

# The neglected morula/compact stage embryo transfer

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**BACKGROUND:** This retrospective study analysed the outcomes of 339 embryo transfers on either day 3 ( $n = 97$ ) or day 4 ( $n = 242$ ), and proposed a grading system for morula/compact embryos. **METHODS:** The morula/compact embryo grading was based on: (i) the proportion of blastomeres undergoing the compaction process; (ii) the morphology of the compacted multicellular mass; (iii) the embryo quality on day 2 and 3; and (iv) the amount of fragmentation. Embryo transfers were classified into groups as follows: group I: transferred with zero 'good' embryos; group II: one 'good' embryo; group III: two or more 'good' embryos. **RESULTS:** Patients on day 4 were transferred with significantly fewer embryos in groups II and III ( $2.58 \pm 0.9$  and  $2.35 \pm 0.6$  respectively) when compared with the correspondent day 3 transfers ( $3.81 \pm 1.4$  and  $4.07 \pm 0.9$  respectively) ( $P < 0.05$ ), but had the same or higher implantation and pregnancy rates. Analysing the patients who had transfers with all 'good' embryos, day 4 transfer achieved a significantly higher implantation rate compared with day 3 transfer (46.4 versus 21.4%,  $P < 0.01$ ), but the number of embryos transferred on day 4 was significantly lower than day 3 ( $2.1 \pm 0.5$  versus  $3.5 \pm 0.9$ ,  $P < 0.01$ ). **CONCLUSIONS:** The morula/compact embryos had great value for embryo selection, which significantly reduced the number of embryos needed for transfer.

**Key words:** compact/embryo grading/implantation/morula/selection

## Introduction

With the first IVF baby success (Steptoe and Edwards, 1978), it was found that human pregnancy could be achieved following transfer of early stage embryos to the uterus, such as the pre-zygote stage, or more commonly the cleavage stages, i.e. the 4-cell (day 2) and 8-cell stages (day 3). The human embryonic genome activates at the 4–8-cell stage (Braude *et al.*, 1988) and early embryo quality evaluation does not accurately predict further growth potential. Because of the poor prediction of day 2 and day 3 embryo growth potential, an average of three or four embryos are routinely placed into the uterus. For women aged  $>40$  years, even more embryos may be transferred.

Transfer with a high number of embryos may increase the pregnancy rate (Tan *et al.*, 1990), but it also increases the risk of multiple implantation in the pregnant population (Hershlag *et al.*, 1990). It is a great challenge to achieve an acceptable pregnancy rate and eliminate multiple gestations, especially triplets or more. To reach this goal, fewer embryos with a high growth potential should be selected. Postponing embryo transfer to later stages can provide better embryo selection and may significantly solve this problem. The blastocyst stage is the last embryonic stage before implantation, and has been believed to give the best embryo selection (Buster *et al.*, 1985; Olivennes, 1994; Gardner *et al.*, 1998). However, only a limited number of embryos reach the blastocyst stage *in vitro*, even with the most advanced sequential culture media. In

clinical practice, not every patient has healthy blastocysts on day 5 or 6. Therefore, blastocyst transfer is only suitable for a selected group who have at least four or five 'good' embryos on day 2 or 3 (Rijnders and Jansen, 1998; Balaban *et al.*, 2000; Graham *et al.*, 2000a).

The embryo at the morula/compact stage has not had enough attention paid to it as a transfer option in assisted reproductive technology. Recent reports of transferring embryos on day 4 were mostly limited to cases that underwent embryo biopsies on day 3 (Grifo *et al.*, 1998; Gianaroli *et al.*, 1999). In terms of embryo selection, the morula/compact embryo should have better selection value compared with the earlier cleavage stages. Not only do the compacted embryos have the activated embryonic genome, but they also indicate that the embryo reaches a further advanced stage, a compaction stage. A grading system for the day 4 embryo, i.e. the morula/compact stage, is proposed in this article. The outcomes of day 4 embryo transfer are compared with routine day 3 transfer and the advantages of the morula/compact embryo transfer are discussed.

