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ASSISTED REPRODUCTION TECHNOLOGIES

Elective single blastocyst transfer in advanced maternal age

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Abstract
Purpose The purpose of this study was to investigate reproductive outcomes following elective single blastocyst transfer (eSBT) compared with those of double blastocyst transfer (DBT) in advanced maternal age.
Methods This was a retrospective cohort study performed at an academic fertility center. All women aged 40 and over for whom in vitro fertilization (IVF) cycles were performed and in whom embryo culture was extended to the blastocyst stage were reviewed for possible inclusion. Exclusion criteria included the following: women with >3 previous IVF cycles, the use of donor or frozen oocytes, preimplantation genetic diagnosis/preimplantation genetic screening cycles, and cycles in which embryos did not reach the blastocyst stage on day 5. The study included 310 women; 148 were included in the eSBT group and 162 were included in the DBT group. Live birth rate (LBR) was the main outcome. Outcomes were analyzed using logistic regression, controlling for confounders. These confounders were embryo expansion, embryo quality, and the number of previous IVF cycles.
Results The mean age of the whole group was 41 ± 0.91 years, and the LBR was 21.6%. The eSBT group and the DBT group achieved similar clinical pregnancy rates (33 vs. 33%) (OR 1.04; 95%CI, 0.62–1.75) and LBRs (20 vs. 22.8%) (OR 1.43; 95% CI, 0.78–2.64). The multiple birth rate was lower in the eSBT group (0 vs. 16%, $p = 0.02$). The subgroup of women who had elective DBT (eDBT) achieved a higher LBR (20 vs. 30.6%) (OR 2.32; 95% CI, 1.16–4.68) and a higher multiple birth rate (0 vs. 22%, $p = 0.001$). Cycles with early blastocyst transfers were associated with lower LBRs compared with cycles with fully expanded blastocyst transfers (11 vs. 24%, $p = 0.02$).
Conclusion The results of this study indicate that eSBT is associated with similar LBRs compared to the entire DBT cohort; however, when supernumerary blastocysts are available for cryopreservation, eDBT is associated with both higher LBRs and a higher number of multiple births. Studies assessing the cumulative LBR in advanced maternal age after single blastocyst transfer and subsequent frozen-thawed blastocyst transfers are needed.

Keywords Elective single blastocyst transfer · Advanced maternal age · Multiple birth rate · Live birth rate

Introduction

In the last two decades, advances in assisted reproductive technologies (ARTs), particularly in embryo culture and cryopreservation, have resulted in improved live birth rates (LBRs) [1, 2]. Concurrently, during the same time period, there has been a shift in the practice of ART from the primary goal of achieving pregnancy to a practice that aims to optimize maternal and neonatal safety. Multiple pregnancy is considered the most significant adverse event associated with ART and is linked to an increased risk of maternal and neonatal morbidity [3]. Limiting the number of transferred embryos, specifically adopting the practice of elective single embryo transfer (eSET), has been shown to be the most effective strategy to decrease the risk of multiple pregnancy [4, 5]. The Practice

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Committee of the American Society for Reproductive Medicine recommends that eSET should be performed in women aged <35 and should be considered in women aged 35–40 when top-quality embryos are present [4]. Despite these recommendations, a recent study by the Center for Disease Control and Prevention (CDC) has indicated that in the USA in 2013, eSET was performed in 17% of women younger than 35 and in 8% of women aged 35–37 [6]. Another survey in the USA showed that 41% of clinical pregnancies achieved in 2013 by ART were multiple gestations [7].

The reluctance to adopt SET in practice can be explained by the desire of both clinicians and patients to ensure pregnancy and to reduce the costs of multiple ART cycles, especially in countries where ART is not publically funded. Indeed, in a 2013 meta-analysis, it was shown that in young good-prognosis women, eSET was associated with a lower LBR compared with double embryo transfer (DET) in fresh cycles [8]. However, the incidence of multiple births was reduced following eSET, and similar cumulative LBRs were achieved when subsequent vitrified-warmed embryo transfer cycles were analyzed [8]. Moreover, a recent study showed that the practice of eSET increased when insurance coverage for ART was present [9].

