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ORIGINAL ARTICLE

The addition of a low-quality embryo as part of a fresh day 3 double embryo transfer does not improve ongoing pregnancy rates

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STUDY QUESTION: Does the addition of a low-quality embryo in fresh Day 3 double embryo transfer (DET) affect the ongoing pregnancy rate (OPR) and multiple gestation rate in patients with only one or no high-quality embryos available?

SUMMARY ANSWER: In patients with only one- or no high-quality embryo available, the addition of a low-quality embryo in fresh Day 3 DET does not improve the OPR but increases multiple gestation rates in fresh DET.

WHAT IS KNOWN ALREADY: Pregnancy rates after DET are considered to be higher compared to single embryo transfer (SET) when analyzed per first embryo transfer only. However, these conclusions are based on RCTs in which mostly patients with two or more high-quality embryos were included, and can therefore not be applied to patients with only one or no high-quality embryo available. This is particularly relevant since it has been suggested that low-quality embryos could impair the implantation of simultaneously transferred embryos by paracrine signaling. Hence, we investigated in patients with only one or no high-quality embryo available whether the addition of a low-quality embryo in DET affects the OPR, multiple gestation rate and miscarriage rate.

STUDY DESIGN, SIZE DURATION: This was a retrospective cohort study of 5050 patients receiving 7252 fresh embryo transfers on Day 3 after fertilization in IVF/ICSI cycles from 2012 to 2015 in two academic hospitals.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We included all women that received fresh SET or DET with any combination of high-quality embryos (7, 8 or 9 blastomeres, with equal to or <20% fragmentation) or low-quality embryos (all other embryos). Outcomes were OPR (primary outcome, defined as a positive fetal heartbeat by transvaginal ultrasound at least 10 weeks after oocyte retrieval), miscarriage rate and multiple gestation rate. We used a generalized estimating equations model adjusting for maternal age, number of oocytes retrieved, center of treatment and the interaction between maternal age and number of oocytes retrieved. Other baseline characteristics, including infertility diagnosis, fertilization method and the number of consecutive fresh embryo transfers per patient, did not contribute significantly to the GEE model and were therefore excluded, and not adjusted for.

MAIN RESULTS AND THE ROLE OF CHANCE: Compared to SET with one high-quality embryo, DET with two high-quality embryos resulted in a higher OPR (adjusted odds ratio (OR) 1.38, 95% CI 1.14–1.67), while DET with one high- and one low-quality embryo resulted in a lower OPR (adjusted OR 0.65, 95% CI 0.49–0.90). However, SET in patients with only one high-quality embryo available resulted in a lower OPR compared to SET in patients with two or more high-quality embryos available (adjusted OR 0.52, 95% CI 0.39–0.70). After adjusting for this confounding factor, we found that both DET with two high-quality embryos (adjusted OR 0.99, 95% CI 0.74–1.31) and DET

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with one high- and one low-quality embryo (adjusted OR 0.78, 95% CI 0.47–1.27) resulted in a not significantly different OPR compared to SET with one high-quality embryo. If only low-quality embryos were available, DET did not increase the OPR as compared to SET with one low-quality embryo (adjusted OR 0.84, 95% CI 0.55–1.28). Multiple gestation rates were higher in all DET groups compared to SET (DET with ≥1 high-quality embryo(s) compared to SET with one high-quality embryo; DET with two low-quality embryos compared to SET with one low-quality embryo; all comparisons $P < 0.001$). Miscarriage rates were not different in all DET groups compared to SET (DET with ≥1 high-quality embryo(s) compared to SET with one high-quality embryo; DET with two low-quality embryos compared to SET with one low-quality embryo; all comparisons $P > 0.05$).

LIMITATIONS REASONS FOR CAUTION: Limitations to this study include the retrospective design and possible bias between study groups related to embryo transfer policies between 2012 and 2015. Consequently, we may have underestimated pregnancy chances in all DET groups. Furthermore, the OPR was calculated as a percentage of the number of fresh embryo transfers in each study group, and not the total number of started IVF/ICSI cycles. Therefore, the reported pregnancy outcomes may not truly reflect the pregnancy chances of couples at the start of treatment. A possible confounding effect of maternal age in our study is acknowledged but we could not compare clinical outcomes in different age groups separately owing to small sample sizes. Analysis of pregnancy outcomes in lower prognosis patients (higher maternal age, fewer oocytes retrieved) separately is an avenue for future research.