## Materials and methods

### Patients

In total, 339 patients under the age of 39 years, who had either day 3 or day 4 transfers, were analysed for this study. Institutional Review

Board approval was not obtained since this was a retrospective review of case records. The data were pooled from two private IVF centres. Analysis of the data was confined to all cases that underwent day 3 or day 4 fresh embryo transfers regardless of embryo quality. The decision of day 3 or day 4 transfer was not randomly determined since these were consecutive cases during different periods of practice. In the first centre, the practice of day 3 transfers all occurred in a time period prior to the time period when day 4 transfers became the normal procedure. Due to the physician retirement in the first centre, the main embryologist moved to the second centre where embryo transfers were all performed on day 4. Both centres employed similar stimulation protocol. The culture medium, protein supplement, incubator environment and transfer method were all the same. The clinical pregnancy rates of day 4 embryo transfers were similar in both centres, 49% (23/47) versus 60% (117/195) respectively. Since the purpose of this study was to evaluate day 4 embryo selection, seven transfers that were postponed to day 5 and four cancelled transfers due to very poor embryo quality were not included in the data analysis.

Patients were started with ovarian suppression. Leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL, USA) was administered in the midluteal phase at dosage of 0.5–1.0 mg per day. After confirmation of adequate pituitary suppression by transvaginal ultrasound (no ovarian cysts >10 mm) and serum oestradiol ( $E_2$ ) level (<50 pg/ml) on cycle day 2, the leuprolide acetate was either discontinued in some patients or decreased by 1/2 to 1/16 in others until HCG administration. Fertinex or Gonal-F (Serono Laboratories, Norwell, MA, USA) or Follistim (Organon, West Orange, NJ, USA) was used for gonadotrophin therapy at the dosage of 2–8 ampoules per day depending upon estimated patient response. Follicular development was monitored with serial pelvic ultrasound examinations and serum  $E_2$  measurements were conducted starting 4 days after gonadotrophin therapy was initiated. After the serum  $E_2$  level reached a plateau (increase less than 10%) or the lead follicles reached 20–22 mm, patients were administered 5000–10 000 IU of HCG i.m. The transvaginal ultrasound-directed oocyte retrieval was performed 36 h later.

Oocytes were identified and then cultured in microdrops (50  $\mu$ l) of equilibrated P1 medium (Irvine Scientific, Santa Ana, CA, USA) with 10% Serum Substitute Supplement (SSS; Irvine Scientific) covered with mineral oil (Sigma, St Louis, MO, USA; or Sage BioPharma, Bedminster, NJ, USA) in an environment of 37°C with 5%  $CO_2$ . Mature oocytes were either inseminated with sperm 5–6 h later at the concentration of 150 000 to 200 000 motile sperm per ml or microinjected with a single spermatozoon. About 18–20 h later, fertilization was assessed. Fertilized oocytes were moved to new microdrops and individually cultured in the same condition until the embryo transfer.

### **Embryo development and grading**

Individual embryo morphology and development were recorded daily. The qualities of embryos were graded from 1 to 4. The grade of 4 represented the best quality. Embryos graded as 4 and 3 were considered to be 'good' embryos, and grades 2 and 1 were 'poor' quality embryos. Day 2 embryo grading was performed at ~46–48 h after insemination or sperm injection, i.e. after the second cell division. Day 2 embryos with four or five even-sized blastomeres and <5% fragmentation were defined as grade 4. Embryos with slightly different-sized blastomeres and/or 5–25% fragments were classified as grade 3. Grade 2 embryos were those with blastomeres with moderate size differences and/or 25–50% fragments. Grade 1 embryos were the poorest, these embryos were composed of markedly different-sized blastomeres and/or had >50% fragments. Slow-grow-

ing embryos, those remaining at the 2-cell stage, and embryos with multinuclei were classified as grade 1 or 2. Day 3 embryo grading was performed at ~70–72 h after insemination. Criteria for day 3 embryo grading were similar to those of day 2 except that the number of blastomeres was expected to be six to eight.

