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REVIEW

Early Serum hCG in IVF: Are We Trending in the Right Direction?

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Abstract

Human chorionic gonadotropin (hCG) measurements may be the earliest indicator of fertility cycle success, available several weeks before an ultrasound would be diagnostic for pregnancy. Outcomes of these cycles are high stakes for a couple, and the earliest reassurance of a normal pregnancy would be beneficial for their well-being. Additionally, earlier diagnosis can allow for more rapid management by providers in the case of abnormal pregnancies. Therefore, establishing normal values for initial hCG level and early hCG kinetics is of great interest. There are many factors involved in assisted reproductive techniques that may lead to alterations in hCG kinetics when compared with spontaneous pregnancies. We aim to characterize normal hCG values for in vitro fertilization (IVF) pregnancies and review how different aspects of the IVF process may alter these trends in order to establish how best to counsel patients during the waiting period.

Keywords In vitro fertilization · Embryo transfer · Early pregnancy · hCG value · Patient counseling

Introduction

Early diagnosis of pregnancy following assisted reproductive technology cycles is critical both for the emotional well-being of the couple as well as for management guidance for their health care providers. While transvaginal ultrasound is a useful tool in diagnosis of early pregnancies, it is typically non-diagnostic before 5–6-week gestation. Serial human chorionic gonadotropin (hCG) measurements in early pregnancy can allow for diagnosis, monitoring, and potential early differentiation of normal versus abnormal pregnancies. Ideally, this would lead to reduced stress surrounding uncertainty of treatment success and to swift management to avoid delaying further attempts at conception if desired.

Fertility clinics may follow different protocols, but initial serum hCG measurement typically occurs around 11 days following transfer of cleavage stage embryos or 9 days following transfer of blastocysts, a time that correlates with the 14th day following oocyte retrieval. Serum hCG can then be followed prior to scheduling an ultrasound. In spontaneously conceived normal pregnancies, the rate of hCG rise has been reported to be at least 53% in 2 days, with a median increase of 50% in 1 day and 124% in 2 days [1]. In order to characterize the rate of hCG rise for pregnancies conceived through IVF, a retrospective cohort study in 2005 demonstrated that the average rate of rise in pregnancies that resulted in live birth was 50% in 1 day and 124% in 2 days, similar to spontaneous pregnancies [2]. There was, however, an earlier plateau in IVF pregnancies (38 gestational days at concentration of approximately 3000 mIU/mL) than in non-IVF pregnancies. The authors reported the slowest confirmed rise for a normal pregnancy was 30% in 2 days.

As a normal rise is predicted in hCG levels following in vitro fertilization cycles, it is possible to identify those pregnancies that may be abnormal earlier than otherwise detected by ultrasound by using the initial hCG level or by following the hCG trend. Abnormal levels could occur in biochemical pregnancies (15%) [3], nonviable pregnancies, ectopic pregnancies (1.6%) [4], and in other clinical scenarios. There may, in fact, be factors related to the IVF techniques themselves or the infertile patient population that leads to alterations of hCG dynamics. This paper aims to provide a review of the potential alternate causes of abnormal hCG rise following assisted reproductive technology.

Methods

A review of the published literature was done to find the role of early hCG monitoring on pregnancy outcomes in assisted

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reproductive technology (ART) treatment cycles (Table 1). A literature search was performed using PUBMED and Google Scholar to find articles about hCG levels following assisted reproductive technology treatments.

The search was conducted using the following keywords: "hCG," "human chorionic gonadotropin"; β-hCG," "assisted reproductive technology"; "IVF," "in vitro fertilization"; "ICSI," "intracytoplasmic sperm injection"; "single-embryo transfer"; "multiple embryo transfer"; "embryo morphology"; "singleton"; "multiple gestation"; "twin gestation"; "vanishing twin"; "pregnancy outcome"; "live birth"; "maternal age"; "aneuploidy"; "trophoblast"; "embryo stage"; "cleavage stage"; "blastocyst"; "embryo biopsy"; "trophectoderm biopsy"; and "PGT." The abstract and purpose of the articles were reviewed, and the publications selected included the abstracts, original articles, case reports, cohorts, or case series published between 1980 until time of publication which discussed the hCG levels and pregnancy outcomes following ART. Only articles written in English were included.

hCG Levels and Pregnancy Outcomes

The most clinically important motivation for evaluating early hCG levels following IVF pregnancies is to predict pregnancy outcomes. Either a single initial hCG value or an hCG rise that is indicative of normal vs. abnormal pregnancy outcomes would be of great interest in counseling after pregnancy is achieved through IVF.

An analysis of 6021 pregnancies published in 2009 characterized the initial hCG level and typical rise of serum hCG levels that correlated with live birth following in vitro fertilization [6]. These authors found that initial hCG was predictive for delivery rates both for the population as a whole and when broken down by age group. The percent rise over 2 days was added to the predictive value, and it was found that delivery rate increased progressively as the percent rise went from 0 to 100%, with no increased chance of delivery over 100% rise. The lowest initial level of hCG that resulted in live birth was 5 mIU/mL.

Another study published in 2007 looked specifically at outcomes of pregnancies with low initial hCG level, which they considered to be < 150 mIU/mL on day 13 after embryo transfer [7]. This group found that an initial hCG level < 150 mIU/mL was correlated with unfavorable outcomes (biochemical or ectopic pregnancy or spontaneous abortion), and this persisted across treatment protocol, fertilization method, and type of embryo transfer (frozen or fresh). Likelihood of delivery dropped from 77.8% in patients with an initial hCG > 150 mIU/mL to 35.2% in those with hCG < 150 mIU/mL. Of those with an initial low hCG level that reached delivery, there were no increased adverse outcomes.