WIDER IMPLICATIONS OF THE FINDINGS: The decision to perform DET rather than SET in order to increase the OPR per fresh embryo transfer seems not to be justified for those patients with only one or no high-quality embryo(s) available. However, owing to the limitations of this study, prospective RCTs are needed that specifically investigate pregnancy outcomes in patients with only one or no high-quality embryo(s) available in SET and DET.

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Key words: embryo quality / embryo transfer / double embryo transfer / single embryo transfer / IVF / ICSI / ongoing pregnancy rate

WHAT DOES THIS MEAN FOR PATIENTS?

Single embryo transfer is increasingly offered to people going through IVF as multiple pregnancy is the biggest risk from the treatment. Embryos are assessed and graded according to their quality, and sometimes a second embryo is transferred. This paper looks at whether adding a second low-quality embryo as part of a fresh Day 3 transfer increases the chances of pregnancy.

Research has suggested that pregnancy rates are higher when two fresh embryos are transferred rather than just one, but the existing studies have usually focused on people who have at least two good-quality embryos. There have been concerns that when a lower-quality embryo is transferred alongside a high-quality embryo, it may have an impact on the chances of the high-quality embryo implanting. The researchers looked back at data from two centres to compare the outcomes and they found that putting back one high-quality and one low-quality embryo did not increase pregnancy chances as compared to transferring just one high-quality embryo. Also where there were no high-quality embryos, transferring two poor quality embryos did not lead to a higher pregnancy rate than just using one.

The researchers say that the results do need to be treated with some caution because of the way the work was carried out and have called for further research, but they say that putting back an additional low-quality embryo does not appear to improve the chances of a successful outcome.

Introduction

In the early days of IVF up to three or four embryos were transferred simultaneously in order to achieve satisfactory pregnancy rates (Edwards and Steptoe, 1983). With increasing efficiency of the IVF procedures, this eventually led to high multiple gestation rates, and consequently high maternal and neonatal morbidity and mortality (Steptoe *et al.*, 1986; Kingsland *et al.*, 1990; Bergh *et al.*, 1999; ESHRE, 2000). Over the past few decades, the efficiency of cryopreservation techniques improved considerably, which resulted in an increased use of cryopreserved supernumerary embryos in consecutive frozen-thawed embryo transfer cycles (Mastenbroek *et al.*, 2011; Wong *et al.*, 2014). This has enabled professionals to transfer fewer embryos, with double embryo transfer (DET) and single embryo transfer (SET) being the most common strategies in recent years (Technology and Medicine, 2012; Calhaz-Jorge *et al.*, 2016; Dyer *et al.*, 2016).

To achieve optimal live-birth rates in both SET and DET, it is important to select the embryo(s) with the highest quality for transfer. To do this, regular morphological assessment of embryo development and quality is being used to rank and subsequently select embryos for transfer (Ebner *et al.*, 2003). The developmental stage of each embryo is determined by the number of cells at a given time-point, ranging from the consecutive cleavage stages to the morula and blastocyst stage. Embryo morphology at Day 3 after fertilization is determined by assessing number, size, symmetry, multinucleation and cellular fragmentation of the blastomeres (Puissant *et al.*, 1987; Van Royen *et al.*, 1999; Balaban *et al.*, 2011). Based on a prediction model for embryo

selection, the embryos with the highest quality at Day 3 after fertilization are considered to be cleavage stage embryos with 7–9 blastomeres and the smallest percentage of fragmentation (van Loendersloot *et al.*, 2014). Transferring embryos with a deviating number of blastomeres or of lower-quality results in lower pregnancy rates (Balaban *et al.*, 2011; van Loendersloot *et al.*, 2014). Moreover, recent evidence suggests that embryos of low morphological quality may actively impair implantation by activating an oxidative stress response in endometrial stromal cells and by inhibiting the secretion of various implantation factors (Teklenburg *et al.*, 2010; Brosens *et al.*, 2014; Macklon and Brosens, 2014). By interrupting endometrial cell function, a low-quality embryo may not only alter its own implantation chances but also those of a simultaneously transferred high-quality embryo.

