Trophectoderm Biopsy of Blastocysts for a Preimplantation Genetic Test does not Affect Serum β -hCG Levels in Early Pregnancy: A Study using Propensity Score Matching

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Research

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Abstract

Background: Although preimplantation genetic test (PGT) has been used worldwide, few studies investigated the effect of trophectoderm biopsy of the blastocysts on early embryo development. This study aimed to investigate whether trophectoderm (TE) biopsy of blastocysts for a PGT affected serum β -human chorionic gonadotropin (hCG) levels 14 days after transfer.

Methods: This was a retrospective cohort study conducted at the Third Affiliated Hospital of Guangzhou Medical University. The study population comprised pregnant women who underwent the transfer of single vitrified-warmed blastocysts after PGT between January 1, 2018, and July 30, 2020. The control group had non-PGT cycles with other inclusion criteria identical to those for the study group. Propensity score matching was used to screen a group of patients so that the baseline characteristics were similar between the two groups. Serum β -hCG levels were compared between the PGT and non-PGT cycles. Multiple linear regression was applied to analyze the influence of PGT on serum β -hCG levels, while receiver operating characteristic curves (ROC curves) were plotted to predict pregnancy outcomes using serum β -hCG levels.

Results: Serum β -hCG levels were comparable between the PGT and non-PGT patients: live birth: 2478 ± 1675 mIU/mL vs 2303 ± 1300 mIU/mL ($P = 0.361$); clinical pregnancy: 2244 ± 1537 mIU/mL vs 2270 ± 1387 mIU/mL ($P = 0.634$); and ongoing pregnancy: 2391 ± 1600 mIU/mL vs 2360 ± 1372 mIU/mL ($P = 0.852$) respectively. Multiple linear regression analysis indicated no impact of PGT on the serum β -hCG level (standardized coefficients = -0.016 , $P = 0.756$). For clinical pregnancy, the cutoff value was 482 mIU/mL and 277 mIU/mL for PGT and non-PGT patients, respectively. The threshold to predict live birth was 1345 mIU/mL and 1113 mIU/mL in the PGT and non-PGT cycles, respectively.

Conclusion: Trophectoderm biopsy of blastocysts for PGT did not affect the serum β -hCG level 14 days after transfer.

Background

Preimplantation genetic test (PGT) has been used worldwide, with the main indications of monogenetic diseases, chromosomal abnormalities, recurrent pregnancy loss, and recurrent implantation failure. Removing 5–10 trophectoderm (TE) cells in the blastocyst stage is one of the procedures of PGT. However, few studies investigated the effect of TE cell reduction on early embryo development. Human chorionic gonadotropin (hCG), which represents the function of TE, is secreted by syncytiotrophoblast cells of the TE from the time of implantation.

A previous study on blastocysts cultured *in vitro* indicated that the mean cumulative hCG was inversely proportional to the number of removed TE cells. However, removing fewer than 10 TE cells did not significantly affect the secreted hCG levels[1]. Two other studies investigated the influence of PGT on the development of embryos in the early stage *in vivo*. Cho et al. explored the influence of blastomere biopsy on serum β -hCG levels of early pregnancy. They found that the mean serum β -hCG level was lower in PGT

cycles than in intracytoplasmic sperm injection cycles for the fresh embryo transfer (ET). However, doubling times were comparable between the two groups during the time of post-ovulation day (POD) 12 and 21[2]. Hobeika et al. found that the serum β -hCG level 9 days after blastocyst transfer was higher in PGT cycles than in non-PGT cycles[3]. In the study by Cho, the biopsy of blastomeres was performed in the cleavage stage, which was quite different from the current procedures in PGT. In addition, fresh embryos were not transferred on the same day after oocyte retrieval, which affected serum β -hCG levels[4]. In the study by Hobeika, one to two embryos were transferred, resulting in vanishing twin syndrome. Although the analysis was performed according to the number of embryos transferred, the sample size was relatively small, resulting in a bias. Therefore, the present study was conducted to investigate whether the TE biopsy of blastocysts for PGT affected serum β -hCG levels 14 days after transfer.

