Prognostic utility of serum beta human chorionic gonadotropin level following single euploid embryo transfer for live birth

Emily A. Clarke, M.D.,[a] Charlotte Jones, B.A.,[b] Jensen Reckhow, M.D.,[a] Kerry Flannagan, Ph.D.,[c] Diana Shaari, B.A.,[a] Morgan Baird, M.P.H.,[b] Joseph A. Lee, B.A.,[b] Lucky Sekhon, M.D.,[b] Alan B. Copperman, M.D.,[a,b] and Phillip A. Romanski, M.D.[a,b]

[a] Department of Obstetrics, Gynecology, and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, New York; [b] Reproductive Medicine Associates of New York, New York, New York; and [c] US Fertility, Rockville, Maryland

Objective: To evaluate live birth outcomes on the basis of initial serum human chorionic gonadotropin (hCG) level following single euploid embryo transfer.
Design: Retrospective cohort study.
Subjects: Single center study that included patients who underwent single euploid embryo transfer with initial positive hCG (>2.5 mIU/mL), measured nine days after transfer. Cycles were grouped by hCG range: Group 1 (hCG 2.5 to <11 mIU/mL), group 2 (11 to <25), group 3 (25 to <50), group 4 (50 to <75), group 5 (75 to <100), and group 6 (≥100).
Exposure: Initial serum hCG level.
Main Outcome Measures: The primary outcome was live birth per positive hCG. Subgroup analysis was performed by blastocyst biopsy day (days 5, 6, or 7). Poisson regression models were used to estimate stepwise comparisons between adjacent hCG groups; receiver operating characteristic analysis was performed to determine the predictive value of initial hCG level for live birth.
Results: A total of 6,410 single euploid embryo transfer cycles with initial positive serum hCG were included. Overall live birth after positive hCG was 71.0%. Higher initial hCG level increased live birth chance in a stepwise fashion, with lowest chance of live birth in group 1 (hCG 2.5 to <11) (1.6%), and highest in group 6 (hCG ≥100) (87.8%). Adjusted analysis demonstrated increasing probability of live birth between adjacent groups: group 2 vs. 1 aRR 6.76 [3.02–15.11], group 3 vs. 2 aRR 3.08 [2.27–4.19], group 4 vs. 3 aRR 1.80 [1.59–2.04], group 5 vs. 4 aRR 1.16 [1.08–1.25], and group 6 vs. 5 aRR 1.15 [1.10–1.21]. Live birth was similar across blastocyst biopsy subgroups, except in group 3 (25 to <50 mIU/mL), where day 5 blastocysts had the lowest live birth (28.8%) vs. days 6 and 7 blastocysts (42.7%, 41.9%). The area under the receiver operating characteristic curve was 0.83 (0.81–0.84), with an hCG of 75.7 mIU/mL representing the optimal threshold.
Conclusion: Initial serum hCG level after single euploid embryo transfer is a strong predictor of live birth, with a stepwise relationship between rising hCG thresholds. Findings from this study provide a valuable counseling tool for both physicians and patients utilizing single euploid embryo transfer. (Fertil Steril® 2026;■:■–■. ©2026 by American Society for Reproductive Medicine.)

Key Words: Single euploid embryo transfer, early pregnancy, human chorionic gonadotropin, live birth

INTRODUCTION

Serum levels of human chorionic gonadotropin (hCG), produced by proliferating trophoblast cells, is one of the earliest indicators of successful implantation following embryo transfer. Early serum hCG levels reflect trophoblastic mass and function, and increase proportionally during early gestation, making early measurements a useful biomarker of implantation and placental development. It is well established that initial serum hCG levels after embryo transfer correlate with pregnancy outcomes, with higher hCG levels predictive of a greater likelihood of live birth (1–15).

However, existing studies on early hCG levels include heterogeneous in vitro fertilization (IVF) populations, including patients undergoing either fresh or fresh and frozen embryo transfers (FET), cleavage-stage and blastocyst transfers, and double embryo transfers (DET) (1–10, 12, 14, 15). The heterogeneity of existing literature limits application to contemporary IVF practice in which frozen single blastocyst transfer is most commonly

Received January 23, 2026; revised April 27, 2026; accepted April 28, 2026.
Data regarding any of the subjects in the study have not been previously published unless specified. Data will be made available to editors of the journal for review or query upon request, pre and/or post publication. The appropriate checklist for this study design was followed.
Correspondence: Emily Clarke, M.D (E-mail: emilyclarkemd@gmail.com).

