hCG 14 Days After Blastocyst Transfer

Yixuan Wu[1234], Haiying Liu[1234]

Correspondence author: Haiying Liu, email: liuhaiying0606@163.com;

[1]Department of Reproductive Medicine, the Third Affiliated Hospital of Guangzhou Medical University, No. 63, Duobao Road, Guangzhou, Guangdong, China

Full list of author information is available at the end of the article

Abstract

Background: Although many previous study investigated the prediction of pregnancy outcomes by serum β-hCG levels after blastocyst transfer, no study focused on the pregnancy outcomes of patients with initially low serum β-hCG levels. The purpose of the study was to investigate the pregnancy outcomes of patients with low serum β- level 14 days after blastocyst transfer.

Methods: A retrospective study was performed in the Third Affiliated Hospital of Guangzhou Medical University. The purpose was to investigate the patients whose serum β-hCG levels were between 5-299 mIU/ml 14 days after frozen blastocyst transfer. Rates of live birth, early miscarriage, biochemical pregnancy loss and ectopic pregnancy were analyzed according to the female patients' age by chi-squared test. Receiver operating characteristic (ROC) curves were plotted to explore the threshold for prediction of clinical pregnancy and live birth.

Results: A total of 312 patients had serum β-hCG levels <300 mIU/ml

14 days after frozen blastocyst transfer, among which 18.6% were live birth, 47.4% were early miscarriage, 22.8% were biochemical pregnancy and 9.6% were ectopic pregnancy. Pregnancy outcomes were comparable between the patients aged <38 years and ⩾38 years. ROC curve analysis showed that the predicted value of β-hCG for clinical pregnancy was 58.8 mIU/ml with the AUC of 0.752(95%CI :0.680-0.823), sensitivity of 95.0% and specificity of 53.5%.The threshold for live birth was 108.6mIU/ml with the area under the ROC curve (AUC) of 0.649(95%CI:0.0.583-0.715), sensitivity of 93.1% and specificity of 37.0%.For the β-hCG fold increase over 48 hours, the cut-off for clinical pregnancy was 1.4 with the AUC of 0.899(95%CI :0.801-0.996), sensitivity of 90.3% and specificity of 77.8%; the threshold for live birth was 1.9 with the AUC of 0.808(95%CI :0.724-0.891), sensitivity of 88.5% and specificity of 64.5%.

Conclusions: Initially low serum β-hCG level 14 days after frozen blastocyst transfer indicated minimal likelihood of live birth. For patients having initial β-hCG >58.8 mIU/ml, luteal phase support is suggested to continue. Another serum β-hCG test and ultrasound should be performed one week later. If the initial serum β-hCG is < 58.8 mIU/ml, luteal phase support is suggested to discontinue and measurement of serum β-hCG and ultrasound can be arranged one week later.

Keywords: assisted reproductive technology; human chorionic gonadotropin; pregnancy; live birth; blastocyst

Introduction

Human chorionic gonadotropin (hCG) is secreted by syncytiotrophoblast cells from the time of implantation. Since it can be detected in maternal serum as soon as 6-8 days after fertilization, β-hCG is widely used in clinic as a marker of pregnancy. In normal conception, β-hCG levels are doubled every 48 hours, and thus the this increase pattern is applied to discriminate the normal pregnancy from the pathological pregnancy[1].

It is routine to have serum β-hCG tests 9-14 days after embryo transfer to confirm the diagnosis of pregnancy. Many previous studies have investigated the relationship between serum β-hCG levels and pregnancy outcomes. In fresh embryo transfer cycles, the thresholds of serum β-hCG levels to predict clinical pregnancy and live birth were 111-213 IU/L and 160-222.8 IU/L respectively 10-12 days after transfer[2-7]. For the frozen embryo transfer, the cut-off value was 137-399 IU/L for clinical pregnancy and 212-411 IU/L for live birth 11-14 days after embryo transfer[6-8]. Higher β-hCG levels are

indicative of better pregnancy outcomes such as higher rates of clinical pregnancy and live birth [9].

