Abnormal human chorionic gonadotropin (hCG) trends after transfer of multiple embryos resulting in viable singleton pregnancies

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Abstract

Purpose The purpose of this study is to investigate whether abnormal hCG trends occur at a higher incidence among women conceiving singleton pregnancies following transfer of multiple (two or more) embryos (MET), as compared to those having a single embryo transfer (SET).

Methods Retrospective cohort study was performed of women who conceived singleton pregnancies following fresh or frozen autologous IVF/ICSI cycles with day 3 or day 5 embryo transfers between 2007 and 2014 at a single academic medical center. Cycles resulting in one gestational sac on ultrasound followed by singleton live birth beyond 24 weeks of gestation were included. Logistic regression models adjusted a priori for patient age at oocyte retrieval and day of embryo transfer were used to estimate the Odds Ratio of having an abnormal hCG rise (defined as a rise or $< 66\%$ in 2 days) following SET as compared to MET.

Results Among patients receiving two or more embryos, 6.1% ($n = 84$) had abnormal hCG rises between the first and second measurements, compared to 2.7% ($n = 17$) of patients undergoing SET (OR 2.16, 95% CI 1.26–3.71). Among patients with initially abnormal hCG rises who had a third level checked (89%), three-quarters had normal hCG rises between the second and third measurements.

Conclusions Patients who deliver singletons following MET were more likely to have suboptimal initial hCG rises, potentially due to transient implantation of other non-viable embryo(s). While useful for counseling, these findings should not change standard management of abnormal hCG rises following IVF. The third hCG measurements may clarify pregnancy prognosis.

Keywords Embryo transfer · Human chorionic gonadotropin · Chemical pregnancy · In vitro fertilization

Introduction

In patients undergoing in vitro fertilization (IVF), initial serum hCG levels measured approximately 2 weeks following embryo transfer (ET) are vital to diagnosing pregnancy and monitoring these early pregnancies at gestational ages for which ultrasound is not yet helpful (up to 5–6 weeks of gestation) [1]. Serial measurements of hCG are the mainstay of monitoring these early pregnancies. Abnormal hCG trends, commonly defined as an hCG rise less than 66% in 2 days, are concerning for failing or ectopic pregnancy [2, 3]. This finding generates significant patient anxiety and may result in interventions that could potentially interrupt a highly desired pregnancy, including uterine evacuation and/or methotrexate.

Patients may develop abnormal hCG trends after IVF without ultrasound evidence of the pregnancy, such as in chemical

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pregnancies (15%) or, less commonly, presumed ectopic pregnancies (1.6%) [4–6]. It stands to reason that patients with transfer of more than one embryo who develop singleton pregnancies may have had coincidental transient implantation of other non-viable embryo(s), in the uterus or less commonly in the fallopian tube, without ever developing ultrasound evidence of these events.

To our knowledge, no studies to date have assessed early hCG trends according to whether several or only one embryo was transferred in patients conceiving viable singleton pregnancies. This study was designed specifically to ask the question whether patients who conceived singleton pregnancies after multiple embryo transfer (MET) are at higher risk of abnormal hCG trends than patients undergoing single embryo transfer (SET). In the setting of abnormal hCG trends, both patient anxiety and physician concern may result in closer surveillance and potentially unnecessary intervention of an otherwise viable pregnancy.

Materials and methods

Patient population

Institutional Review Board approval was obtained from our medical center. Records were reviewed for all women who underwent fresh or frozen ET from 2007 through 2014 at our academic infertility practice ($n = 11,498$). Fresh or frozen autologous IVF/intracytoplasmic sperm injection (ICSI) cycles with day 3 or day 5 ET were included if only one gestational sac was detected on ultrasound with a singleton live birth at or beyond 24 weeks of gestation. Stimulation protocols included those using gonadotropin-releasing hormone (GnRH) antagonists, downregulation protocols using GnRH agonists, and poor responder protocols using low-dose GnRH agonist flare or estradiol priming [7–10]. MET was defined as the transfer of two or more embryos. Frozen cycles were supported with oral estradiol (2 mg twice per day), titrated to a serum level of 200 pg/mL. With few exceptions, luteal support was provided in fresh cycles using daily applications of vaginal micronized progesterone gel (Crinone 8%, Actavis Pharma, Inc., Parsippany, NJ), while frozen cycles were supported with 50 mg of intramuscular progesterone in sesame oil, adjusted for a target serum level of at least 20 ng/mL, per protocol at our center. Luteal support was continued through 10 weeks of gestation.

