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ORIGINAL ARTICLE

Evaluation of possible criteria for elective single embryo transfer

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Abstract
Purpose A major problem of assisted reproductive technology (ART) is multiple gestation, which impacts neonatal and perinatal medicine. The literature contains a number of reports that elective single embryo transfer (eSET) is effective for the control of multiple pregnancies; however, to date, uniform criteria have not been established.
Methods Using logistic regression analysis based on the results of ART in our department from January 2005 to July 2006, our eSET criteria were established. We conducted a comparative study of the clinical pregnancy rate, multiple gestation rate, and delivery rate before and after eSET (before-eSET and after-eSET groups, respectively).
Results As a result of the analysis, our eSET criteria included all three of the following: (A) patient age $\leq$37, (B) previous IVF/ICSI trials $\leq$5, and (C) acquisition of two or more good-quality embryos. Based on our criteria, the after-eSET group was not found to have a decrease in the pregnancy rate; however, the multiple gestation rate decreased as compared to the before-eSET group. In addition, as a result of various evaluations of the eSET group, interesting findings were revealed.
Conclusions In the after-eSET group, our eSET criteria achieved a decrease in the multiple pregnancy rate without a decrease in the pregnancy rate.

Keywords Assisted reproductive technology · In vitro fertilization · Elective single embryo transfer · Multiple gestation rate · Clinical pregnancy rate

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Introduction

Recent advances in assisted reproductive technology (ART) have improved the pregnancy rate. Since the first successful birth following in vitro fertilization-embryo transfer was accomplished by Steptoe and Edwards [1], the pregnancy rate has increased as a result of improved reproductive technology techniques (including improved culture solutions and advancement of culture technology). ART is now commonly employed as an infertility treatment; however, the multiple gestation rate has risen as a result. The frequency of twin and triplet pregnancy by ART is reported to be 20 times and 400 times higher, respectively, than that of normal pregnancy [2]. The literature has been systematically reviewed in regard to outcomes of IVF/ICSI multiple gestation, i.e., pregnancy complications, maternal risks, obstetric outcome, and long-term morbidity [3–5], including an increase in pregnancy-induced hypertension and preeclampsia [6]; gestational diabetes [7]; premature deliveries, low birth weight infants, and cesarean delivery [8]; and neurological sequelae (particularly increased risk of cerebral palsy [9]). Decreasing the number of embryos transferred from three to two has been implemented to reduce the multiple gestation rate;

however, with the employment of double embryo transfer (DET), the multiple gestation rate is reported to range from 15 to 25%, much higher than that of normal pregnancy (1.3%) [10–12]. It has been reported that single embryo transfer (SET) must be conducted to reduce the multiple gestation rate. Opponents of SET cite a decreased pregnancy rate [13–15]; however, these reports are limited in that they only present evaluations of the results of cleavage-stage embryo transfers. Recently, via advancement of culture technology (culture to the blastocyst stage), SET of blastocysts has been employed, and the blastocyst stage SET has been reported to have a significantly higher pregnancy and delivery rate than that of cleavage stage SET [16–19]. It is easier to determine embryo quality at the blastocyst stage than at the cleavage stage. Moreover, it has been proposed that blastocyst stage embryo transfer is more in synch with endometrial status. The transfer cancellation rate and the frequency of monozygotic twins are higher in blastocyst stage embryo transfer [20, 21]. Therefore, it is necessary to determine carefully the time of embryo transfer on a case-by-case basis. With this goal in mind, the establishment of uniform criteria is essential. Criteria for elective single embryo transfer (eSET) to control the frequency of multiple gestation have been established in our department, based on the results obtained by using logistic regression analysis of DET from January 2005 to July 2006. We began a prospective study of eSET in January 2007 according to our criteria. We then compared the multiple gestation and delivery rates before and after the introduction of eSET. The cumulative pregnancy rate and the pregnancy rate by age, IVF/ICSI trial number were examined between January 2007 and December 2008 based on the clinical results of eSET using our criteria.

