Elective single versus double blastocyst-stage embryo transfer in women aged 36 years or older

He Cai[1], juanzi Shi[1], Ben Mol[2], Stephan Gordts[3], and Hui Wang[1]

[1]Affiliation not available
[2]Monash University Medical Centre
[3]Life Expert Centre

March 07, 2024

Abstract

Objective: To evaluate if elective single-blastocyst transfer (eSBT) could be adopted in women aged 36 or older? Design: Retrospective cohort study. Setting: Reproductive medicine center at a tertiary hospital. Population: Women aged [?]36 years received IVF ovarian stimulation cycles and had [?] two blastocysts. Out of 429 women, 240 underwent eSBT and 189 double-blastocyst transfer (DBT) in the first transfer cycle. The subsequent frozen-thawed embryo transfer cycles were a combination of single- and double- blastocyst transfers. Methods: Analysis was stratified for patients in age groups 36-37, 38-39 and [?]40, taking into account the quality of the blastocyst transferred, as graded by morphological examination. Main outcomes measures: Cumulative livebirth rate (cLBR) from all transfers (fresh and frozen) accruing from a single oocyte retrieval. Results: The cLBR was 74.2% (178/240) versus 63.0% (119/189) after eSBT versus DBT, respectively (aOR: 1.09 (0.68, 1.75)). Time to live birth did not vary significantly between the two groups (HR: 0.85 (0.68,1.08)). The total number of children born was 194 after eSBT (162 singletons and 16 pairs of twins) versus 154 (84 singletons and 35 twins) pairs of after DBT. The odds ratios for preterm birth (<37 weeks' gestation) (0.37 (0.21-0.64)), and low birthweight (<2.5 kg) (0.31 (0.16, 0.60)) were all lower in eSBT group than in DBT group. Conclusions: In women aged [?]36 years old with at least two blastocysts, cLBR following single- versus double- blastocyst transfer was comparable while the odds of multiple livebirths and adverse perinatal outcomes were reduced.

Elective single versus double blastocyst-stage embryo transfer in women aged 36 years or older

Running title

Elective single-blastocyst transfer in women aged [?]36 years

He Cai, M.D.,[a] Juanzi Shi, M.D., Ph.D.,[a] Ben W. Mol, M.D., Ph.D.,[b,c,d] Stephan Gordts, M.D.,[e] Hui Wang, M.D.[a]

Corresponding author

Hui Wang, M.D.

Assisted Reproduction Center, Northwest Women's and Children's Hospital, Xi'an, People's Republic of China.

Mail: 73#, Houzaimen North Street, Xi'an, China

E-mail: wanghuiart@126.com

[a] Assisted Reproduction Center, Northwest Women's and Children's Hospital, Xi'an, People's Republic of China.

[b] Medicine Department, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia.

[c] University of Melbourne Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Victoria, Australia

[d] Pregnancy Research Centre, Department of Maternal-Fetal Medicine, Royal Women's Hospital, Parkville, Victoria, Australia

[e] Leuven Institute for Fertility & Embryology, Schipvaartstraat 4, 3000 Leuven, Belgium.

Objective: To evaluate if elective single-blastocyst transfer (eSBT) could be adopted in women aged 36 or older?

Design: Retrospective cohort study.

Setting: Reproductive medicine center at a tertiary hospital.

Population: Women aged [?]36 years received IVF ovarian stimulation cycles and had [?] two blastocysts. Out of 429 women, 240 underwent eSBT and 189 double-blastocyst transfer (DBT) in the first transfer cycle. The subsequent frozen-thawed embryo transfer cycles were a combination of single- and double- blastocyst transfers.

Methods: Analysis was stratified for patients in age groups 36-37, 38-39 and [?]40, taking into account the quality of the blastocyst transferred, as graded by morphological examination.

Main outcomes measures: Cumulative livebirth rate (cLBR) from all transfers (fresh and frozen) accruing from a single oocyte retrieval.

Results: The cLBR was 74.2% (178/240) versus 63.0% (119/189) after eSBT versus DBT, respectively (aOR: 1.09 (0.68, 1.75)). Time to live birth did not vary significantly between the two groups (HR: 0.85 (0.68,1.08)). The total number of children born was 194 after eSBT (162 singletons and 16 pairs of twins) versus 154 (84 singletons and 35 twins) pairs of after DBT. The odds ratios for preterm birth (<37 weeks' gestation) (0.37 (0.21-0.64)), and low birthweight (<2.5 kg) (0.31 (0.16, 0.60)) were all lower in eSBT group than in DBT group.

Conclusions: In women aged [?]36 years old with at least two blastocysts, cLBR following single- versus double- blastocyst transfer was comparable while the odds of multiple livebirths and adverse perinatal outcomes were reduced.

Keywords : Infertility: Assisted conception; Multiple pregnancy

Tweetable abstract

In women [?]36 years with at least two blastocysts, eSBT could be considered as the preferred treatment in the first transfer cycle.

