In order to facilitate clinical decision-making, numerous investigators have attempted to identify a specific factor or a combination of factors that are associated with IVF outcomes. Clinical parameters that positively correlated with IVF success include the total number of oocytes retrieved (Bouckaert *et al*., 1994; Hunault *et al*., 2002; Sharma *et al*., 2002; Hunault *et al*., 2008), endometrial thickness (McWilliams and Frattarelli, 2007; Richter *et al*., 2007; Traub *et al*., 2009), number of embryos transferred (Sharma *et al*., 2002; Pandian *et al*., 2009) and good quality embryos available for transfer (Minaretzis *et al*., 1998; Burke *et al*., 2000; Strandell *et al*., 2000; Terriou *et al*., 2001; Sharma *et al*., 2002; Thomas *et al*., 2010). In contrast, factors associated with a reduced likelihood of IVF success include advanced maternal age (Creus *et al*., 2000; Chuang *et al*., 2003; Ottosen *et al*., 2007; Hunault *et al*., 2008; Nelson and Lawlor, 2011), elevated basal FSH levels (Creus *et al*., 2000; Ottosen *et al*., 2007; van Loendersloot *et al*., 2010), duration of infertility (Templeton *et al*., 1996; Lintsen *et al*., 2005; Nelson and Lawlor, 2011), low antral follicle number (Hendriks *et al*., 2005; Khairy *et al*., 2008), premature luteinization (Bosch *et al*., 2003; Ou *et al*., 2008) and low serum E2 levels during controlled ovarian stimulation (Phelps *et al*., 1999; Khalaf *et al*., 2000).

While serum E2 levels remain an integral component of assessing response to controlled ovarian stimulation, the prognostic value of any particular change in E2 levels before or after hCG administration remains unclear. In fact, the serum E2 concentration alone on the day of hCG has been reported to be a poor predictor of IVF outcome (Kyrou *et al*., 2009). A few studies have assessed the significance of change in E2 levels on the day following hCG administration on IVF cycle outcome with conflicting results (Laufer *et al*., 1986; Meyer *et al*., 1999; Chiasson *et al*., 2007). All these studies are hampered by small sample size and only one study examines the key IVF outcome of live birth rates (Chiasson *et al*., 2007). In an effort to address these limitations, our study was designed to evaluate the relationship between change in E2 levels in response to hCG administration and IVF outcomes including clinical pregnancy and live births. We hypothesize that patients with a decrease in post-hCG E2 levels will have lower clinical pregnancy and live birth rates.

# Materials and Methods

## Patient population

In this retrospective cohort study, all women aged 21–45 years who underwent fresh IVF non-donor cycles at Penn Fertility Care between 1999 and 2008 were identified. Analysis was limited only to first IVF cycles for which complete clinical data with pregnancy outcomes were available ($n = 1712$), representing 57.4% of the total cycles during this period. We excluded those cycles without accurate medical record numbers and missing data (9.8% of fresh, non-donor, first cycles). Patient clinical characteristics and details of the IVF cycle, including stimulation protocol, total gonadotrophin dosage, number of oocytes retrieved, use of ICSI or assisted hatching, number of embryos transferred, implantation rate and pregnancy outcome, were obtained from the Penn Fertility Care IVF database. Diminished ovarian reserve (DOR) was characterized with the presence of at least one of the following: serum day 3 FSH value $\geq$11.4 mIU/ml (Esposito *et al*., 2002), total antral follicle count $\leq$4 or day 3 E2 value $\geq$80 pg/ml (Molinaro *et al*., 2009). Serum FSH value of 11.4 mIU/ml represents the upper limit of the normal range according to the assay used in our endocrinology laboratory. Serum hormone values were obtained from databases of the Pathology and Endocrine Laboratories of the Hospital of the University of Pennsylvania. Approval for the study was obtained from the institutional review board of the University of Pennsylvania.