Materials and methods

Patients and study design

To introduce eSET for the control of multiple gestation, we examined 212 cycles of DET (cleavage-stage embryo transfer: $n = 184$; blastocyst-stage embryo transfer: $n = 28$) within 501 cycles of enforced IVF/ICSI in our department from January 2005 to July 2006. Logistic regression analysis was applied to age, length of infertility, embryo grade, and number of previous IVF/ICSI trials; all of the foregoing are factors that may influence the multiple gestation rate (Table 1).

We compared the clinical pregnancy rate, multiple gestation rate, pregnancy loss rate, and delivery rate before and after eSET [before-eSET group (2005–2006), $n = 583$; after-eSET group (2007–2008), $n = 504$; Table 2]. All of these 1,087 cycles were examined retrospectively.

Table 1 Logistic regression analysis of this study (January 2005–July 2006)

	P value
Age	0.43673852
Duration of infertility	0.89789496
IVF/ICSI trial number	0.02973469
Embryo's quality grade	0.00164118

Become a multiple pregnancy in patients that got pregnant by DET

Table 2 Patient's characteristics of before-eSET and after-eSET groups

	Before-eSET (2005–2006)	After-eSET (2007–2008)
N	583	504
Age (years)	36.5 ± 0.4	36.2 ± 0.4
Cause of infertility, no. (%)		
PCOS	36 (6.2)	40 (8.0)
Tubal	114 (19.6)	104 (20.6)
Endometriosis	91 (15.6)	89 (17.6)
Male	156 (26.8)	125 (24.9)
Unexplained	158 (27.1)	130 (25.8)
Others	28 (4.8)	16 (3.1)
Duration of infertility (years)	5.8 ± 0.4	5.1 ± 0.4
Total gonadotropin dose (IU)	1949.8 ± 81.8	1963.1 ± 106.8
No. of oocytes retrieved	4.9 ± 0.6	5.1 ± 0.3
No. of fertilized oocytes	2.6 ± 0.5	3.1 ± 0.2

No significant difference was found between two groups

In addition, we prospectively compared the eSET group ($n = 159$) that fit our eSET criteria between 2007 and 2008, the DET group ($n = 97$) that did not fit our eSET criteria between 2007 and 2008 [the remaining 248 cases during the period in which SET or three embryo transfer was compulsory (2007–2008) were excluded], and the elective DET (eDET) group ($n = 112$; 2005–2006) in which two good-quality embryos were transferred (Table 3). We explained our eSET policy to all the patients considering ART and selected eSET only for patients who had provided informed consent.

Ovarian stimulation and IVF/ICSI-ET

Ovarian stimulation was performed with a gonadotropin-releasing hormone agonist (400 μg/day intranasal nafarelin acetate; Nasanyl, Astellas Pharma, Tokyo, Japan). The treatment was begun in the mid-luteal phase of the previous cycle (long protocol) or on day 2 of the current cycle (short protocol) for down-regulation; the treatment was

Table 3 Patient's characteristics of eSET, DET, and eDET group

	eSET (2007–2008)	DET (2007–2008)	eDET (2005–2006)
n	159	97	112
Age (years)	33.6 ± 0.4	34.8 ± 0.4	33.8 ± 0.4
Cause of infertility, no. (%)			
PCOS	18 (11.3)	5 (5.2)	9 (8.0)
Tubal	44 (27.7)	19 (19.6)	24 (21.4)
Endometriosis	30 (18.9)	24 (24.7)	23 (20.5)
Male	27 (17.0)	27 (27.8)	29 (25.9)
Unexplained	40 (25.1)	22 (22.7)	27 (24.1)
Duration of infertility (years)	4.6 ± 0.3	6.1 ± 0.4	5.1 ± 0.4
Total gonadotrophin dose (IU)	1,941.9 ± 90.6	1,988.4 ± 119.2	2,161.4 ± 101.6
No. of oocytes retrieved	6.4 ± 0.5	5.2 ± 0.4	5.9 ± 0.4
No. of fertilized oocytes	4.9 ± 0.4	3.8 ± 0.3	4.1 ± 0.3