The numerous RCTs that have been conducted to compare SET and DET are summarized in a recent Cochrane review (Pandian *et al.*, 2013). When analyzed per first transfer only, live-birth rates after DET were higher when compared to SET, with a live-birth rate of 45% after a single cycle of DET and a live-birth rate between 24 and 33% after a single cycle of SET. However, cumulatively, live-birth rates per cycle were similar for repeated SET compared to DET. Moreover, DET was associated with a higher multiple gestation rate. However, most of these RCTs only included patients with two or more high-quality embryos available (Gerris *et al.*, 1999; Thurin *et al.*, 2004; Martikainen *et al.*, 2001). Some RCTs included patients with a wider range in embryo quality, but pregnancy outcomes were not separately reported for patients with only one or no high-quality embryo(s) available (Gardner *et al.*, 2004; Lukassen *et al.*, 2005; van Montfoort *et al.*, 2006). Hence, it is unknown whether the addition of a low-quality embryo in such patients increases ongoing pregnancy and multiple gestation rates, or alternatively, whether it disturbs implantation of a simultaneously transferred high-quality embryo and, subsequently decreases ongoing pregnancy and multiple gestation rates.

We retrospectively analyzed data on fresh embryo transfers on Day 3 after fertilization from two academic medical centers over a period of 4 years and investigated ongoing pregnancy rates (OPR), miscarriage rates and multiple gestation rates in relation to embryo quality in patients receiving SET or DET.

Materials and Methods

We retrospectively analyzed all fresh embryo transfers on Day 3 after fertilization that were performed between January 2012 and December 2015 in the Academic Medical Center (AMC) and between January 2012 and December 2014 in the VU University Medical Center (VUmc) in Amsterdam, The Netherlands. Clinical data were retrieved from the electronic patient databases of both academic medical centers. Under the legal requirements for clinical research in The Netherlands, this study was exempt from institutional review board approval. Therefore, informed consent from patients whose data was used in this study was not required.

Study population

The female age limit for the start of IVF/ICSI was 43 years in both centers. Indications for IVF/ICSI were determined after a basic fertility workup. IVF/ICSI was offered immediately for the following indications: bilateral tubal pathology, severe endometriosis or severe oligozoospermia (post-wash total motile sperm count <3 million). IUIs in at least six cycles were applied before starting IVF/ICSI for the following indications: unilateral tubal pathology, minimal endometriosis, cervical hostility, mild male oligozoospermia or unexplained subfertility.

Treatment procedures

Embryo culture

Embryos were cultured individually in 25 µl pre-equilibrated medium drops under oil in sequential Sage medium (Quinn's advantage protein plus fertilization medium, cleavage medium and blastocyst medium, Cooper Surgical Inc., CT, USA). In the VUmc embryos were cultured in incubators at 36.8°C, with 5% CO_2 and atmospheric O_2 levels, and in the AMC in incubators at 37°C, with 5% CO_2 and 5% O_2.

Morphological grading

Pronuclear formation was scored 17–22 h after fertilization and early cleavage was scored 23–28 h after fertilization. Subsequently, on Days 2 and 3 after fertilization, the morphology (i.e. number of blastomeres, size and symmetry of blastomeres, and the degree of fragmentation) was assessed for each embryo (Puissant *et al.*, 1987).

Embryo transfer

Embryos were selected for transfer on Day 3, based on the morphological scores on Days 1, 2 and 3 according to local protocols. Embryo transfer was performed with a trans-cervical catheter (AMC: Wallace Classic Embryo Replacement Catheter, Smiths Medical, Rosmalen, The Netherlands; VUmc: K-JETS-70190-SIVF; Cook IVF, Eight Miles Plains, Queensland, Australia). Cryopreservation of good-quality embryos was performed on Day 4 after fertilization. To determine pregnancy, an hCG blood test was performed at 14–18 days after oocyte retrieval.