## Controlled ovarian hyperstimulation protocols

IVF protocols used were luteal phase GnRH agonist protocol, GnRH antagonist protocol or 'microdose flare' protocol. Stimulation protocol selection was based on patient age, infertility diagnosis and ovarian reserve testing results. In patients undergoing the luteal phase GnRH agonist protocol, leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL, USA) was started at a dose of 10 U (0.5 mg) daily, 7–8 days after the LH surge. After the subsequent menses, patients underwent baseline ultrasound and serum E2 testing to confirm ovarian suppression and exogenous gonadotrophins were initiated and the dose of leuprolide acetate was decreased to 5 units (0.25 mg) daily. Exogenous gonadotrophins utilized by patients included recombinant FSH (rFSH, Gonal-F; Serono, Norwell, MA, USA or Follistim; Schering-Plough, Kenilworth, NJ, USA) or highly purified human menopausal gonadotrophins (hMG; Menopur or Repronex; Ferring Pharmaceutical, Parsippany, NJ, USA).

For patients utilizing the antagonist protocol, exogenous gonadotrophins were initiated by cycle day 2 after menses. Ganirelix acetate (Ganirelix acetate injection; Schering Plough, Kenilworth, NJ, USA) or Cetrotide (cetrorelix acetate injection; EMD Serono, Rockland, MA, USA) was initiated when serum E2 levels reached >300 pg/ml or on Day 5 of stimulation, and hMG supplementation was added. Patients undergoing the microdose flare protocol started leuprolide acetate 40 μg twice daily on cycle day 1–2 which was maintained throughout the cycle and exogenous gonadotrophins were started on Day 3.

Criteria for hCG administration were the same for all patients, regardless of protocol used. hCG of 10 000 IU (Novarel; Ferring, Parsippany, NJ, USA) was administered when at least two ovarian follicles were $\geq$18 mm in mean diameter. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h after hCG administration, and embryo transfer was performed on the third or fifth day post-retrieval. Daily progesterone 50 mg IM provided luteal phase support.

## Blood samples and endocrine hormone measurements

Day 3 FSH values were obtained within 6 months of the fresh IVF cycle. If multiple values were available, then the highest Day 3 FSH value was used for analysis. Follicular growth was monitored by serial transvaginal ultrasounds and serum E2 levels. Additionally, serum E2 and LH levels were measured on the morning of hCG administration and repeated 10–12 h after hCG administration (post-hCG E2 and LH). Serum hCG test was performed 12–14 days following embryo transfer. Serum E2, LH and hCG were measured using the Immulite 2000 (Siemens; Deerfield, IL). The sensitivity of the E2, LH and hCG assays are 15 pg/ml, 0.05 mIU/ml and 0.4 mIU/ml, respectively, and all have intra-assay and inter-assay

variation coefficients ranging from 2 to 7%. To reduce the assay variability at high serum values, in our endocrinology laboratory, all samples with E2 levels >1300 pg/ml are diluted before repeating the assay to obtain results within the range of maximum accuracy. Linearity under dilution is verified every 6 months to determine the maximal curve slope for E2.

## Micromanipulation and embryo transfer

Criteria for ICSI included the use of non-ejaculated sperm, total motile count <2.5 million, motility <20% and morphology <30%. Assisted hatching was performed in patients >38 years. The majority of patients underwent Day 3 embryo transfers with few having Day 5 embryo transfer. Patients with failed fertilization, arrest of embryo development and severe ovarian hyperstimulation syndrome did not undergo embryo transfer. Embryo cryopreservation was performed on Day 1 if >6 embryos fertilized and on Day 3 if there were residual embryos after transfer. Daily progesterone 50 mg IM provided luteal phase support. During the study period, the number of embryos transferred or luteal phase progesterone support was not altered based on post-hCG E2 levels.

## Definition of outcomes

Pregnancy outcomes and the associated rates were defined using standard SART definitions as follows. The *implantation rate* reflects the number of gestational sacs visualized on transvaginal ultrasound divided by the number of embryos transferred. A *clinical intrauterine pregnancy* (CIP) was identified with the presence of an intrauterine gestational sac confirmed by transvaginal ultrasonography and is expressed per cycle start. *Spontaneous abortion* was defined as spontaneous pregnancy loss after sonographic visualization of an intrauterine gestational sac and is expressed per CIP. *Live birth rate* was classified as those cycles resulting in the delivery of a viable infant after 24 weeks gestation and expressed per cycle.

