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ASSISTED REPRODUCTION TECHNOLOGIES

Blastocyst expansion score and trophectoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer (eSET): a national study

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Abstract
Purpose To determine which characteristics of blastocyst embryo morphology may predict clinical pregnancy and live birth rates.
Methods A retrospective analysis of data from 3,151 cycles of fresh, non-donor eSET cycles from 2008 to 2009 was performed. Data were obtained from the Society for Assisted Reproductive Technologies (SART) underwent. All eSET were performed at the blastocyst stage. Main outcome measures were clinical pregnancy and live birth rates.
Results Trophectoderm morphology, embryo stage and patient age are highly significant independent predictors of both clinical pregnancy and live birth. Neither inner cell mass morphology nor embryo grade predicted clinical pregnancy or live birth.
Conclusions Better trophectoderm morphology, younger patient age and further blastocyst progression all result in higher clinical pregnancy and live birth rates. Therefore, trophectoderm morphology and blastocyst stage should preferentially be used as the most important factors in choosing the best embryo for transfer.

Keywords Trophectoderm morphology · Embryo grading · Inner cell mass · SART · IVF

Capsule Live birth and clinical pregnancy rates after elective single embryo transfer are predicted by trophectoderm morphology, patient age and blastocyst expansion score.

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Introduction

In Vitro Fertilization (IVF) has emerged as the most successful type of Assisted Reproductive Technology (ART) in the past decade, leading to focused research efforts to improve pregnancy rates with the least potential adverse reproductive outcomes. To maintain acceptable pregnancy rates, transfer of multiple embryos has been employed routinely, which often results in twin and high order multiple pregnancies. Preterm delivery is a known complication of ART, occurring in both singletons and higher order multiple pregnancies after IVF [12]. Pingborg et al., using a large Danish national cohort, demonstrated a 10-fold increased risk of preterm delivery in twin pregnancies after IVF when compared to singleton pregnancies after IVF [12]. Multifetal pregnancy also increases the risk of both maternal and fetal morbidity and mortality with a higher incidence of low birth weight, neonatal intensive care unit admission, cesarean delivery and gestational diabetes [14]. Recent guidelines by leading fertility societies advocating a maximum number of embryos to transfer have led to a decrease in the incidence of more than three embryos transferred, from 70 % in 1999 to 39 % in 2008. (http://www.cdc.gov/ART/ARTReports.htm).

These guidelines have resulted in an increase in Double Embryo Transfer (DET); however DET still frequently results in a twin gestation [14]. The transition to elective Single Embryo Transfer (eSET), defined by the Society for Assisted Reproductive Technologies (SART) as "an embryo transfer in which more than one high quality embryo exists, but it was decided to transfer only 1 embryo" is limited by the ability to select the embryo most likely to result in a pregnancy [13].

We hypothesized that characteristics of blastocyst morphology such as the trophectoderm (TE) morphology, inner cell mass morphology (ICM), embryo grade and stage would serve as significant predictors of both live birth (LB) and clinical pregnancy rates (CP), as analyzed using an SET model from a large cohort of all SART IVF centers in the United States.

Materials and methods

A retrospective cohort IRB-approved study was performed using de-identified data from 3,151 cycles of fresh, non-donor SET cycles reported to the Society for Assisted Reproductive Technologies (SART) from January 1, 2008 to December 31, 2009. The data included only elective single embryo blastocyst transfers on day 5. All patients had at least one embryo available for cryopreservation. The SART database reported patient age, weight, height, infertility diagnosis, days of gonadotropin stimulation, number of oocytes retrieved, pregnancy outcome (CP and LB), embryo trophectoderm morphology (good, fair, poor), embryo inner cell mass morphology (good, fair, poor), embryo grade (good, fair poor), embryo stage (early, expanded, hatching blastocyst) and whether Assisted Zona Hatching (AZH), preimplantation genetic diagnosis (PGD) or intracytoplasmic sperm injection (ICSI) were performed.

