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Original Article

Day 4 good morula embryo transfer provided compatible live birth rate with day 5 blastocyst embryo in fresh IVF/ET cycles

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ABSTRACT

Objective: Embryo transfers during cleavage stage (day 2 or day 3) and blastocyst stages (day 5 or day 6) are common in current daily practice in fresh IVF/ET cycles. Data regarding transferring day 4 embryos, morula/compact stage, is still restricted and the grading system is also inconsistent, as between IVF clinics. This study provided a new detailed classification system for morula/compact stage embryos and compared successes rates between day 4 and day 5 ET.

Materials and methods: This was a retrospective study. A review of medical records from January 1st, 2013, to December 31st 2015, performed for all conventional insemination and ICSI cycles with a GnRH-antagonist protocol at the Infertility Division of MacKay Memorial Hospital in Taipei City, Taiwan.

Results: There were 427 cycles included in our study, 107 in study group (day 4 MET) and 320 in control group (day 5 BET). Pregnancy rates and live birth rate were compatible, as between morula embryo transfer (MET) and blastocyst embryo transfer (BET). The implantation rate (36.3% *vs.* 39.6%, respectively, $p = 0.500$), clinical pregnancy rate (49.5% *vs.* 51.9%, respectively, $p = 0.737$), and live birth rate (42.1% *vs.* 45.6%, respectively, $p = 0.574$) were statistically insignificant between groups. The term birth rate was statistically higher in the MET group than in the BET group (95.7% *vs.* 79.5%, respectively, $p = 0.006$). When the clinical outcomes between day 4 good MET and day 5 good BET were compared, the results were compatible. The implantation rate (48.8% *vs.* 41.1%, respectively, $p = 0.335$), clinical pregnancy rate (55.0% *vs.* 53.2%, respectively, $p = 0.867$), and live birth rate (47.5% *vs.* 47.1%, respectively, $p = 1.000$) showed no significant difference. The term birth rate was also higher in day 4 good MET group than in day 5 good BET group (100% *vs.* 78.3%, respectively, $p = 0.025$).

Conclusion: In this study, we performed day 4 MET avoid BET on Sunday. The grading system we provided was more detailed for embryo selection and it was easier to remember. Our data showed that morula embryo transfer might be a flexible, easier and applicable method for embryo transfer in daily routine.

Introduction

Reproductive medicine and reproductive technology have been seen significant advances since the first in-vitro-fertilization (IVF) baby was born in 1978. The selection of the embryo remained one of the key steps to the success of IVF. Although Zygote Intrafallopian Transfer (ZIFT) was widely applied in the early years of IVF, the common trend was to transfer the embryo into the uterus on the 2nd, 3rd or 5th day after Transvaginal Ovum Retrieval (TVOR) in modern days. Embryo quality classification for cleavage stage embryos between the 2nd to 3rd days after TVOR was widely accepted in common [1]. Blastocyst embryo classification for day 5/6 after TOVR proposed by Gardner and Schoolcraft in 1999 [2] is

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also a standard nowadays. However, up to now, there was no general agreement concerning the embryo quality classification of morula/compact stage embryo on the 4th day after TVOR.

In recent years, patients underwent TVOR on Tuesday would receive blastocyst embryo transfer on Sunday. This situation, to work on Sunday, would unfortunately against the Labor Standards Act in Taiwan. Due to the lack of well-established criteria regarding morula/compact stage embryo (on the 4th day after TOVR), it wound be difficult for clinicians and embryologists to select appropriate embryos to transfer on Saturday. The aim of this study is to establish a new quality grading system of morula/compact stage embryo in order to avoid embryo transfer on Sunday. Furthermore, the clinical outcome of day 4 (morula/compact stage) embryo transfers (MET) and day 5 blastocyst embryo transfers (BET) will be compared.

Material and methods

Study design

This is a retrospective analysis. A review of medical records from January 1st, 2013, to December 31st 2015, was performed for all IVF-ICSI cycles with a GnRH-antagonist protocol at the Infertility Division of Mackay Memorial Hospital in Taipei City, Taiwan. The Institutional Review Board Committee of MacKay Memorial Hospital approved the study protocol.