Study groups

All fresh embryo transfers were allocated to study groups based on embryo morphology of the transferred embryos at Day 3 after fertilization. A high-quality embryo was defined as an embryo with 7, 8 or 9 blastomeres with equal to or less than 20% of fragmentation. Low-quality embryos were all other embryos. The study groups were: SET with one high-quality embryo, SET with one low-quality embryo, DET with two low-quality embryos, DET with two high-quality embryos, and DET with one high- and one low-quality embryo.

Outcome measures

The primary outcome of this study was OPR per fresh embryo transfer, defined as a positive fetal heartbeat by transvaginal ultrasound at least 10 weeks after oocyte retrieval. Secondary outcomes were miscarriage rate, calculated as the number of biochemical pregnancies that did not result in an ongoing pregnancy, and multiple gestation rate, defined as a positive fetal heartbeat by transvaginal ultrasound of at least two fetuses at least 10 weeks after oocyte retrieval. Biochemical pregnancy was defined by a positive hCG blood test.

Statistical analysis

The following baseline characteristics were compared between the study groups: maternal age, number of oocytes retrieved, indication for ART, consecutive number of fresh embryo transfers per patient and center of treatment. Significance was tested by using Wald Chi-square tests and one-way ANOVA with Tukey's *post hoc* test. OPR was calculated as a percentage from the total number of fresh embryo transfers per study group, multiple gestation rate was calculated from the total number of ongoing pregnancies per study group, and miscarriage rate was calculated from the total number of biochemical pregnancies per study group. We used a

generalized estimating equations (GEE) model to predict the primary and secondary outcomes adjusted for maternal age, number of oocytes retrieved, center of treatment, the number of fresh consecutive embryo transfers per patient, indication for IVF/ICSI and the interaction between maternal age and the number of oocytes retrieved. Unadjusted and adjusted odds ratios (OR) and corresponding 95% CIs were calculated, and Wald Chi-square tests were used to test for significance. In all cases, SPSS Statistics 23 software was used (IBM, NY, USA).

Results

Study groups and baseline characteristics

Between January 2012 and December 2015, 5050 patients received a total of 7266 fresh embryo transfers on Day 3 after fertilization. Thirteen cases, of which the embryo morphology score at Day 3 was missing, were excluded from the analysis. One case was excluded because pregnancy outcomes were not reported. All included fresh embryo transfers ($n = 7252$) were allocated to one of the following groups: SET with one high-quality embryo ($n = 4653$, 64%), SET with one low-quality embryo ($n = 1102$, 15%), DET with two high-quality embryos ($n = 780$, 11%) or DET with one high- and one low-quality embryo ($n = 383$, 5%) and DET with two low-quality embryos ($n = 334$, 5%) (Table I). Patients that received DET were older compared to patients that received SET ($P < 0.001$; Table I). Patients that received SET with one high-quality embryo or DET with two high-quality embryos had more oocytes retrieved compared to patients that received SET with one low-quality embryo, DET with two low-quality embryos or DET with one high- and one low-quality embryo (all comparisons $P < 0.001$; Table I). Compared to patients that received SET with one high-quality embryo, all patients in other study groups received more consecutive fresh embryo transfers (all comparisons $P < 0.05$; Table I). Additional baseline characteristics of the study groups are summarized in Table I.

Pregnancy outcomes in DET compared to SET

Univariate and multivariate analyses

First, pregnancy outcomes were assessed and compared by univariate analysis. To best analyze embryo transfer strategies in daily clinical practice, we compared SET with one high-quality embryo to DET with two high-quality embryos or DET with one high- and one low-quality embryo, and SET with one low-quality embryo to DET with two low-quality embryos. SET with one high-quality embryo resulted in an OPR of 30.6% (1423/4653), while DET with two high-quality embryos resulted in a not significantly different OPR of 29.1% (227/780; unadjusted OR 0.93, 95% CI 0.79–1.10). DET with one high- and one low-quality embryo resulted in a lower OPR of 15.1% (58/383; unadjusted OR 0.41, 95% CI 0.30–0.54) (Table II, top panel). Furthermore, SET with one low-quality embryo resulted in an OPR of 13.5% (149/1102), while DET with two low-quality embryos resulted in a lower OPR of 9.3% (31/334; unadjusted OR 0.65, 95% CI 0.44–0.99) (Table II lower panel).