Limiting the incidence of multiple pregnancy is particularly important in older women undergoing ART due to the increased risk of obstetrical and neonatal complications associated with advanced maternal age [10]. Although the risk of multiple gestation decreases with advanced maternal age, the multiple birth rate was 28% in women aged 38–40 when two embryos were transferred and additional embryos were available for cryopreservation [7]. Therefore, eSET remains an attractive option for older good-prognosis women.

The incidence and risk factors of multiple gestation in women aged 40 and over undergoing ART are not well defined. Identifying older good-prognosis women who can benefit from SET practice is important. Extending embryo culture to the blastocyst stage can increase the odds of transferring viable, genetically normal embryos and can be an important tool for better embryo selection in advanced maternal age [11]. A previous study showed that the probability of achieving a blastocyst-stage embryo decreases with increasing maternal age [12]. This can increase the risk of cycle cancellation if extended culture is applied to unselected older population. However, a previous study showed low cycle cancellation rates when there were at least four good-quality cleavage-stage embryos in culture [13]. To the best of our knowledge, the feasibility and outcomes of elective single blastocyst transfer (eSBT) in good-prognosis older patients who had at least three good-quality day-3 embryos and in whom embryo culture was extended to the blastocyst stage are still unknown. The purpose of this study is to compare the reproductive outcomes following eSBT with double blastocyst transfer (DBT) outcomes in women aged 40 and over.

Materials and methods

This was a retrospective cohort study conducted at an academic fertility center. The study was approved by the local ethics committee. All fresh in vitro fertilization (IVF) cycles performed in women aged ≥40 from January 2012 to June 2015 were reviewed for possible inclusion. According to local guidelines, women can be treated with autologous oocytes up to the age of 43. In order to be included in the study, women had to be at least 40 years old at the beginning of ovarian stimulation, have embryos cultured to day 5 after fertilization, and have a fresh blastocyst transfer on day 5. Exclusion criteria included the following: women with more than three previous IVF cycles, oocyte donation cycles, PGD/PGS cycles, cycles in which embryos did not reach the blastocyst stage on day 5, cycles that included transfer of cleavage-stage or morula-stage embryos, and cycles with freeze-all embryos. The first ovarian stimulation cycle performed in each subject during the study period was included in the analysis. Each subject was included only once in the study.

Stimulation protocol and embryology procedures

Ovarian stimulation was performed under pituitary suppression. One of three ovarian stimulation protocols was used: a micro-dose flare protocol (initiation of GnRH agonist on days 2–3 of the cycle after oral contraceptive-induced withdrawal bleeding and gonadotropins started on the third day of GnRH agonist); a fixed antagonist protocol (gonadotropins started on days 2–3 of the cycle and GnRH antagonist on the sixth day of stimulation); or a mid-luteal long agonist protocol (GnRH agonist in the mid-luteal phase and gonadotrophins after 2 weeks of downregulation). Final oocyte maturation was induced with urinary/recombinant hCG when at least two follicles were ≥17 mm in diameter. Oocyte collection was performed 35–38 h after hCG triggering. Insemination of retrieved oocytes was done by conventional IVF or intracytoplasmic sperm injection (ICSI). Fertilization was assessed 16–18 h after insemination for the appearance of two distinct pro-nuclei and two polar bodies.

The zygotes were cultured in a cleavage medium (COOK Medical, Sydney, Australia). Embryonic development was assessed daily. If there were at least three good-quality embryos on day 3 (8 cells and <20% fragmentation), embryos were cultured to the blastocyst stage in the blastocyst medium (COOK Medical) and transferred on day 5. According to provincial guidelines, up to two blastocysts can be transferred in this age group, with the preference being to transfer a single blastocyst. The decision about the number of transferred blastocysts was made by the treating physician and the couple and was dependent on clinical factors such as the outcome of previous cycles, the number of blastocysts available, embryo

quality, and the couple's wishes. Remaining supernumerary blastocysts of good quality (Gardner grade ≥ 3bb) were cryopreserved on day 5 or 6.

