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Single Vitrified Blastocyst Transfer Maximizes Live born Children per Embryo while Minimizing Preterm Birth

Kate Devine, M.D.[1,2*], **Matthew T. Connell, D.O.**[1,**], **Kevin S. Richter, Ph.D.**[2,**], **Christina I. Ramirez, M.D.**[3], **Eric D. Levens, M.D.**[2], **Alan H. DeCherney, M.D.**[1], **Robert Stillman, M.D.**[2], and **Eric A. Widra, M.D.**[2]

[1]National Institutes of Health, Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development

[2]Shady Grove Fertility Reproductive Science Center

[3]Walter Reed National Military Medical Center, Department of Obstetrics and Gynecology

Abstract

Objective—To compare live birth rates, blastocyst to live birth efficiency, gestational age, and birth weight in a large cohort of patients undergoing single versus double thawed blastocyst transfer.

Design—Retrospective cohort study.

Setting—Large private ART practice.

Patient(s)—All autologous transfers (FBT) of one or two vitrified-warmed blastocysts from January 2009 through April 2012. Only supernumerary blastocysts with good morphology (BB or better) were vitrified.

Intervention(s)—Single or double FBT.

Main outcome measure(s)—Live birth, blastocyst to live birth efficiency, pre-term birth, low birth weight

Results—1696 FBTs were analyzed. No differences were observed in patient age, rate of embryo progression, or post-thaw blastomere survival. Double FBT yielded a higher live birth per transfer; however, 33% of births from double FBTs were twins versus only 0.6% of single FBTs. Double

[2*]Corresponding authors' present address: Shady Grove Fertility, 2021 K St NW #701, Washington, DC, kate.devine@nih.gov; kate.devine@integramed.com.

[**]Authors should be considered similar in author order.

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FBT was associated with significant increases in preterm birth and low birth weight, the latter of which was significant even when the analysis was limited to singletons. 38% of blastocysts transferred via single FBT resulted in a live born child versus only 34% with double FBT. This suggests that two single FBTs would result in more live born children with significantly fewer preterm births, when compared to double FBT.

Conclusions—Single FBT greatly decreased multiple and preterm birth risk while providing excellent live birth rates. Patients should be counseled that a greater overall number of live born children per couple can be expected when thawed blastocysts are transferred one at a time.

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Keywords

vitrification; frozen embryo transfer; single embryo transfer; preterm birth; low birth weight

Introduction

The modern treatment goal for the infertile patient is the birth of one healthy child at a time. In the past, multiple embryos were transferred to increase pregnancy and live birth rates per fresh embryo transfer procedure. High rates of multiple gestation result from this practice. The increase in multiple births observed over the last 25 years is largely attributable to ART, with the proportion of twin births attributable to IVF conception rising steadily through 2011 (1). Many consider multiple births to be an adverse outcome in ART due to associated maternal and neonatal morbidity as well as the economic impact (2, 3). Risks associated with multiple pregnancy include prematurity, intrauterine growth restriction, low birth weight, cerebral palsy, learning disabilities, and developmental delay (4).

Strategies to curb the multiple birth epidemic aim to increase implantation via blastocyst transfer (5) and to improve cumulative live births per oocyte retrieval, via elective single embryo transfer coupled with future transfer of frozen-thawed embryos (6, 7). The practice of elective single embryo transfer is rising in the U.S., having increased from 1% in 2002 to 12% in 2011; however, the practice is underutilized in comparison to European countries such as Sweden (73.3%) (8,9). The number of frozen-thawed embryo transfers performed in the U.S. increased by 82.5% from 2006 to 2012 (10). It is well established that single embryo transfer reduces multiple birth and improves neonatal endpoints while providing acceptable live birth rates among single versus double *fresh* embryo transfer (6,11,12, 13, 14). However, there is a paucity of data comparing single versus double frozen-thawed blastocyst transfer, and study endpoints have been limited to pregnancy outcomes (clinical pregnancy, live birth, multiple birth, miscarriage, and ectopic) (15, 16). Recently, there has been a call for more substantial reporting of neonatal outcomes as opposed to ART cycle outcome alone (17, 18).