Fertil Steril® Vol. ■, No. ■, ■ 2026 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2026.04.025

utilized. With only one prior study identified that examines early serum hCG dynamics in a preimplantation genetic testing for aneuploidy (PGT-A) tested cohort, which was limited in size and by its inclusion of DETs, a modern study focused specifically on single euploid embryo transfer is essential to clarify the relationship between early hCG levels and live birth outcome (9).

Moreover, the influence of blastocyst development stage on initial hCG dynamics remains poorly characterized. Most studies compare cleavage-stage with day 5 embryo transfers, but few assess blastocysts cultured to day 6 or 7 (3, 4, 6). Despite evidence that delayed embryo development correlates with suboptimal blastocyst quality and decreased reproductive potential, existing studies that include day 5, 6, and/or 7 blastocysts do not compare outcomes across different blastocyst stages (10, 11, 16, 17). To date, no studies have compared initial hCG levels by blastocyst stage within a single euploid embryo transfer cohort.

Interpretation of the initial serum hCG levels can create anxiety for patients undergoing IVF, highlighting the need for clearer prognostic data in those undergoing a single euploid embryo transfer with day 5, 6, and 7 blastocysts. Further, in addition to estimating chance of live birth, initial hCG level can be used to predict other outcomes, including miscarriage or ectopic pregnancy, or multiple gestation resulting from monozygotic twinning. Lastly, it is unknown whether a critical initial hCG threshold exists for live birth. Therefore, the primary objective of this study was to determine the likelihood of live birth on the basis of initial serum hCG levels after a single euploid embryo transfer, and secondarily to evaluate whether this effect is modified by the day of blastocyst development, providing a more nuanced framework for counseling patients.

MATERIALS AND METHODS

Participants and study design

This was a retrospective cohort study conducted at a single academic-affiliated fertility center that included patients who underwent a single euploid embryo transfer and observed a positive initial serum hCG ($\geq$2.5 mIU/mL) after transfer from September 2016 to June 2024. Initial serum hCG was measured 9 days after embryo transfer for all patients; patients who did not have an hCG level measured 9 days after transfer were excluded. Patients who utilized embryos derived from both autologous and donor oocytes were included.

Transfer cycles were categorized by initial serum b-hCG level: Group 1 (hCG 2.5 to <11 mIU/mL), group 2 (hCG 11 to <25 mIU/mL), group 3 (hCG 25 to <50 mIU/mL), group 4 (hCG 50 to <75 mIU/mL), group 5 (hCG 75 to <100 mIU/mL), and group 6 (hCG $\geq$100 mIU/mL). The highest hCG group (group 6, $\geq$100 mIU/mL) was selected on the basis of prior studies demonstrating a high likelihood of live birth after single FET when initial hCG exceeds $\geq$100 mIU/mL (10, 11, 15). Increments of 25 mIU/mL were applied for Groups 3–5 to provide finer resolution in the midrange; the two lowest groups (Groups 1 [2.5 to <11] and 2 [11 to <25]) were further subdivided to enhance sensitivity in detecting differences between cycles with the lowest initial hCG levels.

Patient characteristics and cycle variables of interest were collected from the electronic medical record. Patient information included: patient age at embryo transfer (years), body mass index (BMI) (kg/m^2), and serum antimüllerian hormone (AMH) (ng/mL) level at time of oocyte retrieval. Embryo transfer cycle information included type of endometrial preparation (natural or programmed), endometrial thickness before progesterone initiation, oocyte age (years), oocyte source (autologous or donor), and embryo grade. Embryo grade was characterized using a modified Gardner score, based on degree of expansion and grades of the inner cell mass (ICM) and trophectoderm (TE) (17). Embryos were categorized as "good quality" (modified Gardner grading with ICM and TE of AA, AB, or BA), "moderate quality" (BB), or "fair quality" (AC, CA, BC, CB, or CC).

Procedures

In vitro fertilization stimulation protocols were chosen by the treating physician based on age and ovarian reserve. Patients underwent controlled ovarian hyperstimulation as previously described (18, 19). Trigger shot of human chorionic gonadotropin, gonadotropin-releasing hormone agonist, or both were administered for final oocyte maturation when at least 2 follicles $\geq$18mm in mean diameter were attained (18, 19). Vaginal oocyte retrieval was performed 36 hours after trigger shot administration (18, 19). Oocytes that reached the metaphase II stage were then fertilized using intracytoplasmic sperm injection. Laboratory procedures regarding embryo culture and biopsy techniques are previously described (17). When embryos reached blastocyst stage appropriate for biopsy and cryopreservation, they were given an embryo grade as described above, biopsied, and cryopreserved by vitrification. Biopsied samples were sent for PGT-A using next-generation sequencing (NGS).