Introduction

Although there has been a gradual decrease in multiple births after assisted reproductive technology (ART) across the world, that decrease is mostly in developed countries.[1]Twin delivery rates in China were 27.9% and 27.2% after in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) respectively,[2] which are higher than that of many other countries. Elective single embryo transfer (eSET) has been appeared to be the most effective approach to reduce multiple births associated with IVF/ICSI.[3-5] eSET policy has primarily been recommended in women of younger reproductive age with a good prognosis, which is not routinely offered to women aged beyond 35 years old.[6-8] There is fear that the adoption of eSET in unselected patients will lower pregnancy rates per embryo transfer and extend the time needed to achieve live birth.

Advanced maternal age is associated with a decreased chance of pregnancy after IVF and a higher risk of fetal loss, but older women are still at risk for multiples.[9,10] Increasing age aggravates the risks of unfavorable obstetrics outcomes (e.g. preterm delivery, low birth weight and perinatal mortality), and this is further so in cases of multiple pregnancy.[11,12] However, patients at advanced age and physicians are still hesitant to embrace eSBT and wish to maximize their chance of pregnancy with the transfer of more than one embryo. Several trials that did recruit older women aged over 36 to make such a comparison possible.[13,14] These two studies, however, were restricted to cleavage-embryo transfer, impact of blastocysts transfer on the implications of the policy in that age group has been fully studied.

Aneuploid embryos, often age dependent, are less likely to continue development to the blastocyst stage,[15] hence blastocyst culture theoretically would assist the selection of the most viable embryo. A number of studies have demonstrated significant differences in implantation and pregnancy rates in favor of extending the embryo culture to blastocyst-stage transfer.[16-18]

As extending embryo culture has been a routine practice in many clinics, it remains to be proven if it is appropriate to consider the transfer of one blastocyst-stage embryo for women at advanced age. We carried out the retrospective cohort study to compare the effectiveness of eSBT versus DBT on the cumulative live birth rates (cLBR) (fresh and frozen transfers accruing from a single oocyte retrieval) and perinatal outcomes. We have also assessed the applicability of eSBT by exploring whether its practice is associated with extending the time to live birth (TTLB).

Materials and Methods

We performed a retrospective cohort study between Jan 2015 and Oct 2018 at the Northwest Women and Children's Hospital, China. This study was approved by the institutional research ethics review board (2019013).

We included women aged 36 years or older who received IVF ovarian stimulation cycles and who had at least two blastocysts of any grade available for transfer. Couples undergoing treatment with preimplantation genetic testing (PGT) or donor oocytes were excluded. Patients were included in the study only once. Demographic and IVF cycle characteristics data were obtained from our assisted reproductive center. The results were analyzed per IVF/ICSI cycle, including both fresh and frozen embryo transfers. Given the retrospective nature of the work, no specific consent was required from the patients. Figure 1 shows the flowchart of patient selection.

Included women were divided into two comparison groups based on the number of blastocysts used

in their fresh transfer. The women in the eSBT group had one single-blastocyst stage embryo transferred in the fresh cycle, and one or more blastocysts cryopreserved. In the DBT group, all women had two blastocysts transferred in the fresh IVF/ICSI cycle. The subsequent frozen-thawed embryo transfer cycles were a combination of single- and double- blastocyst transfers, more commonly the latter.

Laboratory procedures

For a full description of the IVF protocols, luteal phase support, and laboratory procedures please refer to our previous publication.[19] If two good-quality cleavage-stage embryos were found on day 3, embryos were transferred to blastocyst-stage media and cultured through day 5-6, at which time they were re-evaluated for blastocyst formation. The blastocyst quality was assessed according to the criteria of Gardner and Schoolcraft.[20]

Good-quality blastocysts were [?]3Bb. All other blastocysts including early blastocysts were graded as fair-quality. If two blastocysts were transferred, the quality of the best embryo was used for analysis. The embryo transfer was performed on Day 5 of development under ultrasound guidance. All remaining blastocysts viable were vitrified.

Transfer strategies and patient education

All patients were extensively counseled on the chances of pregnancy and risk of multiple gestation pregnancies before ART treatment. The updated pregnancy and livebirth outcomes were posted on the wall in the participants' waiting area, with the rationale of single embryo transfer in our institution. Information on the pregnancy rate and complications associated with multiple pregnancy were reiterated for a second time regarding their blastocyst quality and embryo transfer options. At our institution, a mandatory single-blastocyst transfer policy was implemented for young patients ([?]35 years) with good-prognosis. However, older patients were encouraged to accept eSBT which was offered as the primary recommendation for their transfer, with DBT presented as our secondary recommendation, as long as there was no medical contraindication for a multiple pregnancy. The final decision on the number of blastocysts to transfer was made by the couples after collaboration with the embryologist, physicians. '

Frozen embryo transfer (FET) cycles

The subsequent FET cycles were a combination of single- and double- blastocyst transfers. In the eSBT group, single blastocyst transfers were performed in women with at least one good quality cryopreserved blastocyst or those did not want to have multiple pregnancies. If a patient had no good quality blastocyst left or failed in the first two transfer attempts, we usually performed a DBT. In the DBT group, we usually performed double- blastocysts transfers unless a patient with only one blastocyst left.