Next, we used a multivariate GEE model adjusting for maternal age, number of oocytes retrieved, center of treatment and the interaction between maternal age and number of oocytes retrieved. Other baseline characteristics, including infertility diagnosis, fertilization method and the number of consecutive fresh embryo transfers per patient, did not contribute significantly to the GEE model and were therefore excluded, and were not adjusted for. We found that compared to SET with one high-quality embryo, the OPR was higher in patients that received DET with two high-quality embryos (adjusted OR 1.38, 95% CI 1.14–1.67), but lower in patients that received DET with one high- and one low-quality embryo (adjusted OR 0.65, 95% CI 0.47–0.90) (Table II top panel). DET with two low-quality embryos resulted in a not significantly different OPR (adjusted OR 0.84, 95% CI 0.55–1.28) compared to SET with one low-quality embryo (Table II lower panel).

Multiple gestation rates were higher in all DET groups compared to each respective SET group in both univariate (unadjusted) and multivariate (adjusted) analyses (DET with ≥1 high-quality embryo(s) compared to SET with one high-quality embryo; DET with two low-quality embryos compared to SET with one low-quality embryo; all comparisons $P < 0.001$; Table II). Additionally, miscarriage rates were similar in all DET groups compared to each respective SET group in both univariate and multivariate analyses (DET with ≥1 high-quality embryo(s) compared to SET with one high-quality embryo; DET with two low-quality embryos compared to SET with one low-quality embryo; all comparisons $P > 0.05$; Table II).

Number of high-quality embryos and pregnancy outcomes

Patients that received SET with one high-quality embryo may have had multiple high-quality embryos available, similar to patients that received DET with two high-quality embryos, whereas patients that received DET with one high- and one low-quality embryo had by definition only one high-quality embryo available. Therefore, we investigated whether the OPR was related to the number of high-quality embryos that were available per patient. Data on embryo morphology of all cultured embryos per patient were only available from the AMC. In this subset of the data, SET with one high-quality embryo resulted in an OPR of 33.2% (340/1024) in patients with multiple high-quality embryos available, and a significantly lower OPR of 19.5% (84/430; unadjusted OR 0.49, 95% CI 0.37–0.64) in patients with only one high-quality embryo available (Table III). This difference persisted after adjusting for maternal age, number of oocytes retrieved and the interaction between maternal age and number of oocytes retrieved (adjusted OR 0.52, 95% CI 0.39–0.70; Table III). Additionally, SET with one high-quality embryo resulted in a miscarriage rate of 25.5% in patients with multiple high-quality embryos available, and a higher miscarriage rate of 35.6% (119/467; adjusted OR 1.64, 95% CI 1.07–2.51) in patients with only one high-quality embryo available.

Subsequently, pregnancy outcomes in DET were compared to SET in this subset of the data, but now only clinically plausible comparisons between study groups were tested. Specifically, DET with two high-quality embryos was only compared to SET with one high-quality embryo in patients with two or more high-quality embryos available. Furthermore, DET with one high- and one low-quality embryo was only compared to SET with one high-quality embryo in patients that had only one high-quality embryo available. In this comparison, DET with two high-quality embryos resulted in a not significantly different OPR of 29.1% (148/509; unadjusted OR 0.83, 95% CI 0.66–1.04; adjusted OR 0.99, 95% CI 0.74–1.31) (Table IV top panel) while DET with one high- and one low-quality embryo resulted in a lower OPR of 13.2% (29/220; unadjusted OR 0.63, 95% CI 0.40–0.99), which was no longer statistically significant after adjusting for maternal age,

Table I Baseline characteristics of patients receiving SET or DET on Day 3 with low- or high-quality embryo(s).