Our aim was to compare live birth rates, blastocyst-to-live birth efficiency, clinical pregnancy and multiple pregnancy rates, as well as preterm birth and birth weight in a large cohort of patients undergoing single versus double vitrified-thawed blastocyst transfer.

Materials and methods

Study Design

We performed a retrospective cohort study of all autologous single and double vitrified-thawed blastocyst transfers with known live-birth outcomes performed at our center from January 2009 through April 2012. The study was performed at the Shady Grove Fertility and Reproductive Science Center in Rockville, Maryland. Schulman Associates Institutional Review Board approved the retrospective review and analysis of data collected during routine clinical care.

Patients

All transfers of one or two autologous vitrified-warmed blastocysts from January 2009 through April 2012 were analyzed. Transfers of more than two embryos were excluded.

Vitrification/Warming

Modified Gardner and Schoolcraft grading was used to assess developing blastocysts (19). One of two senior embryologists reviewed all embryo grading, as is routine clinical practice at our center. Supernumerary blastocysts with an inner cell mass/trophectoderm grade of greater than or equal to BB by day 5 or 6 post oocyte retrieval underwent vitrification. Over the duration of the study, all embryo cryopreservation-thawing at our center was performed via a vitrification-warming method, performed as previously described (20).

Endometrial preparation protocol

Patients underwent ovarian and uterine suppression using combined hormonal oral contraceptive pills. After baseline hormonal assessment and transvaginal ultrasound documenting no ovarian cysts and a thin endometrium, patients were started on intramuscular estradiol valerate 4 mg every third day. When serum estradiol reached a level greater than 200 pg/mL and the endometrial double thickness was greater than or equal to 8mm on transvaginal ultrasound, patients were started on 50mg daily intramuscular progesterone in oil.

Embryo selection

Number of blastocysts transferred was determined by patients and their physicians as per routine clinical practice. Decisions regarding the number of cryopreserved blastocysts to transfer at our center are generally made based on a number of factors, including but not limited to, age of the patient at the time of cryopreservation, prior birth history; previous unsuccessful embryos transfers; the outcome of fresh embryo transfer cycles from which cryopreserved embryos were derived; the number of cryopreserved embryos available; medical and uterine factors; and infertility diagnosis. Though these are the primary factors generally considered in counseling, they did not all result in statistically confirmed differences in number of embryos transferred. Single embryo transfers were more likely to be performed in patients with a history of prior birth (both in general and specifically in the cycle from which cryopreserved embryos were derived), with fewer previous failed embryo transfers, and with uterine factor infertility. Single embryo transfers were also more

common among patients with fewer cryopreserved embryos available, in part because in some cases, lack of multiple embryos precluded a choice.

Blastocyst quality grading at the time of vitrification did not play a role in decisions regarding how many embryos to transfer. All embryos achieved fully expanded blastocyst stage (expansion grade 4) prior to vitrification, and cryopreservation was limited to embryos with minimum grades of B for both the inner cell mass and trophectoderm. Thus, all cryopreserved blastocysts were considered good quality embryos with similarly high implantation potential. In addition, pre-vitrification grades were not linked to individual cryopreserved embryos, so this information was not available for use at the time of warming and transfer.

Embryo transfer

On the sixth day of progesterone replacement, ultrasound-guided blastocyst transfer was performed using the after-load technique, in which the outer sheath of the transfer catheter is left in place to maintain access to the uterine cavity.

Outcomes and Definitions

The primary outcome was live birth. Secondary outcomes were blastocyst-to-live birth efficiency, biochemical pregnancy (detectable serum hcg), clinical pregnancy, multiple gestation, gestational age at birth, and birth weight. Clinical pregnancy was defined as an intrauterine gestational sac on ultrasound. Live birth was defined as birth of a live infant greater than or equal to 24 weeks' gestation. Blastocyst-to-live birth efficiency was calculated for each transfer as the number of live infants born greater than 24 weeks' gestation divided by the number of blastocysts transferred. Blastocyst-to-live birth efficiency for each group was calculated as the mean of the per-transferred embryo efficiencies.

