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

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Abstract

Purpose Few published articles have compared initial hCG values across all different types of ART cycles, including cycles with fresh or frozen embryo transfer. No articles have compared initial hCG values in cycles utilizing preimplantation genetic screening (PGS). The purpose of this study is to compare initial hCG values after fresh embryo transfer, frozen embryo transfer, and after PGS.

Methods This was a single-center retrospective cohort study at an academically affiliated private IVF center. All fresh and frozen embryo transfers between January 2013 and December 31, 2015 were included. We compared mean initial serum hCG values 14 days after oocyte retrieval for fresh cycles and 9 days after frozen embryo transfer. We examined cycles of single embryo transfer (SET) and double embryo transfer (DET).

Results Two hundred eleven IVF (fresh embryo transfer), 128 FET (frozen embryo transfer cycles, no PGS), and 111 PGS

cycles (ovarian stimulation with embryo cryopreservation, PGS, and frozen transfer in a subsequent estrogen-primed cycle) with initial positive hCG values were analyzed. In patients achieving a positive hCG after SET, initial hCG values were higher after PGS compared to FET (182.4 versus 124.0 mIU/mL, $p = 0.02$) and IVF (182.4 versus 87.1 mIU/mL, $p < 0.001$) as well as FET compared to IVF (124.0 versus 87.1 mIU/mL, $p < 0.01$). After DET, initial hCG values were higher after PGS (222.8 mIU/mL) compared to FET (182.1 mIU/mL, $p = 0.02$) and IVF (131.1 mIU/mL, $p = 0.001$).

Conclusions Our study suggests that initial serum hCG values are higher after using PGS and higher after the transfer of a frozen embryo compared to a fresh embryo. This suggests that initial hCG values relate to the chromosomal status of embryos. Initial hCG values may help determine intervention and monitoring later in pregnancy.

Keywords hCG · Single embryo transfer · Frozen embryo transfer · Preimplantation genetic screening

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Background

Despite millions of successful births through assisted reproductive technology (ART), concern for perinatal outcome still remains. Initial ART research focused almost exclusively on methods to increase pregnancy rates. As pregnancy rates improved, the focus shifted to limiting multiple pregnancies and to perinatal outcome. Investigative efforts to delineate perinatal morbidity first compared in vitro fertilization (IVF) outcomes compared to spontaneous conception [1–3]. Comparisons between IVF and intracytoplasmic sperm injection (ICSI) became popular [4] and then attention refocused on examining ART to non-ART outcomes. The diagnosis of infertility itself confers

worse outcomes regardless of mode of conception [4–9]. Blastocyst embryo transfer (ET), elective single embryo transfers (eSET), and improvements in embryo cryopreservation techniques have decreased perinatal morbidity from ART. In many studies, perinatal morbidity from ART compares favorably to natural conceptions [10–12].

Frozen embryo transfer (FET) cycles have become more prevalent as primary treatment and success rates from FET cycles are comparable or superior to fresh IVF [13–17]. Improved perinatal outcomes have also been demonstrated after FET [18–20]. Gestational age at delivery, preterm labor rates, birth defect rates, and perinatal mortality are generally comparable between fresh and frozen transfer cycles. However, FET yields higher birth weights and fewer low birth weight and small for gestational age neonates [21]. Some unadjusted data shows FET of blastocyst embryos compared to cleavage stage embryos yields higher rates of preterm birth but lower risk of SGA and IUGR [22]. Simultaneous to improvements in cryopreservation, preimplantation genetic screening (PGS) for aneuploidy has underscored the prevalence of aneuploidy [23–26]. The combined model of IVF with complete embryo cryopreservation, PGS, and FET in a future cycle may maximize live birth rate, minimize multiple pregnancies, eliminate unnecessary embryo transfers [27, 28], and improve perinatal outcomes.

Beta human chorionic gonadotropin (hCG) early in pregnancy has been an important biomarker of pregnancy outcomes for decades [29, 30]. HCG can indicate healthy placental implantation and predict perinatal morbidity, including preeclampsia, antepartum hemorrhage, and preterm birth. Low maternal hCG concentrations very early in pregnancy may be associated with severe preeclampsia [31]. Initial hCG values have been shown to predict pregnancy viability—increased risk of miscarriage and ectopic pregnancy with lower initial hCG values and live birth prediction with higher initial hCG levels [30]. Similar findings have been corroborated after IVF cycles [32]. Recently, higher initial hCG values after fresh IVF were predictive of ongoing pregnancy [33–35]. Blastocyst embryos yield higher initial hCG values than cleavage stage embryos [36–38]. No studies have ever compared initial hCG values after PGS compared to non-PGS treatments. Very few studies, and none with data from the last 5 years, have compared initial hCG values after fresh compared to frozen embryo transfer.

