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Single blastocyst transfer yields similar pregnancy rates compared with multiple cleavage embryo transfer, with reduced twin rate, in patients with low number of fertilized oocytes

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Abstract

Background: In patients with low numbers of embryos, there is not yet consensus on whether to extend culture to the blastocyst stage, especially due to the risk that some or all of the embryos will not make it to the blastocyst stage. The objective of our study was to evaluate pregnancy outcomes in patients with a low number of fertilized oocytes (< 4), comparing single blastocyst transfer to one or more cleavage embryo transfer.

Results: We analyzed 6795 cycles from the 2014–2105 Society for Assisted Reproductive Technology (SART) registry. All patients were ≤ 38 years old, had less than four fertilized oocytes, and were undergoing first fresh in vitro fertilization (IVF) transfer. Primary outcomes were clinical pregnancy (CP), live birth (LB), and miscarriage rate in both cleavage stage transfer and single blastocyst transfer. A secondary outcome was the rate of twin gestation. The comparison of interest in day of transfer included (1) single blastocyst vs single cleavage and (2) single blastocyst vs multiple cleavage stage. The association between day of transfer and primary outcome was investigated using logistic regression, controlling for the age, race/ethnicity, BMI, smoking, gravidity, parity, infertility diagnoses, and assisted hatching.
Single blastocyst transfer was associated with an increased odds of CP (adjusted OR 2.03) and LB (adjusted OR 1.86) when compared to single cleavage transfer, and no statistically significant association was observed when comparing single blastocyst transfer to multiple cleavage embryo transfer for CP (adjusted OR 0.94) and LB (adjusted OR = 0.88). The odds of having twins among single blastocyst transfer was significantly lower compared to those odds that among multiple cleavage stage transfer (unadjusted OR 0.09).

Conclusions: While pregnancy outcomes are similar between single blastocyst and multiple cleavage embryo transfer, the twin rate is reduced significantly among the single blastocyst transfers in patients with a low number of fertilized oocytes.

Keywords: In vitro fertilization, Live birth, Clinical pregnancy, Poor responder

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Background

In the current era of in vitro fertilization (IVF), many practitioners choose to extend embryo culture to the blastocyst stage as blastocyst transfers have shown an association of an improved clinical pregnancy (CP) rate and live birth (LB) rate [1]. In a 2016 Cochrane review of 27 randomized clinical trials, the LB and CP rates were significantly higher in the blastocyst versus the cleavage group after a fresh transfer [2]. In a randomized control trial by Papanikolaou et al. in patients under age 36, higher clinical pregnancy and live birth rates were seen with a single blastocyst compared to a single cleavage stage transfer [3].

Blastocyst transfer is also associated with other additional benefits. Foremost, with blastocyst transfer, there is a decreased risk of multiple gestation as less embryos can be transferred with a similar live birth rate. Blastocyst transfer is also more physiologic, as this is the stage when the embryo normally enters the uterus. Finally, blastocyst transfer allows for better selection of a viable embryo by morphology and also allows for the possibility of preimplantation genetic testing through embryo biopsy. As such, a single blastocyst transfer is now the preferred method of transfer, as it leads to improved pregnancy rates and a reduced risk of multiple gestation.

In extending culture to blastocyst stage, there is a risk that some or all of the embryos will not make it to blastocyst stage. In a study of 142 patients that received extended culture to the blastocyst stage, 22.5% of the embryos did not survive the extended culture [4]. In a meta-analysis by Papanikolaou et al., clinical pregnancy and live birth rates were higher in patients after a blastocyst transfer; however, these patients also had a higher cancelation rate [5]. In a prospective randomized study by Levitas et al., blastocyst transfer had a higher implantation rate in patients who failed to conceive in 2 or more day 2/3 transfers, though a higher cancelation rate was seen [6]. It is of interest that in this study, the cancelation rate was reduced if the decision to transfer was made on day 3, a post hoc conclusion.

In patients with low numbers of embryos, there is not yet consensus on whether to extend these embryos to blastocyst. The objective of our study was to evaluate pregnancy outcomes in patients with a low number of fertilized oocytes (< 4), comparing single blastocyst transfer to one or more cleavage embryo transfer.