P value comparing the three groups was not statistically significant across each parameter

conducted in combination with follicle-stimulating hormone (FSH; Fertinom P, Sereno, Tokyo, Japan) and human menopausal gonadotropin (HMG, HMG-nikken-150; Nikken Chemicals, Tokyo, Japan, and/or HMG Injection Teizo; Aska Pharmaceutical, Tokyo, Japan). The HMG injection varied from 150 to 300 IU according to the degree of follicle growth. Ovarian response was confirmed by transvaginal sonography (TVS). When at least two follicles reached a diameter ≥16 mm, ovulation was induced with 5,000–10,000 IU of human chorionic gonadotropin (HCG; Aska Pharmaceutical). Oocyte retrieval by transvaginal ultrasound-guided puncture was performed from 35 to 36 h post-HCG administration.

Oocytes were inseminated using either IVF or ICSI between 4 and 6 h after oocyte retrieval. ICSI was carried out for patients with a severe male factor including one or more of the following: sperm concentration $<20 \times 10^6$/ml, progressive motility <50%, and normal morphology <30%.

Embryos were cultured in Fertilization Solutions (Cook Medical, Bloomington, IN) and Cleavage Solutions (Cook Medical) under an atmosphere of 6% CO_2, 5% O_2, and 89% N_2 at 37°C in an incubator (ASTEC, Fukuoka, Japan) for 48 h. In order to obtain blastocysts, cleavage stage embryos were cultured in BlastAssist System 1, 2 (MediCult; Jyllinge, Denmark) for an additional 72–96 h.

The cleavage stage embryos were transferred into the uterine cavity using a FS-ET catheter (Kitazato Supply, Shizuoka, Japan) on day 2 or day 3 via transvaginal ultrasound-guided oocyte retrieval. Conversely, blastocyst embryo transfer was carried out on day 5 or day 6 by the same method. Surplus good-quality embryos were frozen using the Cryotop method. This method for oocyte vitrification was used as previously reported by Kuwayama et al. [22].

Luteal phase support and evaluation of pregnancy

Luteal function was supported with the injection of 2,000 IU HCG at 0, 3, and 6 days after embryo transfer. Clinical pregnancies were diagnosed by a urine HCG test (Sanwa Kagaku Kenkyusho, Nagoya, Japan) 16 days after embryo transfer and confirmed by the presence of a gestational sac by TVS at 5–6 weeks of gestation.

Statistical analysis

The calculations were performed with the Stat View (Hulinks, Tokyo, Japan) and Stat Mate III (ATMS, Tokyo, Japan). Variables in this study groups were compared using logistic regression analysis and the χ^2 test. A P value of <0.05 was considered to be statistically significant.

Results

With logistic regression analysis, we found a significant difference in embryo grade ($P < 0.01$) and IVF/ICSI trials ($P < 0.05$) (Table 1). Moreover, we examined the relationship between the number of previous IVF/ICSI trials and the multiple gestation rate as well as the theoretical multiple gestation rate in transfer rate for each age group. The following factors were found to be possible causes of multiple gestation: patient age ≤37, number of previous IVF/ICSI trials ≤5, and enforcement of good-quality embryos in DET [cleavage stage: grade 1 or 2 (Veeck's classification); blastocyst stage: grade ≥3AA (Gardner's classification)]. As a result, we determined our eSET criteria to include all of the following: (A) patient age ≤37, (B) previous IVF/ICSI trials ≤5, (C) acquisition of two or more good-quality embryos [cleavage stage: grade 1 or 2 (Veeck's classification);

blastocyst stage: grade ≥3AA (Gardner's classification)] (Table 4).

