Blastocyst morphology score as an indicator of embryo competence for women aged younger than 38 years in in vitro fertilization cycles

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Abstract

Objective: This study aimed to clarify the usefulness of blastocyst scores and female age as embryo competence markers for embryo transfer in in vitro fertilization (IVF) cycles.

Materials and Methods: A total of 352 IVF cycles were investigated. The relevance of blastocyst scores and female age to pregnancy outcome was assessed by logistic regression analysis.

Results: We revealed that, for patients aged < 35 years, the score of the best embryo was the sole factor related to multiple pregnancy, whereas the score of the best two embryos was the only factor relevant to pregnancy. For patients aged 35–37 years, the score of the best three embryos was the sole factor correlated to both pregnancy and multiple pregnancy. As for older patients, the correlation between blastocyst morphology and pregnancy outcome was mainly affected by female age.

Conclusion: The blastocyst score could be used to determine the number of blastocysts transferred to younger patients, but it is less useful for patients aged > 38 years. For older patients, female age is a better indicator to determine the number of transferred blastocysts.

Keywords: age; blastocyst; in vitro fertilization; multiple gestation; pregnancy

Introduction

Blastocyst transfer (BT) is associated with high implantation and clinical pregnancy rates [1–4]. Allowing cleavage (Day 2 or Day 3) embryos to develop for 2–3 additional days facilitates better self-selection of high-quality embryos. Nonetheless, in order to achieve a higher pregnancy rate of in vitro fertilization (IVF) cycles, a tendency to transfer multiple embryos was observed. Due to an increased implantation rate in BT cycles, transfer of multiple blastocysts results in an elevated rate of high-order multiple gestation (HOMG) [1,5].

Multiple gestation has been reported to be associated with increased rates of preterm birth and perinatal morbidity, and higher medical care costs [6]. As a result, efforts have been made to reduce the number of transferred embryos to decrease multiple pregnancy rates [7–10]. Limiting the number of high-quality blastocysts transferred is obviously necessary to avoid multiple gestation. However, in areas or countries where the cost of IVF is not covered by health insurance, maximizing the rate of pregnancy while limiting the rate of multiple gestation remains a major challenge [11].

Major clinical factors related to successful pregnancy by means of IVF with cleavage-stage embryos include the following: age of the patient [12], embryo morphology [13], number of unsuccessful prior attempts, and the number of...
American Society for Reproductive Medicine (ASRM) in 2004 [16] for the number of transferred embryos and groups the patients according to age (<35 years and ≥35 years). The futility of using embryo morphology to predict pregnancy outcome in IVF cycles for women aged ≥35 years has been documented in our previous report [15].

It has been demonstrated that top embryo morphology does not deteriorate with increased maternal age [17], whereas the incidence of aneuploidy has been reported to increase with maternal age [18]. Furthermore, aneuploidy occurs more frequently in embryos with good morphology and development stage than in poorly developed embryos at the cleavage stage [18]. Thus, the weak connection between embryo morphology and pregnancy outcome in IVF with cleavage-stage embryos for women aged ≥35 years might be attributed to a high aneuploidy rate. A lower aneuploidy rate has been reported among blastocysts-stage compared to cleavage-stage embryos for women aged ≥36 years [19,20]. Therefore, BT may be a valuable method to eliminate the effect of female age on IVF programs.

Blastocyst quality is associated with the success of the BT program in IVF cycles [21]. In addition, adequate selection of transferred blastocysts has been proved to improve pregnancy outcome [22]. As a result, an increased number of IVF programs have gradually shifted from cleavage-stage embryo transfer to BT to improve the outcome of IVF cycles. Under such circumstances, we urge establishing or refining our individual algorithms to determine better the number of transferred blastocysts for IVF patients.

The aim of the present study was to develop an algorithm for BT by retrospectively analyzing the relationships among blastocyst quality, chronological age, and pregnancy outcome. Through this analysis, we aimed to ascertain information that could potentially be used for patient counseling and further decrease the number of transferred embryos for the BT program. In addition, we also attempted to elucidate whether blastocyst quality, which featured a lower rate of aneuploidy, could subdue the negative influence of age on IVF outcome.