On day 4, embryos were divided into two categories: compacted and non-compacted. The category 'compacted embryo' included the early compacting, the fully compacted and the late compacted or early blastulation stages. The early compacting embryo was identified by blastomeres which had begun to compact tightly, forming a clustered cell mass. Each individual cell was identifiable but not distinct. At the fully compacted stage, blastomeres compacted completely. At this stage, the cell boundary might not be visible, but nuclei could be identified. At the late compact stage, the cell boundary became visible again, and cell number was significantly increased. Within hours, some spindle-shaped cells could be visualized at the edge of the embryo and the embryo had started to blastulate. Embryo grading on day 4 was mostly performed at the fully compacted or the late compacted stage (about 92–96 h after insemination), because the proportion of blastomeres undergoing the compaction process and the morphology of the compacted cell mass could be easily evaluated at these stages. To observe the growth potential of 'good' day 3 embryos, the percentage of the embryos graded as 'good' on day 3 remaining 'good' on day 4 was calculated.

There is little reference to be found regarding morula/compact embryo grading. In this study, the qualities of the morula/compact embryo were classified into four grades according to the following criteria: (i) the proportion of blastomeres undergoing compaction process; (ii) the morphology of the compacted embryo; (iii) the embryo quality on day 2 and 3; and (iv) the amount of fragmentation. As in cleavage stage embryo grading, embryos graded 4 and 3 were considered as 'good' embryos, those graded as 2 and 1 were 'poor' embryos. The detailed grading criteria are described as follows and summarized in Table I.

The proportion of blastomeres undergoing the compaction process was one of the main factors used to determine day 4 embryo scores because the proportion determines the size of the compacted multicellular mass. When all or the majority of blastomeres undergo the compaction process, a large multicellular mass is expected, which would result in a high score (Figure 1A, B); whereas, if only some blastomeres underwent compaction, that was partial compaction, and other cells remained in a separated status, then a smaller compacted multicellular mass would form and a low score would be given (Figure 1D).

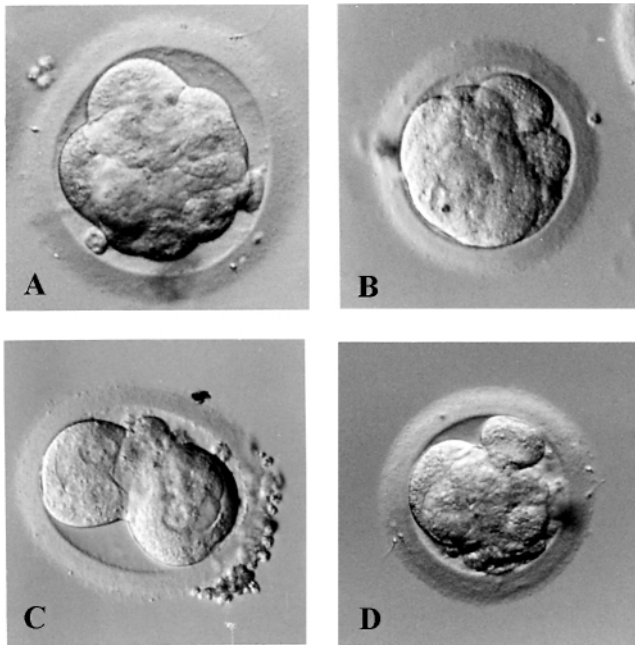
The morphology of the compacted embryo was used as another factor for the grading system. A healthy compacted embryo was identified by a sphere-shaped or close to a sphere-shaped compacted multicellular mass. The profile of a high-scoring embryo was smooth and without deep indentation, i.e. the surface indentation was less than half the diameter of a blastomere. It was not unusual that all or most of the blastomeres would undergo the compaction process; however, an irregular-shaped cell mass with severe surface indentation was formed (Figure 1C). Sometimes, even a lobed compacted cell mass, appearing as two or three small compacted cell masses, was formed, resulting in a low score.

It was observed that the embryo might be able to enter the compaction process on day 4 despite flaws, such as low cell number, or blastomeres with moderate to severe size difference or blastomeres with multiple nuclei on days 2 and 3. To distinguish these embryos from those that were 'good' on day 2 and 3, the cell number, blastomere identity, and multinucleation on day 2 and 3 were taken into account for day 4 embryo grading.

