Elective single-embryo transfer improves cumulative pregnancy outcome in young patients but not in women of advanced reproductive age

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Abstract

Purpose The purpose of the present study is to assess the significance of elective single-embryo transfer (eSET) in older women.

Methods The outcomes of assisted reproductive technology between 2001 and 2013 at single institution were retrospectively evaluated. Cumulative live birth rates (CLBRs) in one oocyte retrieval cycle were compared between those who underwent eSET and multiple embryo transfer (MET) in fresh cycles.

Results The outcomes of 429 eSET cycles and 965 MET cycles were compared. CLBRs in eSET were higher than those of MET in women under 37 and were comparable in women aged 37 and over. The analysis of the outcomes separately in three age subgroups showed a significantly higher CLBR in young eSET (aged under 37) than that in young MET and similar CLBR between older (aged 37–40 and over 40) eSET and MET. Multiple birth rates were lower in eSET in all age groups. Multivariate logistic regression analyses showed that, in women aged under 37, number of frozen embryos, presence of good-quality embryos, and eSET were significantly related to cumulative live birth. In women aged between 37 and 40, age and number of frozen embryos were significantly related, while eSET was not.

Conclusions eSET in women under 37 resulted in increased CLBR compared with MET. In women aged between 37 and 40, CLBR in eSET group was similar with that in MET group. In both age groups, eSET reduced multiple birth rates. The significance of eSET in older women is limited presently, and further research on the strategy to improve cumulative outcomes is necessary.

Keywords eSET · Age · Cumulative live birth rate · Multivariate logistic regression analyses

Introduction

Multiple pregnancy as a result of more than one embryo transfer is one of the most serious complications of assisted reproductive technology (ART), leading to increased maternal morbidity and mortality as well as perinatal complications [1]. In the last 15 years, in proportion as advancements in ART and increased implantation rate, reduce the number of transferred embryos has been of main concern for many of the clinicians involved in ART in the world [2, 3].

Elective single-embryo transfer (eSET) has been widely accepted as a strategy to reduce the risk of multiple birth especially in selected patients. Most of the previous reports regarding the efficacy of eSET have targeted younger patients with enough ovarian reserve, describing a significantly lower rate of multiple pregnancy than multiple embryo transfer cycles, as well as comparable cumulative pregnancy rates including subsequent frozen embryo transfer cycles [4, 5].

On the other hand, the significance of eSET in unselected or older patients is controversial. In a non-randomized study in women aged 36–39 years, clinical pregnancy rate and live birth rate in fresh transfer cycles were similar between eSET and double-embryo transfer (DET) groups [6]. Another

Capsule Elective SET in women aged 37 and over reduced multiple pregnancy rate but did not improve cumulative live birth rate.

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retrospective cohort study showed similar results in women aged 40–44 years [7]. In these two reports, women in eSET groups achieved significantly higher cumulative pregnancy and live birth rates including subsequent frozen-thawed embryo transfer cycles than those in DET groups. However, in a review from a Canadian study group, in women aged 38 years and over, eSET resulted in a significant reduction in live birth rate compared with DET [8]. There have been very few large-scale studies regarding eSET in older patients compared with those in younger patients.

In 2008, the Japan Society of Obstetrics and Gynecology (JSOG) issued guidelines regarding mandatory SET for women less than 35 years of age and in their first and second ETs to reduce multiple pregnancies. Since then, we have recommended patients of all ages to undergo SET according to the instructions of JSOG. We have expanded application of eSET to women of higher age because we considered that multiple pregnancies in older women might be associated with increased maternal morbidity [9] and that frozen embryo transfer after failed fresh transfer cycles could compensate low success rate of eSET in them.

In this retrospective study, the overall effectiveness of eSET was evaluated with cumulative live birth rate (CLBR) after fresh and frozen transfer cycles in one oocyte retrieval. We compared the outcome after eSET with that after multiple embryo transfer (MET) to examine the significance of eSET policy in unselected patients and to readdress the indication of eSET.