Materials and methods

Study participants

This was a retrospective study of IVF outcomes performed by reviewing the medical records of patients undergoing IVF treatment at the National Taiwan University Hospital from January 2007 to December 2008. Only the first or second stimulated cycles of the patients were included for analysis. If both the first and second cycles were performed during the study period, only the first cycle was included for analysis. Cycles for oocyte donation or preimplantation genetic diagnosis were excluded from analysis. Cycles with ≥one blastocyst available for transfer after in vitro culture for 120 hours were included. Consequently, a total of 352 BT procedures met the inclusion criteria and were analyzed in this study.

If only one blastocyst was available for transfer subsequent to in vitro culture, that one blastocyst would be transferred back into the patients, and this situation was compatible with the so-called compulsory single embryo transfer (SET; n = 8). Except for those situations, a total of 344 IVF/intracytoplasmic sperm injection (ICSI) cycles had ≥ two blastocysts available for transfer. Afterwards, all those cycles were recruited for logistic regression analysis, which was performed to determine the clinical factors predictive of pregnancy and multiple pregnancy.

Institutional Review Board approval was not required because the IVF unit at our hospital is licensed and regulated by the Human and Fertilization Authority (Bureau of Health Promotion, Department of Health, Taiwan). There were no interventions other than those involved in standard IVF treatment. Furthermore, the principles outlined in the Declaration of Helsinki were followed in the present study.

Ovarian stimulation protocols and in vitro embryo culture

Stimulation cycles utilizing the long and short protocols for gonadotropin- releasing hormone (GnRH) agonist and multiple dose protocol for GnRH antagonist were recruited in the present study. The formal long and short protocol for controlled ovarian stimulation has been described previously [15]. Briefly, the stimulation procedure commenced with the administration of buserelin (Supromon; Hoechst, Frankfurt, Germany) combined with recombinant follicle-stimulating hormone (rFSH; 200–400 IU/day).

The multidose protocol for GnRH antagonist was performed as previously described [23]. Briefly, the stimulation process commenced with the administration of rFSH (Gonal-F; Serono, Anbunne, Switzerland) (200 IU/day) for 4 days. Cetrorelix (0.25 mg, Cetrotide; Serono, Anbunne, Switzerland) was administered on Day 5 of gonadotropin stimulation.

When two leading follicles (≥ 18 mm in diameter) appeared to be present, rFSH treatment was ceased, and 250 µg human chorionic gonadotropin (hCG, Ovidrel; Serono) was administered on Day 5 of gonadotropin stimulation.

After oocyte collection and IVF/ICSI, all embryos were cultured randomly in one of the two sequential media designed to facilitate blastocyst development. The first system was G series (G1 and G2; Vitrolife, Kungsbacka, Sweden), and the second was Quinns Advantage Cleavage and Blastocyst medium (QAC and QAB; SAGE In-Vitro Fertilization, Inc., Trumbull, CT, USA). Subsequent to morphological grading by the same technician, embryo transfer was performed 120 hours after oocyte retrieval.

After oocyte collection and IVF/ICSI, all embryos were cultured randomly in one of the two sequential media designed to facilitate blastocyst development. The first system was G series (G1 and G2; Vitrolife, Kungsbacka, Sweden), and the second was Quinns Advantage Cleavage and Blastocyst medium (QAC and QAB; SAGE In-Vitro Fertilization, Inc., Trumbull, CT, USA). Subsequent to morphological grading by the same technician, embryo transfer was performed 120 hours after oocyte retrieval.

Serum hCG levels were checked 12 days after ET. A level > 50 IU/L was viewed as an indication of pregnancy. Ultrasonic examination was preformed 3 weeks later in order to assess fetal heart activity in intrauterine gestational sacs. Multiple pregnancy was identified by the observation of activity of more than one fetal heart. A living child 1 week after delivery was defined as a live birth [24].