Methods

Population

This retrospective cohort study included PGT cycles with ET in the Department of Reproductive Medicine of the Third Affiliated Hospital, Guangzhou Medical University (Guangzhou, China) between January 1, 2018, and April 30, 2020. The control group involved the non-PGT cycles with other inclusion criteria identical to those for the study group. This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. The inclusion criteria were as follows: (1) serum β -hCG levels ≥ 25 mIU/mL 14 days after transfer; and (2) single vitrified-warmed blastocyst transfer (Fig. 1). Cycles with donor sperms or oocytes were excluded from the study (Fig. 1).

Ovarian stimulation and oocyte retrieval

Controlled ovarian stimulation was performed using either long protocol or antagonist protocol. On days 2–4 of the cycle, 150–300 IU follicle-stimulating hormone (Gonal-F, Merck Serono, Italy) was used for ovarian stimulation. Oocyte maturation was triggered with 250 μ g recombinant human chorionic gonadotropin (hCG) (Ovidrel, Merck Serono, Italy), and oocyte retrieval was performed 36 h later.

Embryo culture and blastocyst biopsy

All the embryos were cultured to the blastocyte stage (5–6 days after oocyte retrieval), when the TE biopsy was performed on good- and fair-quality embryos. Good-quality embryos were defined as blastocysts of 3–6 AA/AB/BA/BB using the Gardner scoring system. Fair-quality embryos included blastocysts of 4–6 BC/CB [5]. The perforation of zona pellucida was induced with three to five laser pulses (Satum Active Laser System, RI, England). Five to 10 TE cells were aspirated with the biopsy pipet. After washing, the TE biopsy samples were placed in the polymerase chain reaction (PCR) tube with 2 μ L

of PBS, centrifuged immediately, and stored in a refrigerator at -80°C for further processing. The biopsied blastocysts were vitrified using a kit (Kitazato Biopharma Co., Ltd., Shizuoka, Japan).

Whole gene amplification and PGT

Whole gene amplification (WGA) of the biopsied samples and parental reference DNA was performed with amplification equipment (GeneQ, Hangzhou Bioer Technology, China) using a kit (SurePLEX WGA, Basecare, China). Next-generation sequencing and single nucleotide polymorphism (SNP) microarray were used for genome testing, with SNP microarray mainly applied for monogenetic diseases. Data were processed using the Genome Studio program (iScan, Illumina, America).

Frozen-thawed blastocyst transfer

Three protocols were used for endometrial preparation: natural cycle, artificial cycle, and ovarian stimulation cycle. Vaginal progesterone (Crinone, Merck Serono, England), 90 mg once a day, was applied for luteal-phase support. The serum β -hCG test was performed 14 days after ET, and luteal-phase support was continued to the tenth week in the case of intrauterine pregnancy.

Hormonal assays

The immunochemiluminometric assay was performed for testing β -hCG (Architech i2000SR; Abbott Laboratories Inc., IL, USA). The range of detection was between 1.2 and 225,000 mIU/mL. The sensitivity of the assay was 1.2 mIU/mL, and the intraassay coefficient of variation was 7%.

Definitions of pregnancy outcomes

Clinical pregnancy was defined as an intrauterine/extrauterine gestational sac detected by ultrasound with positive serum β -hCG. Biochemical pregnancy loss was defined as serum β -HCG level > 25 mIU/mL 14 days after transferring the embryo, which declined to < 5 mIU/mL at the end without any visible gestational sac on ultrasound. Early miscarriage was defined as fetal growth arrest or no cardiac activity detected in the gestational sac during the first 12 weeks of pregnancy. Ongoing pregnancy was defined as the pregnancy continued beyond 12 weeks with a live fetus. Live birth indicated pregnancy continued after 28 weeks of gestation with a live fetus.