Like embryo grading in the earlier stage, fragmentation was also

**Table I.** Grading criteria for morula/compact embryo

Score	Proportion of blastomere undergoing compaction	Morphology of compacted embryo	Blastomere on days 2 and 3	Fragments
4	All	Full size. Close to sphere with smooth profile	Even-sized blastomere	<5%
3	>75%	>3/4. Close to sphere with shallow indentation	May have slight size difference	<25%
	65–75%	2/3–3/4. Close to sphere with smooth profile		
2	All or partial	Full or >3/4. Irregular with deep indentation.	May have slight to moderate size difference and/or multinuclei	<50%
		1/2 or lobed to two 1/2		
1	All or partial	1/3 or lobed to two to three 1/3	May have moderate to severe size difference and/or multinuclei	Varies



**Figure 1.** Photomicrographs of morula/compact embryos with different scores. (A) Grade 4 embryo. The embryo is nearly fully compacted with all blastomeres undergoing the compact process. Cell boundary is not clear but some nuclei can be identified. (B) Grade 3 embryo. More than three-quarters of blastomeres undergo the compact process. The embryo is close to sphere shape with a smooth profile. (C) Grade 2 embryo. This embryo has an irregular morphology with a deep indentation on the surface. (D) Grade 1. Fewer than half of the blastomeres undergo the compaction process and form a small compacted cell mass. Fragments and non-compacted blastomeres can be identified. Original magnification:  $\times 200$ .

a factor in deciding the embryo score. Embryos with <25% fragments might still be graded as 'good' if a good compacted multicellular mass had formed. When fragmentation was >25%, the embryo would be graded as 'poor' because usually only a small multicellular mass was formed, despite the fact that all blastomeres had entered the compaction process.

Embryos that did not show any sign of compaction on day 4 were considered slow-growing embryos. Sometimes compaction would

occur on day 5 or 6. However, they were still considered slow-growing embryos, and were not evaluated in detail in the present study.

#### Embryo transfer

Routinely, the best embryos were chosen for fresh transfers and the surplus embryos were frozen later. For day 3 transfer, generally a maximum of four embryos was transferred. For those with some embryos of poor quality or having a repeated failed IVF history, five or six embryos were sometimes transferred. Considering that morula/compact embryos were at an advanced stage, initially the maximum number of 'good' quality embryos for transferring on day 4 was determined to be three embryos. However, soon after twin pregnancies were frequently observed, and the number of 'good' morula/compact embryos for transferring was reduced to two embryos. Mechanical assisted hatching was performed on all embryos ~30 min before transfer procedures. Briefly, assisted hatching was performed as follows. An embryo was secured onto a holding pipette at the 9 o'clock position and a microneedle pierced the zona pellucida at the 1 or 5 o'clock position and passed the other side of the zona pellucida. The embryo was released from the holding pipette, and the microneedle that held a portion of the zona pellucida was rubbed against the side of the holding pipette until the zona pellucida was ruptured. The embryo transfer catheter used was a Norfolk intrauterine catheter (Cook, Indiana, IN, USA) assisted with a metal outer sleeve. Progesterone suppositories, 400 mg, were started either on the evening of the oocyte retrieval or the day after the oocyte retrieval. Intravaginal progesterone was used alone or in combination with i.m. administration (50 mg/ml in oil). In some cases i.m. injection of progesterone was used alone. Serum HCG was examined 11 days after day 4 embryo transfer and 12 days after day 3 transfer.

The implantation rate was defined as the number of gestation sacs per number of embryos transferred. Clinical pregnancy was defined as intrauterine sacs identified by ultrasound examination. Ectopic pregnancy was counted as implantation but not as a clinical pregnancy. Monozygotic twins were considered one implantation. To further verify the embryo growth potential, the ratios of the fetal heartbeats to gestation sacs (FHB/sac) were also evaluated, in which the monozygotic twins were regarded as having one FHB.