FETs were done in either a natural cycle or an artificial cycle by the use of estradiol as described in detail in previous publication.[21] FET was scheduled at 5 days after ovulation or 6 days of progesterone supplementation. All embryo transfers were performed using transabdominal ultrasound guidance. Luteal support was continued until 10 weeks of pregnancy.

Outcome measures

The primary outcome was cumulative live birth rates. Secondary outcomes were clinical pregnancy, implantation and miscarriage rate in initial fresh cycles as well as the cumulative multiple live births rates. We also compared rates of low birthweight (<2.5 kg), preterm birth (<37 weeks), cesarean section delivery and congenital anomaly. The cumulative live-birth rate within a cycle was defined as the probability of a live birth from an ovarian stimulation encompassing all subsequent fresh and frozen embryo transfers from that stimulation. Live birth was defined as a living birth after 24 weeks of gestation. Multiple birth was defined as a live birth of multiple infants divided by all live births. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. we defined multiple births as low birthweight if the outcomes applied to any of the live babies.

Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., USA). Categorical data were presented by the number of cases and corresponding percentage and continuous data were presented as the mean value ± SD. Categorical data and continuous data that did not show a normal distribution were analyzed by Pearson's chi-squared test/Fisher's exact test or Kruskal-Wallis test as appropriate. A binary logistic regression model was used to assess the influence of single- versus double-blastocyst transfer on the odds of cumulative live births. Time to live birth for the two groups was estimated by Kaplan-Meier method.P -values <0.05 were considered to indicate statistically significance.

Subgroup analyses

To further investigate the effect of number of blastocyst transfer on cumulative LBR between different subgroups of women, we performed analyses split by certain characteristics. The subgroups were woman's age (36-37,38-39,>40 years old) and blastocyst quality (high and fair). The age of 36 and 38 were chosen as thresholds for stratification, given the embryo transfer guidelines released by the American Society for Reproductive Medicine in 2017, recommending that good prognosis patients under 38 should have a single-embryo transfer.[5,8] The threshold of age 40 years reflected the most commonly used threshold in legislation and guidelines.[22] Given the quality of blastocyst has been shown to be associated with live birth.[20,23] The analysis was divided by the quality of blastocysts into good and fair quality blastocysts, as graded by morphological examination. When two blastocysts were transferred, the higher grade was included in the analysis. For each subgroup, we generated new inverse probability of treatment weights within each imputed dataset. We then used these to weight a logistic regression model to assess the odds of cumulative live births.

Results

Women and cycle characteristics

During the study period, a total of 445 women aged [?]36 years underwent fresh blastocyst transfers at the Infertility Clinics Northwest Women and Children's Hospital. Of them, 16 women had only one blastocyst available for transfer (compulsory SBT), and they were therefore excluded from the analysis. eSBT was performed in 240 cases and extra blastocysts were frozen and DBT was carried out in 189 women (Figure 1).

The baseline and fresh IVF/ ICSI cycle characteristics of both groups are shown in Table1. Women in the eSBT group were significantly younger (P =.001) and had fewer previous number of IVF attempts (P <.001) than those in the DBT group. eSBT was more routinely practiced during the latter two years (2017-2018). Women in the eSBT group had more blastocysts available (4.9 +- 2.0 in eSBT versus 4.4 +- 2.3 in DBT, P =0.011). Other characteristics (body mass index, education level, type and causes for infertility, duration of infertility at the time of IVF and ovarian reserve test including antral follicular count and FSH levels) did not differ significantly between the groups.

Infertility treatment outcomes

We assessed 429 women that yielded 297 livebirths. The CLBR was increased in those who underwent single (74.2%,178/240) compared to double (63.0%,119/189) blastocyst-stage embryo transfer. After adjustment for female age and blastocysts quality, number of blastocyst transfer did not have a significant effect on the chance of live birth (aOR: 1.09 (0.68, 1.75)) (Table 2). The total number of children born was 194 after eSBT (162 from singletons and 16 from twin pregnancies) versus 154 (84 from singletons and 35 from twin pregnancies) after DBT. The cumulative multiple livebirth rate was 9.0% after eSBT (16/178) versus 29.4% after DBT (35/119) (OR: 0.24 (0.12-0.45)). No significant difference in the Kaplan-Meier survival curves for time to probability of livebirth between the two study groups (Hazard ratio: 0.85 (0.68,1.08), P =0.152) (Figure 2).