Early blastocyst have increasing size of fluid pockets, further differentiation of the trophectoderm and the appearance of cells that will form the inner cell mass. Fully expanded blastocyst show the trophectoderm and the inner cell mass clearly, whereas a hatching blastocyst is preparing to escape from the zona pellucida in preparation for implantation. Trophectoderm and inner cell mass morphology are scored as good, fair and poor (Table 1). The embryo grade, also categorized as good, fair and poor, is a combination of scores including the inner cell mass, trophectoderm, fragmentation and symmetry of the embryo [6].

Multivariate logistic regression analysis (Sigmastat 11.0) was performed to evaluate significant independent predictors of CP and LB. Statistical significance was determined by $p<0.05$. The multivariate analysis model included TE morphology, ICM morphology, embryo stage, embryo grade, patient age, performance of assisted zona hatching (AZH), preimplantation genetic diagnosis (PGD) and intracytoplasmic sperm injection (ICSI).

Table 1 Assignment of inner cell mass and trophectoderm score

Score	Trophectoderm score	Inner cell mass score
Poor	- Very few cells forming a cohesive epithelium	-Difficult to discern inner cell mass with few cells
Fair	- Few cells forming a cohesive epithelium	-Easily discernible with many cells that are loosely grouped together
Good	- Many cells forming a cohesive epithelium	-Inner cell mass is easily discernible, with many cells that are compacted and tightly adhered together

Results

From the 3,151 cycles analyzed, the clinical pregnancy rate was 57.4 % (number of clinical pregnancies/number of embryos transferred), the live birth rate was 48.4 % (number of live births/number of embryos transferred) and the miscarriage rate was 15.7 % (number of clinical pregnancies- number of live births/number of clinical pregnancies). Both the clinical pregnancy rate and the live birth rate decreased with increasing patient age (Table 2). The mean age of all patients was 32.0 years (SD+/− 3.76). The mean number of days of stimulation was 11.6 days (SD+/−2.3) and the mean number of follicles retrieved was 18.8 (SD+/−8.8).

We found that clinical pregnancy rates and live birth rates (Table 3) were significantly predicted by TE morphology, embryo stage and patient age. Inner cell mass morphology was not predictive of either live birth rate ($p=0.2653$) nor clinical pregnancy rate ($p=0.9349$). Similarly, embryo grade did not predict live birth rate ($p=0.2374$) or clinical pregnancy rate ($p=0.0583$). Trophectoderm morphology was associated with LB rates of 50 % (good), 41.9 % (fair) and 30 % (poor). Blastocyst stages were associated with LB rates of 50 % (hatching), 49.5 % (expanded) and 36.7 % (early). LB rates in patients <35 years of age remain good across trophectoderm morphology scores. An age-related decline in LB rate is noted in each subsequent age group, with the exception of age greater than 40 with poor trophectoderm score (Table 4).

Finally, we compared LB rates based on embryo stage and TE morphology. The live birth rates were greatest in the hatching blastocyst group [52 % ($n=353$) with a good score, 51.1 % ($n=788$) with a fair score and 43.7 % ($n=80$) with a poor score]. They declined slightly in the expanded blastocyst group [46.5 % ($n=61$) with a good score, 42.7 % ($n=138$) with a fair score and 25.9 % ($n=14$) with a poor score]. Live birth rates were the lowest in the early blastocyst group [31.4 % ($n=11$) with a good score, 32.9 % ($n=12$) with a fair

Table 2 Pregnancy rates by age per elective single embryo transfer

	Clinical pregnancy[a]	Live birth[b]
Age <35	60 % (1397/2326)	51.8 % (1205/2326)
Age 35–37	55.2 % (344/623)	43.3 % (270/623)
Age 38–40	37.9 % (63/166)	26.5 % (44/166)
Age >40	18.2 % (6/33)	15.1 % (5/33)

[a] Data given as % (Clinical pregnancy/Total number of patients)

[b] Data given as % (Live Birth/Total number of patients)

score and 38.7 % ($n=12$) with a poor score], however the lowest live birth rate was in the expanded blastocyst, poor trophectoderm score group.

