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Clinical outcomes of elective single morula embryo transfer versus elective single blastocyst embryo transfer in IVF-ET

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Abstract
Purpose To compare the clinical outcomes of elective single morula embryo transfer (eSMET) versus elective single blastocyst embryo transfer (eSBET) in selected patients.
Methods This study was a retrospective study which analyzed for 271 cycles in women under 37 years of age who are undergoing their first or second trial of in vitro fertilization-embryo transfer (IVF-ET) from January 2008 to December 2009. The eSMET was performed on day 4 (n=130) and the eSBET was conducted on day 5 (n=141).
Results The clinical pregnancy rate (51.5% vs. 51.8%, p=0.97), implantation rate (52.3% vs. 52.5%, p=0.98), and live birth rate (39.2% vs. 44.7%, p=0.36) were similar in the eSMET and eSBET groups, respectively. The miscarriage rate of the eSMET group (23.9%) was slightly higher than that of the eSBET group (13.7%) (p=0.12), without reaching statistical significance. There was only one case of monozygotic twin pregnancy in each group.
Conclusions The clinical outcomes of day 4 eSMET were comparable to those of day 5 eSBET. Therefore, day 4 eSMET is a viable option or an alternative to day 5 eSBET, with no difference in success rates.

Keywords Elective single embryo transfer · Morula · Blastocyst · Monozygotic twins · Live birth

Capsule The Clinical outcomes of elective single morula embryo transfer on day 4 were comparable to those of elective single blastocyst embryo transfer on day 5.

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Introduction

The main goal of in vitro fertilization-embryo transfer (IVF-ET) is the birth of a single healthy baby. However, if two or more embryos are transferred to maintain an acceptable pregnancy rate, it will increase the risk of multiple pregnancies. It has been reported that multiple pregnancies result in adverse outcomes such as higher risks of prematurity, low or very low birth weight, intrauterine growth retardation, pregnancy-induced hypertension, and caesarean section [1–3]. Also, twin pregnancies have a six-fold increased risk of perinatal mortality and morbidity [4] and a four-fold increased risk of cerebral palsy compared to singleton pregnancies [5]. Therefore, there is no doubt that elective single embryo transfer (eSET) will be one of the most obvious ways of minimizing complications due to multiple pregnancies.

In 1999, eSET was first reported by Vilska et al. [6]. However it has been reported that, even though eSET elevates the chance of delivering a healthy baby compared to double embryo transfer (DET) in cleavage stage embryo [7], eSET results in significantly decreased pregnancy and delivery rates per embryo transfer [8, 9]. In contrast to these results, it has been reported that the clinical outcomes of elective single blastocyst embryo transfer (eSBET) are similar to those of double blastocyst embryo transfer (DBET) on day 5 [10].

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Also, some studies have shown that eSBET results in significantly higher pregnancy and delivery rates compared to day 2 or 3 elective single cleavage embryo transfer (eSCET) in selected groups [11–13]. In light of the above studies, eSBET can be one method that can decrease multiple pregnancy rates while maintaining an acceptable pregnancy rate.

Day 4 embryo transfer (ET) was reported in 1994 [14] but mostly limited to cases that were undergoing preimplantation genetic diagnosis on day 3 [15, 16]. According to the results of a retrospective study that analyzed the outcomes in patients with all 'good' embryos [17], day 4 ET achieved a significantly higher implantation rate compared to day 3 ET (46.4 vs. 21.4%, $p<0.01$), while the number of embryos transferred on day 4 was significantly lower than day 3. The morula-stage embryo should have similar advantages compared to blastocyst-stage embryo, because it has both the activated embryonic genome [18] as well as better synchronization between embryos and a better uterine environment compared to the cleavage-stage embryo on day 2 or 3 [19]. Nevertheless, none of studies reported the outcomes of elective single morula embryo transfer (eSMET) on day 4.

This study was carried out to compare the effectiveness of eSMET versus eSBET on outcomes of clinical pregnancy and live births after a fresh embryo transfer in selected patients.