Preterm birth, very preterm birth, and extremely preterm birth were defined as birth prior to 37 weeks, 32 weeks, and 28 weeks, respectively (21). The following definitions were used for birth weight: low birth weight was defined as weight less than 2500 grams, very low birth weight was defined as less than 1500 grams, and extremely low birth weight was defined as less than 1000 grams (22).

Statistical analysis

Chi square analysis and Fisher's exact test were used as appropriate to compare pregnancy, live-birth, and multiple gestation rates between patients who had single versus double thawed blastocyst transfer. Student's t-test was used to evaluate continuous parameters. Sub-analyses of singleton pregnancies were used to control for multiple gestation when assessing intergroup differences in preterm birth and low birth weight. A $p<0.05$ was used for the definition of statistical significance.

Generalized estimating equation (GEE) modeling was used to compare the efficiency of conversion of transferred blastocysts to live born children. This analysis accounted for the potential correlation in outcomes among repeated cycles by the same subjects and estimated the effect of each independent factor as associated with embryo to live birth efficiency, after

adjusting for the other variables in the model. Patient characteristics accounted for in the GEE modeling included age at the time of blastocyst vitrification and at the time of warming and transfer, infertility diagnoses, body mass index, parity, and number of prior unsuccessful transfers. Treatment-associated characteristics accounted for in the GEE modeling included freeze-all cycles, birth outcomes from fresh transfers of embryos from the same cohort, numbers of vitrified blastocysts, use of PGD/PGS, post-retrieval day of vitrification, percentage of blastocysts surviving vitrification and warming, and the percentage of cells that remained intact after warming.

Statistical analysis was performed using SPSS Statistics Version 22 (IBM Corporation, Armonk, NY).

Results

Cycle Characteristics

A total of 1696 transfers were analyzed. Characteristics of single and double transfer groups are presented in Table 1 on a *per transfer* basis. All associated data were updated for each transfer in instances where more than one transfer was performed in the same patient over the course of the study period. Single and double transfer groups were similar with regard to age at vitrification, day (post retrieval) of blastocyst vitrification, proportion of blastocysts surviving vitrification-warming, and mean proportion of intact cells after warming. The double transfer cohort had a younger age at transfer, a higher mean BMI, and were more likely to have PCOS as an infertility diagnosis. Single transfers were more likely to have male factor or uterine factor as an associated infertility diagnosis. However, when analyzed on a *per patient* basis, differences in BMI and uterine factor were no longer statistically significant, indicating the contributions of multiple transfers. Somewhat surprisingly, freeze-all in the antecedent cycle and pre-implantation genetic diagnosis/screening of transferred embryos were more common in the double tranfer group. This phenomenon may be related, in part, to financial considerations.

Cycle Outcomes, Single versus Double Vitrified-Warmed Blastocyst Transfer

Outcomes of vitrified-warmed embryo transfer cycles are depicted in Figure 1. Biochemical pregnancy, clinical pregnancy, and live birth were significantly higher in the double embryo transfer group. Multiple pregnancy was a frequent outcome in the double embryo group and occurred following fewer than 1% of pregnancies resulting from single embryo transfers (42.7% vs. 0.7%, $p<0.0001$).

Blastocyst-to-Live Birth Efficiency, Single versus Double Vitrified-Warmed Blastocyst Transfer

When comparing live birth per embryo transferred, single embryo transfer had significantly more live births per embryo transferred (38.0% vs. 33.8%, $p<0.04$). The relative increase in live birth per embryo transferred was 12% among those transferred one at a time versus those transferred in pairs. The improved live birth efficiency of embryos in the single embryo group was the combined result of higher implantation (gestational sacs per embryo transferred) (50.3 vs. 47.4% $p=0.17$) and a lower rate of embryonic/fetal demise per

implantation (proportion of gestational sacs not progressing to live birth) (24.5 vs. 28.6, p=0.12); though individually these were not statistically significant.