Study participants

Patients who received either day 4 ET or day 5 ET were included in this study. For those patients who underwent TVOR on Tuesday, embryo transfer will be scheduled on Saturday (day 4), to avoid ET on Sunday (day 5) (day 4 study group). For those patients who received TVOR other than Tuesday, embryo transfer will be arranged on day 5 (day 5 control group).

A total of 427 cases were recruited for final analysis. There were 107 cycles receiving day 4 ET (study group) and 320 cycles receiving day 5 ET (control group).

Ovarian stimulation protocols

All patients began ovarian stimulation with a flexible starting dosage of recombinant FSH (Gonal-F; Merck SeronoS.p.A.) dosing from 150 to 225 IU on the 3rd day of menstruation, for three days. The starting dosage was determined by the patient's age, ovarian function, and the response to previous ovarian stimulation. The recombinant FSH dosage was then adjusted according to follicular growth, monitored by serial transvaginal ultrasound. After at least two follicles measured larger than 14 mm in diameter, patients started to receive subcutaneous daily injection of Cetrorelix (Cetrotide; Merck Serono, Baxter Oncology GmbH) 0.25 mg along with the recombinant FSH. When at least two of the largest follicles had extended to at least 18 mm in diameter, final oocyte maturation was dual triggered by 250 mg of recombinant hCG (Ovidrel; Merck SeronoS.p.A.), which was equivalent to approximately 6500 IU hCG according to the manufacturer's data, plus 0.2 mg of Triptorelin (Decapeptyl; Ferring GmbH). TVOR was undertaken 34–36 h after administration of hCG.

Ovum retrieval and embryo culture

The oocytes retrieved were washed with Flushing Medium, product of Origio ®. In Vitro Fertilization (IVF) was induced with conventional insemination procedure, or Intracytoplasmic Sperm Injection (ICSI). Within 16–18 h after fertilization, the oocyte with two pronuclei (2 PN) was considered as normally fertilized. The fertilized embryos were then cultured with global® medium, manufactured by LifeGlobal ®, in Thermo® CO_2 incubator (FORMA/Heracell 240i, CO_2 5.5%, O_2, 5%).

Embryo grading

Day 4 embryo grading

In our study, we established a new grading system for day 4 morula/compact stage embryos. The system we proposed was modified from the 2011 ESHRE Istanbul Consensus for day 4 embryo [1]. There were two different quality scores to each morula/compact stage embryos: 1. The percentage of compaction of blastomere, 2. The formation of blastocele.

Compaction took place during the 4th day of human embryo development. When the outer part of the morula becomes tightly bounded together and the boundaries of the blastomere were indistinguishable, this phenomenon was called compaction.

Embryos, which lose all of the boundaries of the blastomere, are considered to be embryos with complete compaction, and will be classified as grade 1 by the percentage of compaction of blastomere. Embryos with less than 100% but more than 50% of compaction will be rated as grade 2. Embryos with less than 50% compaction will be in grade 3. Embryos without compaction but possessing at least 8 blastomere (≥8 cell) will be classified as grade 4. And those without compaction but with less than 8 blastocele (<8 cell) will be rated as grade 5.

In our grading system, we divided grade 1 into grade 1a and grade 1b according to the formation of blastocele, also called early cavitation. Embryos with full compaction and early cavitation were defined as grade 1a, while those with full compaction but without early cavitation were placed in grade 1b (Table 1).

Day 5 embryo grading

Day 5 blastocyst stage embryo grading was according to Gardner's embryo grading system [2]. The Gardner blastocyst grading system assigns 3 separate quality scores to each blastocyst embryo:

Table 1
Embryo grading classification of day 4 morula/compact stage embryo.

Classification	Compaction	Blastomere	Early cavitation[a]
Grade 1	Full Compaction (100%)		
Grade 1a	Full Compaction (100%)		With early cavitation
Grade 1b	Full Compaction (100%)		Without early cavitation
Grade 2	≥50%, <100 compaction		
Grade 3	<50% compaction		
Grade 4	No Compaction	≥8 blastomere	
Grade 5	No Compaction	<8 blastomere	

[a] Early cavitation = visible blastocele under microscope.