Although many previous study investigated the prediction of pregnancy outcomes by serum β-hCG levels after blastocyst transfer, no study focused on the pregnancy outcomes of patients with initially low serum β-hCG levels. These patients are often in a state of anxiety, worrying about the poor prognosis. In order to follow the pregnancy outcomes, more serum β-hCG tests are required for them, but no appropriate follow-up plans are suggested according to scientific research.

The purpose of the present study is to investigate the pregnancy outcomes of patients with initially low serum β-hCG levels 14 days after frozen blastocyst transfer. Prediction of pregnancy outcomes is performed so as to develop appropriate follow-up suggestions.

Materials and methods

Population

This retrospective study included patients who had frozen blastocyst transfer in the Department of Reproductive Medicine of the Third Affiliated Hospital, Guangzhou Medical University (Guangzhou, China), between January 2014 and October 2019. Patients with serum β-hCG levels between 5 -299 mIU/ml 14 days after transfer were included in the study. This study was approved by the Ethics Committee of the Third

Affiliated Hospital of Guangzhou Medical University.

Assisted Reproductive technology (ART) Techniques and Treatment Protocols

Vitrification and thawing kits (Kitazato Biophama Co.Ltd.Shizuoka,Japan)were applied for blastocyst cryopreservation and thawing. For vitrification, the blastocysts were equilibrated in the Equilibration Solution for 2min and then transferred to the Vitrification Solution, where the embryos would stay for at 45-60s at 37° C. Then the blastocysts were placed into the Cryptop and put into the liquid nitrogen immediately. For thawing of the blastocysts, the top of Cryotop containing the embryos were placed in the Thawing Solution for 1min at 37℃. Then the embryos were transferred sequentially to the Diluent Solution, Washing Solution 1, Washing Solution 2 where they stayed for 3min, 3min and 3min respectively at room temperature.

Endometrium Preparation and Embryo Transfer

Totally there are three protocols for endometrium preparation: natural cycle, artificial cycle and ovarian stimulation cycle. For patients with regular menstruation cycle, natural cycle was the first choice and blastocysts were transferred 5 days after ovulation. For patients without follicular development, artificial cycle was applied. Oral estrogen (Estradiol Valerate, Bayer, Germany) 3mg twice a day was

started from 2-4 days of the cycle and continued for at least 7 days. When the endometrium thickness was ⩾7mm, vaginal progesterone (Crinone, Merck Serono, Germany) 90mg once a day was administered for 5 days and blastocysts were transferred on the 6^{th} day. For stimulation cycle, 37.5-75 U of human menopausal gonadotropin (HMG) was administered from 2-4 days of the cycle. When the dominant follicle was ⩾18mm, 8000-10000 IU human chorionic gonadotropin (HCG) was injected to induce ovulation. Blastocyst transfer was performed 5 days after ovulation. Vaginal progesterone (Crinone, Merck Serono, Germany) 90mg once a day was applied for luteal phase support.Serum β-hCG test was performed 14 days after embryo transfer and luteal phase support was continued to the 10^{th} week if the β-hCG test was positive.

Hormone Measurement

Immunochemiluminometric assay was undertaken for testing of β-hCG (Architech i2000SR; Abbott Laboratories Inc., Chicago, 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 intra-assay coefficient of variation was 7%. Our laboratory is annually checked for qualification by the External Quality Assessment of Clinical Laboratory Center (Ministry of Health of the People's Republic of China, Beijing, China).

Fold increase of β-hCG concentrations over 48 hours were calculated by the formula: fold increase=$\left(\frac{HCG1}{HCG0}\right)^{2/days}$. HCG0 was serum β-hCG

concentrations 14 days after embryo transfer; HCG1 was β-hCG levels in the second test and days represented the interval between the two β-hCG tests.

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 > 5mIU/ml 14 days after transferring the embryo, and declined to < 5 mIU/ml at the end without visible gestational sac by 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. Live birth indicated pregnancy continued after 28 weeks of gestation with live fetus.

Statistics

Statistical analysis was performed by using SPSS 22.0 software (IBM, Armonk, NY, USA). Quantitative variables with homogenous variance were expressed as $\bar{X} \pm SD$ and the means were compared by student's t-test. Quantitative variables with heterogeneous variance were expressed as median (1^{st} quartiles,3^{rd} quartiles) and the medians were compared by Mann-Whitney U test. A chi-squared test was used to compare rates. Serum β-hCG levels and fold change of β-hCG levels over 48 hours were applied to predict clinical pregnancy as well as live birth by

plotting Receiver operating characteristic (ROC) curve. *P*-value < 0.05 was considered statistically significant.