Among the 1696 transfers cycles evaluated, there were 1389 individual subjects (patients), which were included in the GEE model. Subjects underwent a range of 1 to 7 vitrified blastocyst transfer cycles. As shown in Supplemental Table 1, a positive coefficient indicates an association with higher embryo to live birth efficiency, and each coefficient represents the independent contribution of the associated variable. After adjusting for potentially confounding patient and treatment associated characteristics, the percentage of children born per transferred vitrified blastocyst was found to be significantly higher for transfers of one blastocyst compared to transfers of two blastocysts. The adjusted mean percentages of children per transferred blastocyst were 38.08% versus 33.52%. This 4.56% (95% CI 0.33–8.79%; p=0.034) absolute difference translates to a 13.6% relative advantage in embryo to birth efficiency for transfers of one versus two embryos. This difference in embryo to birth efficiency appeared to be primarily the result of a higher implantation rates (i.e. the percentages of confirmed gestational sacs observed through ultrasound observation per transferred embryo) for the blastocysts transferred singly rather than in pairs, although this trend did not reach the level of statistical significance and is thus inconclusive (GEE-adjusted mean implantation rates = 50.9% versus 46.8%, *p*=0.074). An unexpected finding of the model was a significant negative coefficient for parity. The contribution of prior cesarean section, which was not available in our dataset for analysis, may explain this in part. In addition, the inverse relationship between parity and embryo efficiency lost statistical significance when transfers to women with two or more prior births (N=106) were excluded.

Gestational Length, Single versus Double Vitrified-Warmed Blastocyst Transfer

Mean gestational age was one week longer for the single embryo group (38 weeks and 5 days for the single embryo group compared to 37 weeks and 5 days in the double embryo group, p<0.0001). Figure 2 outlines the frequency and severity of prematurity in each group. Overall, rate of preterm birth was lower among the single embryo group (15.1% vs. 25.7%, p< 0.001). Very preterm birth was six times more likely to occur in the double embryo transfer group (p< 0.001). Differences in gestational age at birth were the result of early births in twin gestations. Singletons were born on average three weeks later than twins (38 weeks 5 days vs. 35 weeks 5 days, p <0.0001). There was no difference in preterm birth between the single and double transfer groups when the analysis was limited to singleton deliveries.

Birth Weight, Single versus Double Vitrified-Warmed Blastocyst Transfer

Low birth weight, very low birth weight, and extremely low birth weight were much more common in the double embryo transfer group (Figure 3). More than half of twin infants experienced low birth weight (51.8% vs. 7.4% singletons, p<0.0001). However, twin pregnancy did not fully account for lower birth weight among the double embryo transfer group. When limiting analysis of birth weight to singleton births, the single blastocyst transfer group maintained a higher average birth weight than double blastocyst transfer group (3,426 g vs. 3,245 g, p=0.001).

Discussion

The shared goal of infertile couples and providers of fertility care should be the birth of a healthy infant, rather than a positive pregnancy test. In the current analysis of 1,696 vitrified-thawed blastocyst transfers, embryo-to-live birth efficiency, gestational length and birth weight were improved with single embryo transfer relative to transfer of two thawed embryos. To our knowledge, this represents the largest analysis to date comparing live birth outcomes between single and double frozen blastocyst transfer and the only such analysis to assess gestational length, birth weight, and embryo-to-live birth efficiency.

Extended culture enabling blastocyst transfer and vitrification are two advances in ART that have significantly increased the number of live births per stimulated IVF cycle. Embryo cryopreservation allows for transfer of a limited number of embryos while preserving embryos for future use; however, concerns have persisted regarding the implantation potential of frozen-thawed embryos (23, 24).