Many investigators have sought to determine a "cutoff" value to predict type of clinical pregnancy following in vitro fertilization. A retrospective data analysis published in 2014 analyzed hCG values on day 12 after embryo transfer of 1057 clinical pregnancies and performed receiver operating characteristic curves to determine optimal cutoffs discriminating between singleton and multiple gestation, intrauterine and ectopic pregnancy, and miscarriage and live birth [8]. They determined a cutoff value of 239 IU/L as 69% sensitive and 74.5% specific for multiple pregnancy. For ectopic pregnancy they found a level of 91 IU/L would have a negative predictive value of 98.5%, and for miscarriage a level of 143 IU/L would have a negative predictive value of 90%.

More recently another retrospective cohort investigated initial hCG level and rate of increase to predict pregnancy outcome in hopes to determine a cutoff value or rate of rise for biochemical pregnancy, early pregnancy loss, and live birth of singleton pregnancy [9]. This group found the hCG level measured 14-day post-oocyte retrieval to be the most sensitive with the highest positive predictive value. Specific cutoffs to define biochemical or early pregnancy loss were not calculated, but mean day 14 hCG level for biochemical pregnancy was 38.6 mIU/mL, and for early pregnancy loss was 137.5 mIU/mL. Mean day 14 hCG level for live birth was 197.2 mIU/mL. Receiver-operating characteristic (ROC) curves were performed to determine a cutoff value to predict live birth. In fresh cycles a level of 104.5 mIU/mL was 80.3% sensitive for live birth, with a positive predictive value of 80.8%. In addition, adding the rate of rise improved predictability, with a fold change of 2.78 having a 70.6% positive predictive value for live birth.

Abnormal hCG Levels in Single-Embryo Transfer Cycles

The studies discussed above did not standardize the number of embryos transferred, and many patients included in these studies received multiple embryos. To avoid the potential confounding impact of transfer of multiple embryos on hCG levels, a review of hCG trends following single-embryo transfer is warranted. Al Mamari et al. investigated initial hCG level in a total of 1076 pregnancies resulting from single-embryo transfer between 2013 and 2017 [10]. In those pregnancies with an initial day 16 hCG level of 103 ± 13 IU/L, 50% had a biochemical loss. Biochemical pregnancies were detected in 21% of patients at levels from 136 to 197 IU/L, and 12% at levels 199–252 IU/L. They determined that a cutoff for clinical pregnancy was 190 IU/L, and for live birth was 213 IU/L. While this study did not examine the early hCG trends, this is certainly valuable information in discussing risk of biochemical pregnancy with patients with initial low level hCG.

Table 1 Published literature of early serum hCG levels following IVF

Author	Year	Population	Outcomes measured	Results
Pregnancy outcome				
Shamonki, M. et al.	2009	6021 pregnancies resulting from IVF with detectable early serum hCG after ET at a single large center between 1999 and 2005	Live delivery rate based on initial hCG and rise of hCG	• Progressive increase in delivery rate as the percent rise in hCG went from 0 to 100%. No further enhancement in delivery rates beyond the 100% rise point
Porat, S. et al.	2007	533 IVF cycles that resulted in a positive serum hCG on day 13 after ET at a single center between 1999 and 2004	Favorable vs. unfavorable pregnancy outcome when hCG is <150 or > 150	• Likelihood of delivery dropped from 77.8% to 35.2% in those with initial hCG <150. Of those with initial low hCG that reached delivery, no increased adverse outcomes
Wu, G. et al.	2014	1057 clinical pregnancies resulting from IVF with hCG value on day 12 after ET at a single center	hCG cutoff value for pregnancy outcome determined by ROC curve	• Cutoff of 239 as 69% sensitive and 74.5% specific for multiple pregnancy • Cutoff of 91 with NPV of 98.5% for ectopic pregnancy • Cutoff of 143 with NPV of 90% for miscarriage
Sung, N. et al.	2016	1408 fresh and 598 frozen cycles resulting in biochemical, early pregnancy loss, or live birth of singleton with hCG on postovulatory days 12 and 14 at a single center between 2008 and 2011	hCG cutoff value and degree of increase for pregnancy outcome	• hCG level 14 days post OR most sensitive with highest PPV for pregnancy outcome • Mean day 14 hCG 38.6 for biochemical pregnancy, 137.5 for early pregnancy loss, 197.2 for live birth
Single-embryo transfer cycles				
Al Mamari, N. et al.	2019	1076 pregnancies resulting from SET at a single center between 2013 and 2017	Pregnancy outcome based on day 16 hCG level	• Biochemical pregnancies detected in 50% of patients with initial hCG level of 103, 21% of patients with hCG from 136 to 197, 12% at levels 199–252 • Cutoff for clinical pregnancy of 190 • Cutoff for live birth 213
DeNeubourg, D. et al.	2004	Mean initial hCG levels on day 12 post-ET in 370 single top-quality day 2 or 3 ETs resulting in 192 pregnancies	Pregnancy outcome based on day 8 or 12 hCG level	• hCG value > 45 on day 12 had 75.6% sensitivity and 100% specificity for ongoing pregnancy
Ochsenkühn, R., et al.	2009	230 women with intact intrauterine pregnancy 21 days after OR and term live birth (191) or miscarriage [5]	Pregnancy outcome based on hCG level 11, 13, and 21 days after OR	• Day 21 hCG gave the best prediction of potential viability, with mean hCG levels 107 for miscarriage vs. 2946 for term delivery • Cut-off for probability of live birth of 80 on day 14, and 1500 on day 21, with PPV 93.3% and 91.7%
Transfer of multiple embryos resulting in singleton pregnancy				
Brady, PC. et al.	2018	Cycles resulting in one gestational sac on ultrasound followed by singleton live birth beyond 24-week gestation (629 following SET, 1372 following MET), between 2007 and 2014 at a single academic center.	hCG trends among women conceiving singleton pregnancy following MET compared with SET	• Among women receiving multiple embryos, 6.1% had abnormal hCG rises between first and second measurements compared with 2.7% of patients undergoing SET • Among women with initially abnormal hCG rise, 2/4 had normal rise between second and third measurements
Brady, PC. et al.	2013	Women undergoing fresh IVF/ICSI cycles between 1998 and 2010 with two gestational sacs on early ultrasound and confirmed failure of one twin before 12-week gestation. 503 vanishing twins compared with 2901 normally progressing singletons and 1132 normally progressing twin pregnancies	hCG rise in IVF pregnancies with a vanishing twin compared with normally progressing singleton and twin pregnancies	• Pregnancies with vanishing twins demonstrated a significantly lower mean 2-day percent increase than singletons and twins (114.3% vs. 128.8% vs 125.4%) • Vanishing twins arresting earlier in development demonstrated significantly further reduced hCG level increases