Results

Pregnancy outcomes of patients with low serum β-hCG

A total of 312 patients had serum β-hCG levels <300 mIU/ml 14 days after blastocyst transfer, among which 18.6% were live birth, 47.4% were early miscarriage, 22.8% were biochemical pregnancy and 9.6% were ectopic pregnancy. Pregnancy outcomes were comparable between the patients aged <38 years and ≥38 years(Table 1). Among the 241 clinical pregnancies, 225(93.4%) were singletons and 16(6.6%) were twins(9 monozygotic twins and 7 dizygotic twins).The rate of live birth was 24.9% (56/225) in singletons and 12.5% (2/16) in twins. The lower limits of serum β-hCG level were 64.9 mIU/ml for singleton live birth, 145.1 mIU/ml for twin live birth,15.3 mIU/ml for early miscarriage and 5.3mIU/ml for ectopic pregnancy. Totally, 164 patients had another serum β-hCG test 2-24 days (mean:6.75 days) after the initial measurement, among which 133 had increased β-hCG levels and 31 had decreased values. For patients with decreased β-hCG level, 96.8% (30/31) were biochemical pregnancy loss. The only patient, although presenting with declined β-hCG from 133.4 mIU/ml to 64.5 mIU/ml, eventually developed into an ectopic pregnancy.

Table 1 Pregnancy outcomes of patients with serum β-hCG level < 300 mIU/ml 14 days after blastocyst transfer

Pregnancy outcomes	<38 years % (n)	>38 years %(n)	Total %(n)	*P*
Biochemical pregnancy loss	22.5 (58)	24.1 (13)	22.8(71)	0.800
Live birth	19.0 (49)	16.6 (9)	18.6(58)	0.045
Ectopic pregnancy	10.1 (26)	7.4 (4)	9.6(30)	0.771
Early Miscarriage	46.9 (121)	50.0 (27)	47.4(148)	0.690
Late miscarriage	1.5(4)	1.9 (1)	1.6 (5)	1.000
Total	100.0(258)	100.0 (54)	100.0 (312)	

Pregnancy outcomes of patients with different β-hCG intervals

For patients with β-hCG level of 5-50 mIU/ml, no live birth occurred. Most of them were biochemical pregnancy (77.8%) and the rest were early miscarriage (13.9%) and ectopic pregnancy (8.3%). Among patients with β-hCG level of 51-100mIU/ml, 55.8% were early miscarriage, 25.0% were biochemical pregnancy and only 7.7% were live birth. For patients with β-hCG levels of 101-200 mIU/ml and 201-299 mIU/ml, the likelihood of live birth was about 1/4 (23.7% and 24.5%, respectively) and the probability of early miscarriage was about 1/2(50% and 51.9% respectively) (Table 2).

Characteristics of live birth vs. non-live birth

The baseline characteristics, including female age, male age, number of previous pregnancies and previous transfers, anti-müllerian hormone(AMH), and BMI) were comparable between the two groups. There

were no statistical differences between the two groups in the number of embryos transferred, protocols of endometrium preparation, days of embryo transfer and endometrial thickness. However, the serum β-hCG levels of patients with live birth (median:196 mIU/ml) was significantly higher than that of patients with non-live birth (median: 140 mIU/ml, P=0.000) (Table 3).