Material and methods

This retrospective study included all women who underwent ART treatment in the IVF Unit of University of Tokyo Hospital between January 2001 and December 2013. The Institutional Review Board approval was obtained at University of Tokyo Hospital. Controlled ovarian stimulation (COS) was mainly performed using long protocol. Nafarelin nasal spray (Nasanyl[®]; Pfizer Pharmaceuticals, Japan) was started in the mid-luteal phase. On the third day of the following menstruation cycle, ultrasound examination was performed to confirm pituitary suppression, followed by the administration of human menopausal gonadotropin (hMG) (hMG Teizo[®]; ASKA Pharmaceutical Co. Ltd., Japan) or pure follicle-stimulating hormone (FSH) (Gonapure[®]; ASKA Pharmaceutical Co. Ltd., Japan). In patients with diminished ovarian reserve, clomiphene citrate and/or hMG combined with GnRH antagonist was used. Clomiphene citrate (Clomid[®]; Shionogi & Co. Ltd., Japan) or hMG was started on the third day of a menstrual cycle, followed by the combination with ganirelix acetate (Ganirest[®]; MSD Co. Ltd., Japan) 0.25 mg s.c. When the size of the lead follicle reached

18 mm in mean diameter as measured by transvaginal ultrasound, 10,000 IU of human chorionic gonadotropin (hCG) was administered. Oocytes were retrieved transvaginally with ultrasound guidance 34 h after hCG injection. They were inseminated or injected with husband's spermatozoa, depending on semen quality. Number of cells and morphology of each embryo were evaluated according to the criteria reported by Veeck [10] on day 3 after oocyte retrieval. Seven or eight cell embryos with grade 1 or 2 were defined as good-quality embryos. Other embryos with grades 1–3 were defined as fair-quality and those with grades 4–5 as poor-quality embryos. Embryo transfer was performed under ultrasound guidance on day 3 after oocyte retrieval, and the supernumerary embryos were cryopreserved in day 3 or day 5 using vitrification method. Thawed embryo transfer was performed in natural or hormone replacement cycles. The luteal phase was supported with progesterone suppository (200 mg/day) and transdermal estradiol (Estraderm M[®]; Kissei Pharmaceuticals, Japan). Luteal support was continued up to 7 weeks of gestation, when fetal heartbeat was detected with transvaginal ultrasound.

Between 2001 and 2007, number of embryos transferred was 2 or 3, depending on patients' age, embryo quality, and previous results of treatment. eSET was performed only for parous women or for those for whom multiple pregnancy was contraindicated for medical or other reasons. In and after 2008, women of all ages who had good-quality embryos were strongly recommended to undergo eSET to minimize the risk of multiple pregnancies. They were informed of possible risks associated with multiple pregnancies and of chances of success after subsequent frozen embryo transfer cycles. Double-embryo transfer (DET) was performed for patients under 39 years of age with more than four previous implantation failures and for patients aged 39 years and over with more than three previous failures. When there were no good-quality embryos suitable for transfer, embryo transfer was performed or cancelled after taking the couples' opinion into consideration. In such cases, SET was performed in the patients under 39 years of age with less than three previous failures, and DET was performed in other patients. The supernumerary embryos were incubated until day 5, when they were evaluated again, and blastocysts suitable for transfer were cryopreserved.

Data on fresh embryo transfer and subsequent frozen-thawed embryo transfer were collected as cumulative results in one oocyte retrieval cycle. Cumulative live birth was defined as ever occurrence of a live birth as a result of single oocyte retrieval and treated as a bivariate outcome. Cumulative results were analyzed as of December 2014. Ongoing pregnant cases in more than 12 weeks of pregnancy using frozen embryos were counted for live birth.

Patient selection and statistics

Only women who underwent fresh embryo transfer were included in the present study. The inclusion criterion for eSET group was defined as single-embryo transfer with one or more frozen embryos. The criterion for multiple embryo transfer (MET) group is defined as two or three embryo transfers in fresh cycles. In both groups, women who underwent three or more previous oocyte retrieval (OR) cycles were excluded from the study. Clinical pregnancy and implantation rates of the patients in the study groups in each year were analyzed with Spearman's rank correlation coefficient. In the two study groups, live birth rate following fresh transfer cycles and CLBR were compared in different age groups. According to the result of the first analysis, patients were divided into six groups—young eSET, intermediate eSET, old eSET, young MET, intermediate MET, and old MET. Next, the characteristics and the outcomes of ART following eSET or MET were compared in each age group. Chi-square tests were used to compare insemination method (IVF or intracytoplasmic sperm injection (ICSI)), controlled ovarian stimulation (COS) protocols (long protocol, antagonist protocol or others), presence of good-quality embryos, clinical pregnancy rate, live birth rate, and multiple live birth rate in fresh, frozen transfer cycles and cumulative outcomes. Number of retrieved oocytes was compared using *t* test. Thirdly, significance of eSET in three age groups to achieve cumulative live birth was examined using logistic regression analysis. We adjusted for confounders including year of treatment, maternal age, insemination method, COS protocol, number of retrieved oocytes, number of frozen embryos, and presence of good-quality embryos. In each age group, multilevel univariate analyses were conducted with cumulative live birth as the dependent variable. Variables with $p < 0.20$ in the univariate analysis were selected for variables in subsequent multivariate analysis. Excel Statistics Ver.6.0[®] and Excel Multivariate Analysis Ver. 6.0[®] (Esumi Co. Ltd., Tokyo, Japan) were used as a statistical software program. Statistical significance was defined as $p < 0.05$.