Table 1 (continued)

Author	Year	Population	Outcomes measured	Results
Multiple gestation				
Povoa, A. et al.	2018	177 IVF cycles between 2009 and 2014 (50 singleton births, 50 twin births, 27 with vanishing twins, 43 first trimester singleton losses, and 7 first trimester twin losses)	Predictive value of hCG level at day 13 post ET for clinical pregnancy outcome	• Singleton pregnancies with initial hCG concentration < 85 had an 89% risk of having a first trimester loss. Those with hCG > 386 had 91% chance of live birth • Twin pregnancies with initial hCG < 207 had only 33% chance of delivering twins and 55% risk of vanishing twin. Those with level > 768 had an 81% chance of live twin birth
Embryo stage on day of transfer				
Dahiya, M. et al.	2017	560 women with clinical pregnancy following ET between 2003 and 2014 at a single center	Serum hCG values post ET of cleavage stage ($n = 500$) vs. blastocyst stage ($n = 60$) embryo at day 17 post OR	• hCG levels were not significantly different following transfer of cleavage stage vs. the blastocyst stage embryos (mean 387 vs. 352)
Kumbak, B. et al.	2006	2035 embryo transfer cycles between 2003 and 2005	hCG concentration 12 days post ET and pregnancy outcome with either day 3 or day 5 transfer	• Two-fold increase in hCG values in day 5 transfers compared with day 3 transfers (but measured on day 12 post ET not OR)
Zhang, X. et al.	2003	Embryo transfers between 1999 and 2001 at a single center	Serum hCG levels after day 3 vs day 5 ET at equivalent time intervals post fertilization	• hCG levels were significantly lower in day 5 (102) vs. day 3 (155) embryo transfers
Morphology				
Kuspinar, G. et al.	2019	455 patients undergoing fresh SETs between 2011 and 2016 at a single center resulting in 142 patients with positive hCG	Embryo morphology and day 12 post-ET hCG levels effects on pregnancy and live birth after SET	• Mean initial hCG level similar between cleavage stage and blastocyst transfer (315 vs. 372) • For cleavage stage, no difference between morphology and hCG resulting as positive or negative • For blastocysts, more expanded embryos with higher blastocyst quality score were more likely to result in positive hCG
Girard JM. et al.	2018	441 couples undergoing 455 single blastocyst transfer cycles leading to a positive pregnancy test 12 days after at a single university-affiliated IVF center	Early hCG rise, blastocyst morphology and pregnancy	• hCG kinetics was significantly different according to blastocyst expansion, but with considerable overlap • hCG kinetics were also significantly different according to clinical outcome, with higher values in clinical pregnancies than in other groups
Fresh vs. frozen				
Sites, CK. et al.	2015	322 single embryo transfer cycles between 2007 and 2012 at a single center	Initial hCG level whether fresh or frozen embryo transfer and live birth rate	• Significantly lower median initial hCG level in thawed-warmed cycles compared with fresh (100 vs. 126) • Significant difference between slow freezing and vitrification methods, with median initial hCG levels of 89 vs. 183 • Live birth rates similar between fresh transfers and most methods of frozen other than blastocyst slow freezing
Oron, G. et al.	2017	1130 single blastocyst transfer cycles (789 fresh and 349 vitrified-warmed)	Initial hCG and pregnancy outcome	• hCG levels from cycles resulting in clinical pregnancy were significantly higher after vitrified-warm transfer vs. fresh (383 vs. 334) • Threshold for predicting a clinical pregnancy for a fresh blastocyst was

Table 1 (continued)

