

Investigation of the effects of trophoctoderm morphology on obstetric outcomes in fifth day blastocyst transfer in patients undergoing in-vitro-fertilization

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Abstract

Objective: Trophoctoderm (TE) cells are the first differentiating cells in embryo development and have epithelial features. TE cells, which associate with implantation of the blastocyst into the uterine endometrium, contribute to the formation of the placenta. Inner cells mass (ICM) together with TE cells are used for determining embryo quality. The aim of this study was to investigate the role of TE and ICM cells on pregnancy outcome in 5th day blastocyst transferred in-vitro-fertilization (IVF) pregnancy.

Material and Methods: This was a retrospective study using data from all patients who applied for blastocyst transfer IVF between January 2015 and March 2019 at the Reproductive Endocrinology and Infertility Center of Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology. ALPHA İstanbul consensus evaluation system was used for grading of the blastocyst. The embryo quality, expansion, ICM and TE morphology of the 5th day transferred blastocyst was assessed, together with abortion rate, live birth rate, pregnancy complications, and pregnancy outcomes.

Results: There was a significantly increased risk of preeclampsia (PE) (7.8% vs 1.1%; $p=0.041$), preterm delivery (PD) (36% vs 17.7%; $p=0.037$), and antenatal bleeding rates (13.6% vs 5%; $p=0.021$) in TE-C compared to the TE-A + TE-B blastocysts. Furthermore, a higher rate of obstetric complications was observed in ICM-C compared to ICM-A and B ($p=0.003$). There was a significant correlation between TE morphology and implantation success, ongoing pregnancy rate, and abortion incidence.

Conclusion: These results suggest that TE cell morphology is related to implantation success and pregnancy outcomes, especially in terms of the risk of abortion, PE, PD, and antenatal bleeding. It may be advisable to counsel women concerning possible poor obstetric outcome due to poor ICM quality. Future prospective and controlled studies are needed to clarify this association. (J Turk Ger Gynecol Assoc 2022; 23: 167-76)

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Introduction

Obstetric complications continue to be one of the leading causes of maternal and perinatal mortality and morbidity. Preeclampsia (PE), for example, is responsible for more than 10% of all maternal deaths worldwide (1), and is still a risk factor for perinatal mortality (2). Similarly, multiple pregnancies, fetal growth restriction (FGR), and prematurity are other obstetric complications associated with perinatal mortality and morbidity (3).

In-vitro-fertilization (IVF) is increasingly used as a therapy for infertility. Although the majority of IVF pregnancies have a good perinatal outcome, several studies have found that IVF pregnancies have a greater prevalence of PE, FGR, preterm delivery (PD), and other obstetric issues compared to normal pregnancy (4). However, it is still unclear if these additional complications in IVF pregnancies are related to the cause of infertility or associated with IVF procedures (5).

Blastocoele expansion, organization of inner cell mass (ICM) and trophectoderm (TE) cells are commonly used to assess blastocyst quality (6). There is very limited data investigating the effect of blastocyst quality on the prognosis and outcome of IVF pregnancies (7-12). Although blastocyst morphology has been shown to be closely associated with implantation success (13-16), there is a scarcity of evidence on the impact of blastocyst morphology on obstetric outcome (12,16,17). Therefore, this study was conducted to investigate whether there was a relationship between blastocyst morphology and obstetric outcome in IVF pregnancies.

Material and Methods

This study was conducted at Akdeniz University Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology IVF Center. After Akdeniz University Faculty of Medicine Local Ethical Committee approval (approval number: 1164, date: 11.12.2019), and obtaining informed consent, the files of patients treated at the IVF Center between 01.01.2015-31.03.2019 were reviewed retrospectively. All patients had 5th day blastocyst transfer. Demographic characteristics of the patients, etiology of infertility, the number of previous IVF treatments and controlled ovarian hyperstimulation (COH) protocols were noted. Additionally, the quality information of the embryos was reviewed from an electronic database. Information related to pregnancy, pregnancy weeks at delivery, delivery type, weights and gender of the newborns, and obstetric complications were obtained from Akdeniz University Medical School Hospital's electronic database and from the patients by phone or interview.