Two prior studies have evaluated the impact of single versus double frozen blastocyst transfer. A study by Yanaihara et al. in 2008 comparing single versus double frozen blastocyst transfer in 562 cycles found no statistically significant difference in clinical pregnancy or live birth rates between the two groups. However, ectopic and twin pregnancy rates were higher in the dual blastocyst transfer group, suggesting that single transfer should be the preferred method (15). In contrast, in a 2011 evaluation of 243 cycles, Berin et al. found significantly higher clinical pregnancy, live birth, and twin pregnancy rates with double frozen blastocyst transfer, consistent with the findings of the current study (16). Neither study analyzed embryo-to-live-birth efficiency or included data on preterm birth or low birth weight. Fauque et al. in 2010 evaluated fresh and frozen single versus double cleavage stage embryo transfer. In their subset of 97 frozen-thawed day 2 embryo transfers, no differences in clinical pregnancy, live birth, multiple birth rate, gestational age, or birth weight were found; however, there were only 7 live births for analysis per group (25). A recent study by Ishihara et al. evaluated frozen and fresh single embryo transfer in a large cohort and considered neonatal and maternal outcomes. The authors found that single frozen blastocyst transfer was associated with 66% singleton live birth per transfer, with 93% of these deliveries occurring at term, and that fewer than 4% were small for gestational age (26). However multiple embryo transfers were not included in the analysis for comparison.

The present study adds to this previous literature in that it considers a large, homogeneous cohort, including only transfers of high quality vitrified embryos at blastocyst stage and evaluates neonatal endpoints. Furthermore, this is the first study assessing embryo-to-birth efficiency for single versus double frozen-thawed embryo transfers. As expected, the transfer of two frozen-thawed blastocysts had a modest, but significant increase in biochemical pregnancy, clinical pregnancy, and live birth. Importantly, however, twin pregnancy was also significantly higher in the double blastocyst transfer group, whereas twin pregnancies accounted for less than 1% of those resulting from single blastocyst transfers.

Our analysis of transferred blastocyst to live born child efficiency provides compelling evidence that transferring embryos singly rather than in pairs would result in a significantly higher percentage of live born children per transferred embryo. While the inherent weaknesses of a retrospective study make this a less than ideal method of assessing this possibility, our analytical methods provided rigorous control over potentially confounding factors. The use of GEE modeling, in which the level of analysis was individual patients rather than treatment cycles, controlled for any correlation in outcomes within individual subjects while allowing for valid computation of probabilities without sacrificing power by limiting the analysis to one cycle per patient. The analysis adjusted for patient characteristics including age at treatment, BMI, prior parity and failed transfers, and infertility diagnoses including uterine factor which is well-known to adversely affect implantation. The analysis also adjusted for cycle-specific factors such as the outcome of fresh transfers from the same embryo cohort, cycles in which all viable embryos were cryopreserved, use of PGD/PGS, number of cryopreserved embryos, day of vitrification (indicative of the time needed to develop to the expanded blastocyst stage), percentage of warmed embryos to survive, and the post-warming percentage of intact cells in transferred blastocysts.

One factor that was not included in the analysis was blastocyst grade at the time of cryopreservation. However, our policy of cryopreserving only high quality blastocysts would have the effect of limiting differences in this variable. In addition, this information was not used, and was in fact not available, when embryos were warmed and decisions regarding how many embryos to transfer were made. Blastocyst grade at vitrification was thus effectively randomized with respect to the number of embryos subsequently transferred in cryopreserved embryo transfer cycles, and should not have had a confounding affect on treatment outcomes.

The adjusted estimate indicated an absolute difference of 4.56% (38.08% versus 33.52%) in favor of single embryo transfer in the percentage of children born per embryo transferred, amounting to a relative difference of 13.6% more children per embryo transferred for single compared to double embryo transfers. It should be noted that while statistically significant, the confidence interval for this estimate is wide. Thus, the true advantage of single embryo transfer may be considerably less than (or greater than) this estimate. Ideally, this benefit would be assessed through prospective randomized trials comparing single to double embryo transfer. However, in the absence of this gold standard level of evidence, the statistically significant 13.6% relative advantage derived from this well-controlled retrospective analysis represents our best estimate as to the increased efficiency of single embryo transfer.

This finding of greater embryo to birth efficiency with single embryo transfer is not unexpected. Assuming there exists some variation in cycle-to-cycle endometrial receptivity, as may be suggested by implantation defects associated with suboptimal endometrial thickness (27, 28) and elevated serum progesterone in stimulated cycles (29) and inter-cycle variability in histology (30), transferring embryos one at a time would minimize the potential for all embryos to be transferred to a non-receptive uterus, figuratively avoiding “putting ones eggs all in one basket”. In addition, single embryo transfers might avoid

theoretical competition among embryos for implantation sites, nutrient supply, or other maternal resources, and would therefore maximize their opportunity for development.