Results

During the study period, there were 2624 OR cycles conducted in the IVF Unit of University of Tokyo Hospital. The age of the patients was between 24 and 46 years old. The patient selection chart for the two study groups is shown in Fig. 1. In the study period, there were 1987 fresh embryo transfer cycles, 788 of which were conducted as SET and others as MET. In SET cycles, 437 were accompanied by frozen embryos. Eight of 437 eSET cycles were excluded from the study because of three or more previous OR cycles. Of 1199 MET cycles, 234 cycles with three or more previous OR cycles were

excluded from the further analysis. Accordingly, 429 eSET cycles and 965 MET cycles met the criteria for the present study.

The study period in the present research was as long as 13 years, so we analyzed clinical pregnancy and implantation rates per transfer in fresh cycles of women aged under 37 included in the study in each year (Fig. 2). Clinical pregnancy rate did not change ($r = 0.374$, $p = 0.21$), while implantation rate significantly increased during the study period ($r = 0.769$, $p = 0.002$). Live birth rate in fresh transfer cycles and CLBR of the two groups stratified by age are shown in Fig. 3. In both groups, live birth rates declined according to maternal age. In eSET group, live birth rates in fresh transfer cycles were slightly higher than those of MET group in women aged 36 and under. In ICSI, the differences of live birth rate between eSET and MET in young women (aged under 31) were bigger than those in IVF (Fig. 3a, b). However, in women aged 37 and over, live birth rates in eSET fresh cycles rapidly dropped and fell below those of MET cycles in both IVF and ICSI. Rapid declines of success rate in older women with eSET were also observed in CLBR (Fig. 3a–c). CLBRs in eSET group were higher than those of MET group in women under 37 and were comparable in women aged 37 and over (Fig. 3c).

According to the result of initial analysis, significance of eSET policy was examined separately in three age groups—younger (under 37), intermediate (between 37 and 40), and older (over 40) patients. The characteristics and outcomes of treatment cycles in six groups—young eSET (group A), young MET (group B), intermediate eSET (group C), intermediate MET (group D), old eSET (group E), and old MET (group F)—are shown in Table 1. Insemination methods (IVF or ICSI) were similar between groups A and B, while significantly more women in group D underwent ICSI than those in group C. In all age groups, use of GnRH antagonist was significantly more frequent in eSET cycles than in MET cycles. Number of retrieved oocytes was similar in all age groups. In eSET, significantly more ETs were performed with good-quality embryos in all age groups.

Clinical pregnancy rate in fresh transfer cycles was not significantly different in younger age groups (groups A and B), and live birth rate was significantly higher in group A than in group B ($p = 0.033$). There was no multiple birth in group A while 23.6 % resulted in multiple deliveries in group B. In group A, number of frozen embryos was significantly higher than in group B. Over one third of frozen embryo cycles (35.3 %) resulted in live birth following subsequent transfer cycles, and CLBR reached 66.1 % in group A, significantly higher than those in group B.

In women aged 37–40, clinical pregnancy rate and live birth rate in fresh transfer cycles were significantly lower in eSET group (group C) than in MET group (group D). In group C, significantly more embryos were frozen, and 20.0 % of

Fig. 1 The patient selection chart for the two study groups

frozen cycles resulted in live birth. CLBR in group C was slightly higher than that in group D, though not statistically significant (31.9 vs. 25.3 %, $p=0.13$). Multiple birth rates in fresh transfer cycles, frozen transfer cycles, and cumulative results were significantly lower in group C than those in group D. In women aged over 40, there were no statistically significant differences in live birth rate in fresh transfer cycles or CLBR between eSET and MET. Cumulative multiple birth rate in eSET group (group E) was lower than that in MET group (group F), though not statistically significant (0 and 9.5 %, respectively).

