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Early β-hCG is Predictive of Pregnancy Outcomes after Embryo Transfer in both Fresh and Frozen Single Embryo Transfer Cycles

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Abstract

Purpose: Can a single measurement of β-hCG at day 5-6 post embryo transfer be predictive of pregnancy outcomes in frozen and fresh assisted reproductive cycles?

Methods: This is a single center retrospective study of all fresh and FET cycles utilizing single embryo transfers performed between 2016 and 2021. β-hCG levels were measured 5-6 days after ET, and again on days 9-10 to confirm pregnancy success. A β-hCG value above 5 IU/L was labeled as positive. Patients were followed with serial β-hCG levels, gestational ultrasounds, and pregnancy outcomes were documented.

Results: Early β-hCG levels were associated with pregnancy outcomes. Day 5-6 β-hCG was found to be 97.8% sensitive and 77% specific for fresh ET with an area under curve (AUC) of 0.912, and 91.5% sensitive and 72% specific for FET (AUC = 0.887).

Conclusion: The data suggests that an early measurement of β-hCG on day 5 or 6 post ET is predictive of pregnancy outcomes and should be routinely offered to patients to shorten their wait time.

Keywords: Assisted Reproductive Technology; Early B-Hcg; In-Vitro Fertilization, Frozen Embryo Transfer, Fresh Embryo Transfer

Introduction

Assisted reproductive technology (ART) has improved tremendously over the past decade to become almost comparable to natural conception in terms of pregnancy outcomes [1, 2]. Techniques including blastocyst embryo transfer (ET), single embryo transfers (SET) and frozen embryo transfers (FET) have decreased perinatal morbidity significantly [3]. With the advancement and establishment of safer ART practices, physicians' focus is shifting towards easing the experience for couples undergoing infertility treatment.

The diagnosis of infertility itself is extremely stressful for couples seeking pregnancy. The process of undergoing assisted reproduction not only places physical and financial burdens on the patients and their partners, but also reflects on their mental health and well-being throughout their treatment journey [4]. The most stressful time for patients is the waiting period between embryo transfer and the initial pregnancy test that confirms gestation [5].

The standard practice is measuring β-hCG 10-12 days after blastocyst transfer and 12-14 days after cleavage-stage embryo transfer [6,7]. Few studies have suggested that this wait is unnecessary and that pregnancy can be detected as early as day 5 post embryo transfer [8,9]. Strom et al. developed an assay that detects hyperglycosylated hCG (hhCG), which is an isoform of hCG that is present throughout the first 3 weeks of pregnancy [5]. They found that a single measurement of hhCG is able to identify ongoing and biochemical pregnancies at day 6 post ET. Data from Shapiro et al.'s study of the same year suggested that routine β-hCG can also predict IVF outcomes as early as day 5 post ET [8]. This observation could potentially be a turning point in routine ART practice and would significantly decrease couples' anxiety by documenting successful implantation less than a week after embryo transfer.

The aim of this study is to determine whether routine serum β-hCG measurement at 5-6 days after ET is predictive of ongoing pregnancies in both SET cycles in both fresh and frozen embryo transfers.

Materials & Methods

In the present study, a retrospective review of all fresh and FET cycles performed at the Virginia Center for Reproductive Medicine between 2016 and 2021 was undertaken. All cycles with one embryo transfer that had early β-hCG levels tested on day 5 or 6 were included. Exclusion criteria included cleavage stage cycles, cycles with double embryo transfers (DET), and those that did not have a β-hCG level done on days 5-6 after ET. All women underwent ovarian stimulation using one of two stimulation protocols: long luteal GnRHa or GnRH antagonist. All cycles used a mixed protocol using recombinant FSH (Gonal-F and Follistim, Merck) and HP-HMG (Menopur, Ferring). When at least 3 follicles reached 16-18 mm in diameter, 5,000-10,000 u of urinary HCG (Novarel, Ferring) or a dual trigger (HCG and GnRHa) was administered subcutaneously, and oocyte retrieval was performed 35 hours later. None of the patients received GnRH alone.

