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What is the effect of embryo morphology on serum β-hCG levels?

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ABSTRACT

Objective: To determine the effect of embryonic factors on serum beta human chorionic gonadotropin (β-hCG) levels in pregnancy and live birth resulting after a single fresh cleavage embryo and blastocyst transfer.

Study design: This was a retrospective cohort study conducted at a tertiary care hospital. All fresh single embryo transfers (sETs) between September 2011 and December 2016 were included. The correlation analysis was performed to determine the association of embryo morphological parameters on mean serum β-hCG levels on day 12 after the transfer of a fresh single cleavage embryo and a fresh single blastocyst embryo.

Results: Out of a total of 455 fresh sETs, 60 positive β-hCG results after the transfer of a single fresh cleavage-stage embryo and 82 after the transfer of a single fresh blastocyst. The mean β-hCG level resulting from a single fresh blastocyst ET was 371.7 ± 52.7 IU/L, which was similar to the mean β-hCG level resulting from a cleavage ET (314.5 ± 36.9 IU/L) ($p = .70$). Interestingly, serum β-hCG levels resulting from a single fresh blastocyst ET showed a correlation with day 5 blastocoele expansion, trophectoderm cell number and blastocyst quality score in ongoing pregnancy ($r = .33$, $p = .02$; $r = .29$, $p = .04$; and $r = .31$, $p = .03$, respectively). Moreover, day 5 blastocoele expansion and blastocyst quality score showed a correlation with the serum β-hCG levels resulting from a single fresh blastocyst ET in live birth ($r = .36$, $p = .02$; $r = .31$, $p = .04$, respectively).

Conclusion: Our study suggests that serum β-hCG levels resulting from a single fresh blastocyst ET showed a correlation with day 5 blastocoele expansion and blastocyst quality score in both ongoing pregnancy and live birth.

Introduction

Human chorionic gonadotropin (hCG), a dimer consisting of alpha and beta subunits, is secreted from the trophoblast and appears in maternal circulation 6–8 days after fertilization [1,2]. *In vitro* embryo culture studies also reported that hCG gene expression can be detected as early as the two-cell stage embryo [3,4]. Due to its worldwide usage as an obstetric marker, β-hCG is often regarded as a signal for maternal recognition of pregnancy and is one of the most widely studied markers in embryonic development [5].

In assisted reproductive technology (ART) cycles, embryos can be transferred either 2 or 3 days after fertilization (cleavage-stage embryo) or 5–6 days after fertilization (blastocyst-stage embryo). Compared with cleavage-stage embryos, blastocyst transfers offer the advantages of better viability and developmental potential, better synchronization between the stage of embryonic development and the endometrial environment [6].

In intra-cytoplasmic sperm injection (ICSI) cycles, β-hCG level is routinely measured 12–16 days after embryo transfer (ET) [7]. A single measurement of serum β-hCG concentration, as early as 12 days after embryo transfer, is not only diagnostic but also has good predictive value for pregnancy outcome [8] and is therefore used as part of the routine follow-up after ICSI cycles.

The results of studies comparing β-hCG levels after cleavage-stage or day blastocyst-stage transfers have been mixed, with some studies reporting higher β-hCG levels after the transfer of blastocysts [8,9] and others reporting higher levels after the transfer of cleavage-stage embryos [10,11]. None of the studies have evaluated the correlation of β-hCG levels with embryo morphological parameters resulting in pregnancy and live birth from single fresh cleavage ETs or blastocyst ETs.

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The primary goal of the current study was the evaluation of the effects of embryo morphological parameters on the serum β-hCG levels in pregnancy and live birth. The secondary aim was to compare the level of β-hCG concentrations 12 days after single fresh cleavage ET with those after transfer of a single fresh blastocyst-stage embryo and to determine the predictive value of serum β-hCG levels for ongoing pregnancy and live birth in ICSI cycles.