Blastocysts obtained during IVF were examined using the ALPHA İstanbul consensus evaluation system (18). This system

includes separating the blastocyst from the zona pellucida - hatching (grade 1-4), the size of ICM and arrangement (grade A-C), and the number of TE cell and arrangement (grade A-C). In the blastocyst examination procedure, ICM-A is composed of a large number of tightly packed polygonal cells, ICM-B is composed of a small number of easily distinguished, loosely assembled cells, and ICM-C consists of a small number of hardly distinguishable cells. TE-A is of good quality and consists of a large number of continuous cells, TE-B is of medium quality and cells are loosely arranged, with a small number of cells, TE-C is of poor quality and has been reported to consist of a small number of cells. In all cases, one of the best-quality embryos was transferred, and if there were other good quality embryos among the developing embryos, they were frozen and stored. In contrast, for both fresh and frozen embryo transfers (ET), non-viable embryos (development has been arrested for at least 24 hours, or in which all the cells have degenerated or lysed) were not transferred (18). For frozen ETs, viability below 80% is considered as "not survived" and those embryos were not transferred (19).

Gestational age at delivery, weight and gender of the newborns and any complications, including abortus, PE, eclampsia, FGR, oligohydramnios, polyhydramnios, PD, gestational diabetes mellitus and antenatal hemorrhage, were recorded through the hospital registry system and analyzed.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 20.0, was used for statistical analysis (IBM Inc., Armonk, NY, USA). Continuous variables are presented as mean \pm standard deviation, and categorical variables as numbers and percentages. When the number of independent groups was two, the comparison between groups was investigated by Student's t-test, and the difference between more than two groups was compared with ANOVA. Mann-Whitney U test, Kruskal-Wallis analyses, Pearson chi-square and Fisher's exact tests were used as needed.

The possible relations between obstetric complications and ICM, TE and blastocyst expansion were analyzed by using each grade of these parameters.

Results

A total of 291 patients, who had ET at the blastocyst stage on the 5th day, were included in the study. The mean age of the patients was 32.0 ± 4.5 years. The numbers of patients who had frozen ET and fresh ET, in case of having no frozen ET, were 25 (8.8%) and 266 (91.2%), respectively. Fresh ET was only performed in cases when no frozen ET was available. The mean duration of infertility was 5.0 ± 3.7 years. The rate of biochemical pregnancy was 13%, while abortion in the first 12 weeks was 11.2%, and

abortion between 12-20 weeks was 4%. It was found that 71.8% of the women delivered after 20 weeks, and the live birth rate was 71.4%. Thirty percent of pregnancies were found to have at least one obstetric complication.

The patients were evaluated by comparing grades A, B and C in the ICM and TE parameters, and the degree of expansion under the headings of early blastocyst and blastocyst groups. The ICM-A, B, C groups included 126, 81 and 39 patients and the TE-A, B, C groups consisted of 106, 74 and 66 patients, respectively. Transfers of embryos were performed in the early blastocyst stage in 73 patients and in the blastocyst stage in 217 patients.

The rate of biochemical pregnancy in ICM-A, B, and C groups was 7.1%, 18.5%, and 20.5%, respectively (Table 1). While there was no significant difference between ICM group B and C, less frequent biochemical pregnancy was seen in group A ($p=0.019$ for both). Abortion rates in first 12 weeks and between 12-20 weeks was similar among ICM-A, B, and C groups ($p=0.734$). Delivery incidence over 20 weeks was 76.2%, 66.7% and 61.5% in the ICM-A, B, and C groups, respectively ($p=0.132$). Birth weights of the newborns were not significantly different between the ICM-A, B and C groups (Table 1).

Comparison of pregnancy outcome according to TE grade showed that biochemical pregnancy frequency was strongly related to TE grade and was 7 (6.6%), 11 (14.9%), and 14 (21.2%), in the groups of TE-A, B, and C respectively (Table 2). Abortion rates in the first 12 weeks and 12-20 weeks, delivery frequency

beyond 20 weeks, and live birth rates were not significantly different among the TE-A, B, and C groups (Table 2).