Methods

All autologous first fresh IVF cycles from the 2014–2015 Society for Assisted Reproductive Technology (SART) database were analyzed. We included cycles that were a first fresh IVF cleavage or blastocyst stage transfer and if age was less than or equal to 38. Cleavage stage transfer included cycles with one or more embryos transferred, and blastocyst transfer only included single embryo transfer. Cycles that had less than four fertilized oocytes and that used conventional IVF and intracytoplasmic sperm injection were included while donor oocyte cycles, gestational carrier cycles, any cycles that used PGT-A, and cycles that involved use of a combination of fresh embryos with cryopreserved embryos were excluded.

Demographic information included patient's age, race/ethnicity, BMI (defined as weight in kilograms divided by height in meters squared), follicle stimulating hormone dose, gravity, parity, smoking status, and infertility diagnosis. In addition, cycle characteristics including the number of oocytes retrieved, the number of fertilized oocytes, the number of good quality embryos cryopreserved, and whether assisted hatching was performed are listed.

The IVF and pregnancy outcomes include CP rate (defined by in The International Glossary on Infertility and Fertility Care as the presence of ultrasonographic visualization of one or more gestational sacs or definitive signs of pregnancy), miscarriage rate (defined at the spontaneous loss of a CP before 22 weeks gestational age in which the embryo or fetus is nonviable), live birth (LB) rate (defined as the delivery from a women after 22 weeks completed gestational age), singleton rate (the rate of singleton pregnancies among all cycles with a heartbeat), and twin rate (the rate of twin pregnancies among all cycles with a heartbeat) [7]. All IVF and pregnancy outcomes refer to the first fresh embryo transfer outcome.

Statistical analysis was performed using R version 3.5.3 [8]. Demographic and patient characteristics were summarized using mean with standard deviation (SD), median with interquartile range (IQR), or frequency with percentage by day of transfer (single blastocyst, single cleavage, or multiple cleavage). The comparison groups of interest included (1) single blastocyst transfer vs single cleavage transfer and (2) single blastocyst transfer vs multiple cleavage transfer. Student's *t* test and Wilcoxon rank sum test were used for continuous variables for between groups listed in (1) and (2) above, while Chi-square Goodness-of-Fit test was used between group comparisons of categorical variables. We investigated the association between day of transfer and each primary outcome (CP, LB, and miscarriage) using logistic regression, controlling for age, race/ethnicity, BMI, smoker, gravidity, parity (nulliparous and multiparous), infertility diagnoses (male factor, endometriosis, polycystic ovarian syndrome, diminished ovarian reserve, tubal factor, uterine factor, unexplained, and other reason), and assisted hatching. E-value was calculated for each primary outcome to indicate the strength of residual confounding [9, 10]. The association between missingness in BMI (17.4%), parity (0.5%), and gravida (0.4%) with CP, LB, and miscarriage were assessed using chi-square tests.

Results

The study cohort consisted of 6795 cycles that met the inclusion and exclusion criteria. Among these, 1138

cycles underwent blastocyst stage transfer and 5657 cycles underwent cleavage stage transfer. The patient baseline characteristics are presented in Table 1. The overall mean age of patients was 33.5 (SD = 3.3), and the majority of these women were non-Hispanic White (41%). Common infertility diagnoses were male infertility (36.0%), diminished ovarian reserve (28.5%), and unexplained infertility (15.9%). The median number of available oocytes was 5 [IQR = 3, 8], and the median number of fertilized oocytes was 2 [IQR = 2, 3]. There were very few excess embryos cryopreserved, 89% of the women had no excess embryos.