Pregnancies resulting in any ultrasound evidence of multiple pregnancy (more than one gestational sac) or multiple births were excluded, as were ectopic pregnancies, and cycles utilizing preimplantation genetic diagnosis, donor oocytes, and/or gestational carriers. Records missing key clinical data, including age, cycle parameters including day of ET and number of embryos transferred, hCG levels, and treatment

outcomes, were also excluded. Clinical and laboratory data were extracted from a prospectively maintained departmental database; key data points (including hCG rises below 66% and birth outcomes) were directly verified in the electronic medical record.

hCG value determinations

Initial serum hCG levels were measured approximately 16 days after oocyte retrieval or the equivalent thereof in frozen ETs (range 11–23 days). Subsequent hCG values checked at 2-day intervals whenever possible (range 1–7 days). The hCG measurements were performed at the clinical laboratories of three participating centers. According to policy, serial hCG levels for each patient were measured at the same center. These laboratories are accredited by the College of American Pathologists or inspected by the Joint Commission with Clinical Laboratory Improvement Amendments certification. Inter- and intra-assay coefficients of variation (CV) for hCG laboratories were less than 6%. Results are expressed as milli-international unit per milliliter, standardized against the fourth International Standard for Chorionic Gonadotrophin from the National Institute for Biological Standards and Control (NIBSC) 75/589 [11].

Among patients whose hCG levels were not checked exactly 2 days apart, the 2-day rise was calculated, drawing from prior studies that reported the log-linear nature of early hCG increases, up to 10 weeks of gestation [19]. For this calculation, the difference between the log values of the first and second hCG measures was divided by the log of the first measure. This total percent increase in the log hCG was divided by the number of days between the first and second measure and multiplied by 2. Exponentiation of this value yielded an estimated absolute value of hCG, from which the absolute 2-day percent increase was calculated. A subanalysis was performed limited to patients with serum hCG measured 2 days apart, to avoid calculating the 2-day percent rise.

Statistical analyses

Generalized estimating equations were used to take multiple cycles from a patient into account where indicated. Multivariable-adjustment logistic regression models were used for dichotomous variables to calculate odds ratios (OR) and 95% confidence intervals (CI) of abnormal hCG rise between the first and second and between the second and third measurements (using a binomial distribution and logit link function). Multivariable linear regression was used to assess differences in mean hCG and percent rise in hCG using robust “sandwich” standard errors. Age of the patient at the time of oocyte retrieval was included in the models a priori as a potential confounding variable in order to be consistent with prior studies. Furthermore, the day of ET was included a priori

in the models, as day 5 embryo transfers are associated with higher subsequent serum hCG levels when compared with day 3 embryo transfers [20]. Covariates tested as predictors of abnormal hCG rise and also as potential confounders of the relationship with abnormal hCG trends included recipient age at the time of ET, recipient gravidity and parity, prior ectopic pregnancy, prior chemical pregnancy, prior miscarriage, infertility diagnosis, day 3 follicle stimulating hormone (FSH) and estradiol (E2) levels, body mass index (BMI), race, fresh versus frozen ET, peak estradiol among fresh transfers, transfer of slow frozen versus vitrified embryos among frozen cycles, use of ICSI or assisted hatching, number of good quality embryos transferred, and endometrial stripe thickness at trigger or frozen ET mapping.