Author	Year	Population	Outcomes measured	Results
				111 and for a vitrified-warmed blastocyst was 137
Reljiic M. et al.	2013	775 cycles with positive hCG values 13 days after fresh blastocyst transfer ($n = 568$) or vitrified-warmed blastocyst transfer ($n = 207$)	Predictive values of hCG levels in fresh and vitrified-warmed blastocyst transfer cycles and live birth rate	• Average hCG levels according to pregnancy outcome were not significantly different between fresh and frozen cycles • hCG levels in those that resulted in live birth were still significantly higher than non-viable pregnancies for both groups
Xue, Y. et al.	2014	1131 embryo transfers (797 slow freeze and 334 vitrified)	Median serum hCG levels on day 12 post-ET in pregnancy following FET either by vitrification or slow freezing	• Pregnancy outcomes did not differ between groups • Initial hCG levels for ongoing pregnancies were significantly lower for vitrified-warmed vs. slow frozen embryos (279 vs. 320)
ICSI vs. conventional fertilization				
Gold, RS. et al.	2000	204 pregnancies resulting from in vitro fertilization cycles either from ICSI or conventional fertilization	Initial hCG level on equivalent day post fertilization whether by ICSI or conventional fertilization and difference in rise between day 14 and 16	• No significant difference in hCG levels • No difference in the rise between day 14 and 16
Hoon, K. et al.	2011	A total of 220 clinical pregnancies following COH and IVF/ICSI	hCG levels in twin pregnancies with either ICSI or conventional fertilization	• Significantly higher initial hCG in twin pregnancies resulting from ICSI compared with conventional fertilization • This effect did not persist when controlled for confounding factors
Embryo biopsy for PGT				
Cho, YJ.	2011	129 PGT cycles and 1161 age-matched ICSI cycles resulting in pregnancy	hCG profile starting post-ovulation day 12 following PGT compared with ICSI cycles	• Mean hCG level was consistently lower for those patients who had PGT with no significant difference in biochemical pregnancy rate, miscarriage rate, second trimester loss, preterm delivery, or birth weight • Doubling times were not significantly different
Soriano, LP. et al.	2019	279 PGT cycles compared with 208 cycles without trophectoderm biopsy	Impact of trophectoderm biopsy on hCG dynamics	• No significant difference noted in mean initial or subsequent hCG levels • Significant difference in the rate of rise (247.9% vs. 238.9% in the PGT vs. non-PGT group)
OHSS				
Choux, C. et al	2017	77 patients presenting with clinical pregnancy after IVF and hospitalized for severe OHSS compared with 231 controls in a single center	Pregnancy outcomes (miscarriage, medical abortion, or delivery), hCG values, obstetrical, and neonatal outcomes	• At all time points hCG values were lower in OHSS pregnancies than in controls, as was the relative change of hCG values • In women who underwent paracentesis, rate of hCG rise increased following the procedure • Pregnancy outcomes remained the same
Benor, A. et al.	2019	Single case report of a patient with OHSS following GN/IUI	hCG level in a single case of OHSS	• hCG level only rose 23% in first interval, then 29%. As symptoms resolved, hCG trend normalized and there was a normal pregnancy at 8-week gestation.
Aneuploidy				
	1993	573 patients with pregnancies conceived through infertility treatment between		

Table 1 (continued)

Author	Year	Population	Outcomes measured	Results
Soltes, B. et al.		1984 and 1990 at a single center. 15 with first trimester losses with confirmed abnormal karyotype, 6 with chromosomally normal male abortuses, and 60 with normal term deliveries	Hormonal profiles of chromosomally abnormal pregnancies during the first trimester	• Significant difference in hCG measurements between normal and chromosomally abnormal conceptions • Chromosomally abnormal pregnancies had a linear increase in hCG, compared with normal pregnancies with a curvilinear progression
Placental abnormalities				
Johnson, MR. et al.	1993	9 women who miscarried after FHT detected, 52 normal singleton, and 22 normal twin pregnancies following IVF and ET	Endocrine changes associated with spontaneous miscarriage after fetal heart activity demonstrated	• 4 women had miscarriage between 9- and 12 weeks gestation, and hCG levels were consistently beneath the 10%ile with decrease prior to loss of FHT • 5 women (3 singleton and 2 twin) had loss between 16- and 20-week gestation, with hCG reduced several weeks before fetal demise
Maternal age				
Shamonki, M. et al.	2009	6021 pregnancies resulting from IVF with detectable early serum hCG after ET at a single large center between 1999 and 2005	Live delivery rate based on initial hCG and rise of hCG	• As age increased there was a significant reduction in live delivery rate for a given initial hCG level and a second level drawn 2 days later added predicted value
Grøndahl, ML. et al.	2017	Multicenter cohort from 11,744 IVF/ICSI cycles	Initial hCG 14 days post day 2 embryo transfer as a post-hoc analysis	• hCG value that resulted in ongoing pregnancy was significantly increased with increasing age of the woman in both IVF (429) and ICSI (374) groups

Units: hCG units in mIU/mL or IU/L

Abbreviations: *IVF* in vitro fertilization, *ET* embryo transfer, *hCG* human chorionic gonadotropin, *PPV* positive predictive value, *SET* single embryo transfer, OR oocyte retrieval, MET multiple embryo transfer, *ICSI* intracytoplasmic sperm injection

Mean initial hCG levels were also evaluated on post transfer day 12 in a 2004 study of 370 consecutive single top-quality day 2 or 3 embryo transfers [11]. One hundred and ninety-two pregnancies resulted from these cycles, with 30 biochemical pregnancies, 4 ectopic pregnancies, 23 miscarriages, and 135 ongoing pregnancies at 12-week gestation. A cut-off hCG value of > 45 IU/L on day 12 post transfer was determined by ROC curves to have a 75.6% sensitivity for ongoing pregnancy. This group did evaluate rate of rise of hCG, but over 4 days rather than the 2-day standard.