Materials and methods

Patient population

This retrospective study was approved the Maria Fertility Hospital Institutional Review Board. We analyzed patients for 271 cycles who visited the Daegu Maria Clinic for a first or second IVF-ET treatment from January 2008 to December 2009. They were all under 37 years old, had more than 8 mm of endometrial thickness on the day of human chorionic gonadotropin (hCG) administration, and had more than three good quality embryos on day 3. All patients gave written informed consent for eSMET or eSBET. This study did not include oocyte donation cycles.

Ovarian stimulation and laboratory procedures

Ovarian stimulation was undertaken using the gonadotropin-releasing hormone (GnRH) agonist long protocol and recombinant follicle stimulating hormone (FSH; Gonal-F, Merck Serono, Germany). Oocyte maturation was induced by 10,000 IU of hCG (IVF-C, LG Life Science, Daejon, Korea) when more than two follicles 17–18 mm in diameter were visible on ultrasonography. Oocyte retrieval was undertaken by transvaginal ultrasound-guided aspiration after 36 hours of hCG administration. The retrieved oocytes were washed in MRC#OW medium (Biosupply Co., Seoul, Korea) and then cultured in MRC#D01 medium (Biosupply Co.) until *in vitro* fertilization. *In vitro* fertilization was induced using conventional insemination or intracytoplasmic sperm injection (ICSI). Within 16 to 18 hours after fertilization, the oocyte with two pronuclei and a second polar body was regarded to be normally fertilized.

The embryos were co-cultured with autologous cumulus cells (ACC) in 20 μl of MRC#D16 medium (YS medium [20], Biosupply Co.) containing autologous follicular fluid (AFF). AFF was collected from follicles that produced healthy mature oocytes with a clear corona radiata. AFF was used for culture after inactivation at 56°C for 30 minutes and sterilization with a 0.22 μm filter, followed by centrifuging for 15 minutes at 3000 rpm. ACCs were prepared in a 5 μl micro droplet of an organ culture dish (3536, BD Falcon, USA) under MRC#Oil (Biosupply Co.) by seeding its single cells, followed by excising from a clear corona radiata of healthy cumuli and digesting with MRC#Hyase (Biosupply Co.). The first 48 hours of co-culture was supplemented with 10% AFF, and during the next 48 hours, 20% AFF was added. Culture medium was exchanged for pre-equilibrated fresh medium every morning.

Luteal phase support and embryo selection for transfer

The luteal phase was supported by administration of Crinone gel (90 mg, Merck Serono, Germany) and Utrogestan (100 mg, France) for 14 days after oocyte retrieval. The Crinon gel was taken once a day vaginally, while the Utrogestan was taken orally three times a day.

The quality of cleavage embryos was assessed on the morning of day 3. A good quality embryo day 3 was defined as having more than 7 blastomeres of equal size and less than 20% fragmentation. The quality of morula on day 4 was assessed according to the criteria of Tao et al. [17], and the quality of blastocyst on day 5 was assessed according to the criteria of Gardner and Schoolcraft [21]. The eSMET on day 4 or eSBET on day 5 was completed by transferring a single best embryo into the uterine cavity in each group. After eSMET or eSBET, the surplus embryos were cocultured to day 5 or 6. Only the normal embryos that reached the blastocyst stage were selected for cryopreservation based on the healthiness of trophectoderm cells and the size of the inner cell mass.

Main outcome measures

Serum β-hCG concentration was measured 14 days after oocyte retrieval to verify pregnancy. Clinical pregnancy was judged by observation of the gestational sac (G-sac) on vaginal ultrasonography after 6-7 weeks of gestation. The implantation rate was indicated as the proportion of the gestational sacs to the transferred embryos. Monozygotic

twins were considered as two gestation sac. Ectopic pregnancy was not counted as implantation and clinical pregnancy.