Good quality cleavage-stage (day 3) embryos were defined as having at least 7 cells, fragmentation scores of 0 (0% fragmentation) or 1 (1–9% fragmentation), and symmetry scores of 1 (perfect symmetry) or 2 (moderate asymmetry) [21]. Good quality blastocysts (day 5 embryos) were defined as hatching or hatched blastocysts with fair or good quality inner cell mass and trophectoderm [22]. Patient race (white, black, Asian, Hispanic, American Indian, or other) and stimulation protocol were included as categorical variables. Prior ectopic pregnancy, prior chemical pregnancy, fresh versus frozen cycle, transfer of slow frozen versus vitrified embryos, use of assisted hatching, use of ICSI, and day of ET (day 3 or day 5) were included as dichotomous variables. Recipient and oocyte age (years), body mass index (kg/m^2), gravidity, parity, day 3 FSH (U/L) and E2 (pg/mL), peak E2 (pg/mL), endometrial stripe thickness (mm), and number of good quality embryos transferred were included as continuous variables.

The addition of these covariates separately to the base model did not change the effect estimates by more than 10%, and the covariates were therefore not included in the final model [12]. Analyses were performed using Statistical Analysis Software version 9.3 (SAS Institute, Inc.).

Results

Over the study period, 629 singleton live births following SET and 1372 singleton births following MET were identified. Demographic and cycle characteristics are shown in Table 1. Most notably, those women having SET were an average of 2 years younger than those having MET. The mean number of embryos transferred in the MET group was 2.5 (range 2–10 embryos).

Among patients receiving two or more embryos, 6.1% ($n = 84$) had abnormal rises between the first and second hCG measurements, compared to 2.7% ($n = 17$) of patients undergoing SET (OR 2.09, CI 1.20–3.64) (Fig. 1, Table 2). When analysis was limited to patients whose hCG values were measured 2 days apart ($n = 1543$, 77%), the incidence of

suboptimal 2-day hCG rises following SET (3.1%) was again significantly lower than in MET (6.3%, OR 1.93, 95% CI 1.08–3.45). The lowest 2-day change in hCG resulting in live birth was 28% among SETs and a 4% fall among METs.

The mean initial hCG values were 381.0 and 382.5 mIU/mL, for the SET and MET groups, respectively (linear regression β 0.99, 95% CI – 33.8, 35.6, Table 2). These measurements were drawn a mean of 15 days after oocyte retrieval (or the equivalent thereof in frozen cycles) in both groups (range 11–23 days).

Blastocyst transfer cycles ($n = 547$) accounted for a much higher proportion of SET (52.6%) as compared to MET (15.7%, $p < 0.0001$). Among blastocyst transfers, 3.0% of patients had low initial hCG rises following SET, as compared to 5.1% of patients after MET (OR 1.59, 95% CI 0.62–4.03), a difference which failed to reach statistical significance. However, among women having a day 3 ET ($n = 1454$), there was an increased risk of an abnormal hCG trend after MET as compared to SET (OR 2.56, 95% CI 1.17–5.60).

Fifty four percent of patients had third hCG measurements recorded, of whom 89% had abnormal rises between the first and second hCG measurements. Among patients with abnormal hCG rises between the first and second measurements, 77% had normal hCG rises between the second and third measurements. Abnormal rises between the second and third hCG values were observed in only 3.6% of patients; the incidence was similar for SET and MET groups (2.6% vs. 4.3%, respectively; OR 1.60, 95% CI 0.79–3.28, Fig. 1).

Discussion

In this study, patients who delivered singletons were more likely to have suboptimal initial hCG rises after MET compared with those who had a SET. Such abnormal hCG rises are clinically significant, resulting in patient anxiety and interventions that could jeopardize the pregnancy, such as uterine evacuation and/or methotrexate. When considering the second and third hCG measurements, hCG rises normalized (at least 66% in 2 days) in 77% of patients with initially abnormal hCG rises, indicating the utility of obtaining this third measurement for reassurance. Of note, however, an abnormal rate of hCG rise between the second and third values did not preclude live birth. Among patients with a third hCG value measured, 3.6% had abnormal hCG rises between the second and third hCG values and still went on to have live births.