The frequencies of biochemical pregnancy, abortion in the first 12 weeks, abortion between 12 and 20 weeks, birth beyond 20 weeks, and the live births rates were not significantly different between the early blastocyst and the blastocyst stages groups (Table 3). Delivery weeks and weight of the newborns were also similar between the early blastocyst and the blastocyst groups (Table 3).

The incidence of obstetric complications in the ICM-A, B, and C groups was significantly different at 39 (46.4%), 24 (49%), and 19 (86.4%), respectively (Table 4). After Bonferroni correction, while there was no significant difference between groups A and B, but a higher rate of obstetric complications was found in group C ($p=0.003$).

At least one obstetric complication was seen in 29 (41.4%) pregnancies in the TE-A, 22 (48.9%) in the TE-B, and 31 (77.5%) in the TE-C groups. While the complication frequency was similar in TE-A and B, the TE-C group had significantly higher complication rate when compared to TE-A and B ($p<0.001$). Non-complicated pregnancy frequency was found similar in TE-A and B. However, TE-C group has significantly lower non-complicated pregnancy rate (Table 5).

Early blastocyst and blastocyst groups were similar in terms of obstetric complication rates and there was no significant difference among groups ($p=0.586$).

Table 1. Pregnancy results among ICM groups

	ICM-A, n (%)	ICM-B, n (%)	ICM-C, n (%)	p
Biochemical pregnancy (%)	9 (7.1) ^a	15 (18.5)	8 (20.5)	0.019
Abortion (first 12 weeks)	17 (13.5)	8 (9.9)	5 (12.8)	0.734
Abortion between 12-20 weeks	4 (3.2)	4 (4.9)	2 (5.1)	0.768
Delivery beyond 20 weeks (%)	96 (76.2)	54 (66.7)	24 (61.5)	0.132
Live birth rates (%)	95 (75.4)	54 (66.7)	24 (61.5)	0.172
Birth weight (grams)	3068±782	2909±722	2958±617	0.463
Delivery weeks (days)	261±23	256±27	258±17	0.503

ICM: Inner cells mass. ICM-A, B, C groups included 126, 81 and 39 patients, respectively. ^a: ICM-C vs B and A

Table 2. Pregnancy results between TE groups

	TE-A, n (%)	TE-B, n (%)	TE-C, n (%)	p
Biochemical pregnancy (%)	7 (6.6) ^a	11 (14.9) ^a	14 (21.2) ^a	0.018
Abortion (first 12 weeks)	13 (12.3)	12 (16.2)	5 (7.6)	0.296
Abortion between 12-20 weeks	4 (3.8)	2 (2.7)	4 (6.1)	0.592
Delivery beyond 20 weeks (%)	82 (77.4)	49 (66.2)	43 (65.2)	0.137
Live birth rates (%)	81 (76.4)	49 (66.2)	43 (65.2)	0.189
Birth weight (grams)	3073±826	2979±679	2902±649	0.488
Delivery weeks (days)	260±25	259±24	256±21	0.726

TE: Trophoctoderm. TE grade A, B, C groups included 106, 74 and 66 patients, respectively. ^a: ($p<0.018$), TE grade A vs B, A vs C and B vs C

Obstetric complications did not significantly differ between TE grade A and B (with Bonferroni correction). However, a higher obstetric complication rate was observed in the TE grade C compared to TE grade A and B. For further analysis, ICM grade A and B were grouped together (high quality group) and compared to grade C (poor quality group) for all further comparisons.

Demographic characteristics of the patients, etiology of infertility, COH protocols and ET (fresh/frozen) information was presented in Table 6. The comparison was made between high-quality embryos versus poor quality groups. Paternal age was significantly higher in the ICM poor quality group ($p=0.047$), body mass indexes (BMI) were significantly lower in the ICM poor quality group ($p=0.041$) and maternal age was significantly higher in the TE poor quality group ($p=0.005$) (Table 6). The other demographic characteristics were similar between the ICM and TE high- and poor-quality groups.

ICM and TE groups were compared according to the type of delivery, live birth rates, mean delivery week, gender and weight of the newborns. Both groups were similar in terms of these parameters and there was no statistically significant difference between the two groups (Table 7).