Blastocyst assessment

Blastocysts were evaluated on day 5. Depending on their developmental stage, blastocysts were categorized as fully expanded blastocysts (FEBs) or early blastocysts (EBs). The quality of the FEBs was scored according to the criteria proposed by Gardner et al. [14] and was categorized into four groups—top-quality blastocysts (grade I; ≥3AA), good-quality blastocysts (grade II; ≥3AB and BA), average-quality blastocysts (grade III; ≥3 BB, AC, CA), and poor-quality blastocysts (grade IV; ≥3, BC, CB, CC)—based on the inner cell mass and trophectoderm score.

EBs were graded according to the presence of fragmentation, cell shape, and compaction [15]; top-quality (grade I) had <5% fragmentation and cells were uniform in shape and size with tight compaction. Good-quality EBs (grade II) had 5–20% fragmentation or unincorporated cells, and cells were of uniform shape and size with tight compaction. Average-quality EBs (grade III) contained >20% fragmentation or unincorporated cells and had cells of non-uniform shape and size or loosely compacted cells. Poor-quality EBs (grade IV) had >20% fragmentation or unincorporated cells and contained loosely compacted cells with degenerate or non-viable cells. Our clinic policy is to avoid performing the transfer of poor-quality embryos when better quality embryos are available.

Definitions and outcome measures

1. Elective single blastocyst transfer (eSBT): a single blastocyst was transferred and at least one blastocyst was available for cryopreservation.
2. Elective double blastocyst transfer (eDBT): two blastocysts were transferred and at least one blastocyst was available for cryopreservation.
3. Non-elective double blastocyst transfer (non-eDBT): two blastocysts were transferred and no embryos were available for cryopreservation.

The DBT group included both eDBT and non-eDBT. Pregnancy was defined as a positive βhCG blood test performed 11–14 days after embryo transfer. Clinical pregnancy was defined as a gestational sac seen on a vaginal ultrasound scan by 6 weeks gestation. Live birth was defined as the delivery of a live fetus at the gestational age of ≥24 weeks.

Statistical analysis

Continuous data was confirmed for normal distribution using the Kolmogorov–Smirnov test. All data were normally distributed. Baseline data was compared using a non-paired *t* test or a chi-square test. Clinical pregnancy rates and LBRs were compared among those of the groups using logistic regression analysis, controlling for confounding effects. The confounding effects were embryo stage, embryo quality, and number of previous IVF cycles. Data is presented as percentages or mean ± standard deviation. A two-sided p value of ≤0.05 was accepted as statistically significant. SPSS version 22 (IBM SPSS, USA) was used for data analyses.

Results

During the study period, 436 women had at least three good-quality cleavage-stage embryos on day 3 and had extended embryo culture to the blastocyst stage. Of these women, 86 (19.7%) had only a single blastocyst available by day 5 and 40 (9.2%) had embryos that did not reach the blastocyst stage (either because of embryo development arrest or slow-growing embryos) and were excluded from further analysis.

The remaining 310 women met the inclusion criteria and were included in the final analysis. The mean age of the whole group was 41 ± 0.91 years, and the LBR was 21.6% ($n = 67$). Altogether, 148 women were included in the eSBT group and 162 women were included in the DBT group. Of the 162 women who had DBT, 75 had supernumerary blastocysts available for cryopreservation and were considered as having eDBT. The baseline characteristics of the study groups are presented in Table 1; there were no significant differences in age, infertility diagnosis, baseline follicle-stimulating hormone levels, or antral follicle count. The mean number of previous IVF cycles was higher in the DBT group (1.1 vs. 0.5, $p < 0.01$).