Materials and methods

In this retrospective cohort study, we reviewed 455 fresh single ET cycles performed between September 2011 and December 2016 at the Uludag University, Assisted Reproductive Technology Center, Turkey. Positive serum β-hCG levels (>5 mIU/ml 12 days after ET, biochemical pregnancy) resulting from the transfer of a fresh cleavage-stage embryo (day 2 or 3 after oocyte retrieval) and those of a fresh blastocyst transfer (day 5 after oocyte retrieval) were compared. For standardization of the measurement, we included 142 patients with positive β-hCG values only 12 days after ET (Fig. 1).

Patients were stimulated with 150–375 IU per day of recombinant FSH (rFSH) (Gonal F, Merck-Serono, GERMANY) that was starting on day 2 of the menstrual cycle. Pituitary suppression was achieved with GnRH antagonists (Cetrotide, Merck-Serono, GERMANY) during flexible antagonist protocol. Serum oestradiol (E_2) levels and follicle growth were monitored daily. When at least two or more follicles reached ≥18 mm diameter, 250 μg/0,5 ml recombinant β-hCG (Ovitrelle, Merck-Serono, GERMANY) was administered [12]. Oocyte pick-up (OPU) was performed 34–36 h after ovulation trigger, by vaginal puncture with a 17 mm gauge needle with ultrasound guidance, and cumulus-oocyte complexes (COCs) were collected. After 2–4 h, COCs were removed form oocytes (denuded) with enzymatic treatment with 80 IU/ml hyaluronidase solution to prepare for ICSI. ICSI was performed with oocytes at metaphase II stage, and then oocytes were incubated for pre-embryological development. Fertilization was assessed 16–18 h after ICSI, and embryos with two pronuclei were cultured individually (in separate droplets) and assessed daily by one embryologist in order to reduce bias due to inter-operator differences.

Routinely, the embryo transfer policy was determined on the basis of the quantity and quality of embryos available. Our preference was to transfer a blastocyst stage embryo. However, if the number of good quality embryo was not satisfactory (i.e., if there were no more than four embryos at the 7–8 blastomer stage nor good quality embryos) or, rarely, if the blastocyst ET came during the weekend, a cleavage-stage embryo was transferred. Cleavage-stage embryos were graded as good quality if they had 4 cells on day 2 or 7–8 cells on day 3, <10% fragmentation, and no apparent morphological abnormalities [13]. The cleavage quality score (CQS) was calculated according to Avcı et al. [14]. CQS was the product of the day 3 cell number score, blastomere size score and fragmentation rate score (CQS = day 3 cell number score x blastomere size score x fragmentation rate score [Supplement Table 1]) Blastocysts were classified according to the level of expansion and the grade of inner cell mass and trophectoderm. Good-quality blastocysts were defined as those >3 BB on the basis of a previously published Gardner's classification [15]. Blastocyst quality score (BQS) was calculated according to Rehman et al. [16]. BQS was the product of blastocoele expansion, inner cell mass (ICM) and trophectoderm (TE) grades (BQS = expansion grade x ICM grade x TE grade), where expansion status ranged between 1–6 as above, and ICM and TE grades were each assigned the following numerical values: grade A = 3, grade B = 2 and grade C = 1. When there were two or more good-quality blastocysts, the highest BQS scored embryo was transferred.

Immunoassays during this entire study using the sandwich principle for β-hCG were carried out with i-STAT Total β-hCG test (Abbott Point of Care Inc., USA) at University Hospital, Biochemistry Laboratory. Inter and intra-assay coefficients of variation for β-hCG were less than 10%. Biochemical pregnancy was indicated by a serum β-hCG level of >5 mIU/mL drawn 12 days after ET. Clinical pregnancy was confirmed by transvaginal ultrasound 5–6 weeks