High and poor quality ICM groups were similar in biochemical pregnancy, abortus in the first 12 weeks and abortus between 12-20 weeks of pregnancy and live births frequency. In the high- and poor-quality groups by TE, only the biochemical pregnancy

rate was significantly higher in the poor-quality TE group when compared to high quality group ($p=0.021$, Table 8).

Comparison of preterm birth and low birth weight infants according to ICM and TE groups showed that only the rate of preterm birth before 37 weeks was significantly higher in ICM and TE poor quality groups when compared to high quality group ($p=0.049$ and $p=0.02$, respectively, Table 9).

Pregnancy complication rates in high and poor quality ICM groups was found to be similar. The TE poor quality group had an increased rate of PE ($p=0.041$), antenatal bleeding ($p=0.021$) and PD ($p=0.037$, Table 10).

The findings from multivariate regression analysis for obstetric complications in the ICM groups are presented in Table 11. Analyses showed that the transfer of poor quality ICM grade C embryos still had predictive value in the development of obstetric complications and poor-quality ET increased the risk of obstetric complications by 6.6 times. Paternal age and BMI (Table 6), which had statistically significant differences between high and low quality ICM groups, lost their significance in multiple logistic regression analysis.

In Table 12, the findings from multivariate regression analysis of factors possibly affecting obstetric complications in the TE group is presented. This showed that transfer of poor-quality TE embryos still carried predictive value in the development of obstetric complications and transfers of poor-quality TE embryos increased the risk of obstetric complications 4.3 times. However, maternal age (Table 6), which had a

Table 3. Pregnancy results in the early blastocyst and the blastocyst groups

	Early blastocyst, n (%)	Blastocyst, n (%)	p
Biochemical pregnancy (%)	13 (17.8)	25 (11.5)	0.168
Abortion (first 12 weeks)	9 (12.3)	28 (12.9)	0.899
Abortion between 12-20 weeks	2 (2.7)	10 (4.6)	0.488
Delivery beyond 20 weeks (%)	49 (67.1)	154 (71.0)	0.535
Live birth rates (%)	49 (67.1)	153 (70.5)	0.586
Birth weight (grams)	2924±754	3001±754	0.569
Delivery weeks (days)	257±27	258±24	0.740

The numbers of patients in the early blastocyst stage and in the blastocyst stage were 73 and 217, respectively

Table 4. Obstetric complications in ICM groups

	ICM-A, n (%)	ICM-B, n (%)	ICM-C, n (%)	p
Pregnancy with OC	39 (46.4)	24 (49.0)	19 (86.4) ^a	0.003
Pregnancy without OC	45 (53.6)	25 (51.0)	3 (13.6)	

ICM: Inner cells mass, OC: Obstetric complication, ^a: $p=0.003$, ICM C vs A and C vs B

Table 5. Obstetric complications in TE groups

	TE-A, n (%)	TE-B, n (%)	TE-C, n (%)	p
Pregnancy with OC	29 (41.4)	22 (48.9)	31 (77.5)	0.001*
Pregnancy without OC	41 (58.6)	23 (51.1)	9 (22.5)	

OC: Obstetric complication, TE: Trophoctoderm, *: $p=0.001$, TE C vs A and TE C vs B

statistically significant difference between high- and low-quality TE groups, lost its significance after multiple logistic regression analysis.

Discussion

Since TE mediates the implantation of the embryo and contributes to placental formation, we aimed to investigate

whether TE quality was associated with pregnancy outcome and placental complications in IVF pregnancies. There are some studies investigating the effect of expansion degree, ICM and TE quality on implantation rate and pregnancy outcomes. Thompson et al. (20) reported that TE morphology and the degree of blastocyst expansion were associated with clinical pregnancy and live birth rates in their 3,510 IVF pregnancies.