LBRs in initial fresh cycles for single- versus double- blastocyst groups were also calculated and found to be nearly identical at 52.1% (125/240) and 52.4% (99/189), respectively (Table S1). A statistically significant difference, however, was noted in the rate of implantation (62.9% in SBT vs 45.2% in DBT, P <0.001). Patients undergoing one fresh eSBT had a twinning rate of 4% (5 monozygotic twins /125) compared to a twinning rate of 29.3% (29/99) in those after one fresh DBT (OR= 0.10, 95%CI 0.04 to 0.27). Until Oct 2020, 143 women without livebirth in the fresh cycles went on the subsequent frozen-thawed embryo transfer (FET) cycles (n=185). 53 women of the eSBT group and 20 of the DBT group resulted in live births. More cryopreserved blastocysts were left in eSBT group (3.0 +- 2.2 vs. 2.0 +- 2.2, P <0.001).

Subgroup analyses

Female age

For women aged with 36-37 and 38-39 years old, those who underwent eSBT had slightly higher odds of live birth than those who had DBT, but the differences did not reach statistical significance in each subgroups (Table 2). For women [?]40 years old, it seemed that the use of eSBT over DBT slightly reduced the odds of live birth after treatment weighting (aOR: 0.88 (0.29-2.65)), but the difference was also not statistically.

Blastocyst quality

The findings in the pregnancy outcomes persisted with and without accounting for quality of the blastocyst transferred (Table 2). Numbers of blastocyst transfer did not have a significant effect on the chance of live birth in women across the two categories of quality of blastocyst transfer (aOR: 0.47 (0.19-1.15) in good-quality group and aOR: 1.56 (0.86-2.82) in fair-quality group).

Calendar period

More patients underwent eSBT during the latter calendar period which reflects the change in IVF practice in our clinic. Therefore, we also assessed the impact of the eSBT strategy on older women undergoing IVF/ICSI from 2015 to 2018. Despite a successive rise in eSBT cycles from 39% in 2015 to 45% in 2016 and exceeding 60% in 2017-2018, the cumulative LBRs were maintained at around 50% (varied from 48.9 to 53.5%), while multiple birth rate dropped from 25.7% in 2015 to 12.0% 2018, though the differences between each year categories were not statistically significant (P =.233) (Figure S1).

Perinatal outcomes

Perinatal outcomes after fresh and frozen transfers in women with live birth were shown in Table 3. The odds of preterm delivery (0.37 (0.21-0.64)) and low birth rate (0.31 (0.16, 0.60)) after eSBT were significantly lower than that of those with DBT. There was no significant difference in the risk of cesarean section between eSBT and DBT (OR:0.73 (0.41-1.31).

Discussion

Principal findings

For women aged 36 years and older with at least two blastocysts, there was no significant difference in the odds of cumulative live birth following single- versus double- blastocyst transfer accounting for female age and blastocysts quality. Overall, time needed to achieve live birth did not decrease with transfer of two blastocysts at one time, but the risk of multiple livebirths and adverse perinatal outcomes did increase.

Strengths and limitations

The main strength of this study is in that the female age and quality of blastocysts was taken into account which could introduce bias into results if not adjusted for. Since adverse perinatal outcomes tend to become more common with increasing maternal age, we also assessed the potential effect of eSBT on incidence of preterm birth, low birth weight, and congenital anomalies. Finally, all cycles were performed at the same center, the study population has the advantage of being very homogeneous concerning blastocyst assessment and transfer policy.

Limitations of the present study are its retrospective design, including potential biases in patient selection. Despite that the women received eSBT were chosen much less selectively, good prognosis women in eSET cohort whereas less good prognosis women in DBT group, as evident by having a lower rank of previous IVF cycles. In addition, analysis restricted to individuals with at least two viable blastocysts and may not apply to patients with mandatory SBT. It should be noted that women who intended to proceed to blastocyst transfer but failed to have an embryo replaced on day 5 were not included in the present study and we do not practice a mandatory policy for offering all older patients an SBT. We did not assess the direct cost of the IVF treatment itself or the overall costs associated with multiple births. Cost analyses performed in European countries have been in favor of strategy of eSET.[24,25] A prospective study including health economic analysis has been initiated in our center. Further research on the policy of SBT in women of advanced reproductive age is needed.

Findings in relation to existing literature

Our eSBT patients had a live birth rate per fresh cycle (52.1%) similar to that reported after eSBT in younger women,[26] which might imply that once the blastocysts-stage was achieved, pregnancy outcomes were less effected by maternal age.[27,28] In this regard, despite their age, a subset of these women can still have embryos cultured to blastocyst-stage and can benefit from policy of eSET.

The finding in the present study differs from some previous studies suggesting that eSET can result in a significant lower ongoing pregnancy rate compared with DET (21.4 versus 40.3%) in unselected IVF patients.[29] Nevertheless, only cleavage-stage transfer was included in their study, which may have limited their results. The better outcomes in our work probably reflects marked improvements in IVF over the past decade. Extended culture to blastocyst-stage transfer enables more sophisticated assessment of embryo morphology, with better selection of the embryos more likely to success. Similar to the present study, Davis et al [30] recruited 45 patients older than 35s undergoing eSBT in a small retrospective study. Of them, twenty-three patients (51.1%) have an ongoing pregnancy or live delivery, demonstrating a clear role for SET in this relatively older IVF population. Another retrospective study also confirmed the feasibility of eSBT in women with a narrow maternal age (40-43 years).[31]

Implications for clinical practice

A growing number of women seek IVF over the age of 35 years,[32] specifically as the two-child policy was fully implemented in China, ART has been a beneficial complementary technology and health service for couples with advanced age. Practical questions are encountered: if transferring one embryo at a time is associated with a diminished likelihood of a live birth in women over 35 years? If the woman had two embryos transferred, what is the probability of twin delivering and the potential risks? Providing precise information to answer these questions would help couples to decide how many embryos to transfer.