Embryo transfer cycles were performed in either a natural or programmed endometrial preparation cycle (20, 21). In brief, in programmed cycles, the uterine cavity was prepared with micronized oral estradiol for a minimum of 9 days before reassessment of endometrial lining measurement by transvaginal ultrasound. When adequate endometrial thickness was achieved, progesterone was initiated, either intramuscularly or in combination with oral/vaginal administration (20). In some cases, vaginal, intramuscular, or transdermal estradiol was administered to promote endometrial growth, though only by physician discretion. Embryo transfer was performed on the 6th day of progesterone administration. In natural cycles, patients presented for cycle monitoring approximately 4 days before their expected ovulatory trigger administration based on the length of their natural cycle. Serum hormone levels (estradiol, progesterone, and luteinizing hormone) and transvaginal ultrasound were performed serially until the endometrial thickness was considered adequate and a dominant follicle measured >17 mm, at which point an hCG trigger was administered. The embryo transfer was performed 7 days after hCG trigger, and vaginal progesterone was administered for luteal

support (21). Embryos were thawed using a standard warming process, and embryo transfer was performed under standard conditions as previously described (17, 22).

Pregnancy was assessed 9 days after embryo transfer by serum hCG analysis. Pregnancy was indicated by an initial serum hCG ≥2.5 mIU/mL. Additional hCG levels were measured 48 hours after the initial hCG assessment to evaluate viability of the pregnancy, and transvaginal ultrasound was performed at five weeks gestation to evaluate the location of pregnancy.

Laboratory analysis

Initial serum hCG was assessed 9 days after embryo transfer. Serum hCG concentration was measured using an electrochemiluminescence immunoassay (Cobas e, Roche Diagnostics). The functional sensitivity of the assay was 0.25 mIU/mL and the detection range was 0.2–10,000 mIU/mL. The same standard assay was used for all cycles analyzed.

Outcomes

The primary outcome was live birth, calculated as live birth per single euploid embryo transfer that resulted in positive serum hCG level. Secondary outcomes included clinical pregnancy (presence of an intrauterine gestational sac) per positive hCG, biochemical pregnancy loss (pregnancy loss after positive hCG) per positive hCG, clinical pregnancy loss (pregnancy loss after presence of an intrauterine gestational sac) per positive hCG, and ectopic pregnancy per positive hCG. Monozygotic twinning, defined as the presence of >1 gestational sac or >1 fetal pole (to identify both monoamniotic and diamniotic twin pregnancies), per positive serum hCG was also assessed.

Subgroup analysis was performed on the primary outcome of live birth by day of blastocyst biopsy (day 5, 6, or 7).

Statistical analysis

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Inc., Cary, NC). Patient demographics, embryo transfer cycle variables, and embryo transfer outcomes were compared between groups. Continuous variables were assessed for normality using the Kolmogorov-Smirnoff test, and based on their distribution, were compared using the Kruskal-Wallis test. Continuous data were reported as means with standard deviations. Univariate analysis to compare categorical variables and embryo transfer outcomes used Chi-square test. Categorical data were reported as proportions with Clopper-Pearson 95% confidence intervals (95% CI). A P value of <0.05 was considered significant.

The study used Poisson regression models with a log link, fitted with generalized estimating equations to account for patients with multiple cycles (correlation between cycles from the same patient), to estimate adjusted relative risks (aRRs) with 95% CIs for the association between initial hCG and live birth, as well as other embryo transfer outcomes. To compare outcomes between increasing initial serum hCG levels, we conducted stepwise, pairwise comparisons between adjacent hCG groups where each hCG group was compared with the immediately lower group (ie, group 2 vs. group 1, group 3 vs. Group 2). This approach allowed assessment of the relative change in outcome between successive hCG groups, rather than comparing all groups to a single high-reference group, which is already known to have the highest likelihood of live birth. Regression models were adjusted for oocyte age, BMI at embryo transfer, and treatment year. Blastocyst biopsy day was evaluated as an effect modifier on live birth outcome per initial hCG level. (5,6, or 7)

Receiver operating characteristic (ROC) analysis was performed to evaluate the discriminatory ability of initial serum hCG level for predicting live birth. A logistic regression model with initial hCG as a continuous predictor was fit, and the area under the ROC curve (AUC) was obtained. Test characteristics, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were examined across observed hCG values. The hCG value with the maximum Youden index was reported as the cohort-specific reference threshold.