Cycle characteristics and outcomes are shown in Table 2. The groups did not differ in total dose of gonadotropins used or the number of oocytes retrieved. Embryo quality in the eSBT group was similar to the first embryo in the DBT group but was of better quality than the second embryo transferred. After logistic regression controlling for embryo stage, quality, and number of previous IVF cycles, both groups had similar clinical pregnancy rates (33 vs. 33%, OR 1.04; 95%CI, 0.62–1.75, $p = 0.8$) and LBRs (20 vs. 22.8%) (OR 1.43; 95% CI, 0.78–2.64, $p = 0.2$). In the DBT group, there were six twin deliveries compared to none in the eSBT group (16 vs. 0%, $p = 0.02$).

Considering that the eDBT subgroup had a more favorable prognosis than the other subjects in the DBT group (based on cycle parameters and cryopreservation of supernumerary blastocysts), we compared the reproductive outcomes between eSBT, eDBT, and non-eDBT (Table 3). Table 3 shows that after controlling for confounders, which were embryo stage, quality, and the number of previous IVF cycles, the eDBT group achieved a higher LBR compared with that of the

Table 1 Baseline groups characteristics

	eSBT (n = 148)	DBT (n = 162)	p value
Women age (years)	40.73 ± 0.84	41.15 ± 0.93	0.11
BMI (kg/m^2)	24.5 ± 5	25 ± 5	0.38
AFC	14 ± 9.5	14.2 ± 9.8	0.85
Baseline FSH	7.7 ± 2.6	7.5 ± 3.6	0.57
Number of previous IVF cycles	0.5 ± 0.8	1.1 ± 1	<0.01
Primary infertility	63%	61%	0.47
Infertility diagnosis n (%)			0.64
Unexplained	60 (41)	58 (36)	
Male factor	36 (24)	50 (31)	
PCOS	16 (11)	14 (8.6)	
Mechanical factor	10 (6.7)	13 (8)	
Endometriosis	5 (3.3)	3 (1.8)	
Diminished ovarian reserve	8 (5)	6 (4)	
Combined factors	13 (9)	18 (10.6)	

eSBT elective blastocyst transfer, *DBT* double blastocyst transfer, *BMI* body mass index, *AFC* antral follicle count, *PCOS* polycystic ovary syndrome

$p < 0.05$ was considered statistically significant

Table 2 Comparison of cycle outcomes between and pregnancy outcome between eSBT and DBT

	eSBT (n = 148)	DBT (n = 162)	p value
Total gonadotropin dose (IU)	3250 ± 16,955	3596 ± 1706	0.074
Peak-stimulated E_2 (pg/mL)	2019 ± 937	2060 ± 1053	0.71
No. of oocytes retrieved	11.70 ± 4.7	11.74 ± 4.9	0.94
No. of MII oocytes	9.62 ± 4	9.6 ± 3.8	0.98
Fertilization rate	78%	79%	0.57
No. of zygote formed	7.36 ± 3.14	7.35 ± 3	0.94
1st blastocyst parameters:			
FEB n (%)	128 (86)	126 (78)	0.055
Blastocyst quality n (%)			0.22
Grade 1	14 (9.4)	12 (7.4)	
Grade 2	128 (86.5)	136 84	
Grade 3	6 (4)	14 (9.2)	
2nd blastocyst parameters			
FEB n (%)[a]		90 (55.5%)	<0.01
Blastocyst quality[a]			<0.01
Grade 1		2 (1.2)	
Grade 2		114 (70)	
Grade 3		46 (28.4)	
Pregnancy rate n (%)	62 (42)	80 (49)	0.18
Clinical pregnancy rate n (%)	49 (33)	53 (33)	0.80*
Miscarriage rate n (%)	19 (38)	16 (30)	0.35
Live birth rate n (%)	30 (20)	37 (22.8)	0.20*
Twins delivery rate n (%)	0	6 (16)	0.02

eSBT elective blastocyst transfer, *DBT* double blastocyst transfer, *FEB* fully expanded blastocyst

[a] Comparison of blastocyst stage and quality between embryo from the eSBT group and 2nd blastocyst of DBT group. $p < 0.05$ was considered statistically significant