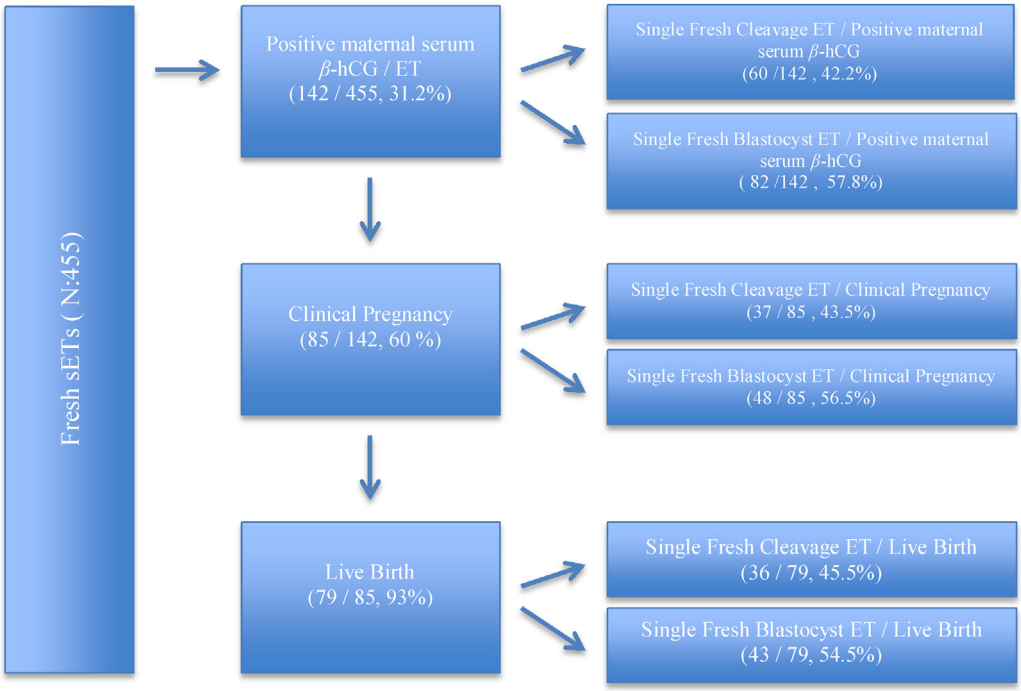

Fig. 1. Fresh sETs allocation diagram and outcome rates.

with fetal heartbeat. The live birth was defined as the birth of a healthy singleton newborn.

The study protocol was approved by Local Institutional Ethics Committee. (2018–9/20)

Statistical analysis

The distribution of data was analysed by using the Shapiro-Wilk test. Study data were summarized using descriptive statistics (mean ± SEM). The continuous variables were compared by using the Mann-Whitney test. Spearman's correlation test was performed to test the relationship between β-hCG and the embryo quality parameters. All the analysis was performed by the SPSS software package for MAC (Statistical Package for Social Sciences, version 23.0, SPSS Inc., Chicago, Illinois, USA). A two-tailed $p < 0.05$ was considered significant.

Results

Out of a total of 455 fresh sETs, 142 resulted in a positive maternal serum β-hCG. There were 60 positive β-hCG results after the transfer of a single fresh cleavage-stage embryo (a total of 209) and 82 after the transfer of a single fresh blastocyst (a total of 246), in which 85 resulted in a clinical pregnancy, and 79 resulted in ongoing pregnancy and subsequent live birth. There were 48 clinical pregnancies resulting from single fresh blastocyst transfer and 37 clinical pregnancies resulting from the transfer of a single fresh cleavage-stage embryo ($p = .63$). There were 43 live births resulting from single fresh blastocyst ET and 36 live births resulting from the transfer of a single fresh cleavage ET ($p = .37$ for both ongoing pregnancy and live birth). There were 149 negative β-hCG results (>5 mIU/ml 12 days after ET) after the transfer of a single fresh cleavage-stage embryo and 164 after the transfer of a single fresh blastocyst. The blastocyst transfer group had lower maternal BMI ($p = .36$), higher β-hCG day E_2 levels ($p < .001$) and higher numbers of total oocytes ($p < .001$) and metaphase II stage oocytes ($p < .001$) than the cleavage-stage transfer group. The maternal age was the same between groups ($p = .51$). In terms of the basal endocrine parameters (FSH, LH, E_2), there was no significant difference between groups (Table 1).

The embryo morphological parameters for cleavage-stage included: day 3 cell number, blastomere size and fragmentation rate as well as the cleavage quality score (CQS). When these morphological parameters between positive serum β-hCG levels resulting from a cleavage ET and negative serum β-hCG levels resulting from a cleavage ET were compared, no statistically significant differences was found (for day 3 cell number, $p = .35$; for blastomere size, $p = .16$; for fragmentation rate, $p = .20$ and *for* CQS, $p = .14$). Consequently, no morphological parameter has been found to be a strong predictor of the implantation status (positive serum β-hCG levels) for the cleavage stage embryo.