## Statistical analysis

Estradiol response was calculated as the percentage change in E2 levels on the day after hCG administration compared with the E2 levels on the day of hCG trigger. Patients were stratified into three groups based on E2 response to hCG administration: >10% increase in post-hCG E2 levels (Group A), plateau (±10% change) in post-hCG E2 levels (Group B) and >10% decrease in post-hCG E2 levels (Group C). These classifications are consistent with previous studies (Meyer *et al*., 1999; Chiasson *et al*., 2007). Analysis of variance and the Kruskal–Wallis test were performed to compare the three groups. Individual associations between demographic and clinical characteristics of the patients according to the E2 response were evaluated by Student's *t*-test (for parametric data) or Mann–Whitney *U*-test (for non-parametric data) for continuous variables, and chi-square test for categorical variables, as appropriate. CIP and live birth were the primary outcomes of interest. Univariate analysis was performed to identify significant individual associations between variables and the clinical outcomes. Multivariable logistic regression models were employed to evaluate the associations between variables of interest and clinical pregnancy and live birth while adjusting for potential confounders. Data analysis was conducted using STATA version 11 (StataCorp, College Station, TX, USA). A *post hoc* power analysis was performed to estimate the effect size. Statistical significance was interpreted as $P$ value $< 0.05$.

# Results

We analyzed a total of 1712 fresh, first IVF cycles (Table I). The majority of IVF cycles had a post-hCG E2 increase of >10% compared with that on the day of hCG administration (Group A, $n = 1065$, 62.2%). The IVF cycles in Group B had post-hCG E2 levels between ±10% ($n = 525$, 30.7%), and IVF cycles in Group C demonstrated a >10% decrease in post-hCG E2 levels ($n = 122$, 7.1%; Table I). Women in Group A were slightly younger than that in Group B (34.8 versus 35.9 years, $P < 0.001$). There were no significant differences in gravidity, prior full-term delivery or prior spontaneous abortion history among the three groups. While the maximum Day 3 serum FSH level for patients in Groups B and C were similar, both groups were significantly higher than Group A ($P < 0.001$). Correspondingly patients in Group A required significantly less exogenous gonadotrophins than patients in Groups B and C ($P < 0.001$). Patients with >10% increase in post-hCG E2 levels were more likely to have PCOS or tubal factor infertility, while those with >10% decrease in E2 levels were more likely to have diminished ovarian reserve or unexplained infertility.

The E2 levels on the day of hCG were not significantly different between the three groups; however, as expected the E2 levels on the day after hCG were significantly different between all three groups (Table II). Groups A and C had similar mean number of oocytes retrieved, micromanipulation with ICSI and assisted hatching, and percentage of cycles with cryopreserved embryos. Group B had a lower mean oocyte yield and fewer cryopreserved embryos compared with Group A only. A similar percentage of cycles did not have an embryo transfer in all three groups. Overall, the majority of patients underwent Day 3 embryo transfers (95.1%). The proportion of patients who had Day 5 transfers was 4.8% (Group A), 4.2% (Group B) and 2.4% (Group C). The mean number of embryos transferred was similar in the three groups. The implantation rate and CIP rate (23.3 and 28.0 versus 38.4%) was significantly lower in Groups B and C compared with Group A, respectively ($P < 0.01$). The spontaneous abortion rates were similar in all three groups but the live birth rates (12.5 and 19.3 versus 27.6%) were significantly lower in both Groups B and C compared with Group A ($P < 0.001$). There was no difference in multiple pregnancy rates in the three groups. We examined the association between Day 3 FSH and IVF cycle outcome. There was a significant correlation between patient's maximum Day 3 FSH and the following variables: number of oocytes retrieved ($r = -0.32$, $P < 0.0001$), number of cryopreserved embryos ($r = -0.19$, $P < 0.0001$), implantation rate ($r = -0.086$, $P = 0.001$), clinical pregnancy ($r = -0.076$, $P = 0.002$) and live birth ($r = -0.081$, $P = 0.001$).