*p value after controlling for confounders that included maternal age, embryo stage, and quality and number of previous IVF cycles

eSBT (30.6 vs. 20%) (OR 2.32; 95% CI, 1.16–4.68, $p = 0.017$), and the eSBT and non-eDBT had similar LBR (20 vs. 15%) (OR 1.1; 95% CI, 0.49–2.4, $p = 0.65$). These results show that in cases where there is a high proportion of good-quality blastocysts, especially when supernumerary blastocysts remain for cryopreservation, DBT can be associated with higher LBRs but with an increased risk of multiple births compared with eSBT (22 vs. 0%, $p = 0.001$). Moreover, eSBT and non-eDBT were associated with a similar number of live births, and the multiple birth rate, although higher in the non-eDBT group (7.7 vs. 0%, $p = 0.2$), did not reach statistical significance.

In order to optimize the comparison between the eSBT and eDBT groups according to the number of blastocysts available, we performed a subgroup analysis of subjects in the eSBT group who had at least two embryos available for cryopreservation ($n = 101$) to subjects in the eDBT group ($n = 75$). Again, after logistic regression controlling for embryo expansion and embryo quality, the eDBT group achieved a higher LBR (30.6 vs. 21.7%) (OR 2.26; 95%CI, 1.07–4.88, $p = 0.03$).

We also compared cycle outcomes when only EBs ($n = 56$) were transferred with cycles in which at least one FEB was transferred ($n = 254$). Cycles with EB transfer only compared with cycles with at least one FEB transfer were associated with a significantly lower LBR (11 vs. 24%, $p = 0.02$) (Table 4).

Discussion

The results of this study indicate that for good-prognosis older women aged 40–43 who had extended embryo culture and blastocysts available for transfer by day 5, the practice of eSBT is feasible and has a significantly reduced risk of multiple births compared with that of DBT. Although eSBT resulted in a similar LBR compared to DBT, this result should be considered with caution because the subgroup that had eDBT achieved a higher LBR compared with the subgroup that had eSBT.

To the best of our knowledge, this study is the first to report the results of eSET at the blastocyst stage in women aged 40 or over. Overall, studies that have evaluated the effectiveness of eSET in older maternal age are few in number. The largest study to date that assessed the feasibility of eSET in this age group was published in 2013 by Niinimaki et al. [16]. In that study, the reproductive outcomes following eSET compared with those of DET of cleavage-stage embryos were retrospectively analyzed in 628 women aged 40–44. The results showed that eSET and DET resulted in similar LBRs (13.6 vs. 11%) and in similar twin delivery rates (0 vs. 7.5%). However, the authors acknowledged that the two groups were not comparable, as eSET was performed in women with a better prognosis who were younger, needed lower doses of gonadotropins, and had more oocytes collected compared with the DET group. In another smaller retrospective study, eSET and DET were performed in 48 and 188 cycles, respectively, in women aged ≥40. The results showed no difference in LBRs (6.3 vs 10.1%) or in multiple births (0 vs. 5.3%) [17]. From these studies, it can be concluded that eSET is not necessarily needed in older women, as it did not result in reduced multiple births. However, our study revealed different results that can be explained by the fact that embryo transfer was carried out at the blastocyst stage and not in the cleavage stage.

In the last 10 years, there has been a shift toward extending embryo culture to day 5 or 6; however, there is ongoing debate about who will benefit from such a strategy. It is well known that embryonic genome activation occurs at the eight-cell stage (day 3) [18]. If this activation does not occur, the embryo is unlikely to continue developing. By extending embryo culture, natural selection of the most genetically competent embryos occurs and can lead to better embryo selection in advanced maternal age. The advantage of extending embryo culture in advanced maternal age was shown in a recent study that implemented PGS on 385 embryos from women with a mean age of 36. It was shown that in women older than 35, 56% of euploid embryos developed to FEB by day 5 compared with 18% of aneuploid embryos. It was also shown that the blastulation rate decreased with the increase in the number of chromosomal abnormalities [19]. In our study, the relatively high LBR (21.6%) can in part be explained by the fact that 73% (344/472) of the total blastocysts transferred were fully expanded on day 5. The clinical advantage of transferring blastocysts in older women was shown in a recent prospective study in which women aged ≥35 achieved higher ongoing pregnancy rates following blastocyst transfer compared with those of cleavage embryo transfer (48 vs. 19.3%, $p = 0.010$). However, this difference was not significant in younger women (33 vs. 37%) [20].