The aim of our study was to compare initial hCG values in women achieving pregnancy after fresh and frozen ET and with the use of PGS.

Methods

We analyzed all ART cycles with a corresponding embryo transfer between January 1, 2013 and December 31,

2015 at Island Reproductive Services. Cycles included fresh IVF (ovarian stimulation with fresh blastocyst embryo transfer), FET (frozen embryo transfer in a cycle prepared with estradiol pills), and PGS (ovarian stimulation with intentional cryopreservation of all embryos, PGS, and subsequent frozen ET in an estrogen-primed cycle). Cycles without embryo transfer were excluded. Embryos were transferred at the blastocyst stage 5 days after egg retrieval, and all cryopreserved embryos were frozen and transferred at the blastocyst stage. Although day 6 blastocysts were cryopreserved in this study, in our clinical practice, they were not used until 2016. This study was approved by an institutional review board at Staten Island University Hospital and North Shore-LIJ Health System.

Oocyte retrieval was performed by transvaginal ultrasound-guided aspiration 35 h after hCG administration. ICSI was performed in a standard fashion for all oocytes 4–6 h after oocyte retrieval with fertilization check 16–18 h later. Embryos were cultured in group culture in 25 µl microdrops global® total® media (Life Global®, USA) with a change of media on day 3. All embryos in every cycle type underwent assisted laser hatching on day 3 (ZILOS-tk® laser, Hamilton Thorne). On day 3, embryos were scored according to the number and regularity of blastomeres, percentage of fragmentation, and multinucleation. Blastocyst embryos were graded based on inner cell mass, trophectoderm, and fragmentation. [39] PGS was performed at the blastocyst stage for the entire study period by trophectoderm biopsy of 7–10 cells by a single embryologist with a noncontact 1.46 µ laser (ZILOS-tk®, Hamilton Thorne, MA, USA). Trophectoderm cells were aspirated by a 20 µ pipet (30° angled flat Humagen pipet, ORIGIO, Cooper Surgical, USA) and loaded into a PCR tube with 3.0 µl PBS. Whole genome amplification was performed for all embryos by a single commercial laboratory using an illumina CGH microarray. All PGS cycles utilized embryo cryopreservation followed by subsequent FET in a future estrogen-primed cycle (no PGS on fresh transfer was attempted).

Excess embryos were cryopreserved by vitrification (LifeGlobal®, USA) at the blastocyst stage 5 days after oocyte retrieval (for PGS and non-PGS embryos alike), which was the standard cryopreservation protocol since 2008. All FET cycles involved embryos previously cryopreserved by the same vitrification protocol. After thaw of frozen embryos for FET, embryos were held and warmed for approximately 4 h prior to FET. FET cycles were all primed using oral 17β-estradiol 2 mg tablets three times daily until endometrial thickness reached at least 8 mm. Luteal support consisted of Prometrium® 200 mg orally twice per day in addition to Crinone® 8% vaginally daily for fresh cycles and twice per day for frozen cycles. No hCG was used for luteal support.

Patient characteristics and cycle parameters were tabulated for all patients. Patient parameters included age, ethnicity,