Existing research has shown that hCG rises are not significantly affected by BMI or any other demographic or cycle factors [13, 14]. While a review of 360 singleton live births after IVF noted a higher rate of hCG rise following frozen ET, this relationship was confounded by perinatal outcomes (which were also associated with rates of hCG rise), and authors noted that the slopes of hCG rise after fresh or frozen ET

Table 1 Demographic and cycle characteristics among single and multiple embryo transfer cycles

Characteristics	Single embryo transfer (<i>n</i> = 629) Mean (SD) Min-Max N (%)	Multiple embryo transfer (<i>n</i> = 1372) Mean (SD) Min-Max N (%)
Patient age at oocyte retrieval (years)	33.4 (3.4)	35.8 (3.9)
Patient age at embryo transfer (years)	33.8 (3.5)	36.0 (3.9)
BMI (kg/m ²)	25.3 (6.4)	25.3 (5.7)
Race		
Caucasian	440 (70.0%)	986 (71.9%)
Asian	80 (12.7%)	136 (9.9%)
African American	24 (3.8%)	54 (3.9%)
Hispanic	29 (4.6%)	49 (3.6%)
American Indian/Native Alaskan	5 (0.8%)	4 (0.3%)
Other/declined/missing	51 (8.1%)	143 (10.4%)
Primary infertility diagnosis		
Male factor	234 (37.2%)	424 (30.9%)
Decreased ovarian reserve	75 (11.9%)	254 (18.5%)
Oligoovulation	107 (17.0%)	168 (12.2%)
Tubal	58 (9.2%)	131 (9.6%)
Uterine	18 (2.9%)	31 (2.3%)
Unexplained	171 (27.2%)	461 (33.6%)
Endometriosis	42 (6.7%)	96 (7.0%)
Other	59 (9.4%)	116 (8.5%)
Gravidity	0.90 (1.19)	1.02 (1.30)
Parity	0.39 (0.63)	0.36 (0.60)
No. of prior ectopic	0.06 (0.28)	0.06 (0.31)
No. of prior chemical pregnancy	0.06 (0.30)	0.05 (0.30)
No. of prior spontaneous abortions	0.32 (0.73)	0.46 (0.86)
Fresh cycle	416 (66.1%)	1093 (79.7%)
ICSI	193 (30.7%)	466 (34.0%)
Use of assisted hatching	78 (26.2%)	732 (63.3%)
Endometrial thickness (mm)	10.7 (2.8)	10.9 (2.7)
Day 3 transfer	298 (47.4%)	1156 (84.3%)
No. of embryos transferred	1	2.5 (1.0)
No. of high quality embryos transferred ^a	0.8 (0.8)	1.3 (1.2)
No. of days post-oocyte retrieval of first hCG check	15.4 (1.2)	15.4 (1.3)

SD standard deviation, *BMI* body mass index, *ICSI* intracytoplasmic sperm injection, *hCG* human chorionic gonadotropin

^a Good quality cleavage-stage (day 3) embryos defined as having at least 7 cells, fragmentation scores of 0 (0% fragmentation) or 1 (1–9% fragmentation), and symmetry scores of 1 (perfect symmetry) or 2 (moderate asymmetry). Good quality blastocysts (day 5 embryos) were defined as hatching or hatched blastocysts with fair or good quality inner cell mass and trophectoderm. References contained in text

were not significantly different [14]. In this present study, no cycle or demographic factors were found to significantly influence the relationship between embryo number and risk of abnormal hCG rise. The number of embryos transferred was the only significant predictor of abnormal hCG rise in this study of women conceiving ongoing singleton pregnancies.

We acknowledge the limitations of this study. Use of a database with many contributors may introduce data entry errors, although the system is periodically audited for outliers.

Key data points, including 2-day hCG rises below 66% and birth outcomes, were directly verified in the electronic medical record. Our study methodology relies on prior studies reporting a log-linear nature of hCG rises up to 10 weeks of gestational age, though subanalysis of patients whose hCG values were drawn exactly 2 days apart confirmed the reliability of this method. As initial hCG values were drawn on a range of days following embryo transfer, conclusions cannot be drawn from this study regarding absolute serum hCG

Fig. 1 Abnormal hCG rises among single and multiple embryo transfer cycles. Red bars = 2-day hCG rise < 66%; blue bars = 2-day hCG rise ≥ 66%. Multivariable logistic regression (with oocyte age included a priori) was used to calculate odds ratios (OR) with 95% confidence intervals (CI) of hCG rise less than 66% in 2 days. When hCG levels were not checked 2 days apart, the 2-day rise was calculated using the log-linear nature of early hCG increases; see text for more details