Discussion

SART collects data from over 85 % of the assisted reproductive technology (ART) clinics in the United States in order to provide guidelines and outcomes to the ART community and the public. Our retrospective study analyzed 3,151 cycles of elective single embryo blastocyst transfers over a 2-year period from the SART database. We show that blastocyst expansion stage, trophectoderm morphology, and patient age significantly and independently predict both clinical pregnancy and live birth. Age remains a strong predictor of outcome of its own accord.

Multiple changes occur at the interface from the cleavage stage embryo to the blastocyst. Transfer at the blastocyst stage is thought to confer an advantage for embryos that may be more likely to implant due to greater synchrony between the embryo and the endometrium [7]. Blastocyst transfer also allows for the selection of more competent embryos with a concomitant decrease in the multiple birth rates, as fewer embryos are needed for transfer in order to achieve pregnancy. A meta-analysis revealed that in well-selected patients with high number of embryos at the 8-cell stage, blastocyst transfer confers both increased pregnancy and live birth rates [4].

Morphology of the expanding blastocyst's trophectoderm (destined to become the placenta) and inner cell mass (destined to become the fetus) has also been used as a predictor of implantation [5]. The morphologic grading system developed by Gardner and Schoolcraft [6] in which the inner cell mass, trophectoderm and blastoceoele status are graded, is commonly used to assess blastocyst. Richter et al. graded embryos by measurement of blastocyst diameter, trophectoderm cell number and inner cell mass size and shape. These investigators found that a larger size of the inner cell mass was more predictive of implantation [18]. There was no difference in trophectoderm cell number between implanting and non-implanting embryos. In contrast, a single center recent study of 1117 single blastocyst transfers concluded that the trophectoderm was the most important predictor of live birth outcome [1]. Even with well-developed grading systems for blastocyst, the most important characteristic of a single embryo at time of transfer has not been identified.

Our data confirms previous data by Ahlstrom et al., Hill et al. and Zaninovic et al. who reported that trophectoderm morphology significantly predicted live birth outcome [1, 8, 21]. Ahlstrom et al. also found that the degree of blastocele expansion (embryo stage) predicted live birth outcome. A recent study of 1,087 frozen elective single embryo transfers by Honnma et al. found that trophectoderm morphology is statistically significantly related to ongoing pregnancy and miscarriage rates [9]. In contrast to our study, they did not find embryo stage to be a statistically significant predictor of ongoing pregnancy. We analyzed over twice as many single embryo transfer blastocyst data as Ahlstrom et al. and four times as many as Hill et al. [1, 8]. Our study remains the largest study to date confirming that trophectoderm morphology and embryo stage are indeed the best predictors of clinical outcomes.

Previous studies have indicated that the inner cell mass is more important in predicting pregnancy outcome than the trophectoderm [3, 18], however recent data including our

Table 3 Trophectoderm score, embryo stage and patient age predict clinical pregnancy and live birth

	Odds of clinical pregnancy	p value (CP)	Odds of live birth	p value(LB)
Trophectoderm score	OR 1.32 [1.10–1.58]	$p=0.0022$*	OR 1.23 [1.04–1.46]	$p=0.0131$*
Embryo stage	OR 1.19 [1.03–1.37]	$p=0.0142$*	OR 1.15 [1.01–1.31]	$p=0.0289$*
Inner cell mass	OR 0.93 [0.68–1.28]	$p=0.9349$	OR 1.18 [0.88–1.57]	$p=0.2653$
Patient age	OR 0.96 [0.94–0.98]	$p=0.0030$*	OR 0.95 [0.94–0.97]	$p<0.0001$*
Embryo grade	OR 1.38 [0.98–1.95]	$p=0.0583$	OR 1.21 [0.87–1.67]	$p=0.2374$

Data given as Odds Ratio (Confidence Intervals).
* denotes significance

Table 4 Live birth rate by age and trophectoderm score

	Good	Fair	Poor
Age <35	54 %(n=973/1802)	46.4 %(n=167/360)	38 %(n=36/95)
Age 35–37	46 % (n=214/466)	33.3 %(n=35/105)	33 %(n=10/31)
Age 38–40	26.4 %(n=28/105)	29.0 %(n=11/38)	21.4 %(n=3/14)
Age >40	14.3 %(n=3/21)	0 %(n=0/6)	40 %(n=2/5)

