European Journal of Obstetrics & Gynecology and Reproductive Biology 212 (2017) 75–79

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Embryo transfer day does not affect the initial maternal serum β-hCG levels: A retrospective cohort study

Mona Dahiya, Karishma Rupani*, Su Ling Yu, Stephanie M.C. Fook-Chong, Diana Chia Siew Fui, Hemashree Rajesh

Singapore General Hospital, Singapore

ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form 29 November 2016
Accepted 7 March 2017

Keywords:
Embryo transfer
Cleavage
Blastocyst
β-hCG
Pregnancy

ABSTRACT

The aim of this study is to compare the serum β-hCG values post transfer of a cleavage stage embryo versus a blastocyst stage embryo at equal time intervals post oocyte retrieval (OR) in clinically pregnant patients, and to ascertain a β-hCG value to predict pregnancy outcomes. This is a retrospective cohort study of 560 women with clinical pregnancy who underwent an embryo transfer performed at either the cleavage stage or the blastocyst stage of embryo development between January 2003 and June 2014 at the Center for Assisted Reproduction (CARE), Singapore General Hospital. The serum β-hCG level was measured on day 17 post OR. The β-hCG values were not significantly different in the cleavage stage versus the blastocyst stage embryos (mean ± SD: 387 ± 486 IU/L D3 vs. 352 ± 268 IU/L D5, $p = 0.96$, median value 297 in both groups). Our study suggests that the initial maternal serum β-hCG values were not affected by the day of transfer of the embryos since assessing the β-hCG at equivalent points after transfer should not lead to a significant difference assuming the progress and development of the embryos occurred as expected.

Introduction

Human chorionic gonadotropin is an early marker of implantation which appears in the maternal serum 6–8 days post fertilization [1–3]. It is secreted by the syncitio-trophoblast and is a dimer consisting of alpha and beta subunits. Its transcription can be detected as early as the two cell stage embryo [4,5]. The primary role of β-hCG is maintenance of the corpus luteum, and progesterone production in the early stages of pregnancy to aid in establishment of implantation. It is universally agreed that β-hCG levels in early pregnancy are predictive of pregnancy outcomes; higher levels correlate with improved outcomes and greater chances of live births [6,7]. However, in spite of this, there is only limited data on the predictability of β-hCG values in IVF cycles. Current literature correlates β-hCG values to certain variables like demographics and the day of embryo transfer (ET) [8,9]. In current practice, embryos from IVF cycles are usually transferred into the uterus 2–3 days post fertilization (as a cleavage stage embryo, D3) or 5 days post fertilization (as a blastocyst stage embryo, D5). Initial prediction of the viability of the day 2–3 and day 5 embryos prior to transfer to a recipient is still challenging and based on morphological parameters and rate of cleavage. Results of studies comparing the β-hCG levels based on day of transfer are conflicting, with some studies reporting higher β-hCG levels after cleavage stage embryo transfers [10], whereas others reporting higher levels after blastocyst transfers [11,12]. The primary objective of our study was to compare the β-hCG levels in cleavage stage vs. blastocyst stage transfers in fresh IVF cycles and to establish β-hCG cut-offs for the prediction of live birth, separately for the cleavage stage and blastocyst stage transfers in fresh IVF cycles, using ROC analysis. The secondary aim of this study is to find out the correlation of the β-hCG levels with various pregnancy outcomes like miscarriage, ectopic, live birth and stillbirth and to find maternal characteristics and cycle parameters predictive of β-hCG. The β-hCG values under these circumstances would be particularly useful for counseling patients early on in the pregnancy.