1. Blastocyst development stage-expansion and hatching status, 2. Inner cell mass (ICM) score, or quality and 3. Trophectoderm (TE) score, or quality. The Expansion and hatching status was graded from grade 1 to grade 6, the inner cell mass quality and the trophectoderm quality were both graded as A, B and C. Blastocyst classified as grade 6 was considered to be better than grade 1. Grade A ICM was better than grade C ICM, and grade A TE was better than grade C TE.

Day of embryo transfer

Extended embryo culture to day 4 or day 5 was a regular daily practice in this study if possible according to embryos' development.

For those patients who underwent TVOR on Tuesday, embryo transfer will be scheduled on Saturday (day 4), to avoid ET on Sunday (day 5) (day 4 study group). For those patients who received TVOR on days other than Tuesday, embryo transfer will be arranged on day 5 (day 5 control group).

Patients who have ET on day 2 and day 3 were excluded in our study. Patients who received at least one embryo transfer on either day 4 or day 5 were included for analysis in this study.

Embryo transfer

Embryos selected for day 4 ET or day 5 ET were according to the morphology in the morning of embryo transfer. In the study group (day 4 ET), we transferred one to three embryos according to patients' age. In the control group (day 5 ET), one to three of the highest quality embryos were transferred according to patients' age. Embryos selected for ET were cultured in UTM^{TM} transfer medium (Origio®) for 30 min before ET. Embryo transferred was performed with the aid of transabdominal ultrasound. Guardia™ AccessET Embryo Transfer Catheter (COOK©) was used for embryo transfer.

Luteal phase support and confirmation of pregnancy

The luteal phase was supported by vaginal gel (Crinone gel 8%, Merck SeronoS.p.A), once daily starting on the day after oocyte retrieval. Serum β-hCG was measured 14 days after oocyte retrieval, and a value above 5 IU/mL was considered to be a positive pregnancy. The luteal support was then continued until the 10th week of gestation after the establishment of luteal-placental shift for all positive pregnancies.

Outcome variables

The study's primary outcome was the live-birth rate per cycle. Other analyzed variables included, the clinical pregnancy rate, the ongoing pregnancy rate, the abortion rate, the implantation rate, the live birth rate per cycle, and the term pregnancy rate. Live birth was defined as delivery of a viable fetus over 23 gestational weeks. Clinical pregnancy was defined as a pregnancy confirmed by ultrasound visualization of the gestational sac. Ongoing pregnancy was defined as viable pregnancy beyond 12 gestational weeks. Abortion was defined as pregnancy terminated spontaneously before 12 gestational weeks. The implantation rate was calculated by dividing the total number of fetal cardiac activity detected by the total number of transferred embryos. The live birth rate per cycle was calculated by dividing the total cycle of live birth by the total number of cycles. The term pregnancy rate was calculated by dividing the total cycle of term birth by the total cycle of ongoing pregnancy.

Statistical analysis

Statistical analysis was performed using MedCalc 10.2 (MedCalc Software). Continuous variables were presented as mean with standard deviation (SD). For categorical variables, the values were presented as raw frequencies with corresponding percentages, and the between-group differences were assessed either by a chi-square test with Yates correction if required, or by the Fisher exact test. $P < 0.05$ was considered statistically significant.

Results

There were 427 cycles included in our study: 107 cycles in study group (day 4 ET) and 320 cycles in control group (day 5 ET). The baseline characteristics and demographics between the control and study groups were not statistically significant. The characteristics of ovarian stimulation, including serum E2/P4 on trigger day, number of oocytes retrieved and number of MII oocytes retrieved, did not differ with any statistical significance between the study group and the control group. The number of embryo transfer was no different between day 4 ET and day 5 ET (2.05 ± 0.21 *vs.* 1.96 ± 0.82, respectively, $p = 0.265$) (Table 2).