Data given as % (Live Births/ Total Number of Patients)

own show the contrary. Trophectoderm morphology may be directly related to aneuploidy. In an analysis of 194 euploid embryos and 238 aneuploid embryos Alfarawati et al. found a 2.5-fold increase in the probability of aneuploidy in embryos with poor trophectoderm morphology as compared to those with good trophectoderm morphology [2]. This effect on aneuploidy is seen at the blastocyst but not the cleavage stage, confirming the advantage of morphologic assessment at the blastocyst stage.

Embryos are usually selected for transfer based on morphological characteristics that many have tried to characterize and standardize, at both the cleavage and blastocyst stage. Some investigators have suggested pronuclear morphology during the zygote stage as a guide to embryo development, specifically the number and distribution of nucleoli in the pronuclei, nuclear size, alignment, nucleoli alignment, position of the nuclei within the zygote [11, 19]. Others use morphological attributes of fragmentation and blastomere number/uniformity during the cleavage stage as a predictor of implantation and successful pregnancy [16, 20]. Vernon et al. validated the SART Day-3 morphology grading system by showing that live birth rates decreased with decreasing embryo grade [20]. Racowsky et al. further assessed live birth rates in regards to cell number, fragmentation and symmetry of the blastomere [16] Live birth rates increased with increasing cell number up to the 8-cell stage. They noted a 24.3 % live birth rate at the 8 cell stage and 16.2 % with greater than 8 cells as compared to 2.9 % with less than 2 cells. Lower live births were noted with greater fragmentation and asymmetry [16]. Balaban et al. used a system that factors in all morphological parameters from the pronuclear zygote to the blastocyst stage with formation and quality and found that cleavage stage quality did not determine blastocyst quality [3].

It is well established that degree of blastocele expansion is an important factor in successful pregnancy outcome [10, 17]. Racowsky et al. [15] found superior embryo viability in expanded (52.3 % viable) and expanding (53.8 % viable) blastocysts in contrast to viability rates in early blastocyst (21.4 % viable) or morulae (14.3 % viable). Our study is the first to look at live birth rates by degree of blastocele expansion and trophectoderm morphology in conjunction. Clinical pregnancy rates remained over 25 % in all groups with the highest rates being demonstrated in the hatching blastocyst group regardless of trophectoderm score. The majority of patients had a hatching blastocyst for transfer. The lowest live birth rate was in the expanded blastocyst with poor trophectoderm score, however it is important to note that the number of patients in that group was very small (n=14). Of note there was one single embryo blastocyst transfer which resulted in live birth of triplets, and 27 of which resulted in twins. This confirms that even with elective single embryo blastocyst transfer the risk of multiple births is not zero.

Conclusions

The large number of patients from ART centers nationwide included in this study confirms and extends the trend in recent data that trophectoderm morphology and embryo stage are the most important predictors of live birth outcome, not the inner cell mass or embryo grade. Strengths of our study include data from multiple IVF centers across the country and a large number of single embryo transfers. As we only included elective single embryo transfer we have clear outcome data on clinical pregnancy and live birth rates as associated with a particular embryo. We were also able to analyze clinical pregnancy as well as live birth data. Limitations to this study include its retrospective nature, and that specific cycle stimulation protocols were not available for analysis. It is possible that embryo grade could be affected by lab quality, and embryos with a poor grade would likely not be chosen for SET. Finally, the majority of the data was in patients less than age 35 who are usually good prognosis patients with higher quality blastocyst, therefore the conclusions of this study may not be directly applicable in older or poor prognosis patients.

While advances in trophectoderm biopsy and array comparative genomic hybridization (array CGH) provide for definitive answers regarding aneuploidy, it is still important to be able to select blastocyst based on morphology if biopsy and array CGH are not available. As elective single embryo transfer becomes the way of the future to decrease multi-fetal births and improve neo-natal outcomes we encourage the development of methods for choosing the best embryo for optimal pregnancy outcome.