To further identify the role of blastocyst quality in practice of eSBT, we stratified our analysis into good- and fair-quality blastocyst transfer. In older women with at least one good-quality blastocyst, the live birth rate was similar between the two cohorts. However, transferring two blastocysts resulted in a large reduction in implantation rate (from 67.9% to 51.0%) and a great increase in multiple birth rate (from 1.2% to 44.1%). This observation supports the hypothesis of embryo-endometrial crosstalk that endometrium may act like a sensor of embryo quality preventing the implantation or sustainment of a low-quality or abnormal embryo.[33]When there was no good-quality blastocyst available, the strategy of eSBT was also feasible, since additional fair-quality blastocyst did not increase the rate of implantation or live birth. These data suggested that in case of two blastocysts were obtained, eSBT should be encouraged, irrespective of blastocyst quality.[34]

For women aged over 36, transfer of two blastocysts after initial fresh IVF/ICSI cycle, the most statistically significant risks are higher rates of low birth weight and preterm labor. Women with DBT are also at higher risk for cesarean delivery, though the odds are less statistically significant. In 2010, a study in Sweden involving more than 25,000 women receiving IVF, showed that the risk of neonatal death and morbidity was significantly reduced following the SET policy.[35] Concerned with the worse perinatal outcomes, there is an urgent need to limit multiple births by reducing embryo transfer from two to one.[36]

Implications for policy

ART has been successfully implemented in mainland China during the past 30 years. Data in 2016, from the first registry report from China showed that more than 300 000 infants born after ART accounting for 1.69% of the total number of children in the same year.[2] Twin delivery rate after IVF/ICSI in Chins have begun to decline slowly but remain at approximately 27.2˜27.9%, which are still far from satisfactory.[2]

Single embryo transfer with cryopreservation is becoming a more widely accepted strategy in reducing multiples. Highest rates of adoption of eSET were reported from Sweden (69.4%), Australia (56.9%), Finland (49.7%) and Belgium (48.0%).[37]Whereas China has lagged behind in adoption of SET, which was less than 40% in 2017.[38] During the study periods, it seems that despite the rate of eSBT in women [?] 36 years increased gradually and steadily a stable live birth rate has been maintained. For many older infertile patients, the fear of lowering their chance to be pregnant may outweigh concerns regarding the adverse outcomes of multiple births.[39] This information could influence on these patients' belief and encourage more couples to move voluntarily to eSBT.

Conclusions

The practice of eSBT, particularly in combination with FET, results in satisfactory live-birth rates and a dramatic reduced risk of adverse perinatal outcomes in women aged 36 years or older, which indicates eSBT can be applied in this age category. This particular set of data may assist policy of eSBT to go further in clinical practice.

Disclosure of interests

BWM is supported by a NHMRC Investigatorgrant (GNT1176437). BWM reports consultancy for Guerbet and ObsEva. BWM has received research funding from Guerbet and Merck. The other authors declare no conflicts of interest.

Contribution to authorship

Juanzi Shi and Hui Wang participated on the design of the study. He Cai and Hui Wang collected the data. He Cai conducted the statistical analyses and drafted the initial version of the manuscript with Hui Wang. BWM and Stephan Gordts provided important guidance throughout the study process and the manuscript preparation. All authors contributed in the interpretation of the data, revised the manuscript and approved the final article.

Details of ethics approval

This study was approved by the Ethics Review Board of the Northwest Women's and Children's Hospital, Xi'an, China (ref. no. 2019013). Written informed consent was obtained from all patients before treatment, and the patients consented to the use of their retrospective data in scientific publications.

Funding

None.

Acknowledgements

The authors thank Dr. Xiaohua Liu for help with data validation and statistical assistance. We are very grateful to our volunteer patients for taking part in our research. Without the efforts, this research would not have been possible.