A 2009 study of 230 pregnancies resulting from single blastocyst transfer evaluated hCG levels on day 11, 14, and 21 following oocyte retrieval [12]. This study did not evaluate the rate of rise of hCG. Pregnancies with multiple gestation were excluded by only including those pregnancies with a single gestational sac on day 21. On day 14, mean hCG levels were 73.1 mIU/mL in the miscarriage group vs. 141.7 mIU/mL in the term delivery group. The day 21 hCG value gave the best prediction of potential viability when ROC curves were used, with mean hCG levels for the miscarriage group being 1075.0 mIU/mL vs. 2946.0 mIU/mL in the term delivery group. They determined cut-off values for probability of live birth of 80 mIU/mL and 1500 mIU/mL on days 14 and 21, respectively, with positive predictive value of 93.3% and 91.7%.

Transfer of Multiple Embryos Resulting in Singleton Pregnancy

Often in assisted reproductive technology cycles the recommendation is made for transfer of more than a single embryo to increase the likelihood of pregnancy based on female age, embryo quality, history of IVF failure, or some other prognostic factor. It is plausible that patients who have more than one embryo transferred may have transient implantation of nonviable embryos that could contribute to hCG concentration without ever developing ultrasound evidence of multiple pregnancy. This could very likely lead to an hCG trend that appears abnormal.

A recent retrospective cohort was designed to investigate this scenario, and results revealed that 6.1% of patients who received two or more embryos compared with only 2.7% of

patients with single-embryo transfer had an abnormal hCG rise (defined as < 66%) between the first and second measurements [13]. In those patients that had hCG values that initially rose abnormally, three quarters then had a normal rise between the second and third measurements.

This same group had previously investigated the hCG rise in IVF pregnancies with a vanishing twin (two gestational sacs detected on early ultrasound but subsequent failure of one twin prior to 12-week gestation). This prior data revealed that pregnancies with a vanishing twin did have a slower initial increase when compared with normally progressing singleton or twin pregnancies (114.3% vs. 128.8% and 125.4%, respectively); however, the rise was still within clinically accepted limits [14].

The phenomenon of vanishing twin is important prognostically for a couple who has achieved pregnancy following IVF. Almog et al. demonstrated worse obstetric outcomes for vanishing twins that resulted from IVF compared with singleton pregnancies resulting from IVF, with increased risk of preterm deliveries and low birth weight in these pregnancies [15]. Additionally, Márton et al. demonstrated worse perinatal outcomes for patients with vanishing twin pregnancies conceived following IVF when compared with spontaneously conceived vanishing twin [16]. These worsened outcomes included growth restriction, placental abruption, and retained placenta. These authors proposed that this could be related to the greater gestational age that vanishing twin is detected in IVF pregnancies compared with spontaneous pregnancies [17] and that later timing of demise would tend to indicate that there is impaired uterine environment or impaired utero-fetal interaction which could subsequently lead to the above mentioned complications.

Multiple Gestation

Given what we know about hCG levels and trends in healthy singleton pregnancies, it may be postulated that multiple gestation resulting from assisted reproductive technology may contribute to an hCG rise that could appear abnormal as there are two (or more) embryos and therefore additional sources of hCG. Perhaps the presence of multiple embryos would lead to a higher initial hCG level, and a steeper rise in hCG levels.

When studied, this has not been observed in multiple gestations resulting from assisted reproductive technology. The previously discussed study that investigated hCG patterns in ART pregnancies did compare trends between singleton, twin, and triplet gestations [2]. The findings revealed that while Initial HCG values were significantly higher for twin and triplet gestations compared with singletons, the rate of rise was unchanged.

Outcomes from 177 IVF pregnancies between 2009 and 2014 were analyzed in a recent retrospective study to determine the hCG value at day 13 after embryo transfer that could predict live birth in singleton and twin pregnancies [18]. Fifty singleton births, 50 twin births, 27 vanishing twin pregnancies, 43 first trimester singleton losses, and 7 first trimester twin losses were included. In singleton pregnancies, a day 13 hCG level of < 85 mIU/mL had an 89% risk of first trimester loss, whereas a concentration > 386 mIU/mL had a 91% chance of live birth. In twin pregnancies, a concentration of < 207 mIU/mL had only a 33% chance of delivery of twins vs. 55% chance of vanishing twin, compared with a level of > 768 mIU/mL which was associated with an 81% chance of live twin birth.

Embryo Stage on Day of Transfer

Embryo transfer following IVF can occur at the cleavage stage (2–3 days after fertilization), or the blastocyst stage (5–6 days after fertilization). The question arises whether transfer at different developmental stage has an impact on early hCG levels and pregnancy outcome. If hCG levels are measured at equivalent time points from oocyte retrieval, there would not be expected to be a significant difference in the two groups as long as embryos progress and develop as expected, unless perhaps there was an impact of longer time spent in vitro culture.

A retrospective cohort study of 560 women with clinical pregnancy following embryo transfer evaluated hCG level following day 3 and day 5 transfers, without demonstrating a significant difference between groups [19]. Initial hCG levels were 387 IU/L for day 3 embryos and 352 IU/L for day 5 embryos on the same day post retrieval. This lack of difference persisted even when subdividing into singleton vs. multiple gestation, although numbers of multiple gestation were small. There were only a limited number of patients in the blastocyst transfer group (only 60 compared with 500 cleavage stage) in this study which may limit the findings. It is possible that the statistically insignificant difference could be due to the fact that embryos develop faster in vivo than in vitro. The cleavage stage embryos transferred perhaps implant and start producing hCG sooner (even by few hours) as compared with the blastocysts transferred.