As shown in Table 1, there were several possible confounding factors in eSET and MET groups.

Therefore, to elucidate the significance of eSET in each age group, we subsequently investigated independent factors

associated with cumulative live birth using logistic regression analysis. Results of univariate analysis in three age groups are shown in Table 2. In women aged under 37, year of treatment, age, number of retrieved oocytes, number of frozen oocytes, presence of good-quality embryos, and eSET were significantly related to cumulative live birth ($p<0.05$). In women aged between 37 and 40, age, ICSI, number of retrieved oocytes, number of frozen embryos, and presence of good-quality embryos were significantly related to cumulative live birth, while eSET was not ($p=0.130$). In women aged over 40, age and number of retrieved oocytes tended to be related to cumulative live birth, though not statistically significant ($p=0.067$ and 0.063 , respectively).

Results of subsequent multivariate logistic regression analysis in each age group using variables with $p<0.20$ in the

Fig. 2 Clinical pregnancy/implantation rates in fresh cycles for women aged under 37 in each year

Fig. 3 Live birth rates in fresh transfer cycles and cumulative live birth rates of the two study groups stratified by age. **a** IVF. **b** ICSI. **c** IVF + ICSI

univariate analysis are shown in Table 3. In women aged under 37, number of frozen embryos, presence of good-quality embryos, and eSET were significantly related to cumulative live birth after adjusting for other factors. In women aged between 37 and 40, age and number of frozen embryos were significantly related to cumulative live birth after adjusting for other factors. However, eSET was not significantly related to cumulative live birth ($p=0.224$). In women aged over 40, none of the factors examined was not related to cumulative live birth.

Discussion

Our results showed that eSET policy with a cleavage stage embryo in women aged under 37 improved CLBR compared with MET, while it did not in women aged 37 and older. In

Table 1 Characteristics and outcomes of treatment cycles in six study groups

	(A) eSET <37	(B) MET <37	(C) eSET 37–40	(D) MET 37–40	(E) eSET >40	(F) MET >40	<i>p</i> value(A vs. B)	<i>p</i> value(C vs. D)	<i>p</i> value(E vs. F)
No. of treatment cycle	221	457	160	320	48	188			
IVF/ICSI	99/122	194/263	57/103	158/162	16/32	75/113	0.56	0.004	0.41
Long/antagonist/others	141/73/7	389/53/15	49/109/2	230/79/11	4/43/1	88/87/13	<0.001	<0.001	<0.001
No. of retrieved oocytes	10.1±5.1	10.6±5.3	7.8±5.0	8.6±4.6	6.1±2.9	7.0±4.4	0.23	0.08	0.08
Cycles with good-quality embryos (%/fresh transfer)	201 (91.0 %)	330 (72.2 %)	141 (88.1 %)	242 (75.6 %)	42 (87.5 %)	122 (64.9 %)	<0.001	0.001	0.002
Clinical pregnancy (%/fresh transfer)	100 (45.2 %)	184 (40.3 %)	33 (20.6 %)	97 (30.3 %)	7 (14.6 %)	34 (18.1 %)	0.22	0.024	0.57
Live birth (%/fresh transfer)	88 (39.8 %)	144 (31.5 %)	19 (11.9 %)	70 (21.9 %)	3 (6.3 %)	19 (10.1 %)	0.033	0.008	0.58
Multiple birth (%/live birth)	0	34 (23.6 %)	0	15 (21.4 %)	0	1 (5.3 %)	<0.001	0.034	0.69
Cycles with frozen embryos (%/fresh transfer)	221	191 (41.8 %)	160	106 (33.1 %)	48	32 (17.0 %)	<0.001	<0.001	<0.001
No. of frozen embryos/freeze cycle	3.4±2.3	2.1±3.2	2.3±1.5	1.4±2.5	1.9±1.1	0.6±1.6	<0.001	<0.001	<0.001
FET clinical pregnancy (%/freeze cycle)	90 (40.7 %)	57 (29.8 %)	48 (30.0 %)	21 (19.8 %)	10 (20.8 %)	5 (15.6 %)	0.022	0.063	0.56
FET live birth (%/freeze cycle)	78 (35.3 %)	45 (23.6 %)	32 (20.0 %)	13 (12.3 %)	4 (8.3 %)	2 (6.3 %)	0.009	0.1	0.73
FET multiple birth	2 (2.6 %)	10 (22.2 %)	2 (6.3 %)	3 (23.1 %)	0 (0 %)	1 (50.0 %)	<0.001	0.136	0.33
Cumulative clinical pregnancy (%/retrieval)	160 (72.3 %)	220 (48.1 %)	78 (48.8 %)	112 (35.0 %)	16 (33.3 %)	39 (20.7 %)	<0.001	0.004	0.066
Cumulative live birth (%/retrieval)	146 (66.1 %)	177 (38.7 %)	51 (31.9 %)	81 (25.3 %)	7 (14.6 %)	21 (11.2 %)	<0.001	0.13	0.51
Cumulative multiple birth (%/CLB)	2 (1.4 %)	46 (26.0 %)	2 (3.9 %)	18 (22.2 %)	0 (0 %)	2 (9.5 %)	<0.001	0.004	0.42