	SET high-quality embryo (*n* = 4653)	SET low-quality embryo (*n* = 1102)	DET high-quality + high-quality embryo (*n* = 780)	DET high-quality + low-quality embryo (*n* = 383)	DET low-quality + low-quality embryo (*n* = 334)
Maternal age, years (SD)	34.3 (±4.4)	34.9 (±5.4)	38.4 (±3.7)*	38.2 (±6.1)*	37.5 (±4.4)*
Infertility diagnosis					
Male factor, *n* (%)	2178 (46.8)	511 (46.4)	331 (42.4)	173 (45.2)	146 (43.76)
Tubal factor, *n* (%)	640 (13.8)	136 (12.3)	97 (12.4)	53 (13.8)	40 (12.0)
Endometriosis, *n* (%)	381 (8.2)	74 (6.7)	32 (4.1)*	21 (5.5)	25 (7.5)
Unexplained, *n* (%)	1477 (31.7)	358 (32.5)	293 (37.5)*	127 (33.2)	127 (38.0)
Ovulatory, *n* (%)	444 (9.5)	118 (10.7)	65 (8.3)	38 (9.9)	25 (7.5)
Cervical, *n* (%)	69 (1.5)	11 (1.0)	5 (0.6)	2 (0.5)	3 (0.9)
Number of oocytes retrieved, *n* (SD)	10.1 (±5.6)	7.3 (±5.2)*	10.4 (±5.3)	7.4 (±4.1)*	8.5 (±5.2)*
Fertilization method					
IVF, *n* (%)	2226 (47.8)	543 (49.3)	346 (44.4)	178 (46.4)	153 (45.8)
ICSI, *n* (%)	2427 (52.2)	559 (50.7)	434 (55.6)	205 (53.5)	181 (54.2)
Number of consecutive fresh embryo transfers per patient, *n* (SD)	1.36 (±0.70)	1.44 (±0.69)*	2.00 (±1.10)*	1.98 (±1.11)*	1.87 (±1.12)*

SET, single embryo transfer; DET, double embryo transfer. The sum of all embryo transfers is 7252 after 13 cases were excluded because embryo morphology score at Day 3 was missing, and one case was excluded because pregnancy outcome was not reported. Values that are significantly different compared to SET with one high-quality embryo are labeled by asterisks (Wald Chi-square tests or one-way ANOVA; *$P < 0.05$).

Table II DET compared to SET in patients with (top panel) or without (lower panel) high-quality embryo(s) available.

≥1 High-quality embryo available	SET high-quality embryo	DET high-quality + high-quality embryo	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	DET high-quality + low-quality embryo	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Embryo transfers, *n*	4653	780	-	-	383	-	-
Ongoing pregnancy, *n* (%)	1423/4653 (30.6)	227/780 (29.1)	0.93 (0.79–1.10)	1.38 (1.14–1.67)	58/383 (15.1)	0.41 (0.30–0.54)	0.65 (0.47–0.90)
Multiple gestation, *n* (%)	26/1423 (1.8)	51/227 (22.5)	15.57 (9.46–25.62)	25.76 (13.47–49.27)	7/58 (12.1)	7.38 (3.05–17.84)	12.31 (4.48–33.81)
Chemical pregnancies, *n* (%)	2079/4653 (44.7)	358 (45.8)	1.05 (0.90–1.22)	1.38 (1.17–1.63)	99 (25.8)	0.43 (0.34–0.55)	0.60 (0.47–0.77)
Miscarriage, *n* (%)	636/2079 (30.6)	124/358 (34.6)	1.20 (0.95–1.52)	0.98 (0.76–1.28)	39/99 (39.4)	1.48 (0.97–2.24)	1.10 (0.71–1.72)

No high-quality embryo available	SET low-quality embryo	DET low-quality + low-quality embryo	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Embryo transfers, *n*	1102	334	-	-
Ongoing pregnancy, *n* (%)	149/1102 (13.5)	31/334 (9.3)	0.65 (0.44–0.99)	0.84 (0.55–1.28)
Multiple gestation, *n* (%)	2/149 (1.3)	4/31 (12.9)	10.89 (1.90–62.43)	16.48 (2.78–97.61)
Chemical pregnancies, *n* (%)	231/1102 (21.0)	56/334 (16.8)	0.76 (0.55–1.05)	0.84 (0.60–1.18)
Miscarriage, *n* (%)	82/231 (35.5)	25/56 (44.6)	1.47 (0.81–2.64)	1.25 (0.67–2.31)

Where indicated, odds ratios (ORs) are adjusted for maternal age, number of oocytes retrieved, center of treatment and the interaction between maternal age and the number of oocytes retrieved.

number of oocytes retrieved and the interaction between maternal age and number of oocytes retrieved (adjusted OR 0.78, 95% CI 0.47–1.27) (Table IV lower panel).