Statistical analysis

Statistical analysis was performed with SPSS 14.0 (SPSS Inc., Chicago, IL, USA) program, and the average value was expressed as the mean ± standard deviation. For comparison of the continuous variables, the Student's *t*-test was used, and for comparison of non-continuous variables, the Chi-square test was used. Results were considered statistically significant if $p<0.05$.

Results

Among 271 cycles, eSMET was performed for 130 cycles on day 4, and eSBET was conducted for 141 cycles on day 5. The demographic characteristics between the eSMET group and the eSBET group are summarized in Table 1. There were no differences between the eSMET and eSBET groups in terms of the age of women, number of previous IVF cycles, endometrial thickness at hCG administration, or duration of infertility. However, the tubal factor rate of the eSMET group was higher than that of the eSBET group, while other factors, including unknown, were lower in the eSMET group compared to the eSBET group in terms of the etiology of infertility. These values were not statistically significant.

The proportion of cycles undergoing ICSI was similar in the two groups (15.4% vs. 17.7% in the eSMET and eSBET groups, respectively), as shown in Table 2. With respect to the laboratory outcomes, there were no differences in the numbers of retrieved, matured, fertilized oocytes and good quality embryos on day 3. However, the number of cryopreserved blastocysts (5.1±3.1 and 6.5±3.5, $p<0.01$) was significantly fewer in the eSMET group compared to the eSBET group.

The clinical pregnancy rate (51.5% vs. 51.8%, $p=0.97$) and implantation rate (52.3% vs. 52.5%, $p=0.98$) were similar in the eSMET and eSBET groups, respectively (Table 3). The ectopic pregnancy rate (3.1% vs. 0.7%, $p=0.15$) and miscarriage rate (23.9% vs. 13.7%, $p=0.12$) was slightly higher in the eSMET group compared to the eSBET group, without reaching statistical significance. In both groups, most of the miscarriages occurred before 10 weeks of pregnancy (14/16 in the eSMET group and 9/10 in the eSBET group). Only one case of a pregnancy with monozygotic twins was found in each group (1.5% vs. 1.4% in the eSMET and eSBET groups, respectively). The eSMET group showed a trend with a lower live birth rate (39.2% vs. 44.7%, $p=0.36$) than the eSBET group, but there was no statistical significance. The rate of low birth weight infants (less than 2,500 g) in the eSMET group was statistically identical to that of the eSBET group. However, the rate of preterm births (before 37 weeks) was slightly higher in the eSBET group (6.4%) compared to the eSMET group (0%), without reaching statistical significance. There was 1 case of monozygotic twin births in both groups, and they were delivered by Caesarean section at 39 weeks in the eSMET group (weights 2.3 kg and 2.4 kg) and 36.5 weeks in the eSBET group (weights 2.5 kg and 2.6 kg).

Discussion

In most human IVF-ET programs, embryos are typically transferred 2 or 3 days after oocyte retrieval or 2 to 3 days later, when they have reached the blastocyst stage. Similarly, most eSET have been mainly performed on day 2, 3, or 5. These studies have shown that the clinical pregnancy and delivery rates were significantly higher after eSBET compared to eSCET in selected patients [11–13]. The present

Table 1 Demographic characteristics of the eSMET and eSBET groups

Variables	eSMET ($n=130$)	eSBET ($n=141$)	p value
Age of women (yrs)	31.6±2.8	31.4±2.8	0.47
No. of previous IVF cycles	0.3±0.4	0.2±0.4	0.82
Endometrial thickness at hCG triggering (mm)	10.6±1.5	10.7±1.4	0.94
Duration of infertility (months)	45.2±26.3	42.9±22.3	0.44
Etiology of infertility			
Tubal	49 (37.7)	38 (27.0)	0.06
Endometriosis	5 (3.9)	4 (2.8)	0.64
Anovulation	7 (5.4)	11 (7.8)	0.43
Male factor	8 (6.2)	10 (7.1)	0.76
Mixed	28 (21.5)	37 (26.2)	0.37
Other, including unknown	33 (25.4)	49 (34.8)	0.09