Statistical analysis

Since this was a retrospective study, the baseline characteristics were different between the PGT and non-PGT groups. Therefore, propensity score matching (PSM) was used to screen a group of patients so that

the baseline characteristics were similar between the two groups. The multiple logistic regression model was used to calculate the propensity score, with PGT as the dependent variable and the variables in Table 1 as independent variables (female age, male age, duration of infertility, number of previous gestations and transfers, AMH level, BMI, endometrial thickness 5 days before transfer, and days of ET). The PSM was performed with 1:2 matching by the nearest neighbor matching, with the caliper width equal to 0.2 of the standard deviation (SD) of the logit of the propensity score (PS). SD was calculated for baseline variables before and after PSM; an absolute value lesser than 0.1 indicated a negligible imbalance.

Statistical analysis was performed using SPSS 22.0 software (IBM, NY, USA). Quantitative variables with homogenous variance were expressed as $\bar{X} \pm SD$, and the means were compared using the Student's *t* test. A chi-squared test was used to compare rates. Multiple linear regression was applied to analyze the influence of PGT on serum β -hCG levels, while receiver operating characteristic curves (ROC curves) were plotted to predict pregnancy outcomes using serum β -hCG levels. A *P* value <0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 143 PGT patients were included in the study, with 2175 non-PGT patients used as control. After the 1:2 PS matching, only 138 PGT patients and 265 non-PGT patients were included in the study. Most of the baseline characteristics were significantly different between the two groups before matching (Table 1). However, most of the aforementioned covariates were balanced between the two groups after matching, except the endometrial thickness and the days of ET (Table 1).

Table 1
Characteristics of patients before and after PS matching

Characteristics	Before matching			After matching		
	PGT	Non-PGT	Standardized	PGT	Non-PGT	Standardized
	N = 143	N = 2175	Difference	N = 138	N = 265	Difference
Female age (years)	30.5 ± 4.5	31.0 ± 4.1	-0.101	30.4 ± 4.4	30.5 ± 4.2	-0.02
Male age (years)	32.5 ± 4.4	33.3 ± 4.9	-0.177	32.3 ± 4.4	32.4 ± 4.8	-0.011
Infertility duration (years)	2.9 ± 2.8	4.5 ± 2.9	-0.556	3.0 ± 2.8	3.1 ± 2.4	-0.049
No. of previous gestation	1.76 ± 1.8	0.83 ± 1.1	0.527	1.60 ± 1.5	1.68 ± 1.4	-0.01
No. of previous transfer	1.43 ± 1.28	1.77 ± 1.04	-0.268	1.43 ± 1.29	1.49 ± 0.71	-0.04
AMH (ng/ml)	5.36 ± 3.96	6.60 ± 4.54	-0.313	5.32 ± 3.99	5.07 ± 4.15	0.034
BMI (kg/m ²)	22.38 ± 3.08	22.12 ± 4.25	0.085	22.02 ± 3.07	22.57 ± 3.55	0.09
EMT ^a (mm)	8.7 ± 1.5	8.9 ± 1.5	-0.122	8.5 ± 1.5	8.6 ± 1.5	0.125
Days of embryo transfer	5.29 ± 0.46	5.16 ± 0.37	0.284	5.36 ± 0.45	5.34 ± 0.48	-0.174
a EMT = endometrium thickness 5 days before transfer.						

Table 2
Pregnancy outcomes of patients after PGT vs. non-PGT after PS matching

Pregnancy outcomes %(n)	PGT	Non-PGT	<i>P</i>
Total ongoing pregnancy ^a	79.7(110)	78.9(209)	0.843
Live birth ^b	56.5(78)	71.3(189)	
Ongoing pregnancy (following up) ^c	20.3(28)	4.5(12)	
Late miscarriage	2.9(4)	2.6(7)	
Fetal death	0(0)	0.4(1)	
Early miscarriage	12.3(17)	17.0(45)	0.218
Biochemical pregnancy loss	8.0(11)	3.0(8)	0.026
Ectopic pregnancy	0(0)	1.1(3)	0.554 ^d
Total	100.0(138)	100.0(265)	
Note: a.Total ongoing pregnancy = live birth + ongoing pregnancy (following up) + late miscarriage + fetal death. b.Live birth only included patients transferred between Jan. 2018 and Dec. 2019. c. Ongoing pregnancy (following up) indicated gestations beyond 12 weeks but follow-up of live birth hadn't been finished. d. Fisher Exact Test was used.			