A larger retrospective cohort including 2035 embryo transfer cycles (1422 on day 3, 613 on day 5) investigated the potential difference in initial hCG and pregnancy outcome [20]. These researchers found an almost two fold increase in hCG values in day 5 transfers compared with day 3 transfers. However, in this study, hCG level was checked 12 days following *transfer* in both groups rather than at the same day post *retrieval/fertilization* and so this difference is not unexpected as the embryos would be at different developmental stages and therefore the length between implantation and hCG testing differed.

In a study that compared levels at the same time post-*fertilization,* hCG levels were actually significantly lower

(102 IU/mL vs. 155 IU/mL) in day 5 embryo transfers compared with day 3 [21]. The authors proposed three possible mechanisms for this finding: first being a difference in very early, subclinical losses; second that delaying to day 5 leads to a gender bias which could relate to rate of embryo development; and third being that the additional 2 days in culture may adversely affect the development of the embryo leading to a subtle delay in hCG production. They went on to explain that their findings were inconsistent with the first two possibilities and concluded that it is most likely related to a potential impact of additional time in culture. We believe it is likely that additional time in culture results in lagging development as discussed above, with potential for these embryos to implant and begin producing hCG later.

Embryo Morphology at Transfer

Embryos are graded with a morphology system that considers number and symmetry of cells and degree of fragmentation for cleavage stage embryos, and expansion and quality of the inner cell mass and trophectoderm for blastocyst stage embryos. As mentioned earlier, studies have compared hCG levels after cleavage stage or blastocyst transfer, with inconsistent results, but only a handful of studies include the impact of morphology.

A recent retrospective cohort was performed with the primary goal of the effect of embryo morphology on serum hCG levels and subsequent live birth, and a secondary goal of determining effect of cleavage vs. blastocyst stage embryo on hCG level and live birth [22]. It included 455 patients who underwent fresh single-embryo transfer cycles between 2011 and 2016 which resulted in 142 patients with positive hCG levels 12 days after transfer, 85 of whom had a documented clinical pregnancy which resulted in 79 live births. The mean initial hCG level for cleavage stage transfer and blastocyst transfer was similar (315 vs. 372 IU/L). For cleavage stage embryo transfers, which were graded by cell number, blastomere size, and fragmentation rate, there was no difference between morphology and hCG resulting as positive or negative on day 12. For blastocysts, which were graded by level of expansion and grade of inner cell mass and trophectoderm, trophectoderm cell number, and blastocyst quality score, more expanded embryos and those with a higher blastocyst quality score were more likely to result in a positive hCG level. Interestingly, blastocyst morphology was shown to have an effect on serum hCG levels in cycles that resulted in ongoing pregnancy and live birth, with blastocyst expansion, trophectoderm cell number, and blastocyst quality score playing a role. However, in this study of relatively young patients (mean age of 30) there was a lower than expected clinical pregnancy and higher than expected biochemical pregnancy rate, so the findings should perhaps be revisited.

A French study published in 2018 aimed to evaluate the association between blastocyst morphology and early hCG rise to further investigate any possible correlation [23]. In this retrospective cohort study, the rate of hCG rise was calculated in 455 single-blastocyst transfer cycles that resulted in positive pregnancy tests. This study revealed that while there was a significant difference attributed to blastocyst expansion, there was considerable overlap between groups (blastocyst quality B1 through B5), and the rate of rise was more affected by ultimate pregnancy outcome.

hCG Levels Following Fresh Vs Frozen Embryo Transfer

Another factor that may potentially impact hCG dynamics in early pregnancy could be whether pregnancy had resulted from a fresh or frozen embryo transfer, and further what technique (vitrification vs. slow freezing) had been used for embryo cryopreservation. Much research has been done regarding the effects of cryopreservation on embryo developmental potential which could be reflected initially by its ability to develop to blastocyst, implant, and produce hCG. For example, lower levels of hCG may reflect a compromised trophoblast.

Sites et al. reviewed their own clinic's single-embryo transfer cycles from 2007 to 2012 to evaluate whether there was an impact of cryopreservation on hCG dynamics and pregnancy outcomes [24]. During this time period they initially used a slow freezing technique and then as technology evolved began using vitrification for cryopreservation of supernumerary embryos, so they have data on each method. They discovered that there was a significantly lower median initial serum hCG level in thawed-warmed cycles compared with fresh cycles (100 vs. 126 mIU/mL). Additionally, a significant difference was noted between slow freezing and vitrification methods, with median initial hCG levels of 89 and 183 mIU/mL, respectively. Despite the difference in initial hCG levels, live birth rates were similar between fresh transfers and most methods of embryo cryopreservation other than blastocyst slow freezing (which had the lowest initial hCG levels). This study was limited by small numbers in some of the groups evaluated as they had changed their methods several times over the study period and included both cleavage and blastocyst stage.

A more recent study of 1130 single blastocyst transfer cycles (789 fresh and 341 vitrified-warmed transfers) found that while overall initial hCG levels were comparable between groups, those pregnancies that resulted in clinical pregnancy had a significantly higher initial hCG level after the transfer of vitrified embryos vs. fresh transfers (383 vs. 334 IU/L), with a higher threshold value for predicting clinical pregnancy required of vitrified embryos (137 vs. 111 IU/L) [25]. These investigators concluded that each clinic should determine its

own threshold but should consider that higher levels of hCG may be expected after a vitrified-warmed transfer cycle. Conversely, a similar study of blastocyst transfer after fresh or vitrified-warmed cycles demonstrated no difference between initial hCG level and type of cycle, although hCG levels in those that resulted in live birth were still significantly higher than non-viable pregnancies for both groups [26].