Materials and methods

The electronic medical records of patients who underwent IVF between 2003 and June 2014 at the Centre for Assisted Reproduction (CARE) at Singapore General Hospital were reviewed retrospectively. The study was approved by the Institutional

* Corresponding author.
E-mail address: sghobgyn15@gmail.com (K. Rupani).

http://dx.doi.org/10.1016/j.ejogrb.2017.03.015
0301-2115/

Review Board. There were 1897 patients who underwent fresh embryo transfer between 2003 and June 2014. Out of these, 560 patients were clinically pregnant, defined by the presence of a gestational sac on transvaginal ultrasound scan at 6–8 weeks. Inclusion criteria for the study were: a day 2/3 (cleavage stage) or day 5 (blastocyst stage) embryo transfer during this period, β-hCG levels 17 days post oocyte retrieval, clinical pregnancy and available information on the pregnancy outcome. All patients entered into the ART program were included regardless of age. Exclusion criteria included: cycle cancelation, embryo transfer on days other than day 2, 3 or day 5, and donor oocytes and frozen embryo transfers. All patients underwent IVF and ET according to conventional protocols. They were assigned to either the Long Agonist, Short Flare or Antagonist protocols. Embryos were evaluated by the embryologist for a day 2 or day 3, or day 5 transfer. Gardner blastocyst grading system [13] was used for grading the blastocysts and an in-house embryo grading system was used to grade the cleavage stage embryos. Depending on the laboratory embryo scores, number of high grade day 2 or 3 embryos and clinical considerations, patients were offered a day 2 or day 3, or day 5 transfer. If on day 2, there were more than 5 embryos at the 4 cell stage with minimal or no fragmentation, the transfer was delayed to day 5. The patients were grouped into: group 1 (cleavage stage) who underwent ET on day 2 or day 3, and group 2 (blastocyst stage) who underwent ET on day 5. Confounding variables between the two groups were age, etiology, protocol used, number of oocytes retrieved and number of embryos transferred. In our study, the two groups were not significantly different in terms of these variables except for maternal age and number of oocytes. A sample of the venous blood was collected 17 days after the oocyte retrieval (OR) in all the cases to analyze the serum β-hCG levels. Pregnancy was indicated by an initial serum β-hCG level > 7 mIU/mL. Additional β-hCG levels were measured to establish the presence of a viable pregnancy, a biochemical pregnancy, or an ectopic pregnancy. Trans-vaginal ultrasound was performed 2 weeks after the initial β-hCG measurement to evaluate the location of pregnancy. The final outcome results of the pregnancy were analyzed as: miscarriage, ectopic, still birth or a live birth.

Laboratory analysis

Serum β-hCG levels were analyzed using an electrochemiluminescence assay (Cobase module, Roche Diagnostics). The functional sensitivity of the assay was 0.6 mIU/mL and the detection range was 0.6–10,000 mIU/mL. The inter- and intra-assay coefficients of variation were 5% and 4% respectively.

Statistical analysis

The Statistical Package for the Social Sciences (version 21.0) and STATA version 13 were used for statistical analyses. The patients were divided into two groups depending on the day of embryo transfer group 1: cleavage stage embryos (day 2 or 3) and group 2: blastocyst stage (day 5). To assess the differences between the two groups, parametric and non-parametric analyses were conducted after determining whether the variables met the normality assumptions. The distribution of data was checked for normality using the normal probability plot and Kolmogorov–Smirnov test. Basic descriptions of parameters were presented either as mean and standard deviation (±SD) for normally distributed data or median and range for data distribution that were skewed and, count and percentage for categorical data. Student *t* test or Mann–Whitney *U* test were conducted to compare continuous and discrete ordinal variables between group 1 and group 2 and categorical data were analyzed with Chi Square tests or Fisher's exact test as appropriate. Next, a multiple linear regression analysis was conducted to assess the factors that significantly predicted higher serum β-hCG. Factors entered in the model were those that were found to be statistically significant in the univariate analysis. The confounding factors were maternal age and number of oocytes. Receiver operating characteristic (ROC) curve analysis was performed for determination of predictive accuracy (area under curve, AUC) of β-hCG values for live birth for each embryo transfer group. ROC curves were performed using a nonparametric distribution method. Receiver operating characteristic curves are a graphic representation of sensitivity (or true positive rate) vs. 1 minus specificity (or false positive rate). The discrimination threshold was chosen on the basis of optimal sensitivity and specificity. The area under the curve (AUC) and its 95% confidence interval were generated for each ROC curve and the AUCs were compared statistically using the method of DeLong (DeLong and Clarke-Pearson, 1988). Additionally, diagnostic accuracy statistics of β-hCG for live birth prediction at different β-hCG values between 90 and 400 IU/L were also presented for comparison purpose.