**Blastocyst scoring system**

The embryos were graded according to the criteria proposed by Gardner and colleagues [25], that is, Grade 1: early blastocyst, wherein the blastocele is less than half the volume of the embryo; Grade 2: blastocyst, wherein the blastocele is greater than or equal to half of the volume of the embryo; Grade 3: full blastocyst, wherein the blastocele completely fills the embryo; Grade 4: expanded blastocyst, wherein the blastocele volume is larger than that of the early embryo and the zona pellucida is thinning; Grade 5: hatching blastocyst, which the trophectoderm has started to herniate through the zona pellucida; and Grade 6: hatched blastocyst, in which the blastocyst has completely escaped from the zona pellucida.

The development of the inner cell mass (ICM) and trophectoderm was also assessed. The ICM grading was as follows: A: many cells that are tightly packed; B: several cells that are loosely grouped; or C: very few cells. The trophectoderm grading was as follows: A: many cells forming a tightly knit epithelium; B: a few cells; or C: very few cells forming a loose epithelium. The blastocyst grading was transformed into a blastocyst score according to the method described by Rehman et al [26]. The scoring system recoded the alphabetical grades as numerical form in the simplest possible manner, that is, A = 3, B = 2, and C = 1. To combine these three values to obtain a single summary score, multiplicative combinations of the three components of blastocyst grading were utilized [26].

In the current study, the Top1 score was equal to the blastocyst scoring of the best blastocyst in transferred embryos. The Top2 and Top3 scores were equal to the summation of the scoring of the best two and three blastocysts in transferred embryos, respectively. The cumulative embryo score was equal to the summation of the scoring of all transferred blastocysts.

**Statistical analysis**

The various biological parameters germane to IVF/ICSI cycles were compared by Student t test, Fisher’s exact test, \( \chi^2 \) test, one-way analysis of variance (ANOVA), or Kruskal–Wallis test according to various conditions. All analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). When a significant change was detected by ANOVA, the Bonferroni test was used for subsequent post hoc multiple comparisons. The differences were considered to be statistically significant when \( p < 0.05 \).

Receiver operating characteristic (ROC) curve analysis was used to estimate the predictive power of the measured variables. The relative capability of embryo scoring systems to predict the IVF outcome was compared by calculating the area under the ROC curve (AUC) and 95% confidence interval (CI). MedCalc software version 9.3 was used to compare the areas under the two ROC curves (MedCalc, Broekstraat, Belgium). Logistic regression analysis was performed to assess the independent effects of individual variables.

**Results**

The clinical parameters regarding the outcomes of IVF procedures for different age groups are summarized in Table 1. Patients in the younger age group revealed significantly higher rates of pregnancy and implantation, despite a significantly lower number of embryos being transferred in the youngest age group. Nevertheless, the highest rates of multiple pregnancies occurred in the group of women aged \(<35\) years. In this analysis, we suggested three types of embryo morphology designation: the summation of the score of the best one, two, and three embryos (Top1, Top2, and Top3, respectively). The Top1, Top2, and Top3 scores were not significantly different among the age groups (Table 1).

Patients were grouped on the basis of age (< 35 years vs. 35 ≤ age < 38 years vs. ≥ 38 years) and ROC AUC comparisons were performed to determine the factors (embryo morphology and patient age) predictive of pregnancy outcome. As shown in Table 2, Top3 scores were more intimately correlated with pregnancy than Top1 and Top2 scores in all groups, except ≥ 38 years group. Interestingly, it was only in the total cohort that age significantly correlated with pregnancy (95% CI of ROC AUC did not include 0.5).

Conditional logistic regression analysis was performed to determine the independence of the following variables: patient age, baseline FSH levels, number of embryos transferred, and embryo morphology (Top1, Top2, Top3, and cumulative embryo score scores). These results are summarized in Table 3. Namely, Top2 score was the sole determinant of pregnancy and Top1 score was the decisive factor of multiple pregnancy in the group < 35 years. Furthermore, embryo morphology (Top3 score) was the sole determinant of pregnancy and multiple pregnancy in the group 35 ≤ age < 38 years. Comparatively speaking, age was a more important determinant of pregnancy than embryo morphology in the ≥ 38 years group.