The outcome did not have any statistically significant difference between the study group and the control group. The implantation rate (36.3% *vs.* 39.6%, respectively, $p = 0.500$), clinical pregnancy rate (49.5% *vs.* 51.9%, $p = 0.737$), ongoing pregnancy rate (43.9% *vs.* 45.6%, respectively, $p = 0.822$), abortion rate (11.3% *vs.* 12.0%, respectively, $p = 1.000$) and live birth rate (42.1% *vs.* 45.6%, respectively, $p = 0.574$) were compatible. The term pregnancy rate was higher in study group than in the control group (95.7% *vs.* 79.5%, respectively, p = 0.006) (Table 3).

We further compared day 4 good morula ET (MET) and day 5 good blastocyst ET (BET). We included the selected patients in either day 4 good MET or day 5 good BET. In day 4 good MET, we included patients who received at least one grade 1 (grade 1a, or 1b) day 4 morula embryos. In day 5 good BET, we included patients who received at least one embryo grading better than 3 CC. By this way, the study group (day 4 good MET) and the control group (day 5 good BET) in the subgroup analysis received comparable graded embryos transferred in the corresponding time.

Table 2
Demographic characteristics of day 4 ET and day 5 ET.

	Day 4	Day 5	*p* value
Total no. of cycles	107	320	
Characteristics of patients			
Age (y/o)	34.46 ± 3.31	34.40 ± 3.27	NS
AMH level (ng/ml)	4.10 ± 2.96	4.66 ± 3.45	NS
Cause of Infertility (%)			
Male factor	18.7	20.3	NS
Tubal factor	39.3	41.3	NS
Ovulation dysfunction	7.5	13.1	NS
Uterine factor	6.5	6.6	NS
Endometriosis	43.0	40.0	NS
Unexplained	8.4	7.5	NS
Proportion of ICSI (%)	68.2	68.8	NS
Characteristics of ovarian stimulation			
E2 on trigger day (pg/ml)	1594 ± 764	1771 ± 966	NS
P4 on trigger day (ng/ml)	1.09 ± 0.70	1.08 ± 0.74	NS
No. of oocytes retrieved	14.61 ± 6.09	15.48 ± 6.40	NS
No. of MII oocytes retrieved	12.67 ± 5.04	13.34 ± 5.67	NS
No. of embryo transferred	2.05 ± 0.21	1.96 ± 0.82	NS

Note 1: Values are expressed as numbers, mean ± standard deviation, or percentages.
Note 2: ET = embryo transfer, AMH = Anti-Müllerian Hormone, ICSI = Intracytoplasmic Sperm Injection, E2 = serum estradiol, P4 = serum progesterone, MII = metaphase II.

Table 3
Outcomes of day 4 ET and day 5 ET.

Outcome	Day 4		Day 5		*p* value
Implantation rate (%)		(36.3)		(39.6)	NS
Clinical pregnancy rate (%)	53/107	(49.5)	166/320	(51.9)	NS
Ongoing pregnancy rate (%)	47/107	(43.9)	146/320	(45.6)	NS
Abortion rate (%)	6/53	(11.3)	20/166	(12.0)	NS
Live birth rate (%)	45/107	(42.1)	146/320	(45.6)	NS
Term birth rate (%)	45/47	(95.7)	116/146	(79.5)	0.006

Note: Values are expressed as numbers (%), ET = embryo transfer.

In this subgroup analysis, there were 40 cycles in day 4 good MET and 293 cycles in day 5 good BET. The baseline characteristics and demographics did not have statistically significant difference between the study group and the control group and the results were similar. The average number of embryo transferred was not different between day 4 good MET and day 5 good BET (2.05 ± 0.22 *vs.* 1.85 ± 0.74, respectively, $p = 0.094$) (Table 4). The clinical pregnancy rate (55.0% *vs.* 53.2%, respectively, $p = 0.867$), the ongoing pregnancy rate (47.5% *vs.* 47.1%, respectively, $p = 1.000$), the abortion rate (13.6% *vs.* 11.5%, respectively, $p = 0.728$) and the live birth rate (47.5% *vs.* 47.1%, respectively, $p = 1.000$) were statically insignificant between the study group and the control group. The term birth rate was higher in day 4 good MET than day 5 good BET (100% % *vs.* 78.3%, respectively, $p = 0.025$) (Table 5).