Pregnancy outcomes

The rates of ongoing pregnancy in the PGT group were not significantly different from

those in the non-PGT group (79.7% vs 78.9%, $P = 0.843$). The rates of early miscarriage (12.3% vs 17.0%) and ectopic pregnancy (0% vs 1.1%) were also comparable between the two groups ($P > 0.05$). However, the rate of biochemical pregnancy loss was significantly higher in the PGT group than in the non-PGT group (8.0% vs 3.0%, $P = 0.026$) (Table 2).

Primary outcomes

The serum β -hCG level of live birth in the PGT group was 2478 ± 1675 mIU/mL, which was not significantly different from that in the non-PGT group (2303 ± 1300 mIU/mL, $P = 0.361$). Serum β -hCG levels from cycles resulting in clinical pregnancy and ongoing pregnancy were comparable between the two groups: 2244 ± 1537 mIU/mL vs 2270 ± 1387 mIU/mL ($P = 0.634$) and 2391 ± 1600 mIU/mL vs 2360 ± 1372 mIU/mL ($P = 0.852$), respectively. The serum β -hCG level of early miscarriage in the PGT group

was 1289 ± 950 mIU/ml, which was not significantly different from that in the non-PGT group (1420 ± 1132 mIU/mL, $P = 0.546$) (Table 3).

Effect of PGT on serum β -hCG levels

Multiple linear regression analysis indicated that PGT was not related to the serum β -hCG level (Standardized coefficients = -0.016 , $P = 0.756$). Variables that affected the serum β -hCG level were BMI and days of ET ($P = 0.009$ and $P = 0.001$, respectively) (Table 4).

Table 3
Comparison of the serum β -hCG level between patients of PGT vs. non-PGT after PS matching

		PGT	Non-PGT	<i>P</i>
All pregnant patients	hCG	2096 ± 1569	2124 ± 1400	0.854
	n	138	265	
Live birth	hCG	2478 ± 1675	2303 ± 1300	0.361
	n	78	189	
Clinical pregnancy	hCG	2244 ± 1537	2270 ± 1387	0.634
	n	127	257	
Ongoing pregnancy	hCG	2391 ± 1600	2360 ± 1372	0.852
	n	110	209	
Early miscarriage	hCG	1289 ± 950	1420 ± 1132	0.546
	n	17	45	
Biochemical pregnancy loss	hCG	388 ± 685	671 ± 1008	0.475
	n	11	8	
Data of live birth only included those from Jan. 2018 to Jun. 2019.				

Prediction of pregnancy outcomes

The ROC curve analysis showed that for clinical pregnancy, the cutoff value was 482 mIU/mL for PGT patients, with the sensitivity of 94.5%, specificity of 90.0% positive predictive value (PPV) of 99.2%, and area under the ROC curve (AUC) of 0.927. The threshold for clinical pregnancy in non-PGT cycles was 277 mIU/mL, with the sensitivity of 96.5%, specificity of 75%, PPV of 99.2%, and AUC of 0.838 (Fig. 2). For ongoing pregnancy, the threshold was 1328 mIU/mL in PGT cycles with the AUC of 0.822 and 1113 mIU/mL in non-PGT cycles with the AUC of 0.758 (Fig. 3). For live

birth, the threshold was 1345 mIU/mL in PGT cycles with the sensitivity of 78.8%, specificity of 73.9%, and PPV of 91.3%, and 1113 mIU/mL in non-PGT cycles with the sensitivity of 85.2%, specificity of 52.5%, and PPV of 54.9% ((Fig. 4, Table 5).

Table 4
Influence of PGT on the serum β -hCG level by multiple linear regression

	Unstandardized coefficients	Standardized coefficients	t	P
BMI	-55.928	-0.130	-2.617	0.009
Endometrium thickness	26.717	0.027	0.544	0.587
Days of embryo transfer	-530.442	-0.171	-3.288	0.001
PGT vs. non-PGT ^a	-48.105	-0.016	-0.318	0.751
a. adjusted for female age, no. of embryo transfer, duration of infertility, AMH, BMI, endometrium thickness and days of embryo transfer.				