Length of gestation and birth weight were also improved with the transfer of one blastocyst. The majority of observed adverse outcomes (preterm birth and low birth weight) occurred among twin gestations, which made up 49.3% of babies born from double blastocyst transfer versus 1.2% of those resulting from single transfer. However, when controlling for multiple pregnancy (i.e. limiting comparison of birth weight to live singleton births where a single gestational sac was seen on initial ultrasound), double blastocyst transfer was still associated with a lower birth weight. Though this phenomenon has not been previously reported among frozen blastocyst transfers, De Sutter et al. found that singletons born after fresh double embryo transfer had a lower birth weight than those born after fresh single embryo transfer. Furthermore, the 120 gram difference the authors reported is similar to the 181 gram difference found in the present study (31). Numerous studies have demonstrated lower birth weight among singletons born from ART (not limited to single embryo transfer) versus spontaneously conceived singletons (32–37). However, De Neubourg et al. observed no difference in the birth weight of spontaneously conceived singletons versus singletons born after fresh ART with single embryo transfer (38), suggesting transfer of multiple embryos as the causative factor of lower birth weight among ART singletons in general.

There exist several possibilities for our observed difference in singleton birth weight. Given the retrospective nature of the study, intrinsic differences in the single and double transfer groups may have played a role. Therefore, we conducted a stepwise multiple regression model of singleton birth weight. With age at vitrification, age at transfer, BMI, infertility diagnosis, day of vitrification (relative to oocyte retrieval), proportion of cells surviving vitrification-warming, infant sex, and number of blastocysts transferred included as potential covariates, only infant sex and number of embryos transferred remained significantly associated with singleton birth weight. The association of double transfer with lower birth weight among singleton pregnancies persisted. Given that our singleton birth weight analysis was limited to pregnancies noted to have one sac from first ultrasound, it is also unlikely that the higher birth weight observed among singletons following single transfer is accounted for by ‘vanishing twin syndrome,’ i.e. the spontaneous miscarriage of one twin in the first trimester (39, 40). In addition, an analysis of initial post-transfer serum hcg level (collected two weeks post transfer) among patients with singleton gestations via single versus double transfer revealed no difference. If early spontaneous reductions of twins to singletons in the double transfer group were the reason for lower observed singleton birth weights, one might expect a higher early hcg level in this group, which we did not find.

Strengths of our study include large, well-matched cohorts, inclusion of neonatal endpoints, and a focus on the clinically-relevant question of what differences in outcome can be expected when choosing between transferring one versus two high quality vitrified-thawed blastocysts. The results are generalizable and relevant to clinical decision-making regarding number of vitrified-warmed blastocysts to transfer. The main limitation is its retrospective design with inherent potential for bias. In general clinical practice, well-counseled patients opting for single embryo transfer may represent a better prognosis group. However, in the current study, the single embryo group was not limited to those with more than one embryo

available. Had the study group included only those patients *electing* for transfer of a single embryo, our finding of increased embryo to live birth efficiency in this group would likely have been amplified.