The embryo morphological parameters for blastocysts-stage included the level of expansion and the grade of inner cell mass and trophectoderm, trophectoderm cell number and also blastocyst quality score (BQS). When these morphological parameters between positive serum β-hCG levels resulting from a blastocysts ET and negative serum β-hCG levels resulting from a blastocysts ET were compared, any blastocyst that was more expanding and having a high scoring BQS and also having more TE cell number had a greater chance of being implanted (positive serum β-hCG levels) than those with lower grades (for day 5 blastocoele expansion, $p = .03$; for BQS, $p = .04$; for TE cell number, $p = .03$). ICM grade and TE grade was not demonstrated to be a significant predictor of implantation (positive serum β-hCG levels) in any of the analyses performed ($p = .25$ $p = .13$, respectively).

The mean β-hCG level resulting from a single fresh blastocyst ET was 371.7 ± 52.7 IU/L, which was similar to the mean β-hCG level resulting from a cleavage ET (314.5 ± 36.9IU/L) ($p = .70$). The embryo morphological parameters for cleavage-stage were day 3 cell number, blastomere size and fragmentation rate. In the simple correlation analysis, serum β-hCG levels resulting from a cleavage ET and the embryo morphological parameters had no associations with pregnancy and live birth (Table 2). The embryo morphological parameters for blastocyst stage were day 5 blastocoele expansion, ICM grade, TE grade, TE cell number and BQS. The serum β-hCG levels resulting from a single fresh blastocyst ET and the embryo morphological parameters for day 5 showed no correlation in biochemical pregnancy. Interestingly, the serum β-hCG levels resulting from a single fresh blastocyst ET showed a correlation with day 5 blastocoele expansion, TE cell number and BQS in ongoing pregnancy (r = .33, $p = .02$; r=.29, $p = .04$; and r=.31, $p = .03$, respectively). Moreover, day 5 blastocoele expansion and BQS showed a correlation with the serum β-hCG levels resulting from a single fresh blastocyst ET in live birth (r = .36, $p = .02$ and r=.31, $p = .04$, respectively) (Table 3).

The ROC curve based on β-hCG level for prediction of clinical pregnancy resulting from cleavage-stage and blastocyst transfers is presented in Figs. 2 and 3. These analyses showed that the β-hCG value of 127.1 IU/L yielded optimal sensitivity (70%) and specificity (75%) for cleavage-stage ET [area under the curve (confidence interval): 0.76 (0.63–0.89)], and the β-hCG value of 173.5 IU/L resulted in optimal sensitivity (65%) and specificity (70%) for blastocyst stage ET [0.74 (0.62–0.86)]. Similar ROC curve analyses were performed to determine β-hCG cut-off values predictive of live birth for cleavage-stage and blastocyst stage ETs. The proposed optimal thresholds predictive for LB were 129.4 IU/L

Table 1
Demographic characteristics of patients with cleavage-stage ET or blastocyst transfer resulted positive maternal serum hCG.

	Cleavage-stage ET (n = 60)	Blastocyst transfer (n = 82)	*p* value
Age (year)	30.5 ± 3.9	29.2 ± 3.7	.51
BMI (kg/m2)	26.1 ± 4.4	24.5 ± 4.1	**.04***
Duration of infertility (years)	6.1 ± 4.3	5.2 ± 3.3	.33
Cycle Number	1.5 ± 0.7	1.6 ± 0.4	.21
Baseline FSH	6.8 ± 3.2	6.2 ± 3.1	.31
Baseline LH	5.5 ± 2.3	6.3 ± 4	.10
Baseline E_2	48.3 ± 35	51.1 ± 45.3	.17
hCG day E_2	1037.8 ± 685.2	1625.9 ± 1056.3	<**.001***
Total oocytes number	9.5 ± 6.1	16.2 ± 8.5	<**.001***
Mature oocytes	6.9 ± 4.9	11.4 ± 6.67	<**.001***
Fertilization rate	.66 ± .28	.68 ± .2	.75
Mean β-hCG levels (IU/L)			
β-hCG levels in biochemical pregnancy	314.5 ± 36.9	371.7 ± 52.7	.70

Data presented as mean ± SD.
* The Mann–Whitney U test for comparison of ranks between two groups.