Of eight patients who received compulsory SET, pregnancy occurred in only one (1/8; 12.5%) with a 3AA (The 3AA represents the morphology scoring of blastocysts according to the paragraph for blastocyst scoring system in the section of Materials and Methods. The “3” means grade 3, the following “AA” means that inner cell mass is grade A and the trophectoderm is grade A) blastocyst (Top1 = 27). A comparison of outcomes between patients aged < 35 years receiving two (\( n = 51 \)) versus three (\( n = 93 \)) versus four (\( n = 43 \)) embryos is shown in Fig. 1. No significant differences in pregnancy rate were found among groups receiving various numbers of embryos. However, a significantly lower Top1 score (\( p = 0.038 \)), multiple pregnancy rate (9/24 vs. 21/60 vs. 2/24, \( p = 0.034 \)) and implantation rate (51/102 vs. 92/279 vs. 26/172, \( p < 0.001 \)) was shown in patients who received four embryos.

The logistic regression models predicting pregnancy and multiple pregnancy for patients aged < 35 years are shown in Fig. 2. If Top1 = 27 (3AA) or 12 (3BB or 2AB) (The 3BB and 2AB also represent the morphology scoring of blastocysts according to the paragraph for blastocyst scoring system in the section of Materials and Methods. The first arabic number represents blastocele volume, the following two uppercase
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Age &lt; 35 y</th>
<th>35 ≤ Age &lt; 38 y</th>
<th>38 ≤ Age &lt; 40 y</th>
<th>Age ≥ 40 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n = 187)</td>
<td>B (n = 93)</td>
<td>C (n = 29)</td>
<td>D (n = 35)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>31.1 ± 2.4</td>
<td>35.9 ± 0.8</td>
<td>38.5 ± 0.5</td>
<td>41.3 ± 2.1</td>
</tr>
<tr>
<td>Baseline FSH (IU/L)</td>
<td>6.5 ± 2.4</td>
<td>6.7 ± 2.8</td>
<td>7.5 ± 2.4</td>
<td>6.6 ± 2.3</td>
</tr>
<tr>
<td>Short GnRH agonist protocol</td>
<td>95/187 (50.8)</td>
<td>40/93 (43.0)</td>
<td>22/29 (75.9)</td>
<td>22/35 (62.9)</td>
</tr>
<tr>
<td>Long GnRH agonist protocol</td>
<td>43/187 (23.0)</td>
<td>27/93 (29.0)</td>
<td>3/29 (10.3)</td>
<td>6/35 (17.1)</td>
</tr>
<tr>
<td>GnRH antagonist protocol</td>
<td>49/187 (26.2)</td>
<td>26/93 (28.0)</td>
<td>4/29 (13.8)</td>
<td>7/35 (20.0)</td>
</tr>
<tr>
<td>Estradiol on the day of hCG (pg/mL)</td>
<td>3980 ± 2381</td>
<td>3493 ± 2230</td>
<td>3166 ± 1735</td>
<td>3610 ± 1948</td>
</tr>
<tr>
<td>Number of retrieved oocytes</td>
<td>18.4 ± 7.8</td>
<td>16.2 ± 7.5</td>
<td>13.9 ± 5.8</td>
<td>16.0 ± 6.9</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>15/8/542 (29.2)</td>
<td>7/2/307 (23.5)</td>
<td>17/96 (17.7)</td>
<td>16/3/33 (12.0)</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>113/187 (60.4)</td>
<td>55/93 (59.1)</td>
<td>17/29 (58.6)</td>
<td>21/35 (60.0)</td>
</tr>
<tr>
<td>Number of cryopreserved embryos</td>
<td>2.9 ± 0.7</td>
<td>3.3 ± 1.0</td>
<td>3.3 ± 0.7</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>Score of the best three blastocysts</td>
<td>6.8 ± 6.4</td>
<td>5.1 ± 5.5</td>
<td>3.6 ± 4.2</td>
<td>4.0 ± 3.9</td>
</tr>
<tr>
<td>Score of the best two blastocysts</td>
<td>10.8 ± 8.5</td>
<td>9.0 ± 8.1</td>
<td>8.1 ± 7.8</td>
<td>10.2 ± 10.5</td>
</tr>
<tr>
<td>Score of the best three blastocysts</td>
<td>17.2 ± 13.7</td>
<td>13.8 ± 11.9</td>
<td>12.9 ± 12.2</td>
<td>15.5 ± 13.6</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>15/8/542 (29.2)</td>
<td>7/2/307 (23.5)</td>
<td>17/29 (58.6)</td>
<td>21/35 (60.0)</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>106/187 (56.7)</td>
<td>47/93 (50.5)</td>
<td>11/29 (37.9)</td>
<td>12/35 (34.3)</td>
</tr>
<tr>
<td>Multiple pregnancy rate (%)</td>
<td>32/187 (17.1)</td>
<td>15/93 (16.1)</td>
<td>4/29 (13.8)</td>
<td>4/35 (11.4)</td>
</tr>
<tr>
<td>Live birth rate (%)</td>
<td>71/187 (38.0)</td>
<td>37/93 (40.0)</td>
<td>6/29 (20.7)</td>
<td>5/35 (14.3)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or percentage.

FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hCG = human chorionic gonadotropin; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

* p < 0.001; ** p = 0.005; *** p < 0.001; ^ p = 0.015 by one-way analysis of variance (ANOVA) test; * p < 0.001; ** p < 0.001; *** p = 0.027; h p = 0.012 by χ² test.

letters mean the inner cell mass grade and the trophectoderm grade, respectively) and two embryos were transferred, the probability of multiple pregnancy was 47.7% or 39.1%, respectively.

For patients aged 35–37 years, the estimated rates of pregnancy and multiple pregnancy are demonstrated in Fig. 3. If Top3 = 27, the estimated rates of pregnancy and multiple pregnancy were 40.4% and 20.2%, respectively.

Discussion

In this retrospective analysis, the probabilities of pregnancy and multiple gestation were calculated with reference to female age and blastocyst morphology scores. By recoding Gardner and colleagues grading system for blastocyst morphology into a cumulative scoring system [26], we were enabled to determine the probability of pregnancy and multiple gestation in IVF/ICSI cycles. However, for women aged > 38 years, it was female age that was the major factor for pregnancy outcome in IVF/ICSI cycle.

The assessment of blastocyst morphology before ET is effective to achieve a high pregnancy rate. Balaban et al have reported that >40% of pregnancies in patients with at least one good blastocyst (Gardner grading > 3AA, i.e., Top1 > 27) were multiple gestation, even if the mean number of transferred embryos was as low as 2.3 [22]. By contrast, only one multiple pregnancy (1/6; 16.7%) occurred and only a low implantation rate (11.8%) was obtained when all the transferred blastocysts had grades < 3AA (Top1 < 27) [22]. A high implantation rate (51.4%) was obtained with the transfer of at least one good blastocyst [22]. In the present study, 47.7% of patients aged < 35 years who received at least one good embryo (Top1 > 27, i.e., Gardner grading > 3AA) resulted in multiple gestation. Our findings indicated that transfer of blastocysts with a high Top1 score resulted in a high rate of multiple gestation, regardless of whether three or two embryos were transferred for women aged >35 years.

The high pregnancy rate in patients with good prognosis (56.7% for women aged <35 years) in the current study is similar to that reported in other studies about women in IVF/ICSI cycles with good prognosis [1,25]. However, the implantation rate (29.2% for young women) revealed in the current study is much lower than those previously reported for the BT program (50.6% for double embryo transfer (DET)) [25].