Values presented as *n* (%) or mean±SD

Table 2 Multilevel univariate analysis in the two age groups with cumulative live birth as the dependent variable

Age	Under 37			Between 37 and 40			Over 40		
	Odds ratio	95 % CI	<i>p</i> value	Odds ratio	95 % CI	<i>p</i> value	Odds ratio	95 % CI	<i>p</i> value
Year	1.11	1.06–1.16	<0.001	1.02	0.96–1.07	0.535	1.08	0.97–1.21	0.169
Age	0.93	0.88–0.99	0.022	0.73	0.61–0.89	0.001	0.70	0.47–1.03	0.067
ICSI	1.01	0.75–1.37	0.928	0.61	0.41–0.91	0.015	1.66	0.70–3.94	0.251
Long protocol	1.09	0.76–1.57	0.641	1.44	0.95–2.17	0.087	1.20	0.54–2.67	0.655
Antagonist protocol	1.12	0.76–1.65	0.557	0.74	0.49–1.13	0.161	1.10	0.50–2.44	0.816
No. of retrieved oocytes	1.06	1.03–1.09	<0.001	1.06	1.02–1.10	0.008	1.08	1.00–1.18	0.063
No. of frozen embryos	1.21	1.14–1.28	<0.001	1.29	1.18–1.41	<0.001	1.14	0.93–1.40	0.208
Good-quality embryo	3.32	2.21–4.99	<0.001	2.64	1.44–4.84	<0.001	1.70	0.66–4.40	0.271
eSET	3.08	2.20–4.31	<0.001	1.38	0.91–2.10	0.130	1.36	0.54–3.41	0.515

women of advanced reproductive age, eSET policy had a modest effect, being associated with decreased multiple birth rate and comparable cumulative outcome compared with MET.

Table 3 Multivariate logistic regression analysis of possible related factors for cumulative live birth in the three age groups: (A) women under 37 years of age, (B) women aged between 37 and 40, and (C) women aged over 40

A			
	Under 37		
	Adjusted odds ratio	95 % CI	<i>p</i> value
Year	1.05	0.97–1.13	0.222
Age	0.94	0.88–1.00	0.065
No. of retrieved oocytes	1.02	0.99–1.06	0.204
No. of frozen embryos	1.12	1.05–1.20	<0.001
Good-quality embryo	2.14	1.38–3.32	<0.001
eSET	1.93	1.10–3.36	0.021
B			
	Between 37 and 40		
	Adjusted odds ratio	95 % CI	<i>p</i> value
Age	0.78	0.64–0.96	0.018
ICSI	0.65	0.42–1.00	0.051
Long protocol	1.18	0.24–5.86	0.836
Antagonist protocol	0.95	0.19–4.73	0.950
No. of retrieved oocytes	1.00	0.95–1.05	0.908
No. of frozen embryos	1.24	1.12–1.38	<0.001
Good-quality embryo	1.58	0.83–3.02	0.166
eSET	1.37	0.82–2.28	0.224
C			
	Over 40		
	Adjusted odds ratio	95 % CI	<i>p</i> value
Year	1.10	0.98–1.24	0.110
Age	0.73	0.49–1.08	0.118
No. of retrieved oocytes	1.09	0.99–1.19	0.075