Conclusions

Patient counseling is an important aspect of reproductive care. The current study demonstrates that in the modern era of ART, where the ultimate goal is a healthy newborn, patients should be counseled that sequential transfer of a single frozen-thawed embryos can be expected to result in a greater number of children born overall. Furthermore, these newborns will be less likely to experience low birth weight and preterm birth, due primarily to the reduction in twin pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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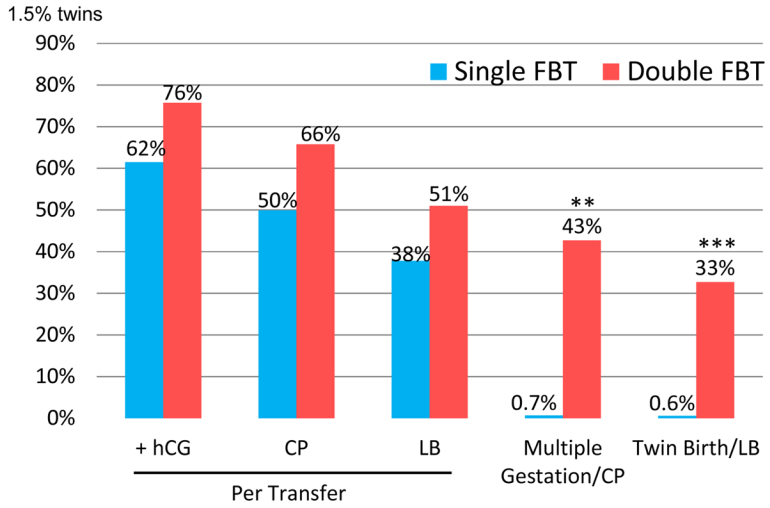

Figure 1.

Pregnancy Outcomes Single vs. Double Frozen Blastocyst Transfer (FBT)*

* p<0.0001 for all comparisons

** 41.5% twins, 0.7% triplets, 0.4% quads in the DFBT group; No high order multiple pregnancies in the SFBT group

*** No high order multiple births in either group (Triplets and quads in DFBT group spontaneously reduced)

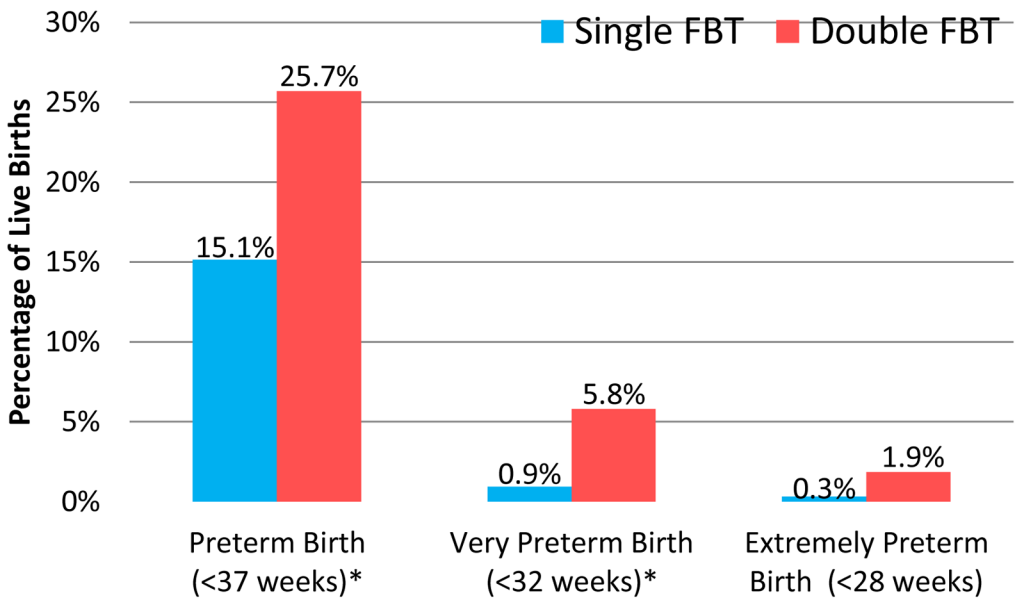

Figure 2.

Incidence and Severity of Prematurity, Single vs. Double Frozen Blastocyst Transfer (FBT)
Mean gestational age at birth was 38w5d ± 13d in the single embryo group vs. 37w5d ± 21d with double transfer ($p<0.0001$). The difference was attributable to increased twin delivery in the double embryo transfer group.