Discussion

Our result showed a compatible success rate between morula embryo transfer on day 4 and blastocyst transfer on day 5. Unexpectedly, the term birth rate was higher in morula embryo transfer than in blastocyst embryo transfer. Thus, this phenomenon suggested that morula embryo transfer on day 4 might be an alternative, flexible and acceptable choice for blastocyst embryo transfer on day 5.

The key aspect to increasing the success of IVF cycles was the ability to choose the most promising embryos, or euploid embryos, for transfer.

Table 4
Demographic characteristics of day 4 good MET[a] and day 5 good BET[b].

	Day 4 good MET[a]	Day 5 good BET[b]	*p* value
Total no. of cycles	40	293	
Characteristics of patients			
Age (y/o)	34.35 ± 2.56	34.33 ± 3.19	NS
AMH level (ng/ml)	4.52 ± 3.07	4.65 ± 3.37	NS
Cause of Infertility (%)			
Male factor	15.0	20.8	NS
Tubal factor	50.0	51.9	NS
Ovulation Dysfunction	10.0	13.3	NS
Uterine factor	5.0	6.8	NS
Endometriosis	45.0	39.9	NS
Unexplained	10.0	7.5	NS
Proportion of ICSI (%)	72.5	68.6	NS
Characteristics of ovarian stimulation			
E2 on trigger day (pg/ml)	1687 ± 798	1780 ± 969	NS
P4 on trigger day (ng/ml)	1.02 ± 0.67	1.06 ± 0.68	NS
No. of oocytes retrieved	15.15 ± 6.13	15.61 ± 6.39	NS
No. of MII oocytes retrieved	13.60 ± 5.17	13.45 ± 5.70	NS
No. of embryo transferred	2.05 ± 0.22	1.85 ± 0.74	NS

Note 1: Values are expressed as numbers, mean ± standard deviation, or percentages.
Note 2: MET = morula embryo transfer, BET = blastocyst embryo transfer, AMH = Anti-Müllerian Hormone, ICSI = Intracytoplasmic Sperm Injection, E2 = serum estradiol, P4 = serum progesterone, MII = metaphase II.
[a] Patients who received at least one grade 1 (grade 1a, or 1b) day 4 morula embryo.
[b] Patients who received at least one day 5 blastocyst embryo grading better than 3 CC.

Table 5
Outcomes of the day 4 good MET[a] group and day 5 good BET[b] group.

Outcome	Day 4 good MET[a]		Day 5 good BET[b]		*p* value
Implantation rate (%)		(36.3)		(39.6)	NS
Clinical pregnancy rate (%)	22/40	(49.5)	156/293	(51.9)	NS
Ongoing pregnancy rate (%)	19/40	(43.9)	138/293	(45.6)	NS
Abortion rate (%)	3/22	(11.3)	18/156	(12.0)	NS
Live birth rate (%)	19/40	(42.1)	138/293	(45.6)	NS
Term birth rate (%)	19/19	(100.0)	108/138	(79.5)	0.025

Note: Values are expressed as numbers (%), MET = morula embryo transfer, BET = blastocyst embryo transfer.
[a] Patients who received at least one grade 1 (grade 1a, or 1b) day 4 morula embryo.
[b] Patients who received at least one day 5 blastocyst embryo grading better than 3 CC.

Several invasive and non-invasive methods have been designed and widely applied in our daily practice to determine whether the embryo was euploidy or aneuploidy. Invasive methods included embryo biopsy from day 3 to day 5/6 with Pre-implantation Genetic Screening (PGS) by Fluorescence i*n situ* Hybridization (FISH) or Next-Generation Sequencing (NGS). Non-invasive methods included static morphology assessment, or kinetic morphology grading.