Table 2

<table>
<thead>
<tr>
<th>Predictive factors with</th>
<th>Age &lt; 35 y</th>
<th>35 ≤ Age &lt; 38 y</th>
<th>38 ≤ Age &lt; 40 y</th>
<th>Age ≥ 40 y</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score of the best blastocyst (Top1)</td>
<td>0.626 (0.552–0.696)</td>
<td>0.503 (0.397–0.608)</td>
<td>0.737 (0.611–0.840)</td>
<td>0.620 (0.566–0.672)</td>
<td>0.620 (0.566–0.672)</td>
</tr>
<tr>
<td>Score of the best two blastocysts (Top2)</td>
<td>0.643 (0.570–0.712)</td>
<td>0.531 (0.424–0.635)</td>
<td>0.722 (0.594–0.827)</td>
<td>0.637 (0.584–0.888)</td>
<td>0.637 (0.584–0.888)</td>
</tr>
<tr>
<td>Score of the best three blastocysts (Top3)</td>
<td>0.658 (0.585–0.725)</td>
<td>0.554 (0.448–0.658)</td>
<td>0.727 (0.600–0.831)</td>
<td>0.649 (0.596–0.700)</td>
<td>0.649 (0.596–0.700)</td>
</tr>
<tr>
<td>Age</td>
<td>0.565 (0.491–0.638)</td>
<td>0.537 (0.431–0.641)</td>
<td>0.625 (0.494–0.744)</td>
<td>0.598 (0.544–0.650)</td>
<td>0.598 (0.544–0.650)</td>
</tr>
</tbody>
</table>

Data are presented as 95% confidence intervals (CIs).

IVF = in vitro fertilization; ROC = receiver operating characteristic.

* p = 0.019; ** p = 0.025; *** p = 0.034; ^ p = 0.003; '# p = 0.018; † p = 0.023; $ p = 0.002; h p = 0.004 by pairwise comparison of ROC curves.
Nonetheless, the implantation rate (50%) of selective DET for women aged < 35 years in the present study was almost equal to that (50.6%) of DET in IVF cycles with good prognosis [25]. The results suggest that some of the embryos transferred were probably of low implantation potential or poor quality. The excess transfer of inferior embryos may be due to the clinicians and/or the patients being uncomfortable with transferring a small number of embryos to achieve pregnancy, especially when the blastocysts were of poor quality.

Elective SET may be the best way to eliminate the risk of multiple gestation [10]. However, some investigators argue that DET is an acceptable approach [27]. Either a successful elective SET or DET program requires optimal patient selection criteria and an efficient embryo cryopreservation program [28,29]. Individual centers could establish certain criteria for embryo and patient selection to decrease the rates of multiple gestation, while maintaining maximal pregnancy rates. The current study results were in accordance with the guidelines proposed by the ASRM in 2009 [30]; namely, for patients aged < 35 years, SET should be performed for cycles with good

Table 3
Odds ratio for conditional logistic regression model based on the age of women and the score of the best one, two, or three embryos (Top1, Top 2, or Top3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age &lt; 35 y (n = 187)</th>
<th>35 ≤ Age &lt; 38 y (n = 93)</th>
<th>Age ≥ 38 y (n = 64)</th>
<th>Total (n = 344)</th>
</tr>
</thead>
<tbody>
<tr>
<td>For pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>—</td>
<td>—</td>
<td>0.659 (0.437–0.993)</td>
<td>0.906 (0.844–0.973)</td>
</tr>
<tr>
<td>Top2</td>
<td>1.061 (1.021–1.099)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Top3</td>
<td>—</td>
<td>1.033 (1.012–1.054)</td>
<td>—</td>
<td>1.034 (1.014–1.054)</td>
</tr>
<tr>
<td>For multiple pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Top1</td>
<td>1.110 (1.045–1.180)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Top3</td>
<td>—</td>
<td>1.030 (1.009–1.053)</td>
<td>—</td>
<td>1.028 (1.007–1.048)</td>
</tr>
</tbody>
</table>

The odds ratios of significant coefficients are shown as 95% confidence intervals (CIs).