Results

Overall outcome

We analyzed all the fresh embryo transfers done from 2003 to June 2014. Of the 1897 cycles that were analyzed, 560 resulted in clinical pregnancy. Out of these, 500 patients had a cleavage stage embryo transfer (day 2 or day 3; group 1) and 60 patients had blastocyst transfer (day 5; group 2). The mean (±SD) age in group 1 was 33.6 ± 3.6 years and group 2 was 35 ± 3.7 years. The β-hCG value in the 2 groups was similar: median (range); group 1: 297 IU/L (18, 8437), group 2: 297.5 IU/L (12.9, 1359). The difference between the two groups was not statistically significant ($p = 0.960$). A post hoc power analysis at 2-sided $\alpha = 0.05$ showed that the power of our study was 83% to detect either a 40% increase or decrease of 120 IU/L in blastocyst stage as compared to cleavage stage arm that had a median β-hCG = 297 IU/L. Overall SD for the two arms ∼=300 IU/L and the ratio of sample sizes in our study, used for calculation was 60 blastocyst stage cases to 480 cleavage stage cases, i.e. a ratio of 1:8. To detect a difference of 30%, i.e. delta = 90 IU/L, the power was 59%. Out of the 560 patients, 307/500 patients (61.4%) had singleton pregnancy in group 1 as compared to 32/60 (53.3%) in group 2. In group 1, 106/500 patients (21.2%) had twins and 4/500 had triplets (0.8%) as compared to group 2 where 11/60 had twins (18.3%) and 2/60 had triplets (3.3%). The β-hCG levels were correlated with the ultrasound at 6 weeks. The twin pregnancies in our study were followed up till delivery and there were no vanishing twins. However, the presence or absence of two viable fetus between 4 and 6 weeks and a subsequent subclinical loss of one of them is difficult to estimate and beyond the scope of our study.

On an average, the embryos transferred were of at least grade 2. Gardner blastocyst grading system [13] was used to grade day 5/6 blastocysts. As for cleavage stage embryos, our in-house grading system was used to help us determine which embryos are suitable for transfer and freezing (Table 1).

β-hCG of singletons, twins and triplets

The difference in the β-hCG resulting in singleton pregnancy in group 1 vs. group 2 was not statistically significant ($p = 0.841$). Similarly, comparison in the β-hCG values resulting in twin gestation for group 1 vs. group 2 ($p = 0.313$) and that resulting in triplets for group 1 vs. group 2 ($p = 0.533$) was not statistically significant (Table 2). In addition, within each group β-hCG values

Table 1
The demographic characteristics and cycle parameters of patients with cleavage or blastocyst transfer.

Demographics and cycle parameters		Cleavage stage ($n=500$)	Blastocyst stage ($n=60$)	p value
Age, mean (years)		33.6	35	
Etiology, n (%)	Male factor	370 (74%)	49 (81.7%)	0.785
	Tubal	31 (6.2%)	3 (5%)	
	Endometriosis	38 (7.6%)	3 (5%)	
	PCO	26 (5.2%)	2 (3.3%)	
	Unexplained	35 (7%)	3 (5%)	
Protocol, n (%)	Long	161 (32.2%)	17 (28.3%)	0.129
	Short	40 (8%)	1 (1.7%)	
	Antagonist	299 (59.8%)	42 (70%)	
Number of oocytes retrieved, median (range)		12 (1, 59)	17 (7, 40)	<0.001
Number of embryos transferred, n (%)	1	19 (3.8%)	1 (1.7%)	0.051
	2	379 (75.8%)	55 (91.7%)	
	3	101 (20.2%)	4 (6.7%)	
	4	1 (0.2%)	0 (0%)	
β-hCG, median (range) in IU/L		297 (18.3, 8437)	297.5 (12.9, 1359)	0.960

Table 2
Distribution of β-hCG for various number of LB in cleavage stage and blastocyst stage transfer.