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References

1. Ahlström A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. Hum Reprod. 2011;26(12):3289–96.
2. Alfarawati S, Fragouli E, Colls P, Stevens J, Gutiérrez-Mateo C, Schoolcraft WB, et al. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertil Steril. 2011;95(2):520–4.
3. Balaban B, Urman B, Isiklar A, Alatas C, Aksory S, Mercan R, et al. The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. Hum Reprod. 2001;16(11):2357–61.
4. Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev. 2007;4, CD002118.
5. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000;73(6):1155–8.
6. Gardner D, Schoolcraft W. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Toward reproductive certainty (fertility and genetics beyond 1999). Carnforth: Parthenon Publishing; 1999. p. 378–88.
7. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Hum Reprod. 1998;13(12):3434–40.
8. Hill M, Richter K, Heitmann R, Graham J, Tucker M, Decherney A, et al. Trophectoderm grade predicts outcomes of single–blastocyst transfers. Fertil Steril. 2013;99(5):1283–9.
9. Honnma H, Baba T, Sasaki M, Hashiba Y, Ohno H, Fukunaga T, et al. Trophectoderm morphology significantly affects the rates of ongoing pregnancy and miscarriage in frozen-thawed single-blastocyst transfer cycle in vitro fertilization. Fertil Steril. 2012;98(2):361–7.
10. Kovacic B, Vlaisavljevic V, Reljic M, Cizek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online. 2004;8(6):687–94.
11. Payne JF, Raburn DJ, Couchman GM, Price TM, Jamison MG, Walmer DK. Relationship between pre-embryo pronuclear morphology (zygote score) and standard day 2 or 3 embryo morphology with regard to assisted reproductive technique outcomes. Fertil Steril. 2005;84(4):900–9.
12. Pinborg A, Loft A, Nyobe Anderson A. Neonatal outcome in a Danish national cohort of 8602 children born after in vitro fertilization or intracystoplasmic sperm injection: the role of twin pregnancy. Acta Obstet Gynecol Scand. 2004;83(11):1071–8.
13. Practice Committee of the American Society for Reproductive Medicine; Practice Committee of the Society for Assisted Reproductive Technology. Guidelines on number of embryos transferred. Fertil Steril. 2009;92(5):1518–9.
14. Practice Committee of the American Society for Reproductive Medicine. Multiple gestatation associated with infertility therapy: an American Society for Reproductive Medicine Practice Committee opinion. Fertil Steril. 2012;97(4), April 2012.
15. Racowsky C, Combelles CM, Nureddin A, Pan Y, Finn A, Miles L, et al. Day 3 and day 5 morphological predictors of embryo viability. Reprod Biomed Online. 2003;6:323–31.
16. Racowsky C, Vernon M, Mayer J, Ball D, Behr B, Pomeroy K, et al. Standardization of grading embryo morphology. J Assist Reprod Genet. 2010;27:437–9.
17. Rehman KS, Bukulmez O, Langley M, Carr BR, Nackley AC, Doody KM, et al. Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer. Fertil Steril. 2007;87(5):1041–52.
18. Richter KS, Harris DC, Daneshmand ST, Shapiro BS. Quantitative grading of a human blastocyst: optimal inner cell mass size and shape. Fertil Steril. 2001;76(6):1157–67.
19. Scott L, Alvero R, Leondires M, Miller B. The morphology of human pronuclear embryos is positively related to blastocyst development and implantation. Hum Reprod. 2000;15(11):2394–403.
20. Vernon M, Stern JE, Ball GD, Wininger D, Mayer J, Racowsky C. Utility of the national embryo morphology data collection by the Society for Assisted Reproductive Technologies (SART): correlation between day-3 morphology grade and live-birth outcome. Fertil Steril. 2011;95(8):2761–3.
21. Zaninovic N, Berrios R, Clarke RN, Bodine R, Ye Z, Veeck LL. Blastocyst expansion, inner cell mass (ICM) formation, and trophectoderm (TM) quality: is one more important for implantation? Fertil Steril. 2001;76:S8.