Table 5
Prediction of pregnancy outcomes by serum β -hCG level between patients after PGT and non-PGT

	Clinical Pregnancy		Ongoing Pregnancy		Live Birth	
Parameters	PGT	non-PGT	PGT	non-PGT	PGT	non-PGT
β -hCG level (mIU/ml)	482	277	1328	1113	1345	1113
AUC	0.927	0.838	0.822	0.758	0.759	0.748
95% CI	0.818-1.000	0.653-1.000	0.727-0.918	0.681-0.835	0.687-0.903	0.675-0.822
Sensitivity	94.5%	96.5%	78.2%	85.6%	78.8%	85.6%
Specificity	90.9%	75.0%	78.6%	57.1%	73.9%	52.5%
PPV	99.2%	99.2%	93.5%	88.1%	91.3%	54.9%
NPV	58.8%	40.0%	47.8%	50.0%	50.0%	85.5%
P	3.00E-06	0.001	1.46E-07	2.90E-09	4.50E-13	5.74E-09
PPV = positive predictive value; NPV = negative predictive value;						

Discussion

The present study indicated that the trophectoderm biopsy of blastocysts for PGT did not affect the serum β -hCG level 14 days after transfer. This was confirmed by Dokra et al., who investigated the

influence of TE biopsy on the development of blastocysts cultured *in vitro*. Their study showed that the removal of < 10 TE cells did not significantly decrease the amount of cumulative β -hCG secretion from day 3 to day 14 (87.6 ± 24.8 mIU/mL vs 146.2 ± 23.7 mIU/mL, $P > 0.05$). In the present study, only 5–10 TE cells were removed from the blastocysts for PGT. Therefore, the TE biopsy with < 10 cells had no significant influence on hCG secretion by embryos in the early stage.

A previous study by Cho et al. demonstrated that the removal of one to two blastomeres from embryos in the cleavage stage significantly decreased serum β -hCG levels in PGT patients on days 12, 14, and 21 after ovulation. However, the doubling time of serum β -hCG levels was comparable to that in the control group[2]. Lu et al. demonstrated that the trophectoderm biopsy decreased the serum hCG levels 12 days after blastocyst transfer (635.7 mIU/mL vs 720.2 mIU/mL, $P = 0.005$). However, in their study, baseline characteristics, such as the endometrium thickness on the transfer day, embryo quality, and endometrial preparation protocols, which might affect the serum hCG levels, were different between the biopsy and control groups. In contrast, Hobeika et al. found that the initial β -hCG level 9 days after transfer was significantly higher in PGT cycles compared with those in the frozen ET (FET) and fresh cycles (182.4 mIU/mL vs 124.0 mIU/mL vs 87.1 mIU/mL, $P < 0.05$) [3].

The present study demonstrated that the mean serum β -hCG level 14 days after transfer in PGT cycles was not significantly different from that in non-PGT cycles (2096 ± 1569 mIU/mL vs 2124 ± 1400 mIU/mL, $P = 0.854$). For each type of pregnancy outcomes (biochemical pregnancy loss, early miscarriage, clinical pregnancy, and live birth), the β -hCG levels between the PGT and non-PGT cycles were similar. The multiple linear regression analysis further confirmed that PGT did not affect serum β -hCG levels (Standardized coefficients = -0.016 , $P = 0.756$). The factors that affected the serum levels of β -hCG were BMI and days of ET (day 5 vs day 6). BMI was negatively associated with the serum β -hCG level, which was also confirmed by the previous study[6]. The day 6 blastocysts had a lower β -hCG level for both PGT and non-PGT groups compared with day 5 blastocysts (PGT group: 1730 ± 1358 mIU/mL vs 2240 ± 1628 mIU/mL, $P = 0.085$; non-PGT group: 1790 ± 1346 mIU/mL vs 2302 ± 1399 mIU/mL, $P = 0.004$). This finding further indicated that the day 6 blastocysts were less potent than the day 5 blastocysts and thus grew slowly[7]. Previous studies did not adjust for the covariates that might have an impact on serum β -hCG levels. Therefore, the conclusion that PGT influenced serum β -hCG levels was not reliable. The present study proved that PGT did not influence serum β -hCG levels 14 days after single blastocyst transfer, following propensity score matching and further adjustment for BMI and days of ET.