In addition, we performed univariate analysis to identify individual factors associated with clinical pregnancy and live births, which were used in logistic regression models. Multivariable logistic regression demonstrated that a >10% decrease in post-hCG E2 levels conferred a 51% reduction in the odds of achieving a CIP [unadjusted OR = 0.49, 95% confidence interval (CI): 0.31–0.76] and 62% decreased odds of a live birth (unadjusted OR = 0.38, 95% CI: 0.21, 0.66) compared with patients with a >10% increase in E2. After adjusting for patient age, total gonadotrophin dose, DOR and PCOS diagnosis, number of retrieved oocytes, use of assisted hatching, and the number of transferred embryos, the associations between >10% E2 decrease and clinical pregnancy [adjusted odds ratio (aOR): 0.53, 95% CI: 0.33–0.84, $P = 0.007$] and live birth (aOR: 0.40, 95% CI: 0.23–0.71, $P = 0.002$) remained statistically significant. The inclusion of day of embryo transfer and ovarian stimulation protocol in the multivariate analysis did not change the adjusted OR for clinical pregnancy or live birth. Additionally, these individual variables were not significantly associated with the outcome when adjusting for other

**Table I Patient characteristics in three groups stratified by serum estradiol response to hCG administration.**

| | Group A (>10% rise), $n = 1065$ | Group B (± 10% plateau), $n = 525$ | Group C (>10% fall), $n = 122$ | *P*-value |
|---|---|---|---|---|
| Clinical characteristics (mean ± SD) | | | | |
| Age (years) | 34.8 ± 4.2 | 35.9 ± 4.2 | 35.1 ± 4.2 | 0.0001[a,†] |
| History of prior gravidity (*n*, %) | 525 (49.3) | 241 (45.9) | 61 (50.0) | 0.63 |
| History of prior full term delivery (*n*, %) | 209 (19.6) | 101 (19.2) | 27 (22.1) | 0.96 |
| Number of prior spontaneous abortion (range) | 0.79 ± 1.0 (0–5) | 0.73 ± 0.95 (0–6) | 0.75 ± 0.94 (0–4) | 0.55 |
| Maximum Day 3 FSH (mIU/ml) | 7.4 ± 3.9 | 8.0 ± 3.8 | 8.2 ± 3.3 | 0.04[a,b,†] |
| Total gonadotrophin (FSH) dose (ampules) | 40.0 ± 19.8 | 46.6 ± 19.7 | 46.5 ± 19.2 | 0.0001[a,†,b,†] |
| Infertility diagnosis (%) | | | | |
| Male factor | 33.0 | 30.1 | 29.5 | 0.40 |
| Tubal factor | 25.1 | 22.3 | 14.8 | 0.03[b] |
| Endometriosis | 17.6 | 18.7 | 11.5 | 0.17 |
| Diminished ovarian reserve | 16.9 | 23.4 | 25.4 | 0.002[a,†,b] |
| Polycystic ovarian syndrome | 13.9 | 5.3 | 7.4 | <0.001[a,†,b] |
| Uterine factor | 8.9 | 8.2 | 6.6 | 0.64 |
| Unexplained | 12.5 | 13.3 | 21.3 | 0.02[b,c] |
| Ovarian stimulation protocol (%) | | | | |
| GnRH agonist | 81.0 | 71.2 | 75.4 | <0.001[a,†] |
| GnRH antagonist | 3.8 | 5.1 | 5.7 | 0.37 |
| Microdose GnRHa Flare | 15.2 | 23.6 | 18.8 | <0.001[a,†] |

*Note*: values are the mean ± SD unless otherwise noted.
[a]Group A significantly different from Group B, $P < 0.05$.
[b]Group A significantly different from Group C, $P < 0.05$.
[c]Group B significantly different from Group C, $P < 0.05$.
[†]*P* value ≤ 0.001.

factors, thus were not included in the final model. The use of assisted hatching was significantly associated with CIG and live birth, and was added to the final model, although the results were unchanged. We also found significant associations between E2 plateau (± 10% change) and clinical pregnancy (aOR: 0.73, 95% CI: 0.57–0.94, $P = 0.013$) and live birth (aOR: 0.74, 95% CI: 0.57–0.97, $P = 0.032$) when adjusting for the same factors.