Compared to each respective SET group, multiple gestation rates were higher in DET with two high-quality embryos (20.9% versus 1.5%; unadjusted OR 17.75, 95% CI 6.74–46.77, adjusted OR 45.25,

Table III SET with one high-quality embryo in patients with multiple, or only one high-quality embryo(s) available.

Number of high-quality embryos available	SET high-quality embryo ≥ 2	SET high-quality embryo 1	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Embryo transfers, *n*	1024	430	-	-
Ongoing pregnancy, *n* (%)	340/1024 (33.2)	84/430 (19.5)	0.49 (0.37–0.64)	0.52 (0.39–0.70)
Multiple gestation, *n* (%)	5/340 (1.5)	2/84 (2.4)	1.63 (0.31–8.57)	2.23 (0.41–12.28)
Chemical pregnancies, *n* (%)	467/1024 (45.6)	132/430 (30.7)	0.53 (0.42–0.67)	0.58 (0.45–0.75)
Miscarriage, *n* (%)	119/467 (25.5)	47/132 (35.6)	1.62 (1.10–2.44)	1.64 (1.07–2.51)

Where indicated, ORs are adjusted for maternal age, number of oocytes retrieved and the interaction between maternal age and the number of oocytes retrieved.

Table IV DET compared to SET analysed separately in patients with multiple or only one high-quality embryo(s) available.

≥2 high-quality embryos available	SET high-quality embryo	DET high-quality + high-quality embryo	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Embryo transfers, *n*	1024	509	-	-
Ongoing pregnancy, *n* (%)	340/1024 (33.2)	148/509 (29.1)	0.83 (0.66–1.04)	0.99 (0.74–1.31)
Multiple gestation, *n* (%)	5/340 (1.5)	31/148 (20.9)	17.75 (6.74–46.77)	45.25 (11.37–180.09)
Chemical pregnancies, *n* (%)	467/1024 (45.6)	220/509 (43.2)	0.91 (0.73–1.12)	1.14 (0.87–1.50)
Miscarriage, *n* (%)	119/467 (25/5)	70/220 (31.8)	1.37 (0.96–1.94)	1.26 (0.85–1.86)
1 high-quality embryo available	**SET high-quality embryo**	**DET high-quality + low-quality embryo**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Embryo transfers, *n*	430	220	-	-
Ongoing pregnancy, *n* (%)	84/430 (19.5)	29/220 (13.2)	0.63 (0.40–0.99)	0.78 (0.47–1.27)
Multiple gestation, *n* (%)	2/84 (2.4)	1/29 (3.4)	1.46 (0.13–16.77)	5.93 (0.61–57.27)
Chemical pregnancies, *n* (%)	132/430 (30.7)	54/220 (24.5)	0.73 (0.51–1.10)	0.89 (0.60–1.33)
Miscarriage, *n* (%)	47/132 (35.6)	24/54 (44.4)	1.45 (0.76–2.75)	1.21 (0.60–2.48)

Where indicated, ORs are adjusted for maternal age, number of oocytes retrieved and the interaction between maternal age and the number of oocytes retrieved.

95% CI 11.37–180.09) and not significantly different in DET with one high- and one low-quality embryo (3.4% versus 2.4%, unadjusted OR 1.46, 95% CI 0.13–16.77, adjusted OR 5.93, 95% CI 0.61–57.27) (Table IV).

Discussion

The beneficial effect on live-birth rates of DET compared to SET has been mostly demonstrated in patients with two or more high-quality embryos available (Pandian *et al.*, 2013). Our study suggests that in patients with only one or no high-quality embryo(s) available, the addition of a low-quality embryo in DET may not improve the OPR, while increasing multiple gestation rates. At first, our data suggested that DET with one high- and one low-quality embryo resulted in a lower OPR compared to SET with one high-quality embryo. However, subsequent analysis illustrated an essential distinction among embryos qualified as being of high-quality, irrespective of similar morphological scores. Namely, SET with one high-quality embryo resulted in a lower OPR and a higher miscarriage rate in patients with only one high-quality embryo available, compared to patients with two or more high-quality embryos available. This indicates that the availability of multiple high-quality embryos converts into better individual quality of these embryos, and thus a higher OPR and a lower miscarriage rate. Therefore, DET in patients with either one- or multiple high-quality embryo(s) available should be compared to SET in patients with, respectively, one- or multiple high-quality embryo(s) available. We then found that the OPR was not significantly different in DET compared to SET in patients with only one high-quality embryo available.