FSH, and BMI. Cycle parameters included cycle type (IVF, FET, PGS), maximal endometrial thickness, number of embryos transferred, eggs retrieved, and peak estradiol levels. Clinical pregnancy was defined by the presence of fetal cardiac activity, and implantation rates were defined by the number of fetal heartbeats per embryo transferred. Again, data was analyzed by cycle type with three groups. The first group underwent IVF with fresh ET (IVF). The second group had frozen ET as described above (FET). The third group underwent IVF with preimplantation genetic screening of blastocysts, embryo cryopreservation of all embryos, followed by subsequent FET in a future estrogen-primed cycle (PGS). The study focused on patients achieving a positive pregnancy test, defined as serum hCG level ≥ 5 mIU/mL performed 9 days after day 5 blastocyst embryo transfer (fresh or frozen). All hCG values were performed at the same center with an IMMULITE 1000 analyzer using the corresponding IMMULITE hCG assay. Any patients with an initial hCG performed outside that assay or on the wrong day were excluded ($n = 25$). HCG values were repeated 2–3 days later and then serially until ultrasound was performed at 6 weeks gestation. Live birth data was collected by nurses and physicians by telephone survey at the time of anticipated delivery. HCG outcomes were compared by ANOVA with Bonferroni correction and by Kruskal-Wallis and Dunn's test when appropriate. Two-way ANOVA was used to compare the interaction between hCG and number of pregnancy sacs as well as the interaction between PGS and cycle type. Clinical pregnancy and live birth rates were tabulated and analyzed by chi-square and logistic regression. Stata v10 was used for statistical analysis, and p value <0.05 was considered statistically significant. Figures were created in GraphPad Prism.

Results

Patient demographics and ovarian reserve markers were similar between each of the three groups, and data includes all ages (combined data not shown—Tables 1 and 2 reflect SET

and DET data). There were no overall differences in hCG positivity between cycle types ($p = 0.27$) or in live birth rate ($p = 0.05$) (chi-square test). However, the number of embryos transferred in the PGS group (1.2) was significantly lower than in the IVF (2.0) or FET (1.7) groups (IVF and FET groups were also statistically different, ANOVA, Bonferroni correction, all $p < 0.001$). Implantation rates were higher after PGS compared to IVF (42 versus 28%, $p = 0.001$) and to FET (31%, $p = 0.008$) but were comparable between IVF and FET ($p = 0.88$). Three patients had ectopic after IVF, two after FET and one after PGS. See Fig. 1 for a flowchart on patient data.

Initial hCG value were examined between IVF, FET, and PGS cycle first focusing on patients undergoing SET. Among patients with a positive hCG, baseline characteristics and general cycle parameters were similar between all groups (Table 1). In patients achieving a positive hCG after SET, initial hCG values were higher after PGS compared to FET (182.4 versus 124.0 mIU/mL, $p = 0.02$) and PGS compared to IVF (182.4 versus 87.1 mIU/mL, $p < 0.001$) as well as FET compared to IVF (124.0 versus 87.1 mIU/mL, $p < 0.01$). The same relationships were seen with respect to patients with clinical pregnancy and live birth (Fig. 2). PGS was also found to be a significant independent predictor of higher initial hCG values when adjusting for cycle type (IVF, FET, PGS) (two-way ANOVA, $p < 0.001$). No patient undergoing eSET had a multiple pregnancy.

Next, initial hCG values were assessed among all three groups in patients with double embryo transfer (DET) and a positive hCG value. Baseline characteristics and general cycle parameters were similar between all groups except that peak estradiol levels were on average lower in patients undergoing IVF (Table 2). In patients achieving a positive hCG after DET, hCG values were higher after PGS (222.8 mIU/mL) compared to FET (182.1 mIU/mL, $p = 0.02$) and IVF (131.4 mIU/mL, $p = 0.001$). In patients who achieved clinical pregnancy, hCG values were higher from PGS (262.0 mIU/mL) compared to FET (237.4 mIU/mL, $p = 0.06$) and IVF (153.6 mIU/mL, $p = 0.001$) and were also higher from FET compared to IVF ($p = 0.002$). Finally, hCG values were higher in patients with a

Table 1 Demographic and cycle parameters of patients achieving positive hCG values by cycle type for patients undergoing SET

Characteristic	IVF ($n = 37$)	FET ($n = 47$)	PGS ($N = 79$)	p value
Maternal age, year	35.5 $\pm$ 4.9	34.7 $\pm$ 5.6	35.0 $\pm$ 5.5	0.80
Maximum FSH (mIU/L)	6.7 $\pm$ 1.9	6.8 $\pm$ 2.4	7.5 $\pm$ 2.2	0.13
BMI (kg/m ²)	28.8 $\pm$ 6.8	27.0 $\pm$ 7.2	27.9 $\pm$ 9.2	0.60
Endometrial thickness (mm)	11.3 $\pm$ 2.3	10.2 $\pm$ 2.0	10.6 $\pm$ 2.2	0.11
Peak estradiol (pg/mL) at fresh cycle	2370.5 $\pm$ 1002.7	2470.8 $\pm$ 1156.9	2415.1 $\pm$ 1094.5	0.92
Peak estradiol (pg/mL) at frozen cycle	n/a	409.0 $\pm$ 247.2	463.7 $\pm$ 225.3	0.55