Ethical approval

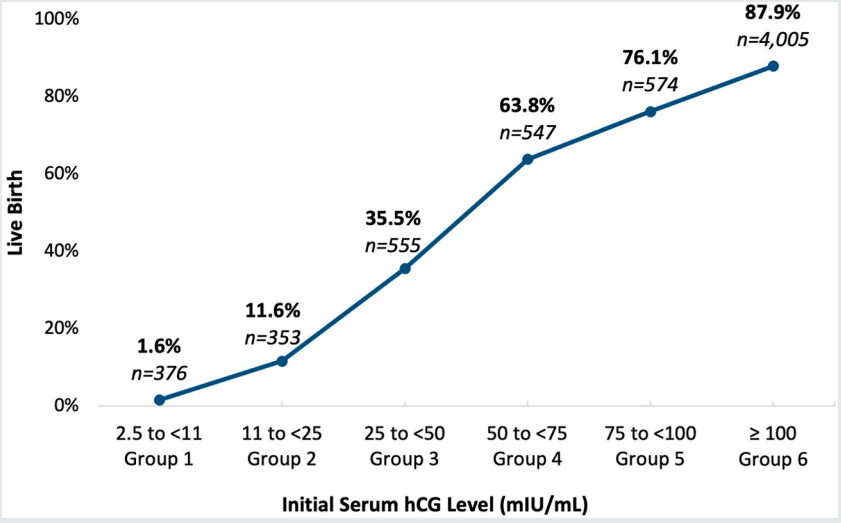
The study was approved by the Mount Sinai School of Medicine Institutional Review Board (STUDY-18-00441-CR005), with a waiver of patient consent for retrospective analysis of deidentified data.

RESULTS

A total of 6,410 single euploid embryo transfer cycles with initial positive serum hCG levels were included. Group 1 (hCG 2.5 to <11 mIU/mL) included 376 cycles; group 2 (hCG 11 to <25) 353 cycles; group 3 (hCG 25 to <50) 555 cycles; group 4 (hCG 50 to <75) 547 cycles; group 5 (hCG 75 to <100) 574 cycles; and group 6 (hCG ≥100) 4,005 cycles. Patient characteristics and embryo transfer cycle parameters in the overall cohort and within each initial hCG group are displayed in Supplemental Table 1, available online. Patient age at embryo transfer, oocyte age, and endometrial thickness were similar across initial hCG groups. Group 6 (hCG ≥100) contained the highest proportion of good quality blastocysts. Lower initial hCG groups more commonly utilized a day 7 blastocyst compared with groups 5 and 6 (hCG 75 to <100, hCG ≥100), whereas the higher initial hCG groups more commonly utilized a day 5 blastocyst. In the overall cohort, 3,769 cycles used a day 5 blastocyst (58.8%), 2,443 cycles used a day 6 blastocyst (38.1%), and 198 cycles used a day 7 blastocyst (3.1%). The majority of cycles utilized autologous oocyte (92.6% of cycles) with programmed endometrial preparation (98.5% of cycles).

Overall live birth per single euploid embryo transfer after observing an initial positive serum hCG was 71.0% [95% CI, 69.8–72.1]. As initial hCG level increased, the chance of live birth increased in a stepwise fashion, with the lowest chance of live birth occurring in group 1 (hCG 2.5 to <11) (1.6%, 95% CI, 0.6–3.4) and highest chance in group 6 (hCG ≥100) (87.8%, 95% CI, 86.8–88.9%) ($P<.01$) (Figure 1, Table 1). The lowest initial serum hCG level that resulted in

FIGURE 1



Chance of live birth by initial serum hCG level after single euploid embryo transfer.

Clarke. Serum human chorionic gonadotropin level after euploid transfer. Fertil Steril 2026.

a live birth was 4.1 mIU/mL. When comparing live birth outcomes between adjacent hCG groups after adjusting for oocyte age, BMI, and treatment year (Table 2), higher initial hCG level was associated with progressively increased probabilities of live birth. Specifically, patients in group 2 (hCG 11 to <25) had a significantly higher chance of live birth compared with those in group 1 (hCG 2.5 to <11) (aRR 6.76, 95% CI, 3.02–15.11). This trend persisted across all stepwise, pairwise comparisons: patients in group 3 (hCG 25 to <50) had a higher chance of live birth compared with those in group 2 (aRR 3.08, 95% CI, 2.28–4.16), patients in group 4 (hCG 50 to <75) higher than group 3 (aRR 1.80, 95% CI, 1.59–2.04), group 5 (hCG 75 to <100) higher than group 4 (aRR 1.16, 95% CI, 1.08–1.25), and group 6 (hCG ≥100) higher than group 5 (aRR 1.15, 95% CI, 1.10–1.21).