We compared the mean difference in serum E2 levels between cycles where a CIP was achieved and those where it was not. The average change in E2 levels of the 577 cycles resulting in a clinical pregnancy was 745.0 ± 916.0 pg/ml post-hCG compared with 552.8 ± 9883.0 pg/ml in the 1113 cycles with no pregnancy ($P < 0.0001$). Of note, similar proportions of cycles underwent egg retrieval without subsequent embryo transfer (Group A: 9.1%, Group B: 10.5%, Group C: 11.5%; $P = 0.52$). Patients in these groups had similar mean age at stimulation (35.3 years, $P = 0.70$), prevalence of DOR diagnosis (26.5%, $P = 0.49$), total gonadotrophin dosage (49.9 ampules, $P = 0.32$), mean number of oocytes retrieved (7.9, $P = 0.19$) and embryos cryopreserved (6.0%, $P = 0.49$).

# Discussion

In this large cohort study, we found a significant association between serum E2 response the day after hCG administration and IVF outcomes including clinical pregnancy and live birth rates. Specifically, the clinical pregnancy rate and live birth rate decreased ~50% in IVF cycles with >10% post-hCG decrease (Group C) in serum E2 levels. Moreover, patients with a plateau in post-hCG serum E2 levels (Group B) showed >25% reduction in clinical pregnancy and live birth rates. Of note, Group B had fewer oocytes retrieved compared with Group A. Both Groups B and C had several indicators of diminished ovarian reserve, such as high Day 3 FSH levels and increased gonadotrophin requirement. Although the mean number of transferred embryos was similar in the three groups, the implantation rates were significantly higher in the group that demonstrated >10% increase in post-hCG E2 levels. Our findings suggest that >10% increase in E2 levels may represent the optimal E2 response on the day after hCG administration and patients should be counseled accordingly.

Our findings are consistent with an early retrospective study by Laufer *et al.* (1986) of women ($n = 162$) with tubal factor infertility who underwent laparoscopic oocyte aspiration. They reported lower pregnancy rates per retrieval in women with a decrease in post-hCG E2 levels (13 versus 19%) with similar fertilization and cleavage rates when compared with the patients with an increase in E2 levels. In fact the authors advocated cancellation of retrieval in patients who displayed a decrease in E2 levels.

**Table II IVF cycle outcomes by serum estradiol response to hCG administration**

| | Group A (>10% rise), $n = 1065$ | Group B (±10% plateau), $n = 525$ | Group C (>10% fall), $n = 122$ | *P*-value |
|---|---|---|---|---|
| Hormone response to hCG | | | | |
| Day of hCG E2 (pg/ml) | 3017 ± 1331 | 3010 ± 1380 | 3194 ± 1430 | 0.56 |
| Post-hCG E2 (pg/ml) | 4051 ± 1898 | 3059 ± 1413 | 2639 ± 1157 | 0.0001[a,†,b,†,c] |
| Day of hCG LH (mIU/ml) | 1.8 ± 1.3 | 1.8 ± 1.3 | 1.5 ± 1.1 | 0.08 |
| Post-hCG LH (mIU/ml) | 1.4 ± 3.2 | 1.4 ± 1.1 | 1.2 ± 0.9 | 0.03[b] |
| Post-hCG E2 absolute difference (pg/ml) | 1034 ± 882 | 49 ± 178 | −555 ± 446 | 0.0001[a,†,b,†,c,†] |
| Post-hCG E2 percent difference (%) | 35.1 ± 28.7 | 1.6 ± 5.4 | −17.0 ± 6.8 | 0.0001[a,†,b,†,c,†] |
| Number of oocytes retrieved | 12.5 ± 6.8 | 11.0 ± 6.2 | 11.8 ± 6.8 | 0.0001[a,†] |
| Micromanipulation | | | | |
| ICSI (%) | 22.3 | 18.9 | 23.0 | 0.26 |
| Assisted hatching (%) | 30.6 | 40.8 | 32.0 | <0.0001[a,†] |
| IVF cycle outcomes | | | | |
| Cycles with embryos cryopreserved (%) | 29.5 | 22.1 | 25.4 | 0.007[a] |
| Number of embryos cryopreserved | 1.8 ± 3.5 | 1.5 ± 3.2 | 1.5 ± 3.0 | 0.005[a] |
| Number of embryos transferred | 2.7 ± 1.0 | 2.8 ± 1.1 | 2.7 ± 1.1 | 0.13 |
| Cycles without embryo transfer (*n*, %) | 97 (9.1) | 55 (10.5) | 14 (11.5) | 0.54 |
| Clinical outcomes per cycle | | | | |
| Number of fetal heart tones (FHTs) | 1.2 ± 0.7 | 1.3 ± 0.7 | 1.2 ± 0.8 | 0.28 |
| Implantation(%) | 20.7 | 16.2 | 14.1 | 0.002[a,†,b] |
| Clinical intrauterine pregnancy (%) | 38.4 | 28.0 | 23.3 | <0.001[a,†,b,†] |
| Spontaneous abortion (%) | 19.7 | 18.7 | 31.8 | 0.55 |
| Live birth (%) | 27.6 | 19.3 | 12.5 | <0.001[a,†,b,†] |
| Multiple gestations per delivery (%) | 31.2 | 36.0 | 35.8 | 0.57 |