Table 2 Pregnancy outcomes of patients with different β-hCG levels 14 days after blastocyst transfer

HCG level mIU/ml	5-50	51-100	101-200	201-299
Biochemical pregnancy loss	77.8(28/36)	25.0(13/52)	13.6(16/118)	13.2(14/106)
Live birth	0.0(0/36)	7.7(4/52)	23.7(28/118)	24.5(26/106)
Early miscarriage	13.9(5/36)	55.8(29/52)	50.0（59/118）	51.9（55/106）
Ectopic pregnancy	8.3（3/36）	9.6（5/52）	11.0（13/118）	8.5（9/106）
Late miscarriage	0.0(0/36)	1.9（1/52）	1.7（2/118）	1.9（2/106）

Prediction of pregnancy outcomes

ROC analysis showed that the predicted value for clinical pregnancy was 58.8 mIU/ml with the AUC of 0.752(95%CI :0.680-0.823), sensitivity of 95.0%, specificity of 53.5%. The threshold for live birth was 108.6mIU/ml with the AUC of 0.649(95%CI:0.0.583-0.715), sensitivity of 93.1%, specificity of 37.0% (Fig.1, Table 4). For the β-hCG fold increase over 48 hours, the cut-off for clinical pregnancy was 1.4 with the AUC of 0.899(95%CI :0.801-0.996), sensitivity of 90.3%,

specificity of 77.8%; the threshold for live birth was 1.9 with the AUC of 0.808(95%CI :0.724-0.891), sensitivity of 88.5%, specificity of 64.5% (Fig.2, Table 4).

Table 3 Characteristics of live birth vs. non-live birth

Characteristics	Live birth	Non-live birth	*P*
N	58	254	
Female age (years)	31.9±4.7	32.5±4.9	NS
Male age (years)	34.8±5.1	34.7±5.1	NS
Infertility duration	4.6±2.7	4.8±3.5	NS
No. of previous			NS
% (n)			
0	48.3 (28)	42.2 (107)	
1-2	48.3 (28)	47.6 (121)	
≥3	3.4 (2)	10.2 (26)	
Causes of			NS
Tubal/ Peritoneal	55.2 (32)	48.0 (122)	
Ovulatory	8.6 (5)	10.2 (26)	
Male factor	10.3 (6)	12.6 (32)	
Others	25.9 (15)	29.1 (74)	
No. of previous	1.8±1.0	2.1±1.3	NS
AMH (ng/ml)	6.80±5.29	6.63±4.54	NS
BMI (kg/m^2)	21.9±2.9	22.3±3.5	NS
Types of cycle			NS
Natural cycle	22.4(13)	27.6(70)	
Artificial cycle	77.6(45)	70.5(179)	
Stimulation cycle	0.0(0)	2.0(5)	
EMT[a] 5 days before	8.6(8.0,10.0)	8.5(7.6,10.0)	NS
Days of embryos % (n)			NS
5	58.6 (34)	67.7(172)	
6	36.2 (21)	31.1 (79)	
both 5&6	5.2 (3)	1.2 (3)	
No. of embryos			NS
1	39.7 (23)	43.7 (111)	
2	60.3 (35)	56.3 (143)	
Serum P[b]	65.8±29.4	53.1±28.7	NS
Serum E_2[b]	1125(479,1386)	891(472,1871)	NS
Serum β-hCG(mIU/ml)	196 (144, 221)	140(84,216)	0.000

a EMT=endometrium thickness. b Only 68 patients tested serum progesterone levels and 62 tested serum estradiol.