To the best of our knowledge, the earliest successful human morula embryo transfer reported in English literature was in the late 1990s [3]. Later, Grifo [4] and Gianaroli [5] both reported successful pregnancy after transferring day 4 morula embryos which received PGD on day 3. Gianaroli [5] also concluded that day 4 morula embryo transfer provided a more convenient environment for patients and clinical staff, since working and waiting until late in the evening of day 3 for ET is no more mandatory.

Nowadays, embryo biopsy with Pre-implantation Genetic Screening (PGS) was the most widely applied invasive methods for assessment for embryo quality. In recent times, the most common practice of PGS in most IVF lab is to biopsy embryos on day 5 or day 6. Compared to other methods, PGS provides the most accurate methods selecting euploid embryos. But there were several disadvantages concerning PGS. First, euploid embryos had to survive from being biopsied, cryopreserved and then thawed before being transferred. Second, PGS required an experienced laboratory embryologist to perform embryo biopsy and such techniques were not widely achievable and easily obtained. Third, inconclusive data after PGS, such as “no signal”, made clinical decisions more difficult. Fourth, the cost of PGS was nearly two-third or equal to the amount of one fresh IVF cycle in most countries. And fifth, until the end of 2016, there were insufficient data regarding PGS without indication, such as parental chromosomal abnormalities, repeated implantation failure or habitual abortion, improved the success rate of IVF. As such, invasive methods such as embryo biopsy with PGS remained a second choice for embryo selection in routine daily work.

On the other hand, kinetic embryo quality grading is now an emerging, interesting, but also frustrating field in embryo assessment. First, there are no consistent data regarding the timing of embryo development, for selecting the best embryos. Second, although computer programming selected the embryo automatically, manual review by human staff is still needed to avoid possible mechanical error. Third, the time-lapse embryo grading system increased the average cost of fresh IVF cycles. Above all, kinetic embryo grading by time-lapse system was also not the first choice of our regular practice.

Thus, static embryo morphologic grading remained the most widely applied methods for embryo selection. In our standard daily operation of embryo culture, the Embryologist observed and graded embryos with regular timing.

The embryo grading system we applied had been well established for cleavage stage embryos on day 2 and day 3, and

blastocyst stage embryos on day 5 and day 6. Blastocyst grading classification for day 5 and day 6 embryos proposed by Gardner was generally adopted worldwide [2]. Despite the well-accepted grading system for cleavage stage embryos (day 2 and day 3) and blastocyst stage embryos (day 5 and day 6), classification for day 4, morula/compact stage, embryos was still inconsistent and indefinite. Till date, there have been only few studies regarding embryo grading for such stages.

In the early twentieth-century, Tao demonstrated successful pregnancy after transferring morula/compact stage embryo on day 4 [6]. Embryos were graded from grade 1, to 4 in Tao et al.'s publication [7]. Grade 4 embryo was considered to be the best, and grade 1 the worst. They described grade 4 embryos as nearly fully compacted with all blastomere having the compaction process. Grade 3 embryos were in spherical shape and more than 75% of blastomere was in compaction. Grade 2 had irregular morphology and with deep indentation on the surface. And grade 1 morula showed that less than 50% of the blastomere was in compaction process. Their study compared the success rate between day 3 ET and day 4 ET. The number of embryos transferred in this study was 2.35 ± 0.6 in day 4 ET (at least two good MET) and 4.07 ± 0.9 in day 3 ET (at least two good cleavage stage embryo transfer). The ongoing pregnancy rate was higher in day 4 ET than in day 3 ET (63.6% *vs.* 44.9%, respectively, $p < 0.05$).

Feil et al. [8] later proposed a similar grading system for morula stage embryos. In their research, embryos were divided into four grades (grade 1, 2, 3 and 4). Grade 1 was defined as early blastocyst with visible cavitation or complete compaction of embryos without morphological anomalies, e.g. vacuolation, excessive fragmentation and self-cavitation within cells. Grade 1 with morphological anomalies was downgraded as grade 2 or with partial compaction. Grade 3 was defined as partial compaction or consisted of more than 8 blastomere. Grade 4 embryos had 8 blastomere or more but without sign of compaction. Single embryo transfer (SET) on day 4 and day 5 took place in their study. There was no difference regarding ongoing pregnancy rates between day 4 SET and day 5 SET (38.7% *vs.* 32.1% respectively, $p = 1.0$).