Similar trends were observed for the outcome of clinical pregnancy per positive hCG, with increasing chance of clinical pregnancy in increasing initial hCG groups (Table 2). Conversely, chance of biochemical pregnancy loss per positive hCG decreased with increasing hCG groups, with 86.4% [95% CI, 82.6–89.7] of cycles resulting as a biochemical loss in group 1 (hCG 2.5 to <11) compared with 1.9% [95% CI, 1.5–2.4] of cycles in group 6 (hCG ≥100) ($P<.01$). Clinical pregnancy loss per positive hCG peaked in the intermediate hCG groups, with declining chance of clinical loss in groups 5 and 6 (hCG 75 to <100, hCG ≥100). Risk of ectopic pregnancy per positive hCG was highest in groups 1 and 2 (hCG 2.5 to <11, hCG 11 to <25) with risks of 6.9% [95% CI, 4.6–10.0] and 7.1% [95% CI, 4.6–10.3], respectively, and lower risk observed in groups 3 to 6 (0.1–0.2) ($P<.01$). Conversely, monozygotic twinning was not observed in group 1 but increased marginally as hCG group increased (groups 2–6, 0.3%–3.7%) ($P<.01$).

When evaluating blastocyst biopsy day (5, 6, or 7) as an effect modifier on live birth per initial hCG level, there were similar chances of live birth among biopsy day in all initial hCG groups, except for group 3 (hCG 25 to <50) (Table 3). In group 3, Day 5 blastocysts demonstrated lower live birth (28.8%, 95% CI, 23.6–34.4) compared with days 6 (42.7%, 95% CI, 36.3–49.2), and 7 (41.9%, 95% CI, 24.6–60.9) blastocysts ($P<.01$). Day 5 blastocysts in group 2 (hCG 11 to <25) also demonstrated lower live birth potential (9.2%, 95% CI, 5.4–14.6) compared with day 6 (14.4%, 95% CI, 9.3–20.8), though this trend did not reach statistical significance.

Lastly, ROC analysis demonstrated a good discriminative ability of initial serum hCG for live birth, with an AUC of 0.83 (95% CI, 0.81–0.84). An hCG value of 75.7 mIU/mL corresponded to the cohort-specific reference threshold with the highest Youden index, with sensitivity of 86.7% and specificity of 66.7%, respectively (Supplemental Figure 1, available online). The PPV was 86.4%, and NPV was 67.3%.

DISCUSSION

In this study of 6,410 single euploid embryo transfer cycles resulting in an initial positive serum hCG, higher hCG levels were associated with progressively increased probability of live birth. A threshold hCG level of 75.7 mIU/mL predicted live birth with an 86.4% PPV, with even higher probabilities of live birth observed as hCG increased beyond this threshold, with 87.9% of patients in the hCG ≥100 mIU/mL group achieving live birth. To our knowledge, this is the first study on early hCG dynamics to evaluate a PGT-A tested cohort of frozen single blastocyst transfers. With single euploid embryo transfer increasingly utilized, this study's characterization of initial hCG level offers physicians and patients a practical framework to interpret early results and set realistic expectations for live birth.

TABLE 1

Single euploid embryo transfer outcomes by initial hCG group.

Outcome	Group 1 2.5 to < 11 n = 376	Group 2 11 to < 25 n = 353	Group 3 25 to < 50 n = 555	Group 4 50 to < 75 n = 547	Group 5 75 to < 100 n = 574	Group 6 ≥100 n = 4,005	*P* value
Clinical Pregnancy (n, %)	27 (7.2)	111 (31.4)	333 (60.0)	466 (85.2)	522 (90.9)	3,936 (98.3)	< .01
Live Birth (n, %)	6 (1.6)	41 (11.6)	197 (35.5)	349 (63.8)	437 (76.1)	3,519 (87.9)	< .01
Biochemical Loss (n, %)	325 (86.4)	219 (62.0)	213 (38.4)	75 (13.7)	50 (8.7)	77 (1.9)	< .01
Clinical Loss (n, %)	18 (4.8)	68 (19.3)	133 (24.0)	114 (20.8)	84 (14.6)	415 (10.4)	< .01
Ectopic Pregnancy (n, %)	26 (6.9)	25 (7.1)	12 (2.2)	9 (1.6)	3 (0.5)	6 (0.1)	< .01
Monozygotic Twinning (n, %)	0 (0.0)	0 (0.0)	4 (0.7)	8 (1.5)	15 (2.6)	113 (2.8)	< .01

hCG = human chorionic gonadotropin; n = number of single euploid embryo transfer cycles resulting in positive hCG.

Clarke. Serum human chorionic gonadotropin level after euploid transfer. Fertil Steril 2026.