Discussion

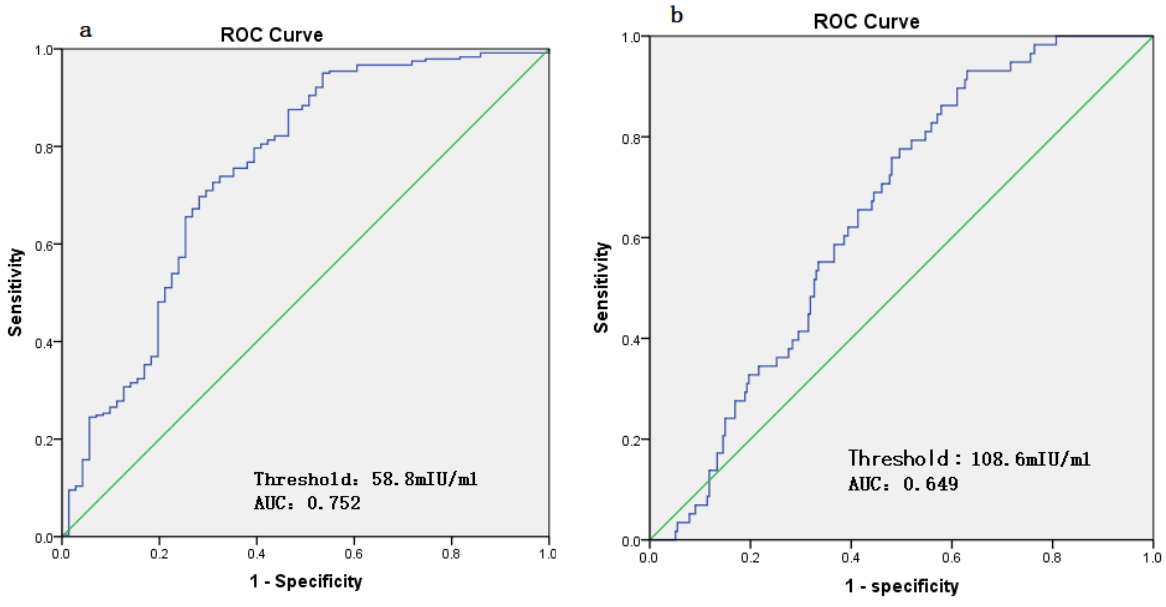
In the present study, pregnancy outcomes of patients whose serum β-hCG levels <300 mIU/ml 14 days after blastocyst transfer were

investigated. Our study shows that the pregnancy outcomes of the patients with initially low serum β-hCG levels were poor, with only 18.6% of live birth. Nearly 50% (47.4%) of the patients were early miscarriage and the rate of ectopic pregnancy was as high as 9.6%.

Table 4 Thresholds of serum β-hCG level for prediction of clinical pregnancy and live birth

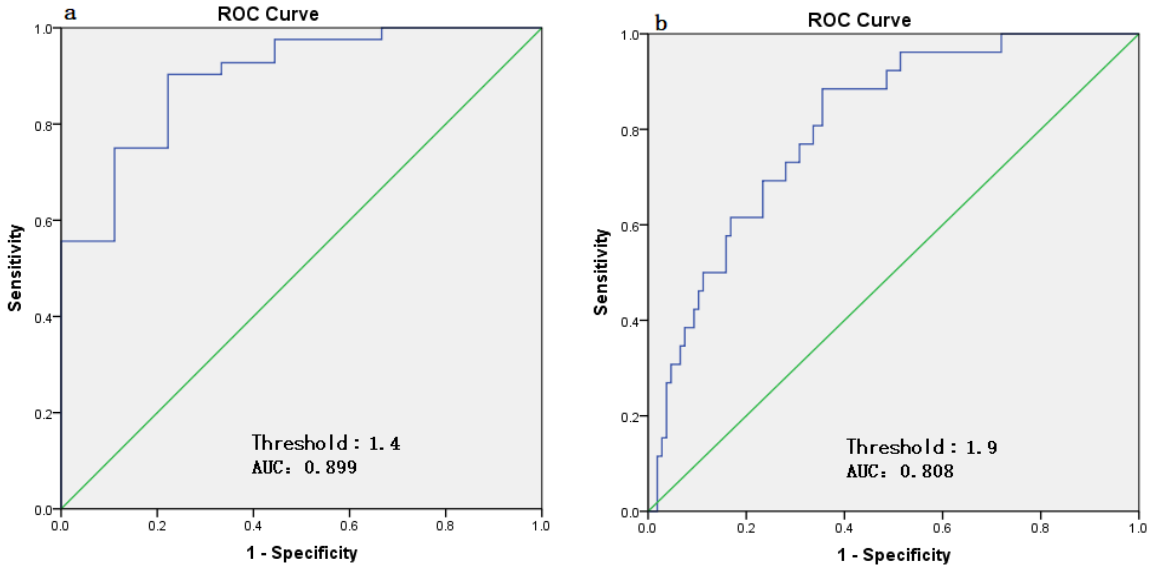
Pregnancy outcomes	Clinical pregnancy	Live birth	Clinical pregnancy	Live birth
	Threshold of β-hCG level (mIU/ml)		Threshold of β-hCG fold increase over 48h	
	58.8	108.6	1.4	1.9
AUC	0.752	0.649	0.899	0.808
95% CI of AUC	0.680-0.823	0.583-0.715	0.801-0.996	0.724-0.891
Sensitivity %	95.0	93.1	90.3	88.5
Specificity%	53.5	37.0	77.8	64.5
PPV %	85.8	25.2	97.4	63.6
NPV %	73.3	95.9	33.3	92.3