Choosing the best embryo to transfer is usually based on embryo morphology. Blastocyst morphology seems to be better correlated with the euploid status of the embryo compared with cleavage embryo morphology. In a study that assessed the embryo morphology of 355 embryos, it was shown that the proportion of euploid and aneuploid embryos carrying lethal genetic abnormalities and that were considered of good quality was similar in the cleavage stage (72 vs. 83%). In contrast, 56% of the top-quality blastocysts were euploid compared with 25.5% poor-quality blastocysts [21]. Taking into consideration these factors, transferring fully expanded, high-quality blastocysts on day 5 is associated with a higher chance of transferring euploid embryos. Moreover, studies have shown that maternal age has no effect on implantation once the embryo is genetically normal [21, 22].

Of all the blastocysts transferred, 27% ($n = 128$) were EBs that did not reach the fully expanded stage on day 5. The

Table 3 Comparison of cycle parameters and clinical outcomes between eSBT, eDBT, and non-eDBT

	(A) eSBT (n = 148)	(B) eDBT (n = 75)	(C) non-eDBT (N = 87)	P (A vs. B)	P (A vs. C)
Women age (years)	40.7 ± 0.8	41 ± 0.8	41.2 ± 1	0.12	0.09
Total gonadotropin dose (IU)	3250 ± 1695	3498 ± 1710	3691 ± 1709	0.30	0.80
Peak stimulated E_2 (pg/mL)	2019 ± 937	2187 ± 1216	1946 ± 874	0.26	0.55
No. of oocytes retrieved	11.70 ± 4.7	13.1 ± 4.8	10 ± 4.7	0.038	0.007
No. of MII oocytes	9.62 ± 4	10.82 ± 4.04	8.5 ± 3.4	0.057	0.03
No. of zygote formed	7.36 ± 3.14	8.7 ± 3.3	6.2 ± 2.2	0.003	0.002
1st blastocyst parameters					
FEB n (%)	128 (86)	58 (77)	67 (77)	0.06	0.043
Blastocyst quality n (%)				0.86	0.04
Grade 1	14 (9.4)	8 (10.6)	4 (4.6)		
Grade 2	128 (86.6)	63 (84)	72 (82.7)		
Grade 3	6 (4)	4 (5.4)	10 (11.4)		
2nd blastocyst parameters[a]					
FEB n (%)		53 (70.6)	35 (40.2)	0.002	<0.001
Blastocyst quality n (%)				0.02	<0.001
Grade 1		2 (2.6)	0		
Grade 2		64 (84.3)	49 (56.3)		
Grade 3		9 (12)	37 (42.5)		
No. of cryopreserved blastocysts	2.29 ± 1.5	2.23 ± 1.8		0.8	
Pregnancy rate n (%)	62 (42)	43 (57)	37 (42.5)	0.03	0.92
Clinical pregnancy rate n (%)	49 (33)	32 (42.6)	21 (24)	0.16*	0.14*
Live birth rate n (%)	30 (20)	23 (30.6)	13 (15)	0.017*	0.65*
Twins delivery rate	0	5 (22)	1 (7.7)	0.001	0.2

eSBT elective blastocyst transfer, *eDBT* elective double blastocyst transfer, *non-eDBT* non-elective double blastocyst transfer, *FEB* fully expanded blastocyst

[a] Comparison of blastocyst-stage quality between blastocyst from the eSBT group and 2nd blastocyst of eDBT and non-eDBT groups. $p < 0.05$ was considered statistically significant