Table 2 Initial hCG values and hCG rises among single and multiple embryo transfer cycles

Outcome	Single embryo transfer <i>n</i> = 629 (31.4%) Mean (SD)	Multiple embryo transfer <i>n</i> = 1372 (68.6%)	Multivariable adjusted effect estimates ^a Linear regression β (95% CI)
First hCG ^b (mIU/mL)	381.0 (299.1)	382.5 (366.6)	0.99 (−33.8, 35.6)
First to second hCG rise ^c (%)	136.8 (42.1)	130.2 (47.8)	−3.51 (−8.18, 1.17)
Second to third hCG rise ^c (%)	133.0 (37.2)	129.6 (42.5)	−3.04 (−8.58, 2.50)
	<i>n</i> (%)		OR (95% CI)
Abnormal hCG rise (< 66%) between first and second values ^{c,d}	17 (2.7%)	84 (6.1%)	2.09 (1.20–3.64)
Abnormal hCG rise (< 66%) between second and third values ^{c,d}	11 (2.6%)	28 (4.3%)	1.48 (0.67–3.27)

hCG human chorionic gonadotropin, SD standard deviation, OR odds ratio, CI confidence interval

^a Analyses are adjusted for patient age at oocyte retrieval and day of embryo transfer

^b First serum hCG levels were measured with a mean of 15 days after oocyte retrieval (or the equivalent thereof in frozen cycles) in both groups (range 11–23 days)

^c When hCG levels were not checked 2 days apart, the 2-day rise was calculated using the log-linear nature of early hCG increases; see text for more details

^d Abnormal hCG rise was defined as the rise of less than 66% in 2 days

levels, only their relative change. Limitations of the study also include the retrospective nature of data collection. For example, clinical protocols changed in November 2012 to require a third hCG level in all patients; as a result, earlier cycles were less likely to have a third hCG measurement obtained. Subgroup analysis of blastocyst transfer cycles ($n = 547$) may have failed to reach statistical significance due to the limited sample size, as compared to cleavage-stage embryo transfer cycles ($n = 1454$). We cannot account for pregnancies that may have been intervened upon (with uterine curettage and/or methotrexate) due to abnormal hCG rises and were thereby excluded from the present study; presumably rare, such cases may have otherwise resulted in an ongoing pregnancy if managed expectantly. Finally, the generalizability of these findings to a non-IVF population is not known.

We cannot account for the possibility of monozygotic twinning from a single embryo, estimated to occur in approximately 2% of transfers [15, 16]. Unidentified monozygotic twinning could theoretically result in a transient implantation of one of the two resulting embryos and an abnormal hCG trend. Risk factors for monozygotic twins include younger patient age, assisted hatching (for day 3 ET), and blastocyst transfer, as well as ICSI [15, 17]. Patients were younger and more commonly received blastocysts in the SET group—in which there was actually a lower incidence of abnormal hCG trends—suggesting that occult, failed monozygotic twinning did not likely constitute a major clinical factor. Assisted hatching was performed more commonly in MET versus SET cycles (63.3 vs. 26.2%, respectively, $p < 0.0001$), which reflects the higher proportion of patients receiving day 3 embryos in the former group. Assisted hatching has been theorized to facilitate earlier implantation as evidenced by earlier detection of serum hCG in patients receiving hatched embryos [18], but assisted hatching has not been shown to affect subsequent hCG rise [13, 18]. Similarly, in this study, assisted hatching was not found to independently influence the relationship between the number of embryos transferred and incidence of abnormal hCG trend.

Conclusions

This is the first study to our knowledge examining the association of abnormal hCG rises and the number of embryos transferred in patients conceiving singleton pregnancies. Patients with multiple embryos transferred who delivered singletons were more likely to have suboptimal early hCG rises, potentially due to transient implantation of other non-viable embryo(s). While these findings are useful for counseling, these findings should not change standard patient counseling regarding risk of ectopic pregnancy and the management of abnormal hCG rises following IVF. Finally, third hCG

measurements may clarify pregnancy prognosis, though a persistently abnormal hCG rise does not preclude live birth.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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