Values are presented as mean ± standard deviation (SD) or number (%); eSMET=elective single morula embryo transfer; eSBET=elective single blastocyst embryo transfer; IVF=in vitro fertilization; hCG=human chorionic gonadotropin

Table 2 Laboratory outcomes in the eSMET and eSBET groups

Variables	eSMET (n=130)	eSBET (n=141)	p value
No. of ICSI attempts	20 (15.4)	25 (17.7)	0.60
No. of retrieved oocytes	15.7±6.4	16.3±6.4	0.41
No. of matured oocytes	12.4±5.8	13.1±5.3	0.28
No. of fertilized oocytes	10.8±4.7	11.8±4.7	0.11
No. of good quality embryos on day 3	7.5±3.8	8.3±4.2	0.09
No. of cryopreserved blastocysts	5.1±3.1	6.5±3.5	<0.01

Values are presented as number (%) or means ± SD; eSMET= elective single morula embryo transfer; eSBET=elective single blastocyst embryo transfer; ICSI= intracytoplasmic sperm injection

retrospective study comparing eSMET to eSBET showed that the clinical pregnancy and live birth rates after eSMET were similar to those after eSBET in women with favorable conditions.

In our clinic, ICSI is performed in about 50% of cycles. It is standard that ET on day 4 or day 5 is performed for patients with more than 3 good quality embryos on day 3. However, most of the patients with embryos produced by ICSI are excluded from this standard, although they have more than 3 good quality embryos, except that they want to be transferred on day 4 or day 5. The reason is because the blastocyst formation rate is lower in the embryos fertilized by ICSI than those fertilized by conventional insemination [22]. Thus, it was found that the attempt ratio of ICSI was very low in both groups (Table 2).

In order to maintain a satisfactory pregnancy and reduce multiple pregnancies, the most successful way is transfer of a single embryo with the highest potential for implantation. However, it would be difficult to accurately select cleavage-stage embryos with the highest implantation potential for ET [23]. On the other hand, some researchers have suggested that blastocyst results in improved selection of developmentally competent embryos compared to cleavage stage embryo, because embryonic genome activation occurs, and embryos with genetic abnormalities have difficulty developing to the blastocyst stage during extended culture [18, 24]. In terms of embryo selection, Tao et al. [17] suggested that the morula/compact embryo with the activated embryonic genome has better selection value compared to cleavage-stage embryos. Also, Harper reported that the embryo travels to the uterine cavity about 3–4 days after fertilization in mammals [19]. This indicates that the morula-stage ET would be more synchronized with in vivo reproductive processes than cleavage-stage ET on day 3. According to the results of our study, it is considered that the transferring of morula-stage embryo on day 4 may have potential advantages similar to blastocyst transfer associated with embryo selection and synchronization between embryo and the uterine environment. Further study is needed to determine whether or not day 4 transfer is efficient.

Gardner et al. [25] have suggested that blastocyst transfer enhances the likelihood of pregnancy. However, it has been associated with an increased risk of cancelled transfer as compared to day 3 ET due to failed blastocyst development. Thus, it has mainly been applied to patients with at least two [26] ~ five [27] top quality embryos on day 3. According to a recent prospective study in France, the rate of ET cancellation was also significantly higher after SBET as compared to DCET (12% vs. 0%, p<0.001) when no top quality embryos were available on day 2 [28]. Our study also included patients with more than three good quality embryos on day 3. Thus, there were no cases of cancelled ET. We did not investigate morula and blastocyst formation rates. However, according to previous studies, 59.2% of good quality embryos on day 3 developed to good quality embryos on day 4 [17], whereas 47% of good quality embryos on day 3 developed to blastocyst on day 5 [29]. These results suggest that the rate of ET cancellation is lower after morula-stage ET