Number of LB	Cleavage stage (total $n=500$), No. (%), median (range) β-hCG	Blastocyst stage (total $n=60$), No. (%), median (range) β-hCG	p-Value
No live birth (0)	$n=70$ (14%), 189 (26.5, 8437)	$n=11$ (18.3%), 117 (12.9, 471)	0.629
Singleton (1)	$n=320$ (64%), 252 (18.3–1499)	$n=36$ (60%), 270 (43.40–641)	0.841
Twins (2)	$n=106$ (21.2%), 450 (21.2–2018)	$n=11$ (18.3%), 656 (120–1359)	0.313
Triplets (3)	$n=4$ (0.8%), 809.5 (520–2209)	$n=2$ (3.3%), 431.5 (182–681)	0.533

LB: live birth

of singleton pregnancies were lower than the values of multiple pregnancies. The minimum β-hCG value resulting in a live birth was 18.3 IU/L in cleavage stage transfer and 43.4 IU/L in blastocyst transfer.

β-hCG of various pregnancy outcomes

All four categories of pregnancy outcome (miscarriage, ectopic, live birth and still birth) were compared between group 1 and group 2 (Table 3). There was no significant difference in distribution of the various pregnancy outcomes between the cleavage and blastocyst transfer ($p=0.390$). Table 3 shows the comparison of the β-hCG values in the various pregnancy outcome groups across group 1 and group 2. The median β-hCG in patients who had live births in group 1 was 314 IU/L as compared with 364 IU/L in group 2 ($p=0.481$). For both cleavage stage and blastocyst stage transfers, the median β-hCG values in cases of spontaneous miscarriage were lower than in cases of live birth (in cleavage stage: $p=0.014$, in blastocyst stage: $p=0.010$). Multiple linear regression of β-hCG shows that β-hCG values are not affected by the transfer day after adjusting for the confounding variables namely age and number of retrieved oocytes (Table 4). ROC curve was plotted for cleavage stage and blastocyst transfer in order to determine optimal β-hCG cut-offs for the 2 groups. The area under the ROC curve (AUC) for each group showed that β-hCG is a predictor for LB (AUC (95% CI)): cleavage stage 0.62 (0.54, 0.69), blastocyst stage 0.75 (0.59, 0.90). AUC was not significantly different between the 2 groups ($p=0.140$, Fig. 1) Table 5 compares the sensitivity, specificity and positive predictive value at different β-hCG cut-offs for the 2 groups. Thus, it can be seen that for cleavage stage transfer a β-hCG of 199 IU/L had optimal sensitivity of 69.5% and specificity of 50% for predicting live birth. For blastocyst transfer, β-hCG of 253 IU/L had optimal 61.2% sensitivity and 63.6% specificity of predicting live birth. At these optimal cut-off points, the positive predictive values (i.e. likelihood of LB) were 89.5% and 88.2% for group 1 and group 2 respectively.

Discussion

Early embryonic development is a precise multistep programmed biologic process. The concentration of β-hCG grows steadily during early stages of pregnancy. Our study shows that when measured at equivalent points after embryo transfer, β-hCG levels are not affected by the day of transfer of embryo. In our study done on 560 clinically pregnant patients, the β-hCG levels measured 17 days post oocyte retrieval were not significantly

Table 3
Distribution of β-hCG in various pregnancy outcomes for cleavage stage and blastocyst stage.