Table 2
Cleavage morphology and β-hCG levels correlations.

Cleavage-stage ET (n = 60)	Ongoing pregnancy (n = 37)		Live Birth (n = 36)	
	Coefficient estimate (r)	*p*	Coefficient estimate (r)	*p*
Day 3				
Cell number	.18	.35	.29	.28
Cell size (even or uneven)	−.01	.95	−.02	.93
Fragmentation rate	.52	.79	.16	.49
CQS	.16	.35	.32	.11

Sperman's Rho test.

Table 3
Blastocyst morphology and β-hCG levels correlations.

Blastocyst transfer (n = 82)	Ongoing pregnancy (n = 48)		Live Birth (n = 43)	
	Coefficient estimate (r)	*p*	Coefficient estimate (r)	*p*
Day 3				
Cell number	.17	.35	.01	.98
Cell size (even or uneven)	.52	.79	.52	.79
Fragmentation rate	.16	.35	.25	.13
CQS	.24	.10	.16	.32
Day 5				
Blastocoele expansion	.33	**.02***	.36	**.02***
ICM grade	.23	.13	.20	.13
TE grade	.12	.43	.14	.35
TE cell number	.29	**.04***	.28	.08
BQS	.31	**.03***	.31	**.04***

* Sperman's Rho test.

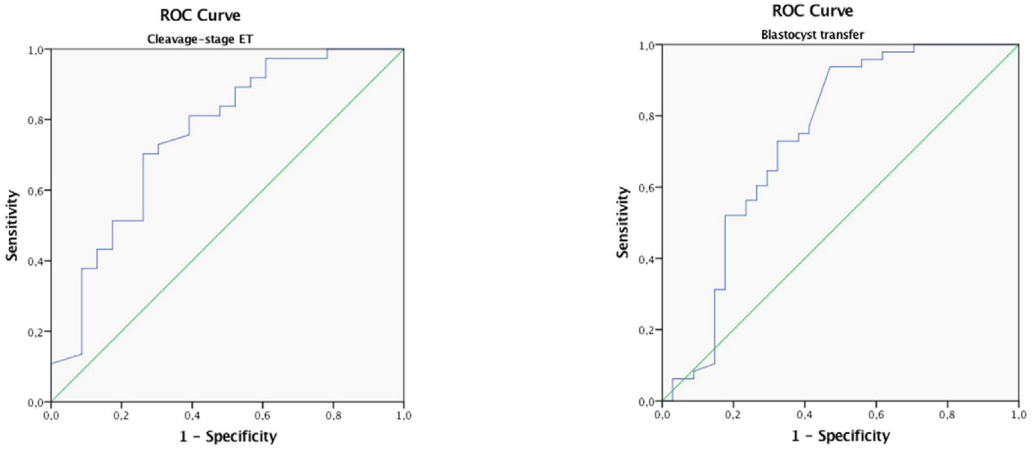

Fig. 2. ROC curve for β-hCG values for the prediction of ongoing precnancy for the cleavage-stage group and the blastocyst group. The X-axis and Y-axis are, respectively, the falsepositive rate (or 1-specificity) and the true positive rate (or sensitivity). These analyses showed that the β-hCG value of 127.1 IU/L yielded optimal sensitivity (70%) and specificity (75%) for cleavage-stage ET [area under the curve (confidence interval): 0.76 (0.63–0.89)], and the β-hCG value of 173.5 IU/L resulted in optimal sensitivity (65%) and specificity (70%) for blastocyst stage ET [0.74 (0.62–0.86)].

and 178.5 IU/L [cleavage-stage: 0.76 (0.64–0.89); blastocyst stage: 0.77 (0.67–0.88)] for cleavage-stage and blastocyst-stage ETs, respectively. Generally, the ROC curve based on β-hCG level for prediction of clinical pregnancy and live birth were performed due to the similar levels of β-hCG after cleavage-stage and blastocyst stage ETs. These analyses showed that the β-hCG value of 131.1 IU/L yielded optimal sensitivity (70%) and specificity (70%) for clinical pregnancy [area under the curve (confidence interval): [0.75 (0.67–0.89)], and the β-hCG value of 133.5 IU/L resulted in optimal sensitivity (70%) and specificity (70%) for live birth [0.76 (0.69–0.85)] (Fig. 4).