TABLE 2

Stepwise comparison of live birth chance by initial hCG group.

hCG Group Comparisons (mIU/mL)	aRR [95% CI]
Group 2 (11 to <25) vs. Group 1 (2.5 to <11) *(ref)*	6.76 [3.02–15.11]
Group 3 (25 to <50) vs. Group 2 (11 to <25) *(ref)*	3.08 [2.28–4.16]
Group 4 (50 to <75) vs. Group 3 (25 to <50) *(ref)*	1.80 [1.59–2.04]
Group 5 (75 to <100) vs. Group 4 (50 to <75) *(ref)*	1.16 [1.08–1.25]
Group 6 (≥100) vs. Group 5 (75 to <100) *(ref)*	1.15 [1.10– .21]

hCG = human chorionic gonadotropin; aRR = adjusted relative risk; CI = confidence intervals. Poisson regression with log link, fitted with generalized estimating equations, adjusted for oocyte age, body mass index at embryo transfer, and treatment year.

Clarke. Serum human chorionic gonadotropin level after euploid transfer. Fertil Steril 2026.

Patients who observed low initial hCG after single euploid embryo transfer experienced an increased chance of biochemical pregnancy loss and ectopic pregnancy; in the highest hCG group (hCG ≥100), only rarely was a biochemical loss (1.9%) or ectopic pregnancy (0.1%) observed. These findings are consistent with other studies (1, 2, 7, 10) that evaluated the relationship between low initial hCG and pregnancy outcomes. This study builds on existing literature using a modern ART treatment approach by restricting the analysis to frozen single euploid embryo transfers (10) and excluding fresh transfers (1, 2, 7). On the basis of this study's findings, an initial hCG level of <25 mIU/mL is associated with a high risk of biochemical pregnancy loss (74.6% overall per positive hCG in Groups 1 and 2), though it also warrants close monitoring due to an increased risk of ectopic pregnancy (7.0% overall per positive hCG in Groups 1 and 2). Data from this study can help physicians set expectations when initial hCG level after single euploid embryo transfer fall within this low range. While live birth occurred in only 1.6% of transfers in the lowest initial hCG group (1, hCG 2.5 to <11), live birth outcomes were observed in this cohort, with 4.1 mIU/mL the lowest initial hCG level that resulted in a live birth. For some patients, this finding is useful to set expectations that while live birth may still result, it remains unlikely.

The present study also evaluated initial hCG level as a predictive value for monozygotic twinning after single euploid embryo transfer. Although previous studies either excluded cycles that resulted in multiple gestation (5–7, 11, 12, 15) or did not assess the association of initial hCG level and multiples as an outcome (2, 9, 10), this study estimated likelihood of monozygotic twinning by initial hCG group. Findings are consistent with prior literature demonstrating higher risk of twinning with higher initial hCG (3.7% per positive hCG in group 6, hCG ≥100) (1, 3, 4, 14), though those prior studies are limited by the use of fresh embryo transfer and a mixture of single and double embryo transfers. Patients can be reassured that after achieving an initial positive hCG level after single euploid embryo

TABLE 3

Chance of live birth by initial hCG group by blastocyst biopsy day

Initial hCG group	Day 5 n = 3,769	Day 6 n = 2,443	Day 7 n = 198	*P* value
Group 1 (hCG 2.5 to <11) (n, %)	4 (2.4)	2 (1.1)	0 (0.0)	0.57
Group 2 (hCG 11 to <25) (n, %)	16 (9.2)	23 (14.4)	2 (10.0)	0.34
Group 3 (hCG 25 to <50) (n, %)	82 (28.8)	102 (42.7)	13 (41.9)	<0.01
Group 4 (hCG 50 to <75) (n, %)	167 (62.3)	160 (65.6)	22 (62.9)	0.74
Group 5 (hCG 75 to <100) (n, %)	226 (75.1)	197 (77.6)	14 (73.7)	0.77
Group 6 (hCG ≥100) (n, %)	2,270 (88.2)	1,189 (87.0)	55 (87.3)	0.58

hCG = human chorionic gonadotropin; n = number of single euploid embryo transfer cycles resulting in positive hCG.

Clarke. Serum human chorionic gonadotropin level after euploid transfer. Fertil Steril 2026.

transfer, chance of monozygotic twinning is low, though reaches 3.6%–3.7% when initial hCG exceeds 75 mIU/mL.