Previous studies indicated a significantly higher ongoing pregnancy rate and a lower miscarriage rate in PGT patients[8–10]. The present study showed that the rates of ongoing pregnancy and early miscarriage were not significantly different in PGT and non-PGT patients once pregnancy was achieved (serum β -hCG ≥ 25 mIU/mL). The explanation for this finding was that non-PGT patients in the present study were young (mean age: 31 years) and mainly transferred D5 blastocysts (65.3%), resulting in a higher pregnancy rate and a lower miscarriage rate.

The cutoff value for clinical pregnancy 14 days after transfer was 482 mIU/mL in PGT cycles, which was higher than that in non-PGT cycles (277 mIU/mL). This might be explained by the higher rate of biochemical pregnancy loss in the PGT group (8.0% vs 3.0, $P = 0.026$), which may result from the perforation of zona pellucida during blastocyst biopsy. The thresholds predicting ongoing pregnancy and live birth were also higher in the PGT group than in the non-PGT group: 1328 mIU/mL vs 1113 mIU/mL and 1345 mIU/mL vs 1113 mIU/mL, respectively. However, the conclusions of the study by Lu et al. were contrary to the findings of the present study. They illustrated that PGT patients had a lower threshold of serum β -hCG levels for live birth compared with the control group (368.55 mIU/mL vs 411.45 mIU/mL) 12 days after transfer[11].

The present study had the following advantages. First, PSM was used to screen a group of patients so that the baseline characteristics were similar between the two groups, thus greatly decreasing the bias that affected serum β -hCG levels. Second, only single blastocyst transfer was included in the study, avoiding the vanishing twin syndrome[12]. Third, all the patients had the serum β -hCG test on exactly the same day after ET in a single laboratory, minimizing the bias from the blood test. However, the study had the following disadvantages. The sample size in the PGT group was relatively small, which may limit the analysis of the effect of PGT on early embryo development. Second, the serum β -hCG test was performed on the 14th day after transfer, which might not be applicable to analyze the effect of PGT on embryo development earlier than this stage[13].

Conclusion

Trophectoderm biopsy of blastocysts for PGT did not affect serum β -hCG levels in early pregnancy.

Abbreviations

PGT: preimplantation genetic test; PSM: propensity score matching; ROC: Receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval; AMH: anti-müllerian hormone. TE: trophectoderm; hCG: human chorionic gonadotropin; ET: embryo transfer; PCR: polymerase chain reaction; WGA: whole gene amplification; SNP: single nucleotide polymorphism; SD: standard deviation; PPV: positive predictive value; NPV: negative predictive value.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. Each patient has signed informed consent on obtaining and analyzing their clinical data prior to the initiation of IVF/ICSI-ET treatment.

Consent for publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: Yixuan Wu, Jianqiao Liu; Methodology: Yixuan Wu; Formal analysis and investigation: Ying Ying; Writing - original draft preparation: Yixuan Wu; Writing - review and editing: Haiying Liu; Funding acquisition: Haiying Liu, Ying Ying; Resources: Yixuan Wu; Supervision: Yixuan Wu.

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Figures

Figure 1

Process of data screening for analysis.

Figure 2

Prediction of clinical pregnancy by serum β -hCG 14 days after blastocyst transfer: a. PGT group; b. non-PGT group.

Figure 3

Prediction of ongoing pregnancy by serum β -hCG 14 days after blastocyst transfer: a. PGT group; b. non-PGT group.

Figure 4

Prediction of live birth by serum β -hCG 14 days after blastocyst transfer: a. PGT group; b. non-PGT group.