Figure 1 ROC curves for prediction of pregnancy outcomes by serum β-hCG level 14 days after blastocyst transfer.



a. ROC curve of β-hCG for clinical pregnancy; b. ROC curve of β-hCG for live birth;

Figure 2 ROC curves for prediction of pregnancy outcomes by fold increase of serum β-hCG over 48 hours.



a. ROC curve of β-hCG fold increase for clinical pregnancy; b. ROC curve of β-hCG fold increase for live birth.

Our research merely included patients with initially low serum β-hCG levels, instead of all the pregnant women, for there have been plenty of studies like that. Patients with low β-hCG values are often very anxious about their pregnancy outcomes. They are often required to take serum β-hCG tests in order to monitor the progress of conception, which will increase the number of visits and thus bring them both psychological and economic stress. For these reasons, it is very important to make individualized follow-up plans according to different serum β-hCG intervals. For patients with initially low β-hCG, the most important thing is to determine whether it is a clinical pregnancy. Our study demonstrated that the initial β-hCG value >58.8

mIU/ml predicted 85.8% of clinical pregnancy while failure to achieve that value led to 73.3% of biochemical pregnancy loss. Among patients having clinical pregnancy, about 60% were early miscarriage and 12.4% were ectopic pregnancy (data not shown). Therefore, for patients having initial β-hCG >58.8 mIU/ml, although luteal phase support is suggested to continue, another serum β-hCG test and ultrasound should be performed one week later to rule out the ectopic pregnancy. If the initial serum β-hCG is < 58.8 mIU/ml, luteal phase support is suggested to discontinue and measurement of serum β-hCG and ultrasound can be arranged one week later, since no live birth occurred in this group of patients.

In our research, we calculated the β-hCG fold increase over 48 hours according to the second test and found that the value of 1.9 was the optimal threshold to discriminate the live birth from the non-live birth. The probability of live birth was 63.6% if the fold increase was > 1.9, compared with the minimal likelihood of live birth (7.7%) in patients with fold increase was < 1.9. For patients with declined serum β-hCG, another serum β-hCG test should be scheduled 7-10 days later, since abnormal conception such as ectopic pregnancy can occur, just as the case of ectopic pregnancy in our study. Shamonki etc. confirmed that declining of serum β-hCG levels almost always led to

a failure to live birth, although they reported 3 cases of live birth with declined serum β-hCG levels in a cohort of 6021 patients[10].

A few previous studies have investigated the prediction of pregnancy outcomes by serum β-hCG levels in various days after vitrified-warmed blastocyst transfer. Oron etc. demonstrated that for β-hCG measured 11 days after single blastocyst transfer, the optimal cut-off value for predicting clinical pregnancy was 137 IU/L with PPV of 85% and NPV of 75%[6]. Zhao etc. found that the single β-hCG value of 399.5 IU/L on day 12 after blastocyst transfer was reliable to predict clinical pregnancy with the PPV of 93.47% and NPV of 67.61%. The single β-hCG value > 410.8 IU/L indicated 76.62% of live birth and the value below that resulted in 80.72% of non-live birth[7]. The study by Xiong etc. determined that optimal thresholds were 152.2 IU/L and 211.9 IU/L respectively for prediction of clinical pregnancy and live birth in patients who had β-hCG tests 14 days after vitrified-warmed blastocyst transfer[8]. The thresholds predicting clinical pregnancy and live birth in these studies were higher than those in our study, which can be explained by the fact than only patients with low β-hCG were included in our research.