There have been a few reports describing the efficacy of eSET in older patients [6, 7]. In those retrospective studies, patients in the study period were divided into eSET group and DET group on the decision by clinicians or patients. These methods could cause selection bias, and the patients with poor prognosis tend to choose or be recommended for DET. In the present study, many patients with recurrent implantation failure or who had only low-quality embryos were recommended for MET rather than SET after eSET has become mandatory in selected patients in Japan. Therefore, we included the patients who underwent MET in the period when MET was exclusively performed and excluded the patients who experienced three or more previous OR cycles from the analysis to exclude the selection bias as much as possible.

There have been a lot of randomized studies comparing the effectiveness of eSET versus DET in young women. In a systematic review, eSET strategy resulted in a lower live birth rate in fresh cycles than DET but achieved a comparable outcome after an additional frozen embryo transfer [5]. In the present study, eSET group in young women achieved a significantly higher live birth rate both in fresh cycles and in cumulative results than MET group. One of the reasons for the difference of live birth rate between the two groups in fresh cycles is that approximately 72 % of the patients in MET group had good-quality embryos, while 91 % of the patients in eSET group did, as shown in Table 1. However, multivariate logistic regression analysis showed that eSET was an independent factor even after adjusting for other indices of embryo quality such as embryo score and number of frozen embryos. Another possible factor associated with superiority of eSET over MET for cumulative live birth is increased number of frozen embryo transfer cycles. Several advancements in ART including COS protocols and culture conditions resulted in increased implantation rate and comparable clinical pregnancy rate with a decreased number of transferred embryos, as shown in Fig. 1. Actually, introduction of eSET strategy

resulted in larger number of frozen embryos with higher quality and more chances of pregnancy with subsequent embryo transfer cycles. In a previous paper, COS has been reported to decrease endometrial receptivity [11] and implantation rate in fresh embryo cycles has been shown to be lower than that of frozen embryo cycles [12]. In most of previous randomized studies, outcomes of eSET with additional one frozen embryo transfer cycles were compared with those of one fresh DET cycles. In the present study, cumulative outcomes of eSET were compared with those of MET. It means that, if there are four embryos suitable for transfer, women in eSET group have four chances of pregnancy—one fresh and three frozen transfer cycles—while those in MET group have only two chances—one fresh and one frozen transfer cycles. Multiple frozen embryo transfer cycles could maximize CLBR in eSET group.

While eSET policy has a marked efficacy in younger women, it reduced live birth rate significantly in fresh cycles in women aged between 37 and 40. It is interesting that, instead of possible selection bias especially in and after 2008, eSET did not improve cumulative outcomes in them. However, CLBR in eSET group was slightly higher than that in MET group of the same age (31.9 vs. 25.3 %, respectively), though not statistically significant. eSET also significantly reduced cumulative multiple birth rate compared with MET (3.9 vs. 22.2 %). These results support the efficacy of eSET in women of this age group and suggest that the embryos from old women are more sensitive to the detrimental effect of COS on the endometrium, while the embryos from younger women might be able to overcome the influences of COS. In multivariate analysis, not eSET but number of frozen embryos was associated with cumulative live birth in women aged 37–40. Recently, a review has been published regarding the efficacy of the freeze-all policy, that is, elective cryopreservation of all viable embryos in fresh cycles [13]. “Freeze-all policy” might be applied for patients of advanced reproductive age in order to avoid the influence of COS and increase number of frozen embryos.

In women aged over 40, introduction of eSET policy resulted in comparable CLBR and decreased multiple birth rate, though not significant. However, none of the examined factors including eSET was significantly related to cumulative live birth. eSET policy in unselected patients of this age group might be difficult, and further examination for better outcomes may be necessary.

In summary, eSET policy in younger women resulted in increased CLBR compared with MET. In women aged 37 and over, CLBR in eSET group was similar with that in MET group. In all age groups, eSET reduced multiple birth rates. The significance of eSET in women of advanced reproductive age is limited presently, and further research on the strategy to

improve cumulative outcomes in them, including all-freeze policy, may be necessary.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review board of our institution.

Informed consent Informed consent was obtained from all individual participants included in the study.

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