Limitations to our study include the retrospective design, and thus the possibility of unascertained confounding factors. For example, contrary to a RCT (Thurin *et al.*, 2004), in this study the OPR was not significantly different in DET compared to SET in patients with two or more high-quality embryos available (Table IV top panel). Presumably, owing to embryo transfer policies between 2012 and 2015, this may be caused by biased patient characteristics in the study groups. Namely, patients with poor prognostic characteristics, such as maternal age of 38 years or over and a history of multiple failed IVF/ICSI

attempts, were offered DET, whereas SET was performed as standard treatment in all other patients. As a result, pregnancy outcomes in all DET groups may have been underestimated in the analyses, despite adjusting for maternal age and the number of oocytes retrieved.

Furthermore, we calculated the OPR per fresh embryo transfer and not per started IVF/ICSI cycle. Since patients were allocated to study groups based on the morphology of the transferred embryo(s), cycles that did not lead to an embryo transfer could not be allocated and were not included in the study. Therefore, pregnancy outcomes could only be calculated as a percentage of the number of fresh embryo transfers in each study group. Consequently, the reported pregnancy outcomes may not truly reflect the pregnancy chances of couples at the start of treatment. Moreover, we only investigated embryo transfers on Day 3 after fertilization and therefore our findings may not be applicable to pregnancy outcomes after blastocyst transfers.

A notable strength of our study is that we used a strict separation of high-quality embryos from all categories of lower-quality embryos, based on commonly accepted morphological characteristics in which top-quality or good-quality embryos on Day 3 have 7–9 evenly sized blastomeres, no multinucleation and up to 20% fragmentation (Balaban *et al.*, 2011; van Loendersloot *et al.*, 2014). Furthermore, we specifically compared pregnancy outcomes in patients with similar prognostic profiles based on embryo quality, i.e. patients with no-, only one- or multiple high-quality embryo(s) available. This distinction is in compliance with clinical practice, in which the availability and the quality of the available embryo(s) determines the embryo transfer strategy. Results from studies that do not make this distinction should be critically appraised and may not be applicable to clinical practice. For example, two recent smaller studies also investigated pregnancy outcomes in DET with one high- and one low-quality embryo compared to SET with one high-quality embryo (El-Danasouri *et al.*, 2016; Wintner *et al.*, 2017). However, within the group of patients that received SET with one high-quality embryo, neither of these studies distinguished patients with only one high-quality embryo available from patients with multiple high-quality embryos available.

Many clinicians worldwide perform DET as a routine treatment to increase the OPR in fresh embryo transfers, regardless of the quality of the embryos available (Calhaz-Jorge *et al.*, 2016). However, the decision to perform DET rather than SET may not be justified for patients that do not have two high-quality embryos available, because our data suggest that DET does not increase the OPR. Furthermore, regardless of embryo quality, any potential increase in OPR should be weighed against an increased multiple gestation rate in DET compared to SET. Additionally, pregnancy outcomes in DET compared to SET may differ depending on prognostic characteristics per patient, i.e. maternal age and the number of oocytes retrieved.

Given the retrospective nature of our study, future prospective RCTs are needed and should investigate separately the pregnancy outcomes in patients with different prognostic profiles and in patients with multiple, only one or no high-quality embryo(s) available for transfer.

Authors' roles

The study was designed by C.B.L., S.R., S.M., C.G.V., R.S., G.H. and R.P.B. Data were provided and collected by A.A.M. and C.G.V. Statistical analyses were performed by R.P.B. and M.W. The original manuscript was drafted by R.P.B. All authors critically reviewed and revised the manuscript and approved the final version.

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Conflict of interest

The authors have no conflicts of interest to declare.

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