The grading system we proposed in this study was modified from 2011 ESHRE Istanbul consensus for day 4 morula/compact stage embryo [1]. This new system provided slightly more details. First, we classified morula stage embryos into five grades. Grade 1 embryos possessed the best quality at this stage, and grade 5 embryos were classified as the poorest. We also raised the standard for grade 1 morula. The best morula embryos had been defined as embryos with more than 75% compaction of blastomere. In our system, embryos with such condition were downgraded and defined as grade 2 only. The best embryo quality in our system had to be with full compaction (100% compaction of blastomere) and with or without blastocele. And we further divided grade 1 morula embryos into two groups, *viz.*, grade 1a (full compaction with blastocele, early cavitation) and grade 1b (full compaction without blastocele, without early cavitation). We also divided low-grade embryos into grade 4 and grade 5. Embryos with more than 8 blastomere were defined as grade 4, and those with less than that was defined as grade 5. This detailed system might provide more information for embryo selection between day 3 and day 4, and between day 4 and day 5.

The results of our study indicated that day 4 ET yielded a compatible outcome with day 5 ET. In the general population, clinical pregnancy rate was 49.5% in day 4 ET, just slightly lower than day 5 ET at 51.9%, although statistically insignificant ($p = 0.737$). And the live birth rate was nearly the same, 42.1% *vs.* 45.6%, also statistically insignificant ($p = 0.574$). This result may concluded that day 4 ET provided the same fertility potential as day 5.

There were several advantages of morula embryo transfer on day 4 compared to cleavage stages embryos. Researchers had found that embryonic gene expression started on day 4, but did not activate before day 3 [9]. After activation of embryonic genome, the apoptosis system and checkpoints of cell cycles might be activated and may result in a reduction of mosaicism from day 4 embryos to the blastocyst stage embryos. And it was also well known that the embryo migrates into the uterine cavity on the 4th day after fertilization in human beings. These evidences suggested that morula stage embryo transfer on day 4 would be more natural than cleavage stages embryo transfer on day 2 or day 3.

Compared with day 5 ET, there is an added advantage. Day 4 MET transferred embryo one day earlier than day 5 BET. Embryos could be exposed to the natural environment, in the uterus, for the maximum time, and minimally *in vitro*, before implantation. This reduction in the time of embryos existing *ex vivo* reduced the susceptibility to the interruption of epigenetic regulatory mechanism. Kang et al. described a compatible pregnancy rate between elective single morula embryo transfer (eSMET) and elective single blastocyst embryo transfer (eSBET) [10]. The live birth rate was slightly below in the eSMET group than the eSBET group (39.2% *vs.* 44.7%, respectively, statistically insignificant, $p = 0.87$). In their publication, they noted that the preterm pregnancy rate was lower in the eSMET group (0/130) than in the eSBET group (4/141) (0% *vs.* 6.4%, respectively, $p = 0.07$). The result might be a benefit from the reduction in embryo transfer time. Thus, they stated day 4 SMET to be a viable option or an alternative choice to day 5 SMET, with no difference in pregnancy rates.

Our data showed a similar result as reported by Kang et al. [10]. The term birth rate was higher in day 4 ET group than in day 5 ET (95.7% *vs*. 79.5%, respectively $p = 0.006$). In our subgroup analysis, the clinical pregnancy rate, the ongoing pregnancy rate, the abortion rate and the live birth rate were comparable in both groups. The term pregnancy rate was also higher in day 4 good MET than in day 5 good BET (100.0% *vs.* 78.3%, respectively $p = 0.025$). The outcome might be benefit from the minimal *in vitro* period, and maximum *in vivo* time. Our data, along with Kang's report, may highlight that morula ET on day 4 might grant a success rate equal to blastocyst ET on day 5, and furthermore, the pregnancy after day 4 embryo transfer will result in term delivery. This concluded that day 4 MET may provide an alternative to day 5 BET and might give an extra flexibility to both patients and faculties in a busy facility.