The pregnancy outcomes are as follows. The CP rate in this study cohort was 39% ($n = 2642$). Among cycles with CP, the miscarriage rate was 15% ($n = 403$). The LB rate for the study cohort was 33% ($n = 2215$). Lastly, among cycles with heart beats, rate of singleton was 83% ($n = 2049/2471$) and rate of twins was 16% ($n = 401/2471$). Among single cleavage stage embryo transfer, singleton rate was 99% ($n = 487/493$) and twin rate was 1% ($n = 5/493$). Among multiple cleavage stage embryo transfer, singleton rate was 74% ($n = 1119/1522$) and twin rate was 25% ($n = 383/1522$). Among blastocyst embryos, singleton rate was 97% ($n = 443/456$) and twin rate was 3% ($n = 13/456$). Of note, 81.5% ($n = 2015/2471$) of cycles were cleavage stage transfers.

The regression results are presented in Tables 2 and 3. Since no statistically significant association between missingness in covariates (BMI, parity, and gravida) and the primary outcomes were observed, complete case analysis was performed. We observed that single blastocyst transfer was associated with an increased odds of clinical pregnancy (OR 2.03; 95% CI = 1.72, 2.41; E value = 2.20) and live birth (OR 1.86; 95% CI = 1.55, 2.22; E value = 2.07) when compared to single cleavage transfer. No statistically significant association was observed

Table 1 Patient demographics and characteristics

Characteristics	Single blastocyst	Single cleavage		2+ cleavage	
	($n = 1138$)	($n = 1925$)	P value[a]	($n = 3732$)	P value[a]
Patient's age, mean (SD)	33 (3.4)	33.6 (3.2)	< 0.0001	33.7 (3.3)	< 0.0001
Race/ethnicity, *n* (%)			0.08		0.03
Non-Hispanic White	52 (4.6%)	138 (7.2%)		234 (6.3%)	
Non-Hispanic Black	74 (6.5%)	126 (6.5%)		196 (5.3%)	
Other (Asian/American Indian)	456 (40.1%)	742 (38.5%)		1586 (42.5%)	
Hispanic/Latino	118 (10.4%)	200 (10.4%)		333 (8.9%)	
Unknown	438 (38.5%)	719 (37.4%)		1383 (37.1%)	
Recipient's BMI, mean (SD)	26 (5.9)	26.1 (6.1)	0.75	26.4 (6.1)	0.09
Parity, *n* (%)			0.09		0.69
Multiparous	197 (17.4%)	383 (20.0%)		668 (18.0%)	
Nulliparous	935 (82.6%)	1532 (80.0%)		3046 (82.0%)	
Gravidity, median [Q1, Q3]	0 [0, 1]	0 [0, 1]	0.31	0 [0, 1]	0.80
Smoker	45 (4.0%)	81 (4.2%)	0.80	155 (4.2%)	0.83
Infertility diagnoses, *n* (%)					
Male infertility	405 (35.6%)	673 (35.0%)	0.75	1,371 (36.7%)	0.50
Endometriosis	140 (12.3%)	198 (10.3%)	0.10	400 (10.7%)	0.15
Polycystic ovarian syndrome	135 (11.9%)	208 (10.8%)	0.40	426 (11.4%)	0.72
Diminished ovarian reserve	279 (24.5%)	602 (31.3%)	< 0.0001	1057 (28.3%)	0.01
Tubal ligation/hydrosalpinx/other	176 (15.5%)	297 (15.4%)	1.00	584 (15.6%)	0.92
Uterine factor infertility	62 (5.4%)	86 (4.5%)	0.26	150 (4.0%)	0.05
Unexplained infertility	174 (15.3%)	286 (14.9%)	0.79	618 (16.6%)	0.33
Other infertility diagnosis	122 (10.7%)	174 (9.0%)	0.14	248 (6.6%)	< 0.0001
FSH dose IU, mean (SD)	3601.9 (1776.8)	3622.6 (1876.3)	0.77	3592.6 (1728.9)	0.88
No. of available oocytes, median [Q1, Q3]	6 [4, 9]	4 [2, 7]	< 0.0001	6 [4, 8]	0.84
No. of excess embryos cryopreserved, median [Q1, Q3]	0 [0, 1]	0 [0, 0]	< 0.0001	0 [0, 0]	< 0.0001
Number of fertilized oocytes, median [Q1, Q3]	2 [1, 3]	1 [1, 2]	< 0.0001	3 [2, 3]	< 0.0001