Oocytes were rinsed and denuded using a hyaluronidase solution combined with mechanical stripping, and ICSI was performed 4-6 hours later. Normally fertilized zygotes were cultured under 37 C in a 5% O_2, 5% CO_2 environment in continuous single culture media (CSC) with 10% protein (Sage, Cooper Surgical) (pre 2019) and CSC with 20% protein (post 2019) (Cooper Surgical) from day 1 until reaching the blastocyst stage at day 5 or 6. Embryos are thought to have better quality and recovery in higher protein percentage media, which is what prompted the protocol modification at the center in 2019. In all fresh ET cycles, a single blastocyst was transferred under ultrasound guidance by a single physician FIS using a Wallace Sure-view catheter (Cooper Surgical). For those cycles undergoing routine preimplantation genetic testing for aneuploidies (PGT-A) for prognosis, day 5 or 6 blastocysts underwent trophectoderm biopsy. Trophectoderm biopsies were conducted under mineral oil in Falcon 60mm Petri dishes in 20 µl drops of CSC media. Excision of 3-6 trophectoderm cells was achieved with the assistance of 5-8 pulses of a Saturn laser (Research Instrument) set at biopsy mode using 0.37 ms pulse width and 7.4-hole size. The biopsied trophectoderm cells were then rinsed in biopsy buffer 3-4 times before loading into pre-labeled samples tubes. Trophectoderm biopsy sample tubes were immediately stored at −20°C to preserve the DNA for genetic analysis. After trophectoderm biopsy, blastocysts were allowed to recover in CSC culture media for a minimum of 1-2 hours and were then vitrified by combining DMSO and ethylene glycol with a vitrification kit (Irvine Scientific).

Non PGT-A embryos underwent the same vitrification protocol without assisted hatching. At the time of ET, all blastocysts underwent assisted hatching before FET under ultrasound guidance by FIS.

In those undergoing fresh ET, all patients received intramuscular progesterone (50 mg) along with vaginal estradiol (2 mg bid) and micronized Progesterone starting the day after oocyte retrieval. All FET cycles underwent a programmed cycle using oral estradiol and IM progesterone which was started when endometrial thickness was ≥ 7 mm.

All patients had their blood drawn at the same reference laboratory (LabCorp) 5-6 days and again on days 9-10 after ET. A β-hCG value above 5 IU/L was labeled as positive. For women with a positive β-hCG, serial measurements were done every 48 hours. Clinical pregnancy was documented by an ultrasound examination at 6-7 weeks of gestation. All patients received luteal support per the clinic's protocol until 10 weeks.

Statistical analysis was performed using X^2. $P < 0.05$ was considered significant. The Institutional Review Board of Virginia Center for Reproductive Medicine approved this retrospective review.

Results

Fresh Embryo Transfer

There were 319 fresh ET between 2016 and 2021. We excluded 194 cases with double embryo transfers and 34 cases where early β-hCG was measured before day 5 or after day 6 of ET. There were 91 cases with SET and β-hCG measured on days 5-6 after ET that were included in our analysis. Of these, 46 patients had an ongoing pregnancy/delivery with an early β-hCG of 31.9±19 IU/L, 9 patients had a spontaneous abortion (SAB) (β-hCG 20.1±16.8 IU/L), 10 patients had a biochemical pregnancy (10.5±7.6 IU/L). and 26 did not get pregnant (2.3±1.9 IU/L) (Table 1). None of the patients who tested negative early had pregnancies later on. None of the patients had ectopic pregnancies.

No significant difference was seen between early β-hCG levels of ongoing/delivered pregnancies and SABs, but a clear trend was noted towards a higher β-hCG level in ongoing/delivered (P=0.08). However, early β-hCG levels were significantly different between ongoing pregnancies/delivery and biochemical pregnancies (P <0.01).

Early β-hCG was found to be 97.8% sensitive and 77% specific (AUC = 0.912) with a positive predictive value of 7mIU/ml for fresh ET (Figure 1).