Table 6. Demographic characteristics, infertility etiology, protocol and ET information of ICM and TE groups in high- and low-quality embryos

	ICM- A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Maternal age	31.7±4.6	33.2±4.1	0.064	31±4.6	33±4.2	0.005
Paternal age	34.6±5.4	36.5±4.4	0.047	34.5±5.5	35.8±4.8	0.093
Body mass index (kg/m ²)	25±4.8	23±6.4	0.041	25.6±4.9	24.2±5.6	0.104
Infertility duration (years)	5±3.6	4.8±4.4	0.869	4.9±3.6	5.1±4.0	0.709
Primary infertility (%)	144 (69.6)	27 (69.2)	0.967	125 (69.4)	46 (69.7)	0.970
Secondary infertility (%)	48 (23.2)	6 (15.4)	0.280	40 (22.2)	14 (21.2)	0.865
Unexplained infertility etiology (%)	76 (36.7)	12 (30.8)	0.477	64 (35.6)	24 (36.4)	0.907
Male factor (%)	34 (16.4)	4 (10.3)	0.328	31 (17.2)	7 (10.6)	0.203
Tubal factor (%)	15 (7.2)	4 (10.3)	0.518	14 (7.8)	5 (7.6)	0.958
Anovulatory (%)	1 (0.5)	0	1.000	1 (0.6)	0	1.000
Decreased ovarian reserve (%)	59 (28.5)	11 (28.2)	0.970	50 (27.8)	20 (30.3)	0.697
Endometriosis	3 (1.4)	1 (2.6)	0.501	1 (0.6)	3 (4.5)	0.060
Protocol (%), GnRH antagonist	159 (76.8)	28 (71.8)	0.501	139 (77.2)	48 (72.7)	0.464
Long GnRH agonist	15 (7.2)	1 (2.6)	0.277	12 (6.7)	4 (6.1)	0.864
Estradiol priming	5 (2.4)	2 (5.1)	0.307	3 (1.7)	4 (6.1)	0.086
Fresh ET (%)	171 (82.6)	31 (79.5)	0.641	146 (81.1)	56 (84.8)	0.498
Frozen ET (%)	21 (10.1)	3 (7.7)	0.776	19 (10.6)	5 (7.6)	0.485

ICM: Inner cells mass, TE: Trophectoderm, GnRH: Gonadotrophin-releasing hormone, ET: Embryo transfers

Table 7. Pregnancy results of patients giving live birth

	ICM, A + B, n (%)	ICM-C, n (%)	p	TE, A + B, n (%)	TE-C, n (%)	p
Type of birth						
Normal delivery	12 (8.8)	2 (9.1)	1,000	10 (9.2)	5 (11.7)	0.907
Cesarian delivery	120 (91.2)	20 (90.9)		107 (90.8)	38 (88.3)	
Gender of live born						
Female	67 (49.6)	12 (52.2)	0.822	61 (52.1)	19 (44.1)	0.364
Male	68 (50.4)	10 (47.8)		56 (47.9)	24 (56.9)	
Birth weeks (day)	259±24	258±17	0.822	259±24	256±21	0.437
Birth weight (grams)	3,009±762	2,958±617	0.760	3,036±770	2,902±649	0.320

ICM: Inner cells mass, TE: Trophectoderm

Table 8. Abortion and live birth results according to ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Biochemical pregnancy (%)	24 (11.6)	8 (20.5)	0.129	18 (10)	14 (21.2)	0.021
Abortion (first 12 weeks) (%)	25 (12.1)	5 (12.8)	0.896	25 (13.9)	5 (7.6)	0.180
Abortion between 12-20 weeks (%)	8 (3.9)	2 (5.1)	0.714	6 (3.3)	4 (6.1)	0.337
Live birth rates (%)	150 (72.5)	24 (61.5)	0.169	131 (72.8)	43 (65.2)	0.244

ICM: Inner cells mass, TE: Trophectoderm

They also reported that there was no relation between ICM quality and IVF success. We also found no relation between the degree of blastocyst expansion (early blastocyst and blastocyst stages) on pregnancy outcomes or obstetric complications.

In contrast, in their large study, Goto et al. (15) reported that blastocyst expansion was significantly correlated with ongoing pregnancy rate, but the TE and ICM qualities were not different between ongoing pregnancy or delivery rate. Although these authors classified blastocyst expansion of the embryos and compared the blastocyst with a 1-6 staging system and found blastocyst stage 1 and 2 had a higher pregnancy and live birth

rate, they did not clearly state which parameters (expansion, ICM or TE) were more important for ongoing pregnancy and delivery rates (15).