Pregnancy outcome	Cleavage stage, No. (%) pregnancy outcome, median (range) β-hCG	Blastocyst stage, No (%) pregnancy outcome, median (range) β-hCG
Abortion	$n=60$ (12%), 210 (27–8437)	$n=11$ (18.3%), 117 (13–471)
Ectopic	$n=8$ (1.6%), 61 (29–853)	NA
Live birth	$n=430$ (86%), 314 (18–2209)	$n=49$ (81.7%), 364 (43–1359)
Still birth	$n=2$ (0.4%), 650 (144–1156)	NA
Total pregnancies	500	60

NA: not applicable because of zero cases.

Table 4
Multiple linear regression analysis to determine the association of potential factors with maternal serum β-hCG level of clinical pregnancies.

Variable	Co-efficient estimate, β	95% CI for β	p value
Cleavage transfer (blastocyst as reference)	45.8	−86.0 to 177.8	0.495
Age	−0.5	−11.1 to 11.0	0.993
No. of oocytes	0.6	−5.3 to 6.5	0.835
No. of embryos			
1	Reference	Reference	Reference
2	107.4	−107.5 to 322.1	0.327
3 or 4	70.9	−154.9 to 296.7	0.538

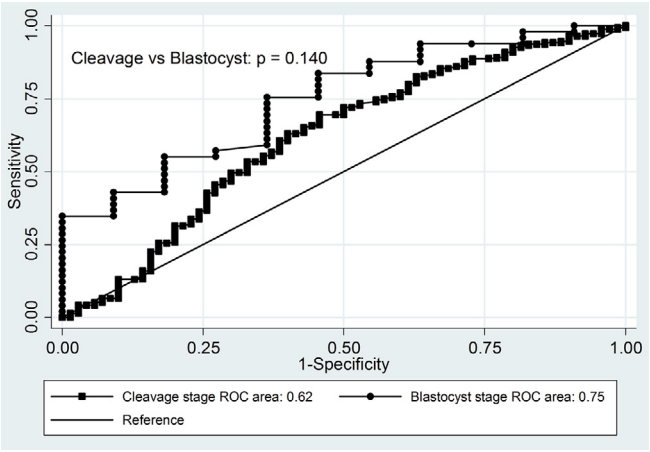

Fig. 1. ROC curves of β-hCG for the prediction of live birth for the cleavage stage and the blastocyst stage.

different in the cleavage stage vs. the blastocyst stage embryo (mean ± SD: 387 ± 486 IU/L D3 vs. 352 ± 268 IU/L D5, $p = 0.96$, median value 297 in both groups). This can be explained by the fact that the transfer involved only reasonably good quality embryos and that the β-hCG was measured at equal time intervals post oocyte retrieval in both the groups. A literature review of studies comparing β-hCG levels on day 3 vs. day 5 embryo transfer reveals conflicting results. Published studies have varied between no difference [14], higher day 3 β-hCG levels [10], or higher day 5 levels [11,12]. There has been published data on serum HCG as a predictor of pregnancy outcome from the western countries. We present our data from the Asian population. Papageorgiou et al. [14] concluded that comparisons of mean values between the day 3 and day 5 embryo transfer groups did not suggest discriminatory values (D3 – 403 mIU/mL vs. D5 – 362 mIU/mL, p value 0.25). Zhang et al. [10] showed that across their analysis of 918 embryo transfers observed that the day 3 β-hCG value was greater than the day 5 β-hCG value D3 (155 ± 76 IU/mL) vs. D5 (102 ± 51 IU/mL) $p < 0.001$ by about 50%. Using evidence from the literature that serum concentrations are shown to be lower in IVF ET pregnancies when compared to those resulting from natural conception [15], they postulated that embryo development or implantation may be impaired by the additional two days in culture, a difference in the rate of early sub-clinical losses; or a gender bias resulting from delay in transfer to day 5. However, in contrast to these results later studies showed that the β-hCG was significantly higher in D5 embryo transfers as compared with D3 embryo transfers [11,12]. The reasons given for this observation were several including significant improvements in the technique of blastocyst culture and the large trophoblastic cell mass in a day 5 embryo contributing to the higher β-hCG production.