Discussion

The results of this study have shown that the mean β-hCG levels resulting from a single fresh blastocyst-stage transfer and cleavage-stage transfer were similar; however, blastocyst morphological parameters had an effect on serum β-hCG levels in

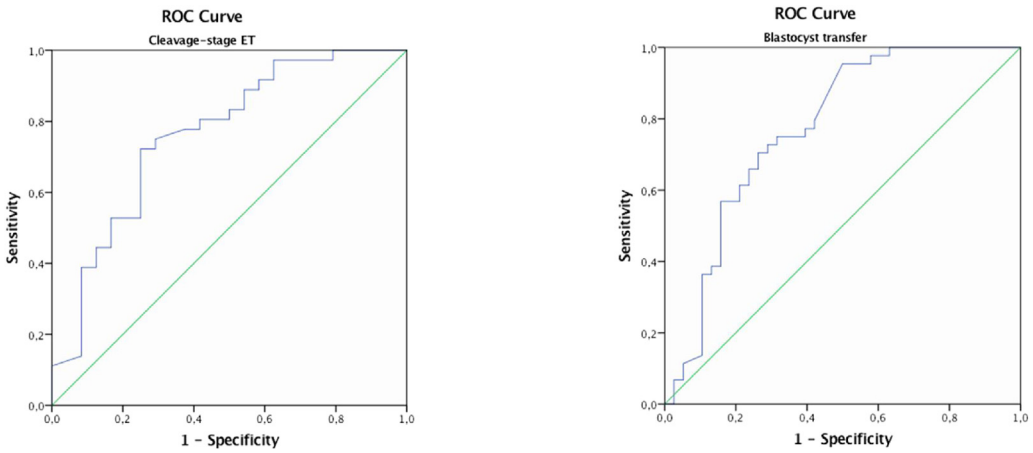

Fig. 3. ROC curve for β-hCG values for the prediction of live birth for the cleavage-stage group and the blastocyst group. The X-axis and Y-axis are, respectively, the false positive rate (or 1-specificity) and the true positive rate (or sensitivity). The proposed optimal thresholds predictive for LB were 129.4 IU/L and 178.5 IU/L [cleavage-stage: 0.76 (0.64–0.89); blastocyst stage: 0.77 (0.67–0.88)] for cleavage-stage and blastocyst stage ETs, respectively.