Zaninovic et al. (21) showed that the expansion degree of the blastocyst (ICM morphology) was not related to the implantation rates. However, they found that an increased implantation rate in TE grade A when compared to grade B and C. Similar to the results of Zaninovic et al. (21) and Thompson et al. (20), our results also showed that blastocyst expansion and ICM morphology were not associated with successful implantation, but degree of TE was associated with implantation success. It

Table 9. Comparison of preterm birth and low birth weight infants according to ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Low birth weight baby <2500 g	16 (11.8)	4 (17.4)	0.136	12 (10.7)	8 (20.0)	0.051
Very low birth weight baby <1500 g	6 (4.5)	0	1,000	5 (4.8)	1 (3)	1,000
Preterm birth <37 weeks	30 (27.3)	10 (47.8)	0.049	26 (23.4)	20 (50)	0.002
Premature preterm birth <32 weeks	4 (7)	0	1,000	6 (6.6)	4 (4.8)	0.859

ICM: Inner cells mass, TE: Trophoctoderm

Table 10. Obstetric complication results of ICM and TE groups

	ICM-A + B, n (%)	ICM-C, n (%)	p	TE-A + B, n (%)	TE-C, n (%)	p
Preeclampsia	8 (3.9)	2 (5.1)	0.662	2 (1.1)	5 (7.8)	0.041
Gestational diabetes mellitus	15 (7.2)	5 (12.8)	0.332	12 (6.7)	8 (12.1)	0.165
Antenatal bleeding	14 (6.8)	4 (10.3)	0.442	9 (5.0)	9 (13.6)	0.021
Preterm delivery	34 (16.4)	10 (25.6)	0.168	32 (17.7)	24 (36)	0.037
Fetal growth restriction	8 (3.9)	4 (10.3)	0.089	8 (4.4)	4 (6.1)	0.739
Oligohydramnios	8 (3.9)	1 (2.6)	0.691	7 (3.9)	2 (3)	0.751
Polyhydramnios	2 (1.0)	0	0.538	2 (1.1)	0	0.390
Fetal anomaly/malformations/genetic diseases	13 (6.3)	2 (5.1)	0.783	8 (4.4)	7 (10.6)	0.127

ICM: Inner cells mass, TE: Trophoctoderm

Table 11. The investigation of the combined effects of all possible factors that may have an effect on obstetric complications in ICM group according to logistic regression analysis

Variable	B	S.E.	OR	95% CI	p
Paternal age	0.002	0.036	1,002	0.934-1,074	0.965
Body mass index	0.024	0.035	0.976	0.911-1,046	0.494
ICM (grade C)	1,891	1,432	6,626	1,823-24,091	0.004

B: Regression coefficient, SE: Standard error, OR: Odds ratio. Reference category: ICM grade A + B. ICM: Inner cells mass, CI: Confidence interval

Table 12. Investigation of the combined effects of all possible factors that may have an effect on obstetric complications in TE group according to logistic regression analysis

Variable	B	S.E.	OR	%95 GA	p
Maternal age	-0.004	0.038	0.920	0.925-1,073	0.965
TE (grade C)	1,471	0.429	4,353	1,879-10,086	0.001

B: Regression coefficient, S.E.: Standard error, OR: Odds ratio, TE: Trophoctoderm, CI: Confidence interval. Reference category: TE Grade A + B.

appears that TE may be an important factor in implantation, as it plays an important role in the attachment of the blastocyst into the endometrium, trophoblastic development and uterine invasion. While the quality of TE may affect blastocyst implantation and survival, blastocyst expansion and ICM morphology may have less influence on implantation success (15,21).

Ahlström et al. (22) stated that the morphological grade of TE cells in patients who underwent IVF was the most important marker in predicting live birth, implantation, and abortion rates. In their study, 1117 fresh, day 5 embryos were transferred and they concluded that the TE cell parameter was more important than the expansion grade and ICM grade in the prediction of live birth, implantation, and abortion rates, in line with our findings. We showed that biochemical pregnancy losses in grade C for both ICM and TE were found to be significantly higher than grade A and B. On the other hand, there was no significant difference among the groups A, B, and C in terms of ICM and TE in terms of abortion (first 12 weeks), abortion less than 20 weeks (12-20 weeks), birth above 20 weeks, and live birth rates. The higher biochemical pregnancy losses in TE grade C embryos suggest that functional TE is more important for the development of pregnancy.