There were several limitations to our study. First, this was not a randomized control study. Patients were not divided into groups at random. In the future, randomized control trial with a larger number of patients recruited will yield more information regarding day 4 embryo transfer.

Second, not every patient in this study received single embryo transfer. Single embryo transfer provided individualized information regarding each embryo. Cycles transferring more than one embryo provided mixed data, and it was difficult to tell each embryo's pregnancy potential from that of another. The main reason we did not arrange single morula embryo transfer was the non-availability of data regarding single morula embryo transfer (MET) in sufficient volumes. To our knowledge, there were only two published documentations regarding single morula embryo transfer [8,10] when we started our investigation.

Conclusion

In conclusion, morula embryo transfer provided success rates similar to blastocyst embryo transfer. And if good morula stage embryos were transferred, it also yielded a pregnancy rate compatible with good blastocyst transfer. The results implied that embryo transfer on either day 4 or day 5 would provide an optimal outcome, and this new classification provided flexibility for the timing of embryo transfer. This flexibility reduced the office

working hours in the holidays or on Sundays for both clinicians and embryologists. The grading system we provided was more detailed for embryo selection and it was easier to remember, especially in selecting the best quality of embryos on day 4, which were classified as grade 1a, or grade 1b. This new grading system was also easily applicable in daily routine. The Embryologist needed less than 5 min to observe the incubated embryos on the 4th day after TVOR, just as we normally performed on day 2, day 3 and day 5.

Our data showed morula embryo transfer providing a flexible, easier and applicable methods for embryo transfer in daily routine. This study also concurred with the few other studies regarding morula embryo transfer documented in the past. However, large size prospective randomized controlled studies are still required in future, to confirm the beneficial effects and applicability of this new grading system in daily routine.

Conflicts of interest statement

L.RS. has nothing to disclose.
H.YM. has nothing to disclose.
L.KK. has nothing to disclose.
L.SH. has nothing to disclose.
L.MH. has nothing to disclose.

References

[1] Alpha Scientists in Reproductive M, Embryology ESIGo. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26:1270–83.
[2] Schoolcraft WB, Gardner DK, Lane M, Schlenker T, Hamilton F, Meldrum DR. Blastocyst culture and transfer: analysis of results and parameters affecting outcome in two in vitro fertilization programs. Fertil Steril 1999;72:604–9.
[3] Huisman GJ, Alberda AT, Leerentveld RA, Verhoeff A, Zeilmaker GH. A comparison of in vitro fertilization results after embryo transfer after 2, 3, and 4 days of embryo culture. Fertil Steril 1994;61:970–1.
[4] Grifo JA, Giatras K, Tang YX, Krey LC. Successful outcome with day 4 embryo transfer after preimplantation diagnosis for genetically transmitted diseases. Hum Reprod 1998;13:1656–9.
[5] Gianaroli L. Advantages of day 4 embryo transfer in patients undergoing preimplantation genetic diagnosis of aneuploidy. J Assist Reprod Genet 1999;16.
[6] Tao J, Tamis R, Fink K. Pregnancies achieved after transferring frozen morula/compact stage embryos. Fertil Steril 2001;75:629–31.
[7] Tao J, Tamis R, Fink K, Williams B, Nelson-White T, Craig R. The neglected morula/compact stage embryo transfer. Hum Reprod 2002;17:1513–8.
[8] Feil D, Henshaw RC, Lane M. Day 4 embryo selection is equal to Day 5 using a new embryo scoring system validated in single embryo transfers. Hum Reprod 2008;23:1505–10.
[9] Vassena R, Boue S, Gonzalez-Roca E, Aran B, Auer H, Veiga A, et al. Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. Development 2011;138:3699–709.
[10] Kang SM, Lee SW, Jeong HJ, Yoon SH, Koh MW, Lim JH, et al. Clinical outcomes of elective single morula embryo transfer versus elective single blastocyst embryo transfer in IVF-ET. J Assist Reprod Genet 2012;29:423–8.