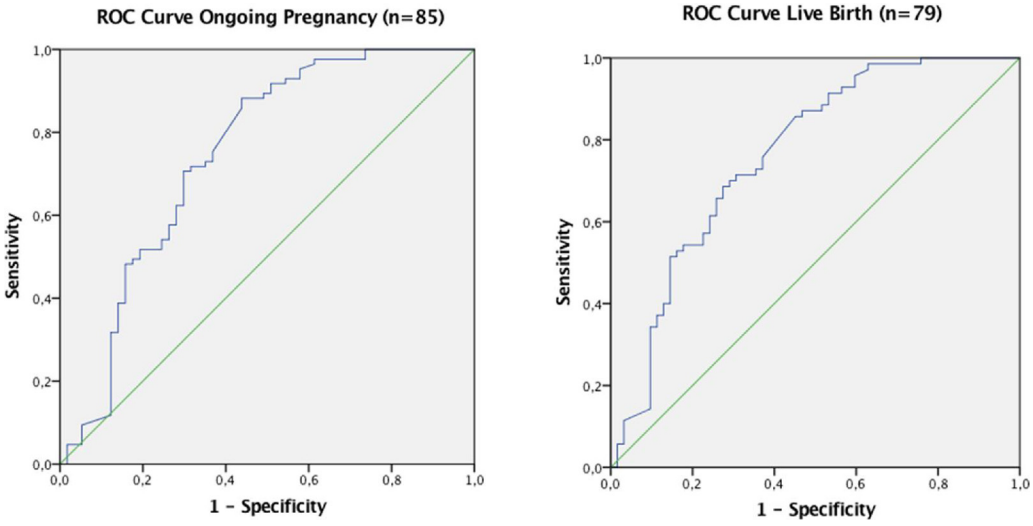

Fig. 4. ROC curve for β-hCG values for the prediction of ongoing precnancy and live birth for all sETs resulting with positive β-hCG values. The X-axis and Y-axis are, respectively, the false positive rate (or 1-specificity) and the true positive rate (or sensitivity). These analyses showed that the β-hCG value of 131.1 IU/L yielded optimal sensitivity (70%) and specificity (70%) for clinical pregnancy [area under the curve (confidence interval): [0.75 (0.67–0.89)], and the β-hCG value of 133.5 IU/L resulted in optimal sensitivity (70%) and specificity (70%) for live birth [0.76 (0.69–0.85)].

cycles that resulted in ongoing pregnancy and live birth. Moreover, our study has investigated the predictive value of serum β-hCG levels resulting in ongoing pregnancy and live birth from a single fresh blastocyst-stage transfer and cleavage-stage transfer.

Previous studies comparing *β-hCG* levels by day of ET have included cycles with more than one ET and have found different results [9–11]. Transfer of more than one embryo makes it impossible to evaluate embryo morphological parameters related to β-hCG levels [17,18]. As expected, multiple gestations are associated with higher initial levels of β-hCG, so multiple gestations resulting from a single embryo transfer were excluded from our study to prevent conflicting results in terms of the evaluation of embryonic factors. Our findings are limited by the retrospective design of the study and the lack of randomization to the study groups. As a result of the embryo transfer preference at the cleavage stage in the cycles where the number of good quality embryos was below four in the second or third day after fertilization, the number of total and metaphase II oocytes (higher values mean to more available embryos to transfer) on the cleavage transfer group was lower than blastocyst transfer group.

The majority of studies investigating the prognostic value of β-hCG thresholds did not separate their analyses according to day of ET. Moreover, in previous studies related to the predictive value of β-hCG, timing of the β-hCG measurement was different; some authors evaluated according to ovulatory (hCG) day, some according to oocyte retrieval day, some according to fertilization, and some according to transfer day. In the present study, serum β-hCG measurement was taken at 12 days after transfer day. Zhang et al. [10] and Papageorgiou et al. [11] previously reported that β-hCG levels on days 13–15 after day 5 blastocyst transfers were lower than after day 3 ETs. They postulated that embryo development or implantation may be impaired by the additional 2 days in culture media. Sites at al. demonstrated similar initial β-hCG levels (9 days after blastocyst transfer or 11 days after cleavage stage transfers) in patients having fresh cleavage compared with fresh blastocyst single embryo transfers. Dahiya et al. [19] reported that β-hCG levels 17 days after the oocyte retrieval were similar in fresh single or multiple blastocyst ET and in fresh single or multiple cleavage ET. Moreover, that study showed that serum β-hCG levels resulting in singleton, twin or triplet pregnancy in fresh single or

multiple blastocyst ET and in fresh single or multiple cleavage ET were not statistically significant. Additionally, multiple linear regression showed that serum β-hCG levels were not affected by number of embryos transferred. Our study shows that day 12 serum β-hCG values are similar after the transfer of a single fresh blastocyst embryo compared with after a single fresh cleavage ET. Considering these data, it is impossible to compare the different levels of β-hCG due to day of measurement. There were some studies that evaluated day 12 β-hCG levels similar to our study. Contrary to our findings, Kumbak et al. [8] and Oron et al. [20] reported that in patients who were given single or multiple embryos on day 5, day 12 β-hCG concentrations were significantly higher than those of patients who were given embryos on day 3 regarding all kinds of pregnancy outcomes. There were several reasons given for that observation including significant improvements in the technique of blastocyst culture and the large trophoblastic cell mass due to higher blastocoele expansion in a day 5 embryo contributing to the higher β-hCG production.