In our study the initial maternal serum β-hCG values were not affected by the day of transfer of the embryos. This can be explained by the fact that assessing the β-hCG at equivalent points after transfer should not lead to a significant difference in the two groups assuming the progress and development of the embryos occurred as expected.

Further comparisons were made in our study by subdividing each of the 2 groups into singleton, twins and triplets. It was observed that β-hCG of day 3 transfer was not significantly different from those of day 5 transfer for each of these subgroups. This further reinforces our hypothesis that at similar stages of embryonic development, the β-hCG produced would be comparable and would not be affected by the initial day of transfer of embryos into the womb. Numerous studies have correlated initial β-hCG levels with the pregnancy outcome [15–21]. For predicting a live birth, we have a cut-off of 199 IU/L for a day 2/3 embryo (sensitivity 69.5%, specificity 50%) and 253 IU/L for a day 5 embryo (sensitivity 58% specificity 63.6%). Kathiresan et al. [11] evaluated the optimal threshold for predicting ongoing pregnancy to be 78 IU/L and 160 IU/L for day 3 and day 5, live births to be 94 IU/L and 160 IU/L and multiple gestations to be 250 IU/L and 316 IU/L respectively. In the study by Oron et al. [12], the β-hCG was 302 ± 158 IU/L for predicting live births in day 2/3 and 345 ± 191 IU/L for predicting live births in day 5 embryos. The β-hCG has been assessed on varying days after the oocyte retrieval: day 14 post OR by Papageorgiou et al. [14], day 13 post OR by Zhang et al. [10], day 15 post OR by Kathiresan et al. [11], day 16 post OR by Oron et al. [12] which may be one of the factors contributing to the difference in the cut-off values. Latest studies show that β-hCG is

Table 5
Prediction of live birth by β-hCG at cleavage stage and blastocyst stage.

Cut-off for β-hCG (IU/L)	Cleavage stage			Blastocyst stage		
	Sensitivity (%)	Specificity (%)	PV (+) (%)	Sensitivity (%)	Specificity (%)	PV (+) (%)
≥90.5	90	21.4	87.6	91.8	36.4	86.5
≥140	81.6	37.1	88.9	79.6	54.6	88.6
≥199	69.5	50	89.5	69.4	63.6	89.5
≥253	58.1	61.4	90.3	61.2	63.6	88.2
≥346	43.6	72.9	90.9	53.1	81.9	92.9
≥410	32.6	77.1	89.7	42.9	90.9	95.5

produced in the culture media as early as the 2 cell stage [4,5]. This is in contrast to earlier findings that human embryos can produce β-hCG as early as 8 days after fertilization [22]. Varying amounts of β-hCG isoforms are present in the serum [23]. The assay system employed in our study can equally recognize most β-hCG isoforms. It is possible that the conflicting results in various studies are due to altered production of various isoforms but this possibility is remote. Our findings are limited by the retrospective design of the study and also by the relatively small number of cases in group 2 (60). Our study was further limited by the fact that the number of twins ($n = 11$) in the blastocyst group and triplets in group 1 ($n = 4$) and group 2 ($n = 2$) were too small to draw any inference.

In conclusion, our study suggests that the day of embryo transfer does not affect the initial serum β-hCG values even after adjusting for confounding variables. Additionally, by comparing the conflicting results of other studies on this topic, we attempt to decipher the reasons behind varying cut-off values for ongoing pregnancy prediction. We are hopeful that our study contributes to the effort to determine the usefulness of β-hCG values for doctors when they interpret these results while counseling anxious group of patients.

References

[1] Braunstein GD, Rasor JL, Engvall E, Wade ME. Interrelationships of human chorionic gonadotropin, human placental lactogen, and pregnancy-specific beta 1-glycoprotein throughout normal human gestation. Am J Obstet Gynecol 1980;138:1205–13.