Table 1: Total number of patients with their mean early β-hCG levels ± standard deviation measured on day 5-6 post ET

	Fresh	All FET	FET PGT-A	FET non PGT-A
Delivered/ Ongoing	46 (31.9±19)	83 (20.6±15.2)	60 (19±13.1)	23 (24.8±19.4)
SAB	9 (20.1±16.8)	13 (17±9)	10 (16.5±9.2)	3 (18.3±10.6)
Biochemical	10 (10.5±7.6)	25 (9.5±8.5)	21 (9±8.9)	4 (12±7)
Negative	26 (2.3±1.9)	74 (1.9±2.3)	55 (1.8±2.5)	19 (2±2.4)

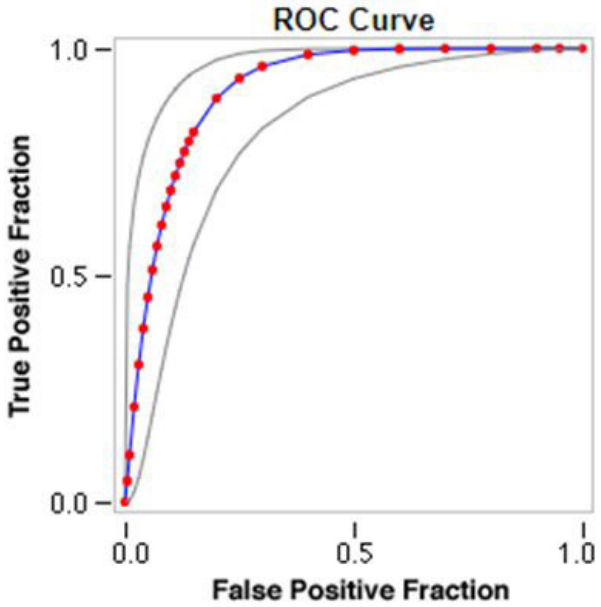

Figure 1: Receiver operating curve (ROC) for fresh transfers with an area under curve (AUC) of 0.912

Frozen Embryo Transfers (FET)

There were 365 FET between 2016 and 2021. Of these, 83 cases with double embryo transfers were excluded, and an additional 87 cases with an early β-hCG measured less than 5 days or more than 6 days following ET were excluded. A total of 195 patients with a SET with serum β-hCG measured 5-6 days after ET were included in the analysis. Among all FET cases, 83 had an ongoing pregnancy/delivery, 13 ended with a SAB, 25 were biochemical pregnancies, and 74 did not get pregnant. The mean β-hCG level was 20.6±15.2 IU/L for ongoing/delivered pregnancies, 17±9 IU/L for SAB, and 9.5±8.5 IU/L for biochemical pregnancies (1.9±2.3 for non-pregnant patients) (Table 1). Two patients out of the 83 who delivered, and another two out of those who had a biochemical pregnancy had initially tested negative for early β-hCG.

No significant difference was noted between the β-hCG levels of ongoing/delivered pregnancies and those of SAB (P=0.26). However, early β-hCG level was found to be significantly different for ongoing/delivered pregnancies when compared to biochemical pregnancies (P<0.01).

Patients were further divided into those who has PGT-A done versus those who have not. Among the group who had PGT-A, 60 patients had a delivery (mean β-hCG 19±13.1 IU/L), 10 had an SAB (β-hCG 16.5±9.2 IU/L), 21 resulted in biochemical pregnancies (β-HCG 16.5±9.2 IU/L), and 55 did not get pregnant (β-HCG 1.8±2.5). In the group that did not undergo PGT-A, 23 patients had a delivery (24.8±19.4 IU/L), 3 resulted in an SAB (18.3±10.6 IU/L), 4 had a biochemical pregnancy (12±7 IU/L), and 19 did not achieve pregnancy (2±2.4 IU/L). Even though there was a difference between the mean early β-HCG levels between PGT-A and non-PGT-A cases, statistical analysis did not yield significant results (P=0.19).

Early β-hCG measured on days 5-6 after ET had a sensitivity of 91.5% (AUC = 0.887) and specificity of 72%, with a positive predictive value of 5mIU/ml for FET (Figure 2).