Note: values are the mean ± SD unless otherwise noted.
[a]Group A significantly different from Group B, $P < 0.05$.
[b]Group A significantly different from Group C, $P < 0.05$.
[c]Group B significantly different from Group C, $P < 0.05$.
[†]*P* value ≤ 0.001.

With the advent of transvaginal ultrasound-guided oocyte retrieval and the development of new stimulation protocols and micromanipulation techniques over the past two decades, it is important to re-evaluate the significance of changes in post-hCG E2 levels in light of current practice. Only two studies have examined this question more recently (Meyer *et al.*, 1999; Chiasson *et al.*, 2007). Meyer *et al.* performed a prospective cohort study of 222 couples undergoing IVF (1999). There was no difference in implantation, ongoing pregnancy or spontaneous abortion rates among the groups with an increase (>10% rise), plateau (±10% change) and decrease (>10% decline) in post-hCG E2 levels. However, the study may not have had adequate power to demonstrate a difference in pregnancy rates given the relatively small sample size. Moreover, their patient population was heterogeneous (recruited from three different centers) with no uniform stimulation protocols or clinical management strategies. Subsequently, Chiasson *et al.* (2007) conducted a retrospective analysis of 844 IVF cycles stratified into four groups by E2 response: >10% increase, ±10% change, 10–30% increase and >30% increase. They observed that decrease in post-hCG E2 levels did not affect the number of oocytes retrieved, the number of mature oocytes, or the fertilization or implantation rates. The clinical pregnancies and live birth rates were similar across the four groups. Given the expense and venipuncture associated with the additional serum testing, these authors did not recommend monitoring post-hCG E2 levels. In contrast to our study, they presented only between-group comparisons without adjusting for any potential confounding variables. While the study had a larger sample size, it may still have been underpowered to show a difference in pregnancy and live birth rates.

One of the main strengths of our study is the large sample size including >1700 fresh IVF cycles within a single institution. A *post hoc* power analysis revealed >96% power to detect the reported difference in clinical pregnancy rate and live births. Secondly, we included end-points that are particularly valuable to patients: CIP and live birth rate. Furthermore, we adjusted our analysis for a number of clinically relevant variables and continued to detect a decrease in both pregnancy and live birth rates in Groups B and C. The limitations of our study are those inherent to retrospective research, which often requires the analysis of data originally collected for reasons other than research purposes (Hess, 2004; Jansen *et al.*, 2004). Our IVF data are, however, collected in an ongoing manner for submission

to the Society for Assisted Reproductive Technologies and verified annually for accuracy and completion. While other authors have investigated the predictive value of serum estradiol curve and progesterone values during ovarian stimulation on IVF outcome (Loumaye *et al.*, 1997; Chen *et al.*, 2003; Kyrou *et al.*, 2012; Wu *et al.*, 2012), we did not collect similar data for this study. In our study the mean E2 and LH levels on the day of hCG were similar across the three groups and premature luteinization is unlikely as the mean LH level was $<2$ mIU/ml in all three groups. However, we did not examine progesterone levels on the day of hCG and recognize this is a limitation of our study.