Table 3 Clinical outcomes in the eSMET and eSBET groups

Variables	eSMET (n=130)	eSBET (n=141)	p value
Clinical pregnancies	67 (51.5)	73 (51.8)	0.97
Ectopic pregnancies	4 (3.1)	1 (0.7)	0.15
Gestational sacs	68 (52.3)	74 (52.5)	0.98
Twin pregnancies	1 (1.5)	1 (1.4)	0.95
Miscarriages	16 (23.9)	10 (13.7)	0.12
Live births	51 (39.2)	63 (44.7)	0.36
Singletons	50 (98.0)	62 (98.4)	0.87
Twins	1 (2.0)	1 (1.6)	0.87
Preterm birth <37 weeks	0	4 (6.4)	0.07
Low birth weight infants <2,500 g	3 (5.8)	3 (4.7)	0.79

The continuous variables are expressed as number (%); eSMET=elective single morula embryo transfer; eSBET= elective single blastocyst embryo transfer

than blastocyst-stage ET. A study on morula-stage ET cancellation has not been conducted, and a prospective, randomized study should be performed to investigate this issue.

In the present study, the pregnancy rate was similar in both groups, whereas the delivery rate was slightly lower in the eSMET group (39.2%) compared to the eSBET group (44.7%), without reaching statistical significance. This difference is related to a higher rate of pregnancy loss in the eSMET group, although the difference was not significant (23.9% vs. 13.7%, p=0.12). It has been known that pregnancy loss before 6-7 weeks of pregnancy is related to poor embryo quality [30]. De Neubourg et al. [31] reported that pregnancy loss before 13 weeks of gestation was increased by age, although a top quality embryo was transferred. However, the patients included in our study were women under 37 years of age who had more than three good quality embryos on day 3. The high miscarriage rate of the eSMET group in our study is not regarded as being related to poor embryo quality and patient's age.

When one embryo is transferred, monozygotic twin pregnancies rarely occur, while dizygotic twin pregnancies could be completely prevented. It has been reported that pregnancies with monozygotic twins have resulted in higher perinatal morbidity and mortality compared to those with dizygotic twins [32]. Da Costa et al. [33] reported that the number of monozygotic twin pregnancies was increased by blastocyst transfer compared to cleavage stage ET due to more damage and hardening of the zona pellucida from an in vitro culture environment. Guerif et al. [11] reported that the monozygotic twin birth rate was slightly higher in the eSBET group than the eSCET group (3.8% vs. 1.6%), but there was no statistical significance. However, Papanikolaou et al. [34] showed that the number of monozygotic twin pregnancies was not increased after eSBET compared to eSCET. In the present study, the proportion of monozygotic twin pregnancies after eSBET was 1.4%. This rate is in accordance with previous studies of monozygotic twin pregnancies rate after eSBET [34]. Also, the rate of monozygotic twin pregnancies after eSMET was 1.5% and identical to the result of eSBET. Our results showed that the number of monozygotic twin pregnancies in both groups was not increased.

Ectopic pregnancy has been reported to occur in approximately 2–5% of clinical pregnancy after IVF-ET [35]. It has been known that ectopic pregnancy is caused by stimulation due to oocyte retrieval, transfer medium injected for transferring embryos, methods of transferring, and uterine contraction. It has been suggested that ectopic pregnancy could be decreased by blastocyst transfer compared to cleavage-stage embryos because of the decreased uterine contractility [36] and larger diameter of the blastocyst [37]. However, Milki and Jun [35] suggested that ectopic pregnancy is not reduced following blastocyst transfer compared to cleavage stage ET. In our study, the ectopic pregnancy rate was slightly higher in the eSMET group (3.1%) compared to the eSBET group (0.7%), without reaching statistical significance.

In conclusion, our study shows that transfer of elective single morula embryo on day 4 results in similar pregnancy and delivery rates compared to transfer of elective single blastocyst embryo in women under 37 years of age who are undergoing their first or second trial of IVF-ET. These results suggest that day 4 eSMET is a viable option or alternative to day 5 eSBET, with no difference in success rates.

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

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