A study by Hill et al. [21] reported that TE grade is the most significant predictor of implantation and live birth among the standard blastocyst morphologic assessments. Therefore, higher grades of TE may translate into more hCG producing cells and higher levels of hCG may lead to a strengthened signalling capacity of the implanted blastocyst; however, the blastocoele expansion stage significantly and independently predicted live-birth rates in a multivariate logistic model for fresh single blastocyst transfer cycles [22]. Goto et al. [23] reported that the grade of blastocoele expansion significantly affected pregnancy outcome, and moreover, neither ICM nor TE affected the pregnancy outcomes for blastocysts of the same expansion degree. Du et al. [24] found that the degree of blastocoele expansion was the only blastocyst morphology parameter that was significantly related to the live-birth rate for fresh single blastocyst ET. In contrast to the previous studies, Richter et al. have reported that the ICM grade is important in predicting clinical outcome. In our study, we showed that day 5 blastocoele expansion, TE cell number and BQS had an effect on day 12 β-hCG levels for blastocyst-stage ETs in ongoing pregnancy cycles. Moreover, day 5 blastocoele expansion and BQS showed a correlation between β-hCG levels in live-birth cycles. The patients with poor-grade blastocysts (<3BB) were excluded from our study because they were more likely to receive double-blastocyst transfer or cancelled. In our study ICM grade and TE grade of the blastocyst ET varied between A or B (small range) so these grades did not suggested as a predictor of implantation (positive serum β-hCG levels) and clinical outcome. Unfortunately, there was no association between cleavage-stage embryo morphological parameters and serum β-hCG levels.

Numerous studies have evaluated initial β-hCG values with the pregnancy outcome. Bjercke et al. [25] found a single β-hCG measurement of 55 IU/l on day 12 after embryo transfer (day 2 cleavage-stage embryo) to be reliable in predicting the occurrence of early pregnancy loss. In another study, 143 IU/l was found as the optimal cut-off value for β-hCG at day 11 post-embryo transfer in differentiating between viable and non-viable pregnancies after assisted reproduction, excluding biochemical pregnancies; however, transferred embryo stage was not mentioned [26]. In another study, a 50 IU/l cut-off value for β-hCG was found on day 14 after embryo transfer in prediction of viable pregnancies [27], similar to Anckaert et al.'s study; however, transferred embryo stage was not mentioned, and furthermore, fresh and frozen ET cycles were evaluated together. Kumbak et al. [8] reported that β-hCG measurements 12 days after transfer of 98 mIU/ml in day 3 transfer cycles and 257 mIU/ml in day 5 cycles were the values most reliably predicting ongoing pregnancy. In our study, ROC curve analysis revealed an β-hCG value of 127.1 IU/L to be the cut-off point for predicting ongoing pregnancy after cleavage-stage ET and 173.5 IU/L to be the cut-off point for predicting ongoing pregnancy after blastocyst-stage ET.

In conclusion, our study suggests that day 12 serum β-hCG values are similar after the transfer of a single fresh blastocyst embryo compared with after a single fresh cleavage ET. To the best of our knowledge, this is the first study in the literature to determine the effect of embryo morphological parameters on serum β-hCG levels in pregnancy and live birth. The blastocyst morphological parameters had an effect on serum β-hCG levels in cycles that resulted in ongoing pregnancy and live birth. The predictive value of serum β-hCG levels might provide a broad generalization for interpreting β-hCG levels and assist clinicians in counselling patients, especially when predicting ongoing pregnancy and live birth. Since β-hCG threshold may vary between different clinics, we recommend that each clinic determine its own threshold.

Authors' roles

G K. contributed to conception and design of the study, review of the manuscript and approval of the final version. I K. contributed to conception and design of the study, surgical intervention, review of the manuscript and approval of the final version. C C. contributed to conception and design of the study, review of the manuscript and approval of the final version. M B A. contributed to conception and design of the study, review of the manuscript and approval of the final version. G U. contributed to conception and design of the study, review of the manuscript and approval of the final version. B A. contributed to conception and design of the study, review of the manuscript and approval of the final version

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None

Conflict of interest

None of the authors have any conflict of interest associated with this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2018.12.001.

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