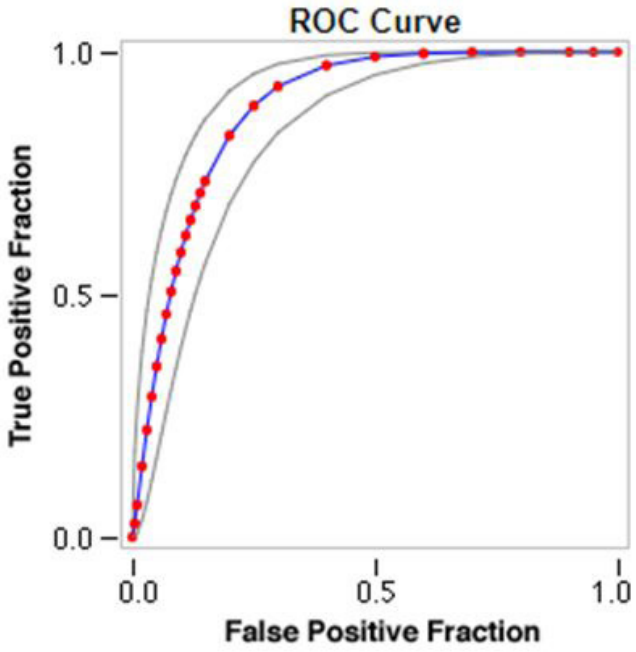

Figure 2: ROC curves for FET with AUC of 0.887

Discussion

Our results showed that early β-hCG measured on days 5-6 after embryo transfer is predictive for clinical pregnancy in both fresh and frozen ET. In fresh ET, an early level of 7mIU/ml was able to detect 97.8% of true pregnancies, while in frozen ET, an early β-hCG level of 5mIU/ml was able to detect 91.5% of true pregnant cases.

A recent study from Brazil assessed the value of day 5 β-hCG after FET in diagnosing biochemical pregnancies [10]. They evaluated 116 frozen-thawed embryo cycles in which patients underwent hormone replacement with estradiol and progesterone to exclude the influence of exogenous hCG. Their cohort included single, double, and triple ET. Their results showed that a day 5 β-hCG level of ≥4 IU/l could predict biochemical pregnancy with a 93.4% sensitivity and a 92.7% specificity (AUC

0.96), however, no other outcomes were reported and no distinction between clinical and biochemical pregnancies was made [10]. Furthermore, even though results were consistent upon sub analysis of single ET to exclude the influence of multiple pregnancies, the SET group only consisted of 32 patients. We validated these results in a bigger sample and found that β-hCG does not only predict biochemical pregnancy but also distinguishes between pregnancy outcomes. In addition, the disappearance rate of exogenous β-hCG measures around 36 hours with a mean half-life of 2.32 days [11]. For this reason, our results reveal that day 5 β-hCG levels remain applicable in predicting pregnancy outcomes regardless of the use of exogenous hCG.

Our findings were consistent with those reported by Shapiro et al in 2012 where they analyzed β-hCG levels at day 5 after transfer of 767 fresh double blastocyst transfer cycles [8]. Their results showed a day 5 mean β-hCG level of 23.4±11.6 IU/l for pregnancies with multiple fetal hearts, and 14.1±8.9 IU/l for those with a single fetal heart. A day 5 β-hCG ≥ 5IU/l was a significant predictor of pregnancy outcomes. Ectopic pregnancy, biochemical pregnancy loss and SAB were predicted by day-5 β-hCG < 5IU/l. Ongoing and multiple pregnancy were predicted by β-hCG ≥ 5IU/l [8]. Shapiro et al. reported a 92% sensitivity and 83% specificity of the day 5 β-hCG assay for detection of ongoing pregnancies following fresh blastocyst transfer. They however included cycles with double fresh embryo transfers in their cohort. Although their work was highly significant in pioneering the idea of earlier pregnancy detection, practice has mostly shifted to frozen SET. Hence, our reproducibility of their results on a frozen SET cohort validates the clinical use of early β-hCG for frozen SET.