SD standard deviation, *Q1* 25th percentile, *Q3* 75th percentile
[a]Compared to single blastocyst

Table 2 Pregnancy outcomes by single blastocyst and single cleavage

Pregnancy outcomes	Single blastocyst ($n = 1138$)	Single cleavage ($n = 1925$)	OR (95% CI)	P value
Clinical pregnancy rate	489 (43%)	526 (27%)	2.03 (1.72, 2.41)[a]	< 0.0001
Live birth rate	398 (35%)	439 (23%)	1.86 (1.55, 2.22)[a]	< 0.0001
Miscarriage rate	84 (17%)	84 (16%)	1.10 (0.76, 1.59)[a]	0.60
Total number of cycles with heart beats	456	493	–	
Singleton rate	443 (97%)	487 (99%)	0.42 (0.16, 1.11)[b]	0.08
Twins rate	13 (3%)	5 (1%)	2.86 (1.01, 8.10)[b]	0.05
Triplets	0	1	–	
Quadruplets	0	0	–	

[a]Adjusted for age, race, BMI, smoker, parity, gravida, infertility diagnoses, and assisted hatching
[b]Unadjusted

when comparing single blastocyst transfer to multiple cleavage embryo transfer for clinical pregnancy (OR 0.94; 95% CI = 0.81, 1.09) and live birth (OR = 0.88; 95% CI = 0.76, 1.03). In addition, the association between day of transfer and miscarriage between single blastocyst transfer and single cleavage embryo transfer or between single blastocyst transfer and multiple cleavage embryo transfer was not statistically significant (OR = 1.10; 95% CI = 0.76, 1.59, and OR = 1.35; 95% CI = 0.99, 1.83), respectively.

In the unadjusted regression model, the odds of having a singleton among single blastocyst transfer was not significantly different than that among single cleavage stage transfer (OR 0.42; 95% CI 0.16, 1.11) but was significantly higher compared to multiple cleavage stage transfer (OR 12.27; 95% CI 6.99, 21.55). On the other hand, the odds of having twins among single blastocyst transfer was significantly higher than that among single cleavage stage transfer (OR 2.86; 95% CI = 1.01, 8.10) and significantly lower than that among multiple cleavage stage transfer (OR 0.09; 95% CI 0.05, 0.15).

Discussion

In patients with less than four fertilized embryos, it is difficult to know whether extending culture to blastocyst will improve the patient's outcome. In our respective cohort study, we found that pregnancy outcomes (CP, LB) are significantly improved with single blastocyst transfer compared to single cleavage embryo transfer and similar compared to multiple cleavage transfer in patients with < 4 fertilized oocytes. We also found that the twin rate is significantly lower with a single blastocyst transfer compared to multiple cleavage stage transfer, decreasing pregnancy complications. Even though blastocyst transfer was not associated with improved outcomes with respect to multiple cleavage embryo transfer, the benefit of blastocyst transfer lies in a reduced multiple rate. In a previous study of our center analyzing SART data, multiple pregnancy rate in patients age 35–37 was 40.5% with a 2 blastocyst transfer versus 1.7% with a single blastocyst transfer and in patients 38–40 was 34% with a 2 blastocyst transfer versus 2.0% with a single blastocyst transfer [11].

Table 3 Pregnancy outcomes by single blastocyst and 2+ cleavage

Pregnancy outcomes	Single blastocyst ($n = 1138$)	2+ cleavage ($n = 3732$)	OR (95% CI)	P value
Clinical pregnancy rate	489 (43%)	1627 (44%)	0.94 (0.81, 1.09)[a]	0.43
Live birth rate	398 (35%)	1378 (37%)	0.88 (0.76, 1.03)[a]	0.12
Miscarriage rate	84 (17%)	235 (14%)	1.35 (0.99, 1.83)[a]	0.06
Number of embryos transferred, median [Q1, Q3]	–	2 [2, 2]	–	
Total number of cycles with heart beats	456	1,522	–	
Singleton rate	443 (97%)	1119 (74%)	12.27 (6.99, 21.55)[b]	< 0.0001
Twins rate	13 (3%)	383 (25%)	0.09 (0.05, 0.15)[b]	< 0.0001
Triplets	0	19	–	
Quadruplets	0	1	–	