Data represents mean $\pm$ SD. ANOVA was used for most patient demographics and cycle parameters and Kruskal-Wallis was used for estradiol values

n/a not applicable

Table 2 Demographic and cycle parameters of patients achieving positive hCG values by cycle type for patients undergoing DET

Characteristic	IVF (<i>n</i> = 164)	FET (<i>n</i> = 81)	PGS (<i>N</i> = 32)	<i>p</i> value
Maternal age, year	35.5 ± 5.0	35.7 ± 4.6	36.0 ± 5.0	0.83
Maximum FSH (mIU/L)	7.4 ± 2.6	7.5 ± 2.4	6.5 ± 1.8	0.12
BMI (kg/m ²)	27.3 ± 6.7	27.9 ± 6.8	28.0 ± 8.0	0.74
Endometrial thickness (mm)	11.0 ± 2.7	10.6 ± 2.2	11.5 ± 2.3	0.17
Peak estradiol (pg/mL) on fresh cycle	1992.0 ± 1142.2	2828.4 ± 1369.5	2654.6 ± 1348.2	<0.01*
Peak estradiol (pg/mL) on frozen cycle	n/a	444.5 ± 216.3	476.0 ± 203.2	0.55
Multiple pregnancy	31% (<i>n</i> = 51)	28% (<i>n</i> = 23)	34% (<i>n</i> = 11)	0.85

Data represents mean ± SD. ANOVA was used for most patient demographics and cycle parameters and Kruskal-Wallis was used for estradiol values.

n/a not applicable

* statistically significant with *p* < 0.05

live birth from PGS (257.2 mIU/mL) compared to IVF (165.1 mIU/mL, *p* = 0.001), comparable between PGS and FET (254.0 mIU/mL, *p* = 0.2), and higher after FET compared to IVF (*p* = 0.002) (Fig. 3). Multiple pregnancy rate was comparable between all groups (Table 2). In order to account for the influence of multiple pregnancy on initial hCG values after DET, hCG was analyzed by two-way ANOVA taking into

account patients with positive hCG, clinical pregnancy, and live birth. In all analyses, cycle type was still predictive of hCG values independent of the number of pregnancy sacs seen (all *p* < 0.001).

Finally, the relationship between hCG and pregnancy outcomes was measured. HCG directly increased with the number of pregnancy sacs independent of cycle type (two-way

Fig. 1 Flow sheet of patient cycles

Fig. 2 Initial hCG values are higher after PGS compared to FET and IVF in patients undergoing eSET. All hCG values are in milli-international units per milliliter. Asterisk denotes statistical significance with $p = 0.001$ in overall comparison by Kruskal-Wallis within each pregnancy

outcomes category. Post hoc Dunn's test shows that within each of the three pregnancy outcomes, hCG values are higher after PGS > FET, PGS > IVF, and FET > IVF, each with $p < 0.05$

ANOVA, $p < 0.001$) for hCG positivity, clinical pregnancy, and live birth. There was a significant relationship between higher hCG values and frozen transfers independent of PGS status (two-way ANOVA, $p = 0.003$). Age (coefficient -2.5 , $p = .04$) and BMI (coefficient -2.02 , $p < 0.01$) were associated with lower hCG values and PGS was associated with higher hCG values (coefficient 66.3 , $p < 0.001$) (multivariable linear regression adjusting for the number of embryos transferred). Finally, after adjusting for age, BMI and number of embryos transferred, higher hCG was significantly associated with live birth outcome (coefficient 94.2 , $p < 0.001$) and negatively associated with biochemical pregnancy (coefficient -112.7 , $p < 0.001$).