*p value after controlling for confounders that included maternal age, embryo stage and quality and number of previous IVF cycles

significance of slow-growing embryos is still evolving. A recent study has shown that EBs that became FEBs on day 6 were less likely to be euploid compared to FEBs on day 5 (23 vs. 41%, $p < 0.05$) [23]. A second study demonstrated similar results, with a higher aneuploidy rate of EBs compared to those of FEBs (70 vs. 61%, $p < 0.05$) [24]. However, other studies showed similar aneuploidy rates between day 5 and day 6 FEBs [15, 22]. In a recent retrospective study that compared the outcomes of fresh vs. frozen blastocyst transfer, it was shown that transferring day 6 FEBs in a frozen/thawed cycle resulted in a better LBR compared with the fresh transfer of slow-growing day-5 embryos. The authors concluded that this lower LBR was the result of embryo-endometrial asynchrony in fresh cycles. Therefore, they recommend freezing all slow-growing embryos that reach the FEB stage on day 6 and transferring them in subsequent frozen cycles [2]. In our study, the LBR of fresh EB transfers was significantly lower than that for FEB transfers. Whether this is the result of increased blastocyst, aneuploidy rates or endometrial-embryo asynchrony should be studied further.

Table 4 Comparison between cycles that included transfer of early blastocysts only and cycles in which at least one fully expanded blastocyst (FEB) was transferred

	Cycles with EB transfer only (n = 56)	Cycles with at least one FEB transfer (254)	p value
Pregnancy rates	21 (37%)	142 (56%)	0.009
Clinical pregnancy rates	12 (21%)	102 (40%)	0.006
Live birth rates	6 (11%)	61 (24%)	0.02

92 EBs were transferred in 56 cycles (1.66 blastocyst/cycle) compared to 380 blastocysts (at least one fully expanded blastocyst) were transferred in 254 cycles (1.49 blastocyst/cycle), $p = 0.025$

Based on the results of this study, the decision about the number of blastocysts to be transferred should be made according to the number and quality of available blastocysts. When at least two or more fully expanded, good-quality blastocysts are achieved, transferring two blastocysts can result in a higher LBR compared with that of eSBT. However, this can be associated with an increased risk of multiple births. Moreover, as the transfer of day 5 early blastocysts results in a reduced LBR, we recommend keeping slow-growing blastocysts in culture until day 6 or 7 until full expansion is achieved and transferring them in subsequent warming cycles. This can result in cycles in which no embryos are available for fresh transfer. In this case, couples should be counseled about the lower LBR associated with slow-growing blastocyst transfer. Studies to assess the feasibility of freezing all embryos in advanced maternal age are needed.

The role of PGS in this age group is still unclear. In a retrospective study that implemented PGS in women aged 40–44, the PGS group had blastocysts available for transfer in 52% of the initiated cycles compared with 95% of cycles without PGS. The LBR per embryo transfer was higher in the PGS cycles (45 vs. 16%); however, the LBR per implanted embryo was similar between the groups (89 vs. 75%) [25].

Considering the high miscarriage rate, which is >30% in both groups, and the superiority of DBT over eSBT when three or more blastocysts are available, makes this subgroup of patients with ≥3 blastocysts a potential candidate that could benefit from PGS to optimize pregnancy outcomes.

Our study has some limitations. The retrospective nature of the study can be a source of bias. Women in the DBT had more previous IVF cycles compared to women in the eSBT group, and the second embryo was of lower quality, which could mean that DBT was performed in women with a poorer prognosis. However, when the eDBT subgroup was analyzed, it clearly showed the DBT in good-prognosis older women is associated with both a higher LBR and a higher twin birth rate compared with eSBT. Studies assessing the cumulative LBRs of fresh and subsequent warmed cycles when eSBT is performed in advanced maternal age are needed.

In conclusion, multiple births are associated with short- and long-term health consequences. Advanced maternal age is known to be a major risk factor for adverse perinatal outcomes. For good-prognosis women of older maternal age, extended embryo culture can improve embryo selection and facilitate the practice of SET.

Compliance with ethical standards The study was performed in accordance with the guidelines of the local ethics committee.