References

1. Adamson GD, Norman RJ. Why are multiple pregnancy rates and single embryo transfer rates so different globally, and what do we do about it? Fertil Steril 2020;114(4):680-9.
2. Bai F, Wang DY, Fan YJ, Qiu J, Wang L, Dai Y, et al. Assisted reproductive technology service availability, efficacy and safety in mainland China: 2016. Hum Reprod 2020;35: 446-52.
3. American Society for Reproductive Medicine. Elective single-embryo transfer. Fertil Steril 2012;97(4):835-42.
4. ESHRE. Prevention of twin pregnancies after IVF/ICSI by single embryo transfer. ESHRE Campus Course Report. Hum Reprod 2001;16:790-800.
5. Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med 2004;351:2392-402.
6. Gerris J, DeNeubourg D, Margelschots K, Van Royen E, Vercruyssen M, Barudy-Vasquez J, et al. Elective single day 3 embryo transfer halves the twinning rate without a decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum Reprod 2002;17(10):2626-31.
7. Martikainen H, Tiitinen A, Tomas C, Tapanainen J, Orava M, Tuomivaara L, et al. One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 2001;16:1900-3.
8. Practice Committee of the American Society for Reproductive Medicine and Practice Committee of the Society for Assisted Reproductive Technology. Guidance on the limits of the number of embryos to transfer: a committee opinion. Fertil Steril 2017;107:901-3.
9. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000; 24;320(7251):1708-12.
10. Engmann L, Maconochie N, Tan SL, Bekir J. Trends in the incidence of births and multiple births and the factors that determine the probability of multiple birth after IVF treatment. Hum Reprod 2001;16,2598-605.
11. Cleary-Goldman J, Malone FD, Vidayer J, Ball RH, Nyberg DA, Comstock CH, et al. Impact of maternal age on obstetric outcome. Obstet Gynecol 2005;105:983-90.

12. Waldenstrom U, Cnattingius S, Vixner L, Norman M. Advanced maternal age increases the risk of very preterm birth, irrespective of parity: a population-based register study. BJOG. 2017;124(8):1235-44.
13. Niinima"ki, M, Suikkari, et al. Elective single-embryo transfer in women aged 40-44 years. Human Reproduction 2013;28(2);331-5.
14. Veleva Z , Vilska S , Christel Hyden-Granskog, et al. Elective single embryo transfer in women aged 36-39 years. Human Reproduction, 2006, 21(8):2098-102.
15. Vega M, Breborowicz A, Moshier EL, McGovern PG, Keltz MD. Blastulation rates decline in a linear fashion from euploid to aneuploid embryos with single versus multiple chromosomal errors. Fertil Steril. 2014;102(2):394-8.
16. Cameron NJ, Bhattacharya S, McLernon DJ. Cumulative live birth rates following blastocyst- versus cleavage-stage embryo transfer in the first complete cycle of IVF: a population-based retrospective cohort study. Hum Reprod. 2020;1;35(10):2365-74.
17. Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with singe blastocyststage versus single cleavage-stage embryos. N Engl J Med 2006;354: 1139-46.
18. Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion. Fertil Steril 2013;1;99(3):667-72.
19. Shi W, Zhang S, Zhao W, Xia X, Wang M, Wang H, et al. Factors related to clinical pregnancy after vitrified-warmed embryo transfer: a retrospective and multivariate logistic regression analysis of 2313 transfer cycles. Hum Reprod 2013;28(7):1768-75.
20. 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;73:1155-8.
21. Liu X, Shi W, Shi J. Natural cycle frozen-thawed embryo transfer in young women with regular menstrual cycles increases the live-birth rates compared with hormone replacement treatment: a retrospective cohort study. Fertil Steril 2020;113(4):811-7.
22. Lawlor DA, Nelson SM. Effect of age on decisions about the numbers of embryos to transfer in assisted conception: A prospective study. Lancet 2012;11;379(9815):521-7.
23. Heitmann RJ, Hill MJ, Richter KS, DeCherney AH, Widra EA. The simplified SART embryo scoring system is highly correlated to implantation and live birth in single blastocyst transfers. J Assist Reprod Genet 2013; 30:563-7.
24. Gerris J, De Sutter P, De Neubourg D, Van Royen E, Vander Elst J, Mangelschots K, Vercruyssen M, Kok P, Elseviers M, Annemans L, Pauwels P, Dhont M. A real-life prospective health economic study of elective single embryo transfer versus two-embryo transfer in first IVF/ICSI cycles. Hum Reprod. 2004;19(4):917-23.
25. Veleva Z, Karinen P, Tomas C, Tapanainen JS, Martikainen H. Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. Hum Reprod 2009;24(7):1632-9.
26. Shapiro BS, Daneshmand ST, Desai J, Garner FC, Aguirre M, Hudson C. The risk of embryo-endometrium asynchrony increases with maternal age after ovarian stimulation and IVF. Reprod Biomed Online. 2016;33(1):50-5.
27. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. Influence of patient age on the growth and transfer of blastocyst-stage embryos. Fertil Steril. 2002;77(4):700-5.
28. Maheshwari A, Griffiths S, Bhattacharya S. Global variations in the uptake of single embryo transfer. Hum Reprod Update 2011;17(1):107-20.
29. VanMontfoort AP, Fiddelers AA, Janssen JM, Derhaag JG, Dirksen CD, Dunselman GAJ, et al. In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. Hum Reprod 2006;21:338-43.
30. Davis LB, Lathi RB, Westphal LM, Milki AA. Elective single blastocyst transfer in women older than 35. Fertil Steril 2008;89(1):230-1.