In a study evaluating the impact of vitrification vs. slow freezing on day 3 embryo transfer cycles, the method for cryopreservation did reveal a significant difference in initial hCG levels [27]. These investigators found that while pregnancy outcomes did not differ between groups, initial hCG levels for ongoing pregnancies were significantly lower for vitrified-warmed cleavage stage embryos than slow frozen embryos (279 vs. 320 IU/L).

Intracytoplasmic Sperm Injection Versus Conventional Fertilization Methods

Given the additional micromanipulation of oocytes that is required during cycles that use intracytoplasmic sperm injection (ICSI) for fertilization, it could be predicted that ICSI could have an effect on early pregnancy dynamics. This additional manipulation had earlier been postulated to potentially lead to possible delayed implantation of the embryo, or even increased degree of embryo fragmentation, which could impact early hCG levels [28, 29].

A retrospective analysis of 204 pregnancies resulting from in vitro fertilization cycles compared initial serum hCG levels of singleton pregnancies resulting either from ICSI or conventional fertilization (IVF) [28]. These authors found no significant difference in hCG levels on day 14 or 16 post embryo transfer, and no difference in the rise between day 14 and 16 whether conventional IVF or ICSI was used.

Another retrospective analysis investigated whether there was an effect from ICSI on twin pregnancies [30]. This group found that there was a significantly higher initial hCG in twin pregnancies resulting from ICSI compared with conventional fertilization, but this effect did not persist when controlled for confounding factors.

Embryo Biopsy for Preimplantation Genetic Testing

Preimplantation genetic testing (PGT) is accomplished through biopsy of the embryo and evaluation of the removed cells for genetic makeup. Given the additional manipulation with this procedure and the physical removal of either blastomere at eight cell stage or trophectoderm cells, it is plausible that there could be decreased initial hCG levels as an effect of a lag in development to blastocyst or decreased trophectoderm cell numbers, respectively. Data are limited but there have been some investigations into early hCG levels following cycles with embryo biopsy for PGT.

In 2011 Cho et al. published their data comparing initial serum hCG levels in 129 pregnancies following PGT with 1161 age-matched controls who underwent ICSI cycles [31]. Their data revealed that mean hCG level was consistently lower for those patients who had PGT: 37.9 mIU/mL vs. 46.9 mIU/mL on day 12 post retrieval, 111.3 mIU/mL vs. 145.4 mIU/mL on day 14 post retrieval, and 2225 mIU/mL vs 3614 mIU/mL on day 21 post retrieval. The doubling times were not significantly different. Despite the lower initial hCG levels, there was no significant difference in biochemical pregnancy rate, miscarriage rate, second trimester losses, preterm delivery rates, or birth weights between groups.

More recently, trophectoderm biopsy has become the standard method for PGT; however, data on the impact of this procedure on initial hCG levels is limited. Soriano et al. recently presented their data on the effect of trophectoderm biopsy on early serum hCG levels in pregnancies that resulted in singleton live birth [32]. Two hundred-seventy-nine PGT cycles were compared with 208 cycles without trophectoderm biopsy, and there was no significant difference noted in mean initial or subsequent hCG levels. There was a statistically significant difference in the rate of rise (247.9% vs. 238.9% in the PGT vs. non-PGT groups respectively); however, this difference may be clinically less significant.

Ovarian Hyperstimulation Syndrome

Ovarian hyperstimulation syndrome (OHSS) is a complication of controlled ovarian stimulation that involves potentially massive fluid shifts from intra to extravascular compartments. Given these fluid shifts, it is reasonable to consider the possibility that serum hCG levels may appear abnormal in pregnancies resulting from in vitro fertilization cycles that were complicated by OHSS.

A recent case control study did demonstrate altered early hCG trends in pregnancies complicated by OHSS without change to obstetrical or neonatal outcomes [33]. This study compared 77 pregnancies complicated by OHSS with 231 IVF-conceived pregnancies without OHSS which resulted in 69 deliveries in the OHSS group and 160 in the control group. At all time-points hCG values were lower in OHSS pregnancies than in controls, as was the relative change of hCG values between time points. Interestingly, in women who underwent paracentesis therapy for OHSS, rate of hCG rise increased following the procedure but then fell again several days following treatment (potentially indicating re-accumulation of ascites). Importantly, pregnancy outcomes remained the same despite the altered hCG kinetics.

Similarly, a case report that was recently published found a similar pattern in a patient suffering from OHSS following a gonadotropin cycle with intrauterine insemination [34]. hCG level in the first interval only rose 23%, and in the next time interval only 29%. As symptoms resolved, hCG trend

appeared to normalize, and ultrasound revealed a normal intrauterine pregnancy at 8-week gestation. Delivery information for this patient was not available at time of publication.

Aneuploidy

Prenatal aneuploidy screening is offered to most pregnant women and various types of screening tests are available at different gestational ages [35]. First trimester screening includes a single maternal serum hCG and pregnancy-associated plasma protein A (PAPP-A) level and nuchal translucency on ultrasound and is the earliest option offered from 11- to 14-week gestation. hCG values are also used in second trimester screening options. While these aneuploidy screening options do include a single hCG level in conjunction with other markers in determining risk of chromosomal abnormalities, information about early hCG rise in pregnancies that result in chromosomally abnormal pregnancies is limited. However, if there were a correlation, this would certainly be good information to have to prompt appropriate counseling and earlier definitive screening when available.