Stone etc. investigated the association between the doubling time of serum β-hCG(β-t2) and ongoing pregnancy in pregnant women after assisted reproductive technology (ART). They illustrated that the β-

t2 on day 12 after embryo transfer was about 1.6 days and the cut-off value of 2.2 days had the optimal PPV of 87% and NPV of 42%[11]. Sung etc. calculated the fold increase between postovulatory day 12 and 14 in frozen-thawed cycles, but they were not able to find a difference between live birth and early pregnancy loss (3.1 ± 0.9 folds vs. 3.0 ± 1.0 folds, $P>0.05$). Nevertheless, they demonstrated that the values of 2.37 and 2.6 folds respectively predicted 89.8% of clinical pregnancy and 72.7% of live birth[1]. In our present study, the optimal threshold of fold increase for clinical pregnancy was 1.4 with the AUC of 0.899, sensitivity of 90.3%, specificity of 77.8% and PPV of 97.4%. The value for prediction of live birth was 1.9, with the AUC of 0.803, sensitivity of 88.5%, PPV of 97.4% and NPV of 92.3%. The fold increase of clinical pregnancy and live birth in our research was lower than those in Sung's study. The possible reasons are as follows: First, the serum β-hCG in our study were measured 14 days after blastocyst transfer, at least two days later than that in Sung's research. The study by Stone etc. showed that the doubling times of serum β-hCG(β-t2) rose from 1.6 days on day 12 to 3 days on day 24 after embryo transfer[11], suggesting that β-hCG doubled more quickly in early pregnancy. Second, the study population in our study was patients with low serum β-hCG level, whose embryos transferred may be less potent than those from patients having normal β-hCG levels.

Our previous study investigated the likelihood of live birth with serum β-hCG <100 mIU/ml 14 days after day 3 embryo transfer, which showed that the live birth rate was only 4.3%. In our present study, the live birth rate was 4.5% when the serum β-hCG was below 100 mIU/ml 14 days after blastocyst transfer (Table 2), which is comparable to that of day 3 embryo transfer[12].

The present research possesses the following advantages. First, we only analyzed patients with low serum β-hCG level instead of investigating all the pregnant patients, which will be helpful to develop appropriate suggestions of follow-up for this group of patients. Second, all the included patients transferred only vitrified-warmed blastocysts and had serum β-hCG level measured exactly the same day after embryo transfer, which would increase the accuracy of serum β-hCG level.

However, the current study contains two disadvantages. First, the patients studied had β-hCG tested 14 days after embryo transfer, which was later than most of the study. This may limit its wide application because serum β-hCG level was not measured in all the patients 14 days after embryo transfer. Second, the number of embryos transferred varied from one to two. This may cause vanishing twin syndrome, which may affect the initial serum β-hCG level. It has been reported that the rate of vanishing twin syndrome was as high as 10% after ART, [13],

which may affect the initial maternal serum β-hCG level. In the present research, the rate of twin pregnancy was only 5.1% (16/312) with initial serum β-hCG level <300 mIU/ml. No vanishing twin syndrome occurred in the twin pregnancy, except for 14 total miscarriages.

Conclusions: Initially low serum β-hCG level 14 days after frozen blastocyst transfer indicated minimal likelihood of live birth. For patients having initial β-hCG >58.8 mIU/ml, luteal phase support is suggested to continue. Another serum β-hCG test and ultrasound should be performed one week later. If the initial serum β-hCG is < 58.8 mIU/ml, luteal phase support is suggested to discontinue and measurement of serum β-hCG and ultrasound can be arranged one week later.

Abbreviations:

ROC: Receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval; ART: Assisted Reproductive technology; HMG: human menopausal gonadotropin; HCG: human chorionic gonadotropin; AMH ： anti-müllerian hormone.

Acknowledgments

The study was performed under the auspices of the IVF unit of the Third Affiliated Hospital of Guangzhou Medical University.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Animal studies

This article did not contain any studies with animals performed by any of the authors.

Authors' contributions

YXW designed research and wrote the manuscript. HYL analyzed the data. All authors read and

approved the final manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (No.81801532, to HYL and No.81701518, to YY) and Scientific Research Program of The Third Affiliated Hospital of Guangzhou Medical University (No.2017Q15, to YXW).

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.

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

The author confirms that the work described has not been published before; that its publication has been approved by all co-authors; that its publication has been approved (tacitly or explicitly) by the responsible authorities at the institution where the work is carried out.

Competing interests

The authors declare that they have no competing interests.