Both Groups B and C that did not have a $>10\%$ increase in E2 had significantly higher Day 3 FSH levels and increased gonadotrophin requirement compared with Group A. These findings also support our data that women with diminished ovarian reserve had a greater likelihood for having a plateau or decrease in the post-hCG E2 levels. All three groups had similar rates of male factor infertility and ICSI; however, Groups B and C had fewer embryos available for cryopreservation. This information may be useful in counseling women with both DOR and decrease in post-hCG E2 levels regarding the outcomes of their IVF cycle.

The underlying cause for the association between pregnancy and live birth rate and a decrease in post-hCG E2 levels is unclear. In natural menstrual cycles, serum E2 levels typically decrease after the initial LH surge (Hillier *et al.*, 1981; Speroff and Fritz, 2005), which is coordinated with optimal monofollicular development and oocyte maturation. In contrast, gonadotrophin-stimulated cycles produce multiple follicles and oocytes at different stages of maturation, and may thereby exhibit different responses to hCG administration. As the follicle develops and matures, the concentration of LH receptors on granulosa cells increases and the use of high doses of long-acting hCG may affect estradiol production and rate of luteinization (Speroff and Fritz, 2005). It has been suggested that the decrease in serum E2 post LH surge may reflect a reduction in androgen production by theca cells and/or concurrent decline in aromatase activity within the granulosa cells (Hillier *et al.*, 1981). A decrease in post-hCG levels of estradiol may also be a response to premature luteinization, which occurs in IVF cycles despite the widespread use of GnRH agonists for pituitary down-regulation and GnRH antagonists for competitive inhibition of pituitary gonadotrophin secretion (Silverberg *et al.*, 1991; Ubaldi *et al.*, 1996; Fisher *et al.*, 2005; Venetis *et al.*, 2007). An increase in serum progesterone levels during ovarian stimulation suggestive of premature luteinization has been reported to occur in 2–41% of cycles (Younis *et al.*, 1996; Bosch *et al.*, 2003; Elnashar, 2010).

In our study the majority of IVF cycles demonstrated an increase in E2 levels post-hCG associated with the improved clinical pregnancy rates and live births. The initiation and development of a successful pregnancy requires the intricate coordination of the developing embryo and the maternal uterine milieu. Implantation, the complex process of cellular and molecular events, is characterized by three sequential stages that correspond to the apposition, adhesion and invasion of the embryo to the endometrium (Loutradis *et al.*, 2008). Despite the mean number of embryos transferred in all three groups being similar, the implantation rates were significantly lower in the two groups that did not have a $>10\%$ increase in post-hCG E2 levels. Our study was not designed to elucidate the mechanism of lowered clinical and live birth rates but these findings suggest that a decrease in post-hCG E2 may be associated with suboptimal oocyte/embryo quality or impaired endometrial receptivity impairing implantation (Paulson *et al.*, 1990; Friedler *et al.*, 2005; Chiasson *et al.*, 2007; Gruber *et al.*, 2007; Kyrou *et al.*, 2009).

In summary, our study suggests that post-hCG E2 level can be used as an additional component, along with patient age, infertility diagnosis and response to COH stimulation, to provide a comprehensive assessment of the outcome of an IVF cycle prior to oocyte *retrieval*. Based on these findings, we propose that post-hCG estradiol response can be used as an additional parameter to counsel patients about the likelihood of success and manage patients' expectations of cycle outcome. Further studies are needed to elucidate the underlying mechanisms that might result in a decrease in post-hCG E2 levels, thereby allowing clinicians to appropriately modify subsequent IVF cycles.

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## Authors' roles

L.A.K., T.A.M., and A.D. developed the study design and performed the data collection, analysis and interpretation. M.D.S. assisted with data analysis and interpretation. L.A.K. and A.D. prepared the manuscript. All authors have seen and approved the final version of the manuscript.

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## Conflict of interest

None declared.

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