31. Tannus S, Cohen Y, Son WY, Shavit T, Dahan MH. Cumulative live birth rate following elective single blastocyst transfer compared with double blastocyst transfer in women aged 40 years and over. Biol Reprod 2017;35:733-8.
32. Kamath MS, Mascarenhas M, Kirubakaran R, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database of Systematic Reviews 2020: CD003416.
33. Mills M, Rindfuss RR, McDonald P, te Velde E; ESHRE Reproduction and Society Task Force. Why do people postpone parenthood? Reasons and social policy incentives. Hum Reprod Update. 2011;17(6):848-60.
34. Macklon NS, Brosens JJ. The human endometrium as a sensor of embryo quality. Biol Reprod 2014;91:1-8.
35. Kallen B, Finnstrom O, Lindam A , Nilsson E, Nygren KG.,et al. Trends in delivery and neonatal outcome after in vitro fertilization in Sweden: data for 25 years. Hum Reprod 2010;25 (4):1026-34.
36. Eapen A, Ryan GL, Ten Eyck P, Van Voorhis BJ. Current evidence supporting a goal of singletons: a review of maternal and perinatal outcomes associated with twin versus singleton pregnancies after in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 2020;114(4):690-714.
37. ESHRE. The European IVF monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2005. Results generated from European Registers by ESHRE. Hum Reprod 2009;24:1267–87.
38. Chinese Society of Reproductive Medicine Annual Report: data analysis of ART in 2017. J Reprod Med 2020,29(02):143-8.
39. Newton CR, McBride J, Feyles V, Tekpetey F, Power S. Factors affecting patients' attitudes toward single- and multiple-embryo transfer. Fertil Steril 2007;87(2):269-78.

Figure legends

Figure 1. Flow chart of the patient selection.

Figure 2. Kaplan- Meier curve for time to live birth.

Figure S1.

Birth rate and multiple birth rate in relation to the proportion of eSBT among fresh blastocyst transfers for women over 36 years from 2015 to 2018.

Table 1. Baseline characteristics of the patients at the start of the IVF/ICSI.

	Elective single blastocyst-stage transfers (n=240)	Double blastocyst-stage transfers (n=189)	P-value
Female age (years)	37.4 ± 1.8	38.1 ± 2.1	**0.001**
36-37, n (%)	152 (63.3)	95 (50.3)	**0.006**
38-39, n (%)	61 (25.4)	54 (28.6)	
[?]40, n (%)	27 (11.3)	40 (21.2)	
BMI (kg/m^2)	22.9 ± 2.9	23.2 ± 2.9	0.279
Primary infertility, n (%)	44 (18.3)	38 (20.1)	0.643
Infertility duration (years)	4.1 ± 3.5	4.9 ± 4.4	0.05
Rank of cycle, Median (range)	1 (1-3)	1 (1-4)	**<0.001**
Level of education, n (%)			
Primary school or lower	19 (7.9)	14 (7.4)	0.389

	Elective single blastocyst-stage transfers (n=240)	Double blastocyst-stage transfers (n=189)	*P*-value
Secondary school	141 (58.8)	123 (65.1)	
University or higher	80 (33.3)	52 (27.5)	
Infertility diagnosis, n (%)			
Tubal factor	123 (51.2)	97 (51.3)	0.098
Ovulatory dysfunction	9 (3.8)	17 (9.0)	
Endometriosis	4 (1.7)	1 (0.5)	
Male factor	35 (14.6)	34 (18.0)	
Unexplained	20 (8.3)	11 (5.8)	
Multiple factors	49 (20.4)	29 (15.3)	
Year of oocyte retrieval, n (%)			
2015	26 (10.8)	41 (21.7)	**<0.001**
2016	40 (16.7)	48 (25.4)	
2017	66 (27.5)	35 (18.5)	
2018	108 (45.0)	65 (34.4)	
Insemination methods, n (%)			
IVF	195 (81.3)	150 (79.4)	0.600
ICSI	39 (16.3)	31 (16.4)	
Mixed IVF and ICSI	6 (2.5)	8 (4.2)	
No. of oocytes retrieved	11.5 ± 4.0	11.1 ± 4.1	0.184
No. of 2PN	8.2 ± 3.1	7.9 ± 3.3	0.359
No. of viable embryos	7.5 ± 2.8	7.3 ± 3.1	0.486
Total blastocysts per patient	4.9 ± 2.0	4.4 ± 2.3	**0.011**
Good-quality, n (%)	159 (66.3)	48 (25.4)	**<0.001**
No. of supernumerary blastocysts for vitrification	3.9 ± 2.0	2.4 ± 2.3	**<0.001**

BMI: body mass index; AFC: antral follicular count, FSH: Follicle-stimulating hormone. Bold indicates *P* -value <0.05.

Table 2. The effect of elective single- versus double blastocyst-stage embryo transfer on the odds of cumulative live birth overall and by subgroup.