In January 2007, we introduced the eSET criteria in our department in an attempt to decrease the multiple gestation rate. When the after-eSET group was compared to the before-eSET group, no significant difference was observed in the clinical pregnancy rate per embryo transfer [164/504 (32.5%) versus 205/583 (35.2%)], delivery rate per embryo transfer [128/504 (25.4%) versus 162/583 (27.9%)], or pregnancy loss rate per pregnancy [36/164 (22.0%) versus 43/205 (21.0%)]. Furthermore, the multiple gestation rate per pregnancy in the after-eSET group was significantly decreased when compared to that in the before-eSET group [6/164 (3.7%) versus 21/205 (10.2%)] (Table 5). In addition, we conducted a comparative study of the following three groups. In regard to the clinical pregnancy rate per embryo transfer, there was no significant difference between eSET and DET; the pregnancy rate for the eDET group was significantly higher than that of the eSET group. The multiple gestation rate per pregnancy in eSET group was significantly lower than those in both the DET and eDET groups, and the multiple gestation rate per delivery in the eSET group (0%, 0/65) was significantly lower than that in the DET (12.8%, 6/47) and eDET groups (19.7%, 14/71) (Table 6). When cleavage stage embryo transfer was compared with blastocyst stage embryo transfer, the clinical pregnancy rate in the eSET group was 18/49 (37%) for cleavage stage embryos and 30/63 (48%) for blastocyst stage embryos. And it did not become a pregnancy by eSET in fresh cycles, and the accumulation pregnancy rate rescued by the frozen embryo transfer is present 67.9% in the point (Table 7). In addition, the pregnancy rate per each 3-year division was similar [26–28: 4/8 (50%); 29–31: 10/26 (39%); 32–34: 13/32 (41%); and 35–37: 21/51 (41%)] (Table 8). The difference in pregnancy rate based on the number of previous IVF/ICSI and eSET cycles was also

Table 4 eSET criteria of this study

(A) Patient age ≤37
(B) Previous IVF/ICSI trials ≤5
(C) The acquisition of two or more good-quality embryos
Cleavage stage embryo: Grade 1 or 2 (Veeck's classification)
Blastocyst stage embryo: Grade ≥3AA (Gardner's classification)
The whole of (A)–(C)

Table 5 The cumulative outcome of before-eSET and after-eSET group

Variable	Before-eSET (2005–2006)	After-eSET (2007–2008)	
No. of transfers	583	504	
Average number of ET	1.66 ± 0.24	1.32 ± 0.18	NS
Clinical pregnancy rate	205/583 (35.2)	164/504 (32.5)	NS
Pregnancy loss rate	43/205 (21.0)	36/164 (22.0)	NS
Delivery rate	162/583 (27.9)	128/504 (25.4)	NS
Multiple pregnancy rate	21/205 (10.2)[a]	6/164 (3.7)[a]	

NS Not statistically significant

[a] $P < 0.05$

Table 6 The cumulative outcome of eSET, DET, and eDET group

Variable	eSET (2007–2008)	DET (2007–2008)	eDET (2005–2006)
No. of transfers	159	97	112
Clinical pregnancy rate	65/159 (40.9)[a]	47/97 (48.5)	71/112 (63.4)[a]
Pregnancy loss rate	14/65 (21.5)	9/47 (19.1)	16/71 (22.5)
Delivery rate	51/159 (32.1)[b]	38/97 (39.2)	53/112 (46.4)[b]
Multiple pregnancy rate	0/65 (0.0)[a,b]	6/47 (12.8)[b]	14/71 (19.7)[a]

[a] $P < 0.01$, [b] $P < 0.05$

Table 7 Rates of pregnancy after the transfer of cleavage stage and blastocyst stage embryos in eSET

Variable	Cleavage	Blastocyst	Thawed	
No. of transfers	49	63	42	
Clinical pregnancy rate	18/49 (36.7)	30/63 (47.6)	18/42 (42.9)	NS
Accumulation pregnancy rate[a]			76/112 (67.9)	
Multiple pregnancy rate	0/18 (0.0)	0/30 (0.0)	0/18 (0.0)	NS

NS Not statistically significant

[a] It has not become a pregnancy by eSET in fresh cycles and was rescued by the frozen embryo transfer

Table 8 Pregnancy rate according to age (in eSET group)

Age (years)	Clinical pregnancy rate
26–28	4/8 (50.0)
29–31	10/26 (38.5)
32–34	13/32 (40.6)
35–37	21/51 (41.2)

P value comparing the four groups was not statistically significant

Table 9 Relation of IVF/ICSI trial number and pregnancy rate (in eSET group)

Trial number	Pregnancy rate
1	22/59 (37.3)
2	10/27 (37.0)
3	7/13 (53.8)
4	5/11 (45.5)
5	4/7 (57.1)

P value comparing the five groups was not statistically significant

similar [1st trial; 22/59 (37%); 2nd trial: 10/27 (37%); 3rd trial: 7/13 (54%); 4th trial: 5/11 (46%); 5th trial: 4/7 (57%)] (Table 9).