References

1. Roque M, Valle M, Guimaraes F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil Steril. 2015;103:1190–3.
2. Wirleitner B, Schuff M, Stecher A, Murtinger M, Vanderzwalmen P. Pregnancy and birth outcomes following fresh or vitrified embryo transfer according to blastocyst morphology and expansion stage, and culturing strategy for delayed development. Human reproduction (Oxford, England) 2016
3. ACOG Practice Bulletin No. 144: Multifetal gestations: twin, triplet, and higher-order multifetal pregnancies. Obstetrics and gynecology 2014;123:1118–32.
4. Elective single-embryo transfer. Fertility and sterility 2012;97:835–42.
5. Sullivan EA, Wang YA, Hayward I, Chambers GM, Illingworth P, McBain J, et al. Single embryo transfer reduces the risk of perinatal mortality, a population study. Human reproduction (Oxford, England). 2012;27:3609–15.
6. Mancuso AC, Boulet SL, Duran E, Munch E, Kissin DM, Van Voorhis BJ. Elective single embryo transfer in women less than age 38 years reduces multiple birth rates, but not live birth rates, in United States fertility clinics. Fertility and sterility 2016.
7. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Warner L, et al. Assisted reproductive technology surveillance—United States, 2013. Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002). 2015;64:1–25.
8. Pandian Z, Marjoribanks J, Ozturk O, Serour G, Bhattacharya S. Number of embryos for transfer following in vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev 2013:Cd003416.
9. Styer AK, Luke B, Vitek W, Christianson MS, Baker VL, Christy AY, et al. Factors associated with the use of elective single-embryo transfer and pregnancy outcomes in the United States, 2004–2012. Fertil Steril. 2016;106:80–9.
10. Prapas N, Kalogiannidis I, Prapas I, Xiromeritis P, Karagiannidis A, Makedos G. Twin gestation in older women: antepartum, intrapartum complications, and perinatal outcomes. Arch Gynecol Obstet. 2006;273:293–7.
11. Harton GL, Munne S, Surrey M, Grifo J, Kaplan B, McCulloh DH, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013;100:1695–703.
12. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. Influence of patient age on the growth and transfer of blastocyst-stage embryos. Fertil Steril. 2002;77:700–5.
13. Papanikolaou EG, D'Haeseleer E, Verheyen G, Van de Velde H, Camus M, Van Steirteghem A, et al. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. Hum Reprod. 2005;20:3198–203.
14. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Human reproduction (Oxford, England). 1998;13:3434–40.
15. Kroener L, Ambartsumyan G, Briton-Jones C, Dumesic D, Surrey M, Munne S, et al. The effect of timing of embryonic progression on chromosomal abnormality. Fertil Steril. 2012;98:876–80.
16. Niinimaki M, Suikkari AM, Makinen S, Soderstrom-Anttila V, Martikainen H. Elective single-embryo transfer in women aged 40–44 years. Human reproduction (Oxford, England). 2013;28:331–5.
17. Fujimoto A, Morishima K, Harada M, Hirata T, Osuga Y, Fujii T. Elective single-embryo transfer improves cumulative pregnancy outcome in young patients but not in women of advanced reproductive age. J Assist Reprod Genet. 2015;32:1773–9.
18. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature. 1988;332:459–61.

19. 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;102:394–8.
20. Fernandez-Shaw S, Cercas R, Brana C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. J Assist Reprod Genet. 2015;32:177–84.
21. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod. 2014;20:117–26.
22. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Human reproduction (Oxford, England). 2014;29:1173–81.
23. Kort JD, Lathi RB, Brookfield K, Baker VL, Zhao Q, Behr BR. Aneuploidy rates and blastocyst formation after biopsy of morulae and early blastocysts on day 5. J Assist Reprod Genet. 2015;32:925–30.
24. Piccolomini MM, Nicolielo M, Bonetti TC, Motta EL, Serafini PC, Alegretti JR. Does slow embryo development predict a high aneuploidy rate on trophectoderm biopsy? Reproductive biomedicine online 2016.
25. Lee HL, McCulloh DH, Hodes-Wertz B, Adler A, McCaffrey C, Grifo JA. In vitro fertilization with preimplantation genetic screening improves implantation and live birth in women age 40 through 43. J Assist Reprod Genet. 2015;32:435–44.