#### Outcome analysis

To indicate the embryo quality and related implantation/clinical pregnancy rates, data are presented based on the number and the quality of the embryo(s) transferred. The data are presented in three

**Table II.** Comparison of day 3 and day 4 embryo transfer

	Transfer day	Group I (0 'good')	Group II (1 'good')	Group III ( $\geq 2$ 'good')
No. of embryos transferred <sup>a</sup>	3	2.25 $\pm$ 0.8 (12)	3.81 $\pm$ 1.4 (16)	4.07 $\pm$ 0.9 (69)
	4	2.56 $\pm$ 1.1 (45)	2.58 $\pm$ 0.9 (57) <sup>b,d</sup>	2.35 $\pm$ 0.6 (140) <sup>b</sup>
Pregnancy (%)	3	3/12 (25.0)	7/16 (43.8)	32/69 (46.4)
	4	13/45 (28.9) <sup>c,d</sup>	29/57 (50.9) <sup>d</sup>	98/140 (70.0) <sup>b</sup>
Implantation rate (%)	3	3/27 (11.1)	12/61 (19.7)	54/281 (19.2)
	4	18/115 (15.7) <sup>d</sup>	37/147 (25.2) <sup>d</sup>	146/329 (44.4) <sup>b</sup>
FHB/sacs (%)	3	2/3 (66.7)	11/12 (91.7)	53/54 (98.1)
	4	14/18 (77.8)	35/37 (94.6)	132/146 (90.4)
Ongoing pregnancy (%)	3	2/12 (16.7)	6/16 (37.5)	31/69 (44.9)
	4	10/45 (22.2) <sup>c,d</sup>	29/57 (50.9)	89/140 (63.6) <sup>b</sup>
Singleton (%)	3	2/2 (100)	3/6 (50.0)	14/31 (45.2)
	4	6/10 (60.0)	24/29 (82.8)	48/89 (53.9)
Twins (%)	3	0/2 (0)	2/6 (33.3)	14/31 (45.2)
	4	4/10 (40.0)	4/29 (13.8)	39/89 (43.8)
> Twins (%)	3	0/2 (0)	1/6 (16.7)	3/31 (9.7)
	4	0/10 (0)	1/29 (3.4)	2/89 (2.2)

<sup>a</sup>Mean  $\pm$  SD. Values in parentheses are the patient numbers.<sup>b</sup>Significant difference compared with the corresponding day 3 group ( $P < 0.05$ ).<sup>c</sup>Significant difference compared with group II ( $P < 0.05$ ).<sup>d</sup>Significant difference compared with group III ( $P < 0.05$ ).

FHB = fetal heart beat.

**Table III.** Comparison of patients transferred with 'good' embryos only

Transfer	<i>n</i>	Age (years) <sup>a</sup>	No. of embryos <sup>a</sup>	Implantation (%)	Clinical pregnancies (%)	Ongoing pregnancies (%)
Day 3	20	33.9 $\pm$ 2.8	3.5 $\pm$ 0.9	15/70 (21.4)	9/20 (45.0)	8/20 (40.0)
Day 4	122	33.0 $\pm$ 3.5	2.1 $\pm$ 0.5	121/261 (46.4)	83/122 (68.0)	76/122 (62.3)
<i>P</i>		NS	< 0.01	< 0.01	NS	NS

<sup>a</sup> Mean  $\pm$  SD.

NS = not significant.

groups: group I transferred with only 'poor' embryos (grade 1 and 2). Group II transferred with one 'good' embryo (grade 3 or 4) plus some 'poor' ones (grade 1 and 2). Group III transferred with two or more 'good' embryos (graded as 3–4) and possibly some 'poor' ones (Table II).

When patients were transferred with mixed 'good' and 'poor' embryos and when this ratio varied between patients, an implantation rate would not provide accurate information for data analysis if the purpose was to estimate the implantation potential of 'good' embryos. To exclude the interference of 'poor' embryos, patients who were transferred with only 'good' embryos either on day 3 or day 4 were selected. The number of embryos transferred, implantation and pregnancy rates are compared in Table III.

### Statistical analysis

Data are presented as mean  $\pm$  SD or percentage. Where appropriate, data were analysed with unpaired Student's *t*-test or  $\chi^2$ -test. Significance was accepted at  $P < 0.05$ .

### Results

The comparison of day 3 and day 4 transfer is presented in Table II. The ages of the patients who had day 3 and day 4 embryo transfers were  $33.1 \pm 3.6$  and  $33.4 \pm 3.3$  respectively ( $P =$  not significant). The numbers of oocytes retrieved on day 3 and day 4 were  $14.5 \pm 7.2$  and  $15.4 \pm 9.9$  respectively

( $P =$  not significant). In the day 4 transfer group, 1356 embryos were graded as 'good' on day 3 in which 803 remained as 'good' quality on day 4 (59.2%).