Discussion

ART has markedly improved the pregnancy rate for infertile couples; however, the multiple gestation rate resulting from ART is a major problem for neonatal and perinatal medicine. In Sweden, the adoption of SET was accomplished in 2003 to decrease the multiple gestation rate with IVF/ICSI [23]. Moreover, clinical guidelines for SET or eSET have been established in the US [24], Britain [25], and other countries. However, only a handful of papers have defined the criteria of eSET, especially in Japan. We extracted four factors according to logistic regression analysis. Clinical guidelines for the above-mentioned countries exhibited almost the same factors as ours.

In January 2007, we introduced our criteria for eSET in our department in an attempt to decrease the multiple gestation rate. As a result, we were able to decrease the multiple gestation rate without a decrease in either the clinical pregnancy rate or the delivery rate. The eSET criteria that we established were found to produce excellent results; however, even if our criteria were used, multiple gestation was not completely prevented. As a result, we will reevaluate and verify our adopted criteria (adjustment age, number of previous IVF/ICSI trials, and selection of a good embryo).

The first enforcement of eSET occurred in Finland; the procedure was performed on a young patient with the goal of the prevention of multiple gestation and the attainment of two or more good-quality embryos [26]. eSET was later performed on older women <36 years of age [26–30]. These studies found a decrease in the multiple gestation rate without a decrease in the pregnancy rate in each case. Furthermore, Van Montfoort et al. [31] reported that eSET was effective for women ≤38 years of age, and Veleva reported that eSET was efficacious for the treatment of infertility patients from 36 to 39 years of age [32]. In our department, eSET was enforced for women ≤37 years of age, and our findings were comparable to those of Veleva. It is widely accepted that the pregnancy rate decreases with increased maternal age [33]. When we examined the pregnancy rate in 3-year age divisions in the eSET group, the pregnancy rate was similar for all divisions; thus, the pregnancy rate did not decrease as age increased (Table 8). The age range for eSET is expected to be expanded in the future.

In regard to the number of IVF/ICSI trials and eSET criteria, the literature contains a number of reports recommending one or two previous trials and a report by Van Montfoort et al. [31] that success was achieved after three previous trials. In a European Society for Human Reproduction and Embryology (ESHRE) consensus meeting, the recommended enforcement frequency of IVF/ICSI and eSET was two or fewer previous trials [4]. With our criteria, the number of previous of IVF/ICSIs and eSETs was set at five or fewer previous trials. We obtained excellent results and did not find a significant difference in the pregnancy rate based on the number of previous trials (Table 9). Expansion of the recommended number of previous trials should be considered.

With our criteria, it might be difficult to evaluate embryo quality, as quality is subjective. It is possible that embryos selected for transfer may not actually be of good quality. In the DET group presented in Table 6, the multiple gestation rate was comparatively high. The possibility exists that the quality of both embryos was excellent, despite the fact that one of them had been evaluated as poor quality. Recently, using scanning electrochemical microscopy, the relationship between mitochondrial oxygen consumption and embryo quality can be evaluated both quantitatively and objectively [34]. However, to date, widespread adoption of this method has not occurred. Hopefully, an objective, quantitative evaluation method of embryo quality will be established in the near future.

In conclusion, it can be inferred from our research that we must pursue further revision of the criteria for eSET in regard to patient age and number of IVF/ICSI cycles with the goal of reducing multiple gestation. We also must take into consideration the underlying cause of infertility. Some researchers favor the expansion of eSET criteria [35], while others oppose such an expansion [36, 37], and there is no common consensus worldwide [38–41]. The development of the technology that can expand the implementation of eSET with strict safeguards should result in an overall improvement of ART.

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