[2] Dor J, Rudak E, Rotmench S, Levran D, Blankstein J, Lusky A, et al. The role of early postimplantation beta-HCG levels in the outcome of pregnancies following in-vitro fertilization. Hum Reprod 1988;3:663–7.

[3] Kadar N, Romero R. Observations on the log human chorionic gonadotropin–time relationship in early pregnancy and its practical implications. Am J Obstet Gynecol 1987;157:73–8.

[4] Jurisicova A, Antenos M, Kapasi K, Meriano J, Casper RF. Variability in the expression of trophectodermal markers beta-human chorionic gonadotrophin, human leukocyte antigen-G and pregnancy specific beta-1 glycoprotein by the human blastocyst. Hum Reprod 1999;14:1852–8.

[5] Ramu S, Acacio B, Adamowicz M, Parrett S, Jeyendran RS. Human chorionic gonadotropin from day 2 spent embryo culture media and its relationship to embryo development. Fertil Steril 2011;96:615–7.

[6] Shamonki MI, Frattarelli JL, Bergh PA, 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.

[7] McCoy TW, Nakajima ST, Bohler HC. Age and a single day-14 beta-hCG can predict ongoing pregnancy following IVF. Reprod Biomed Online 2009;19:114–20.

[8] Porat S, Savchev S, Bdolah Y, Hurwitz A, Haimov-Kochman R. Early serum beta human chorionic gonadotropin in pregnancies after in vitro fertilization: contribution of treatment variables and prediction of long-term pregnancy outcome. Fertil Steril 2007;88:82–9.

[9] Lawler CC, Budrys NM, Rodgers AK, Holden A, Brzyski RG, Schenken RS. Serum beta human chorionic gonadotropin levels can inform outcome counseling after in vitro fertilization. Fertil Steril 2011;96:505–7.

[10] Zhang X, Barnes R, Confino E, Milad M, Puscheck E, Kazer RR. Delay of embryo transfer to day 5 results in decreased initial serum beta-human chorionic gonadotropin levels. Fertil Steril 2003;80(6):1359–63.

[11] 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(6):1362–6.

[12] Oron G, Esh-Broder E, Son WY, Holzer H, 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 2015103(June (6)) 1526–31.e1–2.

[13] Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: toward a single blastocyst transfer. Fertil Steril 2000;73:1155–8.

[14] 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(5):981–7.

[15] Bjercke S, Tanbo T, Dale PO, Morkrid L, Abyholm T. Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization. Hum Reprod 1999;14:1642–6.

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

[17] Schmidt LL, Asch RH, Frederick JL, Rojas FJ, Stone SC, Balmaceda JP. The predictive value of a single beta human chorionic gonadotropin in pregnancies achieved by assisted reproductive technology. Fertil Steril 1994;62:333–8.

[18] Glatstein IZ, Hornstein MD, Kahana MJ, Jackson KV, Friedman AJ. The predictive value of discriminatory human chorionic gonadotropin levels in the diagnosis of implantation outcome in in vitro fertilization cycles. Fertil Steril 1995;63:350–6.

[19] Qasim SM, Callan C, Choe JK. The predictive value of an initial serum beta human chorionic gonadotropin level for pregnancy outcome following in vitro fertilization. J Assist Reprod Genet 1996;13:705–8.

[20] Chen CD, Ho HN, Wu MY, Chao KH, Chen SU, Yang YS. Paired human chorionic gonadotrophin determinations for the prediction of pregnancy outcome in assisted reproduction. Hum Reprod 1997;12:2538–41.

[21] Alahakoon TI, Crittenden J, Illingworth P. Value of single and paired serum human chorionic gonadotropin measurements in predicting outcome of in vitro fertilisation pregnancy. Aust N Z J Obstet Gynaecol 2004;44:57–61.

[22] Lopata A, Hay DL. The surplus human embryo: its potential for growth, blastulation, hatching, and human chorionic gonadotropin production in culture. Fertil Steril 1989;51:984–91.

[23] Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the a- and b-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997;7:15–32.