Discussion

Initial maternal serum hCG values were higher after PGS than IVF or FET cycles and values were higher in FET than in IVF cycles. This is the first study examining differences in initial hCG values across all different types of ART cycles including

the effects of PGS. All patients in our clinical practice, regardless of prognosis, were encouraged to freeze embryos, perform PGS, and utilize a subsequent FET. Those who chose to undergo fresh IVF or to cryopreserve without PGS did so mostly to avoid the cost of PGS. There were some patients whose embryo quality was poor, and therefore, patients chose not to cryopreserve any embryos and this is likely reflected in the lower peak estradiol values during IVF with DET (Table 2). Patients using PGS were encouraged to undergo eSET and most complied—therefore, this was the focus of the analysis. Patients who underwent DET after PGS did so because of lower embryo quality or patient preference.

As previously mentioned, large data sets show that children born from frozen transfers (compared to fresh) are healthier, and that placentation in pregnancy from frozen cycles is improved [18–22]. Multiple studies have linked low hCG values early in pregnancy to biochemical pregnancy and miscarriage [30, 32–35, 40, 41]. HCG levels reflect the health of the pregnancy. But here, we actually compare the initial hCG values between different ways of performing ART where prognosis was generally unrelated to the choice of cycle type. Authors

Fig. 3 Initial hCG values are higher after PGS compared to FET and IVF in patients undergoing DET. All hCG values are in milli-international units per milliliter. Asterisk denotes statistical significance with $p = 0.001$ in overall comparison by Kruskal-Wallis within each

pregnancy outcomes category. Post hoc Dunn's test shows that within each of the three pregnancy outcomes, hCG values are higher after PGS > FET, PGS > IVF, and FET > IVF, each with $p < 0.05$

have theorized that supraphysiological levels of estradiol present in fresh ART cycles harm the endometrial environment thereby harming placentation [42, 43] and even harm embryo development [44]. Even though clinical data support this, there has never been a useful biochemical measurement that differed between ART cycle types which correlated with these clinical outcomes. Previously studies have linked higher initial hCG values to different stages of embryo transfer and most have found higher hCG values after blastocyst rather than cleavage stage transfer [36]. Most embryo transfers now occur at the blastocyst stage which makes data analysis of mixed cleavage stage and blastocyst stage transfers difficult to compare in prior studies [40, 41]. These prior studies combined with the present study suggest that transferring a frozen embryo where estradiol levels are much lower than in the fresh IVF cycle leads to a higher initial hCG, improved placentation, and a healthier pregnancy. We believe this can be predicted by the initial hCG value.

The chromosome status of an embryo has never before been linked to initial hCG values. HCG values later in the first trimester comprise the basis for maternal screening that is the mainstay of first trimester aneuploidy screening [45–48]; however, this has never been applied to the initial hCG value. Even second trimester hCG values have been linked to IUGR, miscarriage, and preterm birth [49]. We believe that there is room to expand the utility of hCG values to the initial one seen in pregnancy after ART.

Initial hCG values may provide an opportunity to stratify patients into different fetal testing protocols. If larger data sets can provide cutoff values after each modality of ART, it may be possible to use the initial hCG value to determine whether one is at risk for IUGR, preeclampsia, abruption, or stillbirth. Then patients may immediately be advised as to the benefit of low dose aspirin, earlier fetal testing, or additional antenatal sonography later in pregnancy.

Our study is limited by its retrospective design. As the study focuses on patients who are already pregnant from their treatment, we believe this is less important. Our study, however, includes a significant number of patients undergoing SET. We believe that this model is the best one to study as it alleviates any potential contribution of hCG value from a second embryo which implanted and failed to achieve viability. It has been well documented that multiple pregnancies correlate with higher hCG values [33, 50]. Also, our study did not include perinatal data which could be further correlated to initial hCG values.

Conclusion

Our study suggests two novel findings. PGS has a significant effect on initial hCG values. Initial maternal serum hCG values are higher after FET relative to IVF. This suggests for

the first time that hCG values may relate to chromosomal status of the embryo. We believe it is important to examine antenatal records to ascertain the incidence of intrauterine growth restriction, oligohydramnios, preeclampsia, and abruption in these pregnancies and then ultimately examine childhood developmental. These data can then be correlated with initial hCG values. We believe an algorithm can be developed by which patients, obstetricians, and pediatricians can use low early hCG values and ART modality to stratify pregnant patients and children into high-risk categories for monitoring.

Compliance with ethical standards

Declarations This study was approved by an institutional review board at Staten Island University Hospital and North Shore-LIJ Health System. No consent for participation was required. We hereby give full consent for publication. The authors declare that they have no conflict of interest. No funding was used. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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