Lastly, this study appears to be one of the first to compare early hCG dynamics among days 5, 6, and 7 blastocysts. Within each initial hCG group, days 5, 6, and 7 blastocysts demonstrated similar chances of live birth, except for in group 3 (hCG 25 to <50), in which day 5 blastocysts had a lower chance of live birth compared with days 6 and 7 blastocysts. Although not reaching statistical significance, in group 2 (hCG 11 to <25), day 5 blastocysts also trended toward lower chance of live birth. One explanation for this observation is that at implantation, day 5 blastocysts are more developmentally advanced, and thus may be expected to produce higher initial hCG due to increased trophoblast proliferation. As such, when a day 5 blastocyst generates lower levels of hCG, it may signal suboptimal development relative to its expected developmental timeline, corresponding with lower reproductive potential and chance of live birth. Still, in Groups 4-6 (hCG ≥50), live birth rates were similar across days 5, 6, and 7 blastocysts, suggesting that as early hCG levels increase, that influence of biopsy day on outcomes diminishes. Although biopsy day is useful to understand an embryo's potential for live birth before embryo transfer, it does not seem to change the relationship between initial hCG and live birth chance; thus, patient counseling after an initial positive hCG should focus primarily on the initial hCG level.

Strengths of this study include its inclusion of only single euploid vitrified-warmed embryo transfer cycles, comparison of days 5, 6, and 7 blastocysts, and inclusion and analysis of monozygotic twinning. Additionally, the study is strengthened by its large sample size and single-center design, with all initial hCG levels determined 9 days after transfer using a single assay.

This study is not without limitations. First, the study is limited by its retrospective design. Next, findings may not be applicable to pregnancies in which initial hCG is not collected 9 days after embryo transfer. However, in centers where initial hCG is obtained slightly earlier or later than day nine, these results may still provide a general framework for counseling, as early hCG values typically rise in a predictable manner, approximately doubling every 48–72 hours. Therefore, values obtained earlier (i.e., 8 days after transfer) may be associated with a more favorable prognosis than suggested by these day nine data, whereas measurements obtained later (i.e., 10 days after transfer) may be associated with a less favorable prognosis relative to the day nine data. The study is also limited by the predominance of programmed endometrial preparation cycles (98.5%), which may limit generalizability of these findings to natural or modified natural cycles. Lastly, although this was an entirely euploid embryo cohort, other factors besides embryonic ploidy may influence pregnancy outcomes, regardless of initial hCG level. Although multivariable adjustment was performed, unmeasured clinical and laboratory factors may have influenced both initial hCG levels and pregnancy outcomes.

CONCLUSION

In conclusion, initial serum hCG level after a single euploid embryo transfer is significantly associated with live birth, with a stepwise relationship between rising hCG thresholds. Lower hCG levels of <25 mIU/mL are associated with biochemical pregnancy loss but also warrant particularly close monitoring for increased risk of ectopic pregnancy. Day of blastocyst biopsy does not appear to be useful when interpreting initial hCG levels. Findings from this study provide a valuable counseling tool for both physicians and patients undergoing single euploid embryo transfer.

CRediT Authorship Contribution Statement

Emily A. Clarke: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Charlotte Jones:** Writing – review & editing, Investigation. **Jensen Reckhow:** Writing – review & editing, Investigation. **Kerry Flannagan:** Methodology. **Diana Shaari:** Investigation. **Morgan Baird:** Resources, Data curation. **Joseph A. Lee:** Writing – review & editing. **Lucky Sekhon:** Writing – review & editing, Conceptualization. **Alan B. Copperman:** Writing – review & editing, Methodology. **Phillip A. Romanski:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Interests

E.A.C. has nothing to disclose. C.J. has nothing to disclose. J.R. has nothing to disclose. K.F. has nothing to disclose.

D.S. has nothing to disclose. M.B. has nothing to disclose. J.A.L. has nothing to disclose. L.S. has nothing to disclose. A.B.C. has nothing to disclose. P.A.R. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.fertnstert.2026.04.025.