	Elective single blastocyst-stage transfers (n=240)	Double blastocyst-stage embryo transfe
Cumulative livebirth rate (%)	178 (74.2)	119 (63.0)
Subgroups		
Age groups (years)		
36-37	120/152 (78.9)	67/95 (70.5)
38-39	42/61 (68.9)	33/54 (61.1)
40	16/27 (59.3)	19/40 (47.5)
Quality of blastocysts		
Good	123/159 (77.4)	40/48 (83.3)

	Elective single blastocyst-stage transfers (n=240)	Double blastocyst-stage embryo transfe
Fair	55/81 (67.9)	79/141 (56.3)

Table 3. Perinatal outcomes of eSBT versus DBT in women with live birth. Figures are numbers (percentages), unless stated otherwise.

	eSBT (n=240)	DBT (n=189)	*P*-value	Odds ratio (95% CI)
Cumulative Livebirths	178 (74.2)	119 (63.0)	**0.013**	1.69 (1.12-2.55)
Low birth weight (<2.5 kg)	17 (9.6)	30 (25.2)	**<0.001**	0.31 (0.16-0.60)
Preterm delivery (<37 weeks)	26 (14.6)	38 (31.9)	**<0.001**	0.37 (0.21-0.64)
Cesarean section	136 (76.4)	97 (81.5)	0.295	0.73 (0.41-1.31)
Congenital anomalies	1	3		
Singleton delivery	162 (67.5)	84 (44.4)	**<0.001**	2.60 (1.75-3.85)
Low birth weight (<2.5 kg)	8 (4.9)	3 (3.6)	0.623	1.40 (0.36-5.43)
Preterm delivery (<37 weeks)	17 (10.5)	12 (14.3)	0.384	0.70 (0.32-1.56)
Cesarean section	120 (74.1)	66 (78.6)	0.437	0.78 (0.42-1.46)
Twin delivery	16[a] (6.7)	35 (18.5)	**<0.001**	0.314 (0.17-0.59)
Low birth weight (<2.5 kg)	9 (56.3)	27 (77.1)	0.129	0.38 (0.11-1.35)
Preterm delivery (<37 weeks)	9 (56.3)	26 (74.3)	0.198	0.45 (0.13-1.55)
Cesarean section	16 (100.0)	31 (88.6)	0.075	1.13 (1.00-1.27)

eSBT= Elective single blastocyst-stage transfers; DBT= Double blastocyst-stage embryo transfers; Each of twin births was counted as one newborn

a Five monozygotic twin births after eSBT

One baby with patent foramen ovale after eSBT; Two babies in DBT group were diagnosed with ventricular septal defect. One boy of twins in DBT group was diagnosed with congenital hypospadias.

Bold indicates *P* - value <0.05.

Table S1.

Pregnancy outcomes in eSBT and DBT groups following initial fresh transfer cycle.

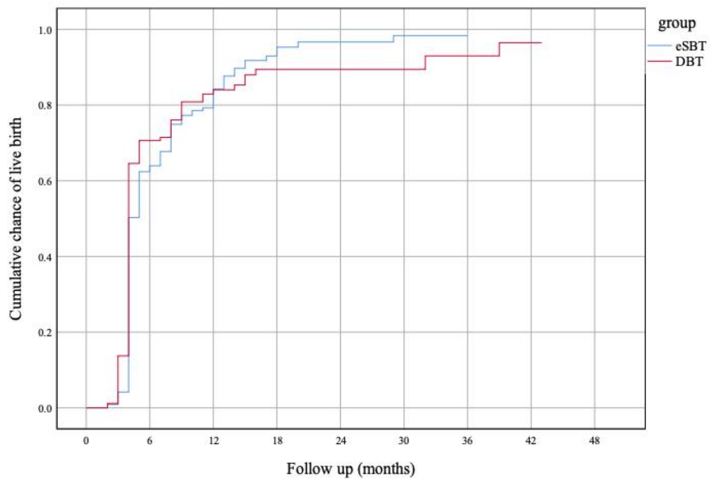
	eSBT (n=240)	DBT (n=189)	*P* -value	OR	*95% CI*
Clinical pregnancies (%)	146 (60.8)	118 (62.4)	0.735	0.94	0.63-1.38
Implantation (%)	151 (62.9)	171 (45.2)	**<0.001**	2.05	1.48-2.86
Spontaneous abortion (%)	21 (14.4)	19 (16.1)	0.699	0.88	0.45-1.72
Live birth (%)	125 (52.1)	99 (52.4)	0.951	0.99	0.68-1.45
Twin birth (%)	5 (4.0)	29 (29.3)	**<0.001**	0.10	0.04-0.27

eSBT: Single blastocyst-stage transfer; DBT: Double blastocyst-stage embryos transfer.

Bold indicates P- value <0.05.

Hosted file

Figure 1.docx available at https://authorea.com/users/349200/articles/710816-elective-single-versus-double-blastocyst-stage-embryo-transfer-in-women-aged-36-years-or-older



Hosted file

Figure s1.docx available at https://authorea.com/users/349200/articles/710816-elective-single-versus-double-blastocyst-stage-embryo-transfer-in-women-aged-36-years-or-older