Q1 25th percentile, *Q3* 75th percentile
[a]Adjusted for age, race, BMI, smoker, parity, gravida, infertility diagnoses, and assisted hatching
[b]Unadjusted

This question of whether a patient with a low number of fertilized oocytes will have a blastocyst to transfer that leads to a live birth is clinically applicable. However, there are very few studies which address this question. A recent study found higher CP and LB rates with cleavage stage transfer versus blastocyst transfer when only one embryo was available for transfer [12]. Instead, many researchers have investigated blastocyst formation and pregnancy outcomes in order to predict which embryo is most capable of producing a pregnancy [1]. Time-lapse morphology has been used to predict which cleavage stage embryos will form blastocysts; however, this technology is often cost prohibitive [13, 14]. Metabolomics and proteomics are currently being investigated to help predict the selection of the optimal embryo, but it is not ready for clinical use [1].

In patients with low numbers of embryos to choose from, the concern is the lack of an embryo to transfer with extended culture. In a study by Jones et al. of good prognosis patients that desired a blastocyst transfer, 7% of patients did not have a blastocyst to transfer after going forward with extended culture [15]. In poor prognosis patients or in patients with few numbers of embryos, a canceled cycle due to no embryo to transfer is of concern.

Strengths of our study include that the data was abstracted from a large national database. This is also one of the first studies to evaluate pregnancy outcomes in patients with either a cleavage stage or blastocyst transfer and a low number of fertilized eggs.

Limitations of our study include primarily limitations of our data set. The major limitation of our study is the absence of data on how many cycles did not have a transfer due to the cleavage stage embryos failing to reach the blastocyst stage. We also acknowledge that a majority of the cycles in this data set (83%) were cleavage stage transfers and not blastocyst transfers. Due to the nature of the SART dataset, there is missing data in the database and there may be errors in data reporting. The data is also from 2014 to 2015, and it is not the most current data available. Our study was a retrospective cohort study in design. A limitation of our data is the difference in the baseline characteristics in our subgroups, such as the younger age in single blastocyst transfers versus cleavage stage transfers, which could be a confounding factor. Another limitation is that with an E value around 2, there must exist a confounder with both the exposure and the outcome with an effect size of at least 2. This confounder or confounders could contribute to the results as listed above.

Another major limitation of our data set is that embryo quality information was not available, especially as this relates to embryo quality on day 3 of the blastocyst transfers. It is possible that the embryos on day 3 that had better morphology were the embryos that underwent extended culture and the embryos that had worse morphology were transferred as a cleavage stage embryo.

It is interesting and important to note that most of the patients in our study did have a cleavage stage transfer rather than a blastocyst transfer (likely due having less than four fertilized eggs). However, the pregnancy outcomes (CP, LB) were still improved in the blastocyst group. This finding does not eliminate the possibility of having no embryos for patients who opted for extended culture. This information could be very important for counseling these patients on their options.

Conclusions

In this retrospective cohort study, pregnancy outcomes (CP, LB) are significantly improved with single blastocyst transfer compared to single cleavage embryo transfer and similar compared to multiple cleavage embryo transfer in patients with < 4 fertilized oocytes. Multiple pregnancy rates are significantly reduced with single blastocyst transfer compared to multiple cleavage transfer. Based on these findings, IVF programs and patients should consider pursuing extended culture with single blastocyst transfer if more than one embryo with good morphology is available on day 3.

Abbreviations

CP: Clinical pregnancy; IVF: In vitro fertilization; LB: Live birth; SART: Society for Assisted Reproductive Technology

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Authors' contributions

SS, KA, and SM designed the study. TT and CP conducted the statistical analysis. All authors contributed to the writing and revising of the manuscript and approved the final version.

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Availability of data and materials

Data for our study can be found in the SART database.

Ethics approval and consent to participate

Approval was granted by the Duke institutional review board for this retrospective cohort study (Pro00089887). No consent was necessary due to the deidentified nature of our data.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

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