In 2012, Strom et al. followed hyperglycosylated hCG (hhCG) levels after embryo and blastocyst transfers of 56 patients and noted that by day 6, serum and urine hhCG levels began to rise in clinical pregnancies and decline in non-pregnancies [5]. A serum hhCG >75pg/ml or urine hhCG >25pg/ml at day 6 had a sensitivity and specificity of 100% for diagnosing pregnancy following blastocyst transfers compared to a 75% sensitivity and 100% specificity following cleavage stage embryo transfers [5]. Further follow up revealed that serum hhCG levels were able to distinguish between clinical and biochemical pregnancies. A serum hhCG level of >300pg/ml at day 6 following a blastocyst transfer identified clinical pregnancies with a 92% sensitivity and 100% specificity and a level between 75 and 300pg/ml most likely means that the pregnancy is biochemical. Patients with hhCG levels <75pg/ml on day 6 are almost certainly not pregnant. Urine hhCG were not able to distinguish clinical from biochemical pregnancies and the data for cleavage stage embryos was promising but not as decisive [5]. Even though these results are highly significant, hhCG assays are not readily available and not used clinically. Another new assay that can detect hCG, hhCG, β-hCG and nicked hCG was developed and was found to be comparable to the hhCG assay on day 4 [9]. However, β-hCG is routinely used to detect and follow pregnancy and our results show that the same test that is widely available can be implemented earlier with confidence without the need to develop and invest in a new one.

Preimplantation genetic testing aids in selecting euploid embryos for transfer to improve outcomes such as implantation, clinical pregnancy, and live birth rates. A trophectoderm biopsy is the most commonly used method to collect cells for PGT-A [12]. However, it is unclear how this affects levels of β-hCG. One study showed that trophectoderm biopsy significantly decreases day 12 β-hCG levels in clinical pregnancies compared to those who have not had PGT-A [13]. In another study, Hobeika et al. compared initial hCG levels in 3 different groups, one undergoing fresh ET, one undergoing FET, and another with PGT-A followed by FET [3]. Initial β-hCG levels were measured 14 days after retrieval for fresh cycles and 9 days after FET. Their results showed significantly higher β-hCG levels in the PGT-A group when compared to both the FET and fresh ET groups, and a higher initial β-hCG level in the FET group when compared to the fresh ET. Hobeika et al. concluded that PGT-A has a significant effect on initial β-hCG values suggesting that β-hCG values may relate to the chromosomal status of the embryo [3]. Our analysis did not reproduce these findings and results were not significant upon comparing PGT-A versus non-PGT-A subgroups in our FET cohort. However, we only analyzed 60 patients with PGT-A on day 5 and while there was a trend towards a lower β-hCG level in the PGT-A group, a bigger sample is needed to truly establish whether or not day 5 β-hCG levels correlate with fetal chromosomal abnormalities. The heterogeneity of data also calls for further investigation of this association.

Day 7 β-hCG levels have also been shown to be predictive of ongoing versus adverse pregnancy and could significantly distinguish between pregnancy and non-pregnancy, viable and non-viable pregnancy, biochemical and clinical pregnancy, as well as single and multiple pregnancy with high sensitivity and specificity [14, 15]. Although this reduces the wait time significantly, our data show that similar results were generated for day 5 β-hCG, reducing the wait time further by 2 days. However, it is noteworthy to mention that our results showed specificities of 77% in fresh embryo transfer and 72% in FET, which leaves a

relatively high rate of false positivity and so, physicians should be cautious in reporting these results to their patients.

Couples undergoing infertility treatment are already under a significant amount of stress pertaining to successful conception. The current standard 10-12 day waiting period from ET to β-hCG measurement is the most anxiety provoking time in patients undergoing assisted reproduction [16]. Emerging studies are challenging this practice and our data further supports earlier testing. As opposed to previous studies, our study is unique in that we only included single embryo blastocyst transfer. As most centers performing ART have shifted nearly completely towards the transfer of a single frozen embryo at the blastocyst stage, we believe our results are clinically valid and applicable to current practice.

Undergoing fertility treatment and assisted reproduction confers a lot of stress on patients and their partners. With more data validating the sensitivity and specificity of early β-hCG in detecting pregnancy, the wait could become shorter and physicians can potentially confirm gestation as early as 5 days post ET, significantly reducing the couple's stress levels.

Declarations

The authors declare no conflict of interest.

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Author Contributions

FIS conceived the study and analyzed the results, MHK wrote the manuscript, ALB & HT performed laboratory techniques, GNM reviewed and edited the manuscript.

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