There are limited data analyzing the effect of ICM and TE on birth weight. Licciardi et al. (23) found that the birth weight of newborns was related to the ICM degree of the blastocyst. They found that for ICM, the more polygonal cells present in the fetus, the more division will occur and results in greater growth of the fetus. In our study, ICM grades were not related to newborn weights, and the weights of the newborns in grade A, B, and C were similar. Although there was an 86% increase in low birth rate in ICM grade C when compared to ICM grade A + B, this difference was not significant. This may be because TE contribute to development of the embryo by transporting substances that are needed for the development of the fetus and its metabolic activity.

Recently, Alfarawati et al. (24) examined human TE biopsy samples and reported that aneuploidy of TE cells was significantly associated with blastocyst morphology. Braude et al. (25) found that decreased TE grade from A to B and C was related to increased aneuploidy rates and they suggested that false gene expression was related to poor quality of TE and prevention of implantation of the embryo. We did not perform genome analysis of the transferred embryos or from the abortion materials. The finding of increasing biochemical pregnancy losses rates by decreasing TE grade, and increasing chromosomal abnormalities in early pregnancy losses need further research to identify the underlying mechanism.

Honnma et al. (26) investigated the effect of low-quality of blastocysts by performing multiple logistic regression analyses

among blastocyst parameters. A significant relationship was found between TE morphology and pregnancy loss. In our study, ICM and TE groups were compared separately according to obstetric complication rates in grade A, B, and C, and obstetric complication rates were found to be close to each other in grade A and B but were much higher in grade C with a similar pattern emerging for TE grade. Therefore; grades A and B for both parameters were combined and a further comparison with grade C was made. When the abortion and live birth results were analyzed according to TE groups, it was observed that 21.2% of pregnancy loss grade C patients had biochemical pregnancy, 7.6% had an abortion, and 65.2% had a live birth, although there was no statistically significant difference. However, it was observed that the live birth rate in TE grade C (65.2%) was around 10% lower than for grade A + B combined. It is thought that the low number of cases may have affected this finding.

Hill et al. (27) found that implantation and live birth rate were related to TE quality but not ICM grade. However, we found that live birth rates were related to both TE quality and ICM grade. While their multiple logistic regression analysis showed that only patient age and TE grade were significantly associated with implantation and live birth, our analyses showed that only paternal age and BMI of the woman were related to ICM grade and only maternal age was related to TE grade. These differences may be due to differences in study sample sizes. The quality of embryos at the time of ET appears to have an effect on pregnancy outcome.

We also investigated the relationship between obstetric complications and the quality of TE. As TE initiates the placenta, obstetric complications due to abnormal placentation may occur in low-quality TE cases (28,29). The placenta is a complex, short-lived, and multi-functional organ that plays an important role in intrauterine development of the fetus. Any irregularity and insufficiency in placental development can lead to serious complications in both the mother and the growing fetus.

In our study, hypertensive diseases of pregnancy developed in 1.1% of the patients in the TE A + B group and 7.8% in the C group, and the difference was significant, while no statistical difference was found between ICM groups A + B vs C. Abnormal trophoblast invasion is thought to be related to the development of PE. It was suggested that the blastocyst needs healthy TE cells for implantation, which is a complex process that requires endometrial invasion in the early stages of embryonic development (30). Furthermore, the more quality of TE increases, the better implantation would be expected. Abnormal maturation and differentiation of the villous syncytiotrophoblast have been suggested to be related to barrier integrity of the placenta and therefore the increase in the release of necrotic, apoptotic trophoblast fragments

contributes to the development of PE (30). Our results and recent literature suggest that the quality of TE may have a role in the development of PE.