The probability (p) of multiple pregnancy and pregnancy can be calculated through $p = e^{\text{Fertility index}}/(1 + e^{\text{Fertility index}})$.

1. Age < 35 y: Fertility index (pregnancy) = 1.254 + 0.032 × Top2.
2. $35 \leq \text{Age} < 38 \text{y}$: Fertility index (pregnancy) = 1.254 + 0.032 × Top3.
3. Age ≥ 38 y: Fertility index (pregnancy) = 16.201 – 0.418 × Age.
4. Age < 35 y: Fertility index (multiple pregnancy) = –2.926 + 0.105 × Top1.
5. $35 \leq \text{Age} < 38 \text{y}$: Fertility index (multiple pregnancy) = –2.185 + 0.030 × Top3.

Fig. 1. The score of the best blastocyst (Top1), and the rates of pregnancy, multiple pregnancy, and implantation for patients aged < 35 years receiving two, three, and four blastocysts (*$p = 0.038$ by Kruskal–Wallis test, **$p = 0.034$, and ***$p < 0.001$ by χ² test).

Fig. 2. Logistic regression model of pregnancy and multiple pregnancy for women aged < 35 years. Top1 and Top2 denote the score of the best blastocyst and the best two blastocysts, respectively.
blastoceysts and the first cycle, while DET should be reserved for other cycles. We suggest that only one blastocyst should be transferred into patients with good quality blastocysts (Top1 > 27) to decrease the rate of multiple conception for women aged <35 years.

The age of the patient, baseline serum FSH levels, the number of embryos transferred, and embryo morphology scores were included for logistic regression analysis in the present study. This analysis did reveal that the transfer of more than one good blastocyst in selected patients (those aged <35 years) increased the rate of multiple gestation. Furthermore, the transfer of more than two embryos did not further elevate the pregnancy or live birth rate after IVF treatment. That is to say, the benefits of transferring multiple blastocysts, in terms of pregnancy outcome in young IVF patients, peak at the number of two [8].

The relationship between age and natural fertility is neither linear nor exponential [31]. It has been suggested that there exists a biphasic association between patient age and IVF outcome [32]. According to the ASRM guidelines, patients are classified as the following ages: <35 years, 35–37 years, 38–40 years, and >40 years [30]. In the present study, we adopted 35 years and 38 years as the cut-off points and found that the impact of female age was not significant for women aged <38 years. Only embryo morphology was closely associated with the rates of pregnancy and multiple gestation for patients aged <38 years. Female age was a more important determinant of pregnancy than embryo morphology for those aged >38 years. The results of the current study were similar to our previous report about a Day 3 ET program [15].

There were no differences among age group in Top1, Top2, or Top3 blastocyst scores. The results are concordant with the observation in cleavage embryos that embryo morphology of the top quality does not deteriorate with increased maternal age [17]. Although extended culture to the blastocyst stage is associated with a low rate of aneuploidy for women with advanced maternal age [19,20], we still revealed the lack of correlation between blastocyst morphology and pregnancy outcome in IVF/ICSI cycles for women aged >38 years. This indicates that normal morphology of blastocysts does not guarantee the status of the chromosome and the subsequent outcome of IVF cycles for advanced age women.

During the years prior to menopause, oocytes in human ovaries undergo an accelerated rate of loss until the store of oocytes is nearly depleted. It has been reported that this accelerated loss is initiated when the total number of oocytes reaches ~25,000, a number or threshold reached in normal women at age 37–38 years [33,34]. If elective SET instead of DET was performed for women aged 35–37 years, they might lose a chance of achieving a second conception. For women in this age group, 1 year of delay for pregnancy results in an accelerated decreased chance of conception [35]. The necessity to split twin pregnancies into singleton pregnancies by means of elective SET for such women has been questioned [35]. Therefore, the risk of twin pregnancy and the benefit of DET should be carefully considered for women aged 35–37 years. The logistic regression model developed in this study could provide a counseling tool to determine the number of transferred embryos.

Rather than embryo morphology, other parameters that better predict the implantation potential for older patients should be