A prospective study between 1984 and 1990 of pregnancies conceived through infertility treatment evaluated hCG levels in early pregnancy and reported subsequent chromosomal abnormalities [36]. Of 573 patients, 15 had first trimester losses with confirmed abnormal karyotype. A significant difference in hCG measurements was noted between normal and chromosomally abnormal conceptions. Normal pregnancies from both natural and stimulated cycles demonstrated a linear and then curvilinear progression of hCG until a plateau of approximately 110,000 mIU/mL at 10-week gestation, whereas chromosomally abnormal pregnancies conceived spontaneously remained linear with a max hCG concentration of 10,000 mIU/mL at 7-week gestation, followed by decline until spontaneous abortion. Similarly, for those women who conceived chromosomally abnormal pregnancies through infertility treatment, hCG concentration rose to a max of 20,000 mIU/mL and then declined. This suggests a difference in the rate of hCG rise for chromosomally abnormal pregnancies whether spontaneous or conceived through IVF.

Placental Abnormalities

hCG is produced by the synctiotrophoblast following implantation, and it follows therefore that if hCG secretion is abnormal, perhaps there is a higher likelihood of placental abnormalities in these pregnancies. In regard to the possibility of trophoblast dysfunction marked by abnormal hCG levels, we can look to early pregnancies with abnormal hCG and subsequent poor pregnancy outcomes.

Johnson et al. followed women who conceived following IVF with weekly serum analytes and ultrasound to characterize the endocrine changes in early pregnancies that result in miscarriage following detection of fetal cardiac activity [37]. Four women were found to have a miscarriage on ultrasound following initial documentation of fetal cardiac activity between 9- and 12-week gestation, and seven had losses between 16- and 20-week gestation. Of the four with earlier losses, three had hCG levels consistently beneath the 10th percentile and all had decrease in serum levels prior to loss of cardiac activity. In the women with later losses, placental protein levels including hCG were reduced several weeks before fetal demise occurred while fetal growth remained intact, suggesting the trophoblast was functioning abnormally prior to the effects on the fetus were seen.

Maternal Factors and hCG Levels After IVF

Age

As more women are delaying childbearing and age-related declines in fertility occur, ART has been increasingly used for this older population, and there is a well-established effect of maternal age on pregnancy outcome. Therefore, interpretation and counseling regarding an initial hCG level should likely be considered in relation to age.

The previously discussed study by Shamonki et al. found that maternal age was an important variable when assessing initial hCG level following IVF [6]. As age increased there was a significant reduction in live delivery rate related to age for a given initial hCG level, and a second hCG level drawn 2 days later added predictive value. This indicates that age should be considered when counseling a patient following their first hCG test.

In 2017 a multicenter cohort study investigating the influence of female age on oocyte, zygote, and embryo morphology in addition to cycle outcome was published [38]. This study included data from 11,744 IVF/ICSI cycles and evaluated initial hCG level in a post-hoc analysis. The initial hCG value (14 days after day-2 embryo transfer) that resulted in ongoing pregnancy was increased significantly with increasing age of the woman in both IVF (429 cycles) and ICSI (374 cycles) groups. This would seem to echo the previously discussed study as age increases a higher initial hCG level would be required for reassurance that pregnancy would be ongoing [6].

It seems that patients with late maternal age deserve specific consideration as outcomes may be more pressing for this group as time may be limited for this population. They are also known to have poorer outcomes with fertility treatment and any early reassurance that could be provided would be beneficial.

Body Mass Index

Two recent retrospective cohort analyses were designed to investigate the association of body mass index (BMI) with

serum hCG levels and rate of hCG increase [5, 39]. The results revealed that while the rate of increase in β-hCG was unaffected by body weight, the initial serum hCG values were significantly negatively correlated with BMI. Since increasing body weight is associated with significantly lower β-hCG, it is plausible that the initial hCG values lacks sensitivity for pregnancy outcome in patients with obesity.

Discussion

The waiting period between embryo transfer and pregnancy test has been shown to be a period of increasing anxiety and depression, with anxiety persisting even in women whose pregnancy test came back positive [40]. Fertility providers may also be emotionally invested in their patients' outcomes, and in addition would benefit from early determination of pregnancy potential in order to counsel patients, manage abnormal pregnancies, and proceed with additional treatment if desired in a timely manner. The earliest test for whether a cycle is successful is the initial hCG value, and this value is typically repeated over a number of days to help provide some insight into whether a pregnancy may be normal or abnormal. Despite this early indication, there are no accepted "cutoff" initial levels or rates of increase that can definitively diagnose pregnancies that will be abnormal and those that will result in live birth, so uncertainty remains. Additionally, the different factors and technologies involved in IVF further complicate the matter, and hCG levels are not necessarily constant across different labs and measuring platforms, which limits universalization. Ultimately, ultrasound still must be performed, but in the early days prior to ultrasound ability to detect a clinical pregnancy, knowledge of normal hCG characteristics may reassure a patient. The aim of this review was to evaluate the current literature in regard to initial hCG values and trends in IVF patients, in order to provide earlier counseling and guidance.

While there will likely never be a single hCG level or rise in hCG that can be entirely reassuring for successful pregnancy following IVF, the review of the above studies hopefully does provide some information as to how a fertility specialist can begin to counsel their patients during the very early stages of pregnancy, and how to identify patients that may need closer monitoring, earlier ultrasounds, or need preparation that their pregnancy may not be normal. It also demonstrates that there can be a wide range of normal initial values that do not necessarily lead to poor outcomes and that alone can provide some reassurance to patients in the uncertain window between embryo transfer, pregnancy test and ultrasound. Repeated, larger studies are required to study these factors in further depth and to provide more support to the trends, although a framework for counseling can be developed based on the information that is currently available.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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