The relationship between quality of blastocyst and the rate of antenatal bleeding were also investigated in our study. Although there was no relation between ICM grade and antenatal bleeding, it was found that TE grade C cases had a more than two-fold increased rate of antenatal bleeding risk when compared to TE A + B grades. Similar to our results, Bouillon et al. (31) also reported that antenatal hemorrhage was related to the quality of blastocyst in cases of IVF. It has been reported that the most common cause of antenatal bleeding is abortion during early pregnancy, and ablation of the placenta and placenta previa in late pregnancy. The common factor in the pathogenesis of these pathologies abnormal placentation (32). It was thought that poor quality of TE may cause insufficient trophoblastic invasion and negative effects on implantation, which may predispose to abortion or placental detachment. The fact that there is no significant difference in the ICM groups in terms of the rate of antenatal bleeding suggests that TE quality is more important for development of the placenta and its function than ICM grade.

Like the mechanisms that initiate normal birth, the pathogenesis of preterm labor is not fully understood. It is thought to be a complex, multifactorial event, in which genetic, pathophysiological, and environmental factors play a role in the pathogenesis of preterm birth. Infection, inflammation, uterine tension, and placental vascular disorders have all been suggested as possible factors for PD (33). In our study, while PD was not related to ICM quality we found PD rates were significantly increased in the TE C group when compared to TE A + B. Decidual hemorrhage has been reported to be related to PD. Maternal smoking, chronic hypertension, PE, and hereditary coagulopathies can cause decidual bleeding and each of these conditions may cause PD by damaging the uterine spiral arteries (34). In light of the recent literature and our results, we suggest that poor TE quality might be a risk factor for PD.

FGR is one of the major obstetric problems associated with impaired early placentation (35). Implantation site disorders appear to be both a cause and a consequence of hypoperfusion in the placental bed (36). This situation is consistent with the relationship between some placental angiogenic factors and hypertensive diseases of pregnancy. Huppertz (36) has suggested that the irregularity of the syncytiotrophoblast cells cause PE, while the irregularity of cytotrophoblast development can lead to FGR. The author stated that the impairment of cytotrophoblast differentiation may impair both villous and extravillous trophoblast expansion,

resulting in failure of trophoblast invasion and incomplete transformation of uterine arteries, which are typical of FGR (36). In our study, FGR was detected in 3.9% of patients in the ICM A + B group and in 10.3% of the patients in the C group. Although FGR was found to be approximately 1.6 times more common in group C than the others, this difference did not reach statistical significance. Although the frequency of FGR increased approximately 38% in the patients in the TE A + B group compared to the patients in the grade C group, this difference was also not significant. Our findings support that the frequency of FGR may be affected by both ICM and TE grading. Once again, the reason for a lack of significant difference may be the low number of cases. Thus, there is a need for larger studies to clarify this issue.

There are no published studies comparing the relationship between obstetric complications and the quality of TE. Studies of this subject have generally been conducted in terms of implantation and live birth rates. The most important strength of our study is the investigation of the relationship between obstetric complications due to placenta and TE quality and it appears that TE quality is an important factor for developing obstetrics complications in IVF-ET cases. The low number of cases and the lack of aneuploidy screening in the blastocyst stage were accepted as limitations of the current study. Once again, larger studies will be necessary to provide more robust evidence concerning this relationship between blastocyst quality and obstetric outcomes.

Study Limitations

There are two main limitations in our study. Firstly, the inclusion of both fresh and frozen ETs in the statistical analyses was a limitation. Thus, we could not perform any statistical comparisons to elucidate the possible differences between fresh and frozen embryos. This limitation could be eliminated in further studies with adequate numbers of both fresh and frozen embryos. Secondly, it is well known that endometrial receptivity plays a very important role in implantation success (37). However, we only investigate the relationship between embryo quality and pregnancy outcomes in this study. In the future, more detailed investigations into the effects of embryo quality and endometrial thickness and vascularity on pregnancy outcomes should be conducted.

Conclusion

The scores of embryo evaluation may have value in assessing the prognosis of pregnancy during the ET process. This study showed that TE may have a greater impact on the development of pathologies of placental origin, especially PE. However, further studies are needed to clarify the relationship between TE quality and pregnancy prognosis in IVF pregnancies.

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