* $P<0.001$

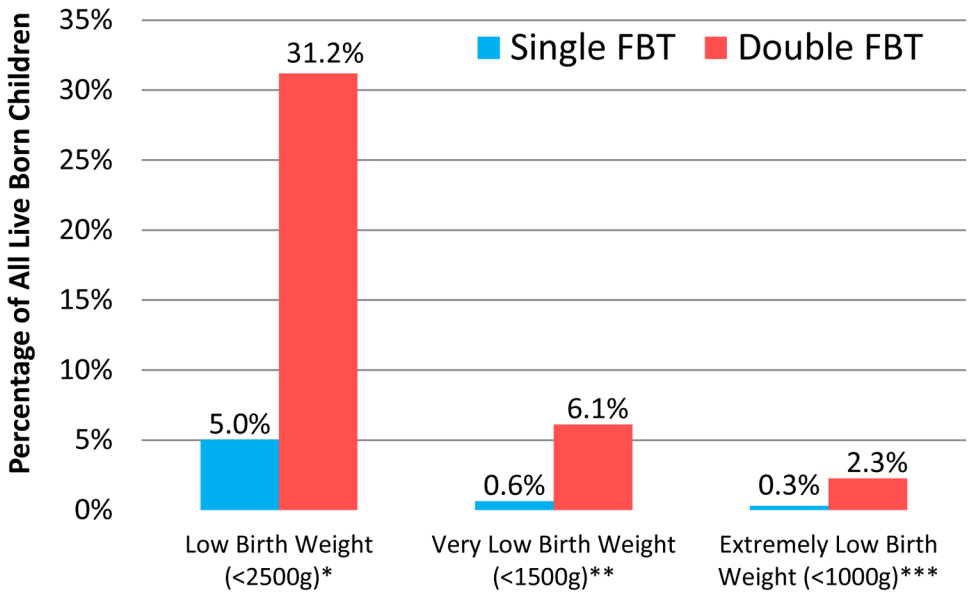

Figure 3.

Incidence and Severity of Low Birth Weight, Single vs. Double Frozen Blastocyst Transfer (FBT)

Mean birth weight was 3418 ± 605 grams in the single embryo group vs. 2837 ± 770 grams with double transfer ($p<0.0001$). A statistically significant decrease in birth weight persisted in the double transfer group when analysis was limited to singleton deliveries.

*P<0.0001; **P<0.001; ***P=0.024

Table 1

Cycle Characteristics Per Transfer

Number of Vitrified-Warmed Blastocysts transferred	**One**	**Two**	***p*-value**
Number of Transfers (Number of Patients)	**845 (711)**	**851 (760)**	--
Patient Characteristics Per Transfer (± SD)			
Age at Vitrification (years)	33.7 ± 3.9	33.5 ± 4.0	0.34
Age at Warming/Transfer (years)	34.5 ± 3.9	34.0 ± 4.0	0.012
Body Mass Index	24.9 ± 5.0	25.4 ± 5.3	0.044
Number Prior Transfers w/o Birth	1.37 ± 1.26	1.40 ± 1.08	0.64
Parity	0.58 ± 0.65	0.36 ± 0.62	<0.0001
Prior Cycle Characteristics Per Transfer; N (%)			
Freeze-all (No transfer) in Fresh Cycle	81 (9.6)	113 (13.3)	0.017
Live Birth from Fresh ET	175 (22.9)	60 (8.1)	<0.0001
Infertility Diagnosis Per Transfer; N (%) transfers carrying diagnosis			
Endometriosis	61 (7.2)	42 (4.9)	0.053
Male Factor	315 (37.3)	277 (32.6)	0.041
PCOS	128 (15.2)	176 (20.7)	0.0030
Tubal Factor	108 (12.8)	110 (12.9)	0.93
Uterine Factor	47 (5.6)	23 (2.7)	0.0030
Blastocyst Characteristics Per Transfer (± SD)			
Day of Vitrification Post-Retrieval/Fertilization	5.64 ± 0.57	5.60 ± 0.54	0.14
Blastocysts Surviving Vitrification/Warming (%)	95.6 ± 14.9	95.5 ± 12.6	0.84
Intact Cells per Transferred Blastocyst (%)	95.5 ± 5.4	95.2 ± 5.2	0.29
Number of Vitrified Embryos Available for Transfer	3.4 ± 3.0	4.6 ± 2.6	<0.0001
PGD/PGS**; N (%)**	54 (6.4%)	82 (9.6%)	0.014