In group I, transferred with zero 'good' embryos, both day 3 and day 4 transfers had similar numbers of embryos,  $2.25 \pm 0.8$  and  $2.56 \pm 1.1$  respectively. The low number of embryos transferred in this group was due to lack of sufficient embryos. The implantation and clinical pregnancy rates of day 3 and day 4 transfers were similar, and low (11.1 versus 15.7%, and 25.0 versus 28.9% respectively). The ongoing pregnancy rates in this group were 16.7 and 22.2% respectively.

In group II (one 'good' embryo), the clinical pregnancy rates in day 3 and day 4 (43.8 versus 50.9%) were similar, as were the implantation rates (19.7 versus 25.2% respectively), but the number of embryos transferred on day 4 was significantly lower than on day 3 ( $3.81 \pm 1.4$  versus  $2.58 \pm 0.9$ ,  $P < 0.05$ ). In group III, in which at least two 'good' embryos were transferred, both clinical pregnancy rate and implantation rate on day 4 transfer were significantly increased (70.0 and 44.4% respectively) compared with the correspondent day 3 group (46.4 and 19.2% respectively) ( $P < 0.05$ ) and the embryo number transferred on day 4 was significantly lower than day 3 ( $2.35 \pm 0.6$  versus  $4.07 \pm 0.9$ ,  $P < 0.05$ ).

In day 3 transfers, transferring of more 'good' embryos did



not significantly increase implantation and pregnancy rates, which indicated the poor prognostication of the day 3 scoring system. However, in day 4 transfers, both implantation and pregnancy rates were significantly increased as more 'good' embryos were transferred, which indicated a good prognostic value of the day 4 scoring system.

Patients transferred with all 'good' embryos were analysed and the results are presented in Table III. Although day 4 transfers had a significantly lower number of embryos when compared with day 3 transfer ( $2.1 \pm 0.5$  versus  $3.5 \pm 0.9$ ,  $P < 0.01$ ), the implantation rate of day 4 transfers was significantly higher than day 3 (46.4 versus 21.4%,  $P < 0.01$ ).

## Discussion

It has been known for many years that human pregnancy can be achieved after transferring cleaved embryos to the uterus 2 or 3 days after IVF. To date, embryo selection for transfer still relies mainly on a morphological evaluation, which is to a certain degree subjective. Moreover, human embryonic genome activation does not occur until the 4–8-cell stage, which makes the value of the morphological judgement of cleaved embryos questionable.

Day 4 embryo transfer has been reported before (Huisman *et al.*, 1994) and was recently being applied to those who had embryo biopsy on day 3 (Grifo *et al.*, 1998; Gianaroli *et al.*, 1999). The data presented here demonstrated that the morula/compact transfer on day 4 improves embryo selection compared with day 3 embryo transfer. In this study, only 59.2% 'good' day 3 embryos developed to 'good' day 4 embryos. Similar results have been reported (Rijnders and Jansen 1998) indicating that 69% of embryos remained 'good' on day 3 and only ~47% of these embryos reached the blastocyst stage when cultured in a mixture of Earle's and Ham's F-10 medium throughout the entire culture period. Recently, a study using P1/blastocyst and G1/G2 media systems (Graham and Han *et al.*, 2000b) has reported that only 48% of embryos chosen for use on day 3 were selected again on day 5 or 6.

An extensive literature search has found little reference to grading criteria for the morula/compact stage embryos. Four criteria were employed for the grading system proposed in this article. The first is the proportion of blastomeres undergoing the compaction process. It is very common to see some but not all of the blastomeres undergo the compaction process. The excluded cells might be 'bad' and excluded by natural selection. It is also possible that these excluded cells were retarded in development and may undergo the compaction process later, which can make grading difficult. The proportion of blastomeres that undergoes the compaction process is related to the size of the compacted embryo. Thus, the smaller the proportion undergoing compaction, the smaller will be the compacted embryo formed. Small morula/compact embryos may indicate an inferior potential.