Author details

1 The Department of Reproductive Medicine, the Third Affiliated Hospital of Guangzhou Medical University, No. 63, Duobao Road, Guangzhou, Guangdong, China; 2 Key Laboratory of Reproductive Medicine of Guangdong Province, No. 63, Duobao Road, Guangzhou, Guangdong, China; 3 Key Laboratory for Major Obstetric Diseases of Guangdong Province, No. 63, Duobao Road, Guangzhou, Guangdong, China;4 Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, No. 63, Duobao Road, Guangzhou, Guangdong, China

References

1. Sung N, Kwak-Kim J, Koo HS and Yang KM. Serum hCG-beta levels of postovulatory day 12 and 14 with the sequential application of hCG-beta fold change significantly increased predictability of pregnancy outcome after IVF-ET cycle. J Assist Reprod Genet. 2016; 33: 1185-94.

2. Kathiresan AS, Cruz-Almeida Y, Barrionuevo MJ, Maxson WS, Hoffman DI, Weitzman VN, et al. Prognostic value of beta-human chorionic gonadotropin is dependent on day of embryo transfer during in vitro fertilization. Fertil Steril. 2011; 96: 1362-6.

3. Zhang X, Barnes R, Confino E, Milad M, Puscheck E, and Kazer RR. Delay of embryo transfer to

day 5 results in decreased initial serum beta-human chorionic gonadotropin levels. Fertil Steril. 2003; 80: 1359-63.

4. Papageorgiou TC, Leondires MP, Miller BT, Chang AS, Armstrong AB, Scott LA, et al. Human chorionic gonadotropin levels after blastocyst transfer are highly predictive of pregnancy outcome. Fertil Steril. 2001; 76: 981-7.

5. Oron G, Esh-Broder E, Son WY, Holzer H and Tulandi T. Predictive value of maternal serum human chorionic gonadotropin levels in pregnancies achieved by in vitro fertilization with single cleavage and single blastocyst embryo transfers. Fertil Steril. 2015; 103: 1526-31 e1-2.

6. Oron G, Shavit T, Esh-Broder E, Weon-Young S, Tulandi T, and Holzer H. Predictive value of serum HCG concentrations in pregnancies achieved after single fresh or vitrified-warmed blastocyst transfer. Reprod Biomed Online. 2017; 35: 272-8.

7. Zhao WE, Li YJ, Ou JP, Sun P, Chen WQ, and Liang XY. Predictive value of initial serum human chorionic gonadotropin levels for pregnancies after single fresh and frozen blastocyst transfer. J Huazhong Univ Sci Technolog Med Sci. 2017; 37: 395-400.

8. Xiong F, Li G, Sun Q, Chen P, Wang Z, Wan C, et al. Obstetric and perinatal outcomes of pregnancies according to initial maternal serum HCG concentrations after vitrified-warmed single blastocyst transfer. Reprod Biomed Online. 2019; 38: 455-64.

9. Zhang Q, Yan J, Tang R, Gao X, Zhang B, and Chen ZJ. Serum human chorionic gonadotropin level measured 17 days after oocyte retrieval can predict final clinical pregnancy outcomes in IVF/ICSI treatment cycles. J Reprod Med. 2014; 59: 285-92.

10. Shamonki MI, Frattarelli JL, Bergh PA and Scott RT. Logarithmic curves depicting initial level and rise of serum beta human chorionic gonadotropin and live delivery outcomes with in vitro fertilization: an analysis of 6021 pregnancies. Fertil Steril. 2009; 91: 1760-4.

11. Stone BA, Vargyas JM, Ringler GE, March CM and Marrs RP. The rate at which serum total beta-subunit human chorionic gonadotropin increases after embryo transfer is a predictor of the viability of pregnancy and an identifier of determinants of pregnancy. Fertil Steril. 2006; 86: 1626-33.

12. Wu Y and Liu H. Likelihood of live birth with extremely low beta-hCG level 14 days after fresh embryo transfer. Gynecol Endocrinol. 2020: 1-6.

13. Poikkeus P, Gissler M, Unkila-Kallio L, Hyden-Granskog C and Tiitinen A. Obstetric and neonatal outcome after single embryo transfer. Hum Reprod. 2007; 22: 1073-9.