REFERENCES

1. Wang Z, Gao Y, Zhang D, Li Y, Luo L, Xu Y. Predictive value of serum β-human chorionic gonadotropin for early pregnancy outcomes. Arch Gynecol Obstet 2020;301:295–302.
2. Dahiya M, Rupani K, Yu SL, Fook-Chong SMC, Siew Fui DC, Rajesh H. Embryo transfer day does not affect the initial maternal serum β-hCG levels: a retrospective cohort study. Eur J Obstet Gynecol Reprod Biol 2017;212:75–9.
3. Kathiresan ASQ, Cruz-Almeida Y, Barrionuevo MJ, Maxson WS, Hoffman DI, Weitzman VN, et al. Prognostic value of beta-human chorionic gonadotropin is dependent on day of embryo transfer during in vitro fertilization. Fertil Steril 2011;96:1362–6.
4. Papageorgiou TC, Leondires MP, Miller BT, et al. Human chorionic gonadotropin levels after blastocyst transfer are highly predictive of pregnancy outcome. Fertil Steril 2001;76:9817.
5. Ozer G. Initial β-hCG levels and 2-day-later increase rates effectively predict pregnancy outcomes in single blastocyst transfer in frozen-thawed or fresh cycles: a retrospective cohort study. Medicine (Baltimore) 2023;102:e35605.
6. Oron G, Esh-Broder E, Son WY, Holzer H, Tulandi T. Predictive value of maternal serum human chorionic gonadotropin levels in pregnancies achieved by in vitro fertilization with single cleavage and single blastocyst embryo transfers. Fertil Steril 2015;103:1526–31.
7. Oron G, Shavit T, Esh-Broder E, Weon-Young S, Tulandi T, Holzer H. Predictive value of serum HCG concentrations in pregnancies achieved after single fresh or vitrified-warmed blastocyst transfer. Reprod Biomed Online 2017;35:272–8.
8. Zhao W-E, Li Y-J, Ou J-P, Sun P, Chen W-Q, Liang X-Y. Predictive value of initial serum human chorionic gonadotropin levels for pregnancies after single fresh and frozen blastocyst transfer. J Huazhong Univ Sci Technolog Med Sci 2017;37:395–400.
9. Hobeika E, Singh S, Malik S, Knochenhauer ES, Traub ML. Initial maternal serum human chorionic gonadotropin levels in pregnancies achieved after assisted reproductive technology are higher after preimplantation genetic screening and after frozen embryo transfer: a retrospective cohort. J Assist Reprod Genet 2017;34:1333–40.
10. Wu Y, Liu H. Possibility of live birth in patients with low serum β-hCG 14 days after blastocyst transfer. J Ovarian Res 2020;13:132.
11. Ueno S, Ezoe K, Abe T, Yabuuchi A, Uchiyama K, Okuno T, et al. Maternal age and initial β-hCG levels predict pregnancy outcome after single vitrified-warmed blastocyst transfer. J Assist Reprod Genet 2014;31:1175–81.
12. Naredi N, Singh SK, Sharma R. Does first serum beta-human chorionic gonadotropin value prognosticate the early pregnancy outcome in an in-vitro fertilisation cycle? J Hum Reprod Sci 2017;10:108–13.
13. Mourad A, Antaki R, Rowen M, Lévesque É, Lapensée L. The POPI-Plus tool: prediction model of outcome of pregnancy in in vitro fertilization from a large retrospective cohort. Fertil Steril 2024;121:489–96.
14. Póvoa A, Severo M, Xavier P, Matias A, Blickstein I. Can early β-human chorionic gonadotropin predict birth of singletons and twins after in vitro fertilization? J Matern Fetal Neonatal Med 2018;31:453–6.
15. Kidera N, Ishikawa T, Kawamura T, Miyasaka N. Serum human chorionic gonadotropin can predict live birth 7 days after frozen-thawed blastocyst transfer. F S Rep 2025;6:455–61.
16. Wirleitner B, Schuff M, Stecher A, Murtinger M, Vanderzwalmen P. Pregnancy and birth outcomes following fresh or vitrified embryo transfer according to blastocyst morphology and expansion stage, and culturing strategy for delayed development. Hum Reprod 2016;31:1685–95.
17. Hernandez-Nieto C, Lee JA, Slifkin R, Sandler B, Copperman AB, Flisser E. What is the reproductive potential of day 7 euploid embryos? Hum Reprod 2019;34:1697–706.
18. Sekhon L, Lee JA, Flisser E, Copperman AB, Stein D. Blastocyst vitrification, cryostorage and warming does not affect live birth rate, infant birth weight or timing of delivery. Reprod Biomed Online 2018;37:33–42.
19. Clarke EA, Hernandez-Nieto C, Bayefsky M, Baird M, Slifkin M, Lee JA, et al. Developing a decision support tool to optimize oocyte thawing for producing at least three euploid blastocysts. Fertil Steril 2025;124:694–700.
20. Bergin K, Borenzweig W, Roger S, Slifkin R, Baird M, Lee J, et al. Effect of postthaw change in embryo score on single euploid embryo transfer success rates. Fertil Steril 2024;122:288–96.
21. Baksh S, Casper A, Christianson MS, Devine K, Doody KJ, Ehrhardt S, et al. Natural vs. programmed cycles for frozen embryo transfer: study protocol for an investigator-initiated, randomized, controlled, multicenter clinical trial. Trials 2021;22:660.
22. Parmegiani L, Beilby KH, Arnone A, Bernardi S, Maccarini AM, Nardi E, et al. Testing the efficacy and efficiency of a single ''universal warming protocol'' for vitrified human embryos: prospective randomized controlled trial and retrospective longitudinal cohort study. J Assist Reprod Genet 2018;35:1887–95.