Morphology of morula/compact embryos was the most important factor applied for the proposed grading system. In some embryos, even if all or most of the blastomeres underwent the compaction process, the morphology of the embryo was severely irregular in shape. Commonly, the embryo presented

as an irregular-shaped multicellular mass with severe surface indentations (Figure 1C). Sometimes the embryo was lobate, appearing as two or three small compacted cell masses. It would be interesting to know whether each small compacted cell mass can develop an individual inner cell mass, or if this phenomenon is an indication of poor quality. Based on limited results, no increased incidence of identical twins was found but only low implantation rates occurred after transferring these embryos.

An embryo quality index, such as the cell number and size identity of blastomeres on day 2 and day 3, was also considered as key factor for day 4 grading. It was not unusual that embryos with low cell number and/or severe uneven size blastomeres on day 2 and 3 underwent the compaction process on day 4, which would have made the embryo grading difficult if the embryos had not been assessed on a daily basis. It is believed that tracking embryo development daily will increase the accuracy of the grading.

Multiple nuclei, usually observed at the 2–4-cell stage (Munné and Cohen, 1993; Kligman *et al.*, 1996), are always considered as a factor to downgrade the embryo score in the proposed grading system. Embryos with multiple nuclei are frequently accompanied by uneven-sized blastomeres and a moderate to severe amount of fragmentation.

Only more than a moderate amount of fragmentation (>25%) was considered meaningful for day 4 embryo grading as in earlier cleavage stages. Small to moderate amount of fragmentation seems to affect the size of the compacted cell mass rather than morphology of the embryos. This supports the findings of other authors that a small amount of fragmentation (<20%) did not significantly impact implantation (Scholtes and Zeilmaker, 1996).

Using the proposed grading system, a 22.2% ongoing pregnancy rate was achieved in group I, who were transferred with zero 'good' embryos on day 4. This 'misdiagnosis' might be due to a non-synchronized blastomere development that caused a partial compaction at the time of grading, or perhaps irregular-shaped compacted embryos in which a prognosis was difficult. Further refinement of the grading system will be needed to improve the accuracy of the grading. However, the significantly lower implantation and pregnancy rates in this group did indicate that the potential of these embryos was poor.

It would be desirable to be able to judge an embryo grading system, especially its accuracy in predicting 'good' embryos. In practice, this is difficult. In many cases, transfers are made with mixed numbers of 'good' and 'poor' embryos. This mixed transfer makes the implantation analysis unreliable. In addition, it is not known exactly how much the non-embryo quality-related factors, such as non-optimized endometrial receptivity, transfer technique and catheter choice, are responsible for failed implantation (Goudas *et al.*, 1998; Ghazzawi *et al.*, 1999; Meriano *et al.*, 2000; Schoolcraft *et al.*, 2001). In Table III, 'poor' quality embryos were eliminated, which makes it possible to compare 'good' embryo implantation probability on day 3 and day 4. The results indicated the considerable prognostic potential of the morula/compact stage grading. Of course, the real implantation rate of these embryos

is still unattainable because of other non-embryo quality-related factors as mentioned before.

Some other advantages may be associated with the morula/compact embryo transfer. The first is that it is easy and safe to perform assisted hatching. Because of the cell compaction, embryos at this stage have the largest perivitelline space during the entire preimplantation stage. In addition, since the extensive cell junctions have been established, it is not necessary to be concerned about losing an individual blastomere after the zona pellucida has been opened.

The second advantage is the ability to delay embryo cryopreservation until after fresh transfers, which provides the assurance that the best embryos are selected for the fresh embryo transfer. In addition, it reduces the possibility of cryopreservation of embryos with poor prognostic value since embryos at the morula/compact stage have better selection value compared with earlier stages. Live births in mice and human beings have recently been reported after transferring thawed morula/compact embryos (Tao et al., 2001a,b).

The outcome of embryo transfer on day 4 appears to correlate with the proposed grading system. The implantation and pregnancy rates of day 4 embryo transfer support the concept that embryos at the morula/compact stage have a good selection value. The validity of this grading system and the advantages of the morula/compact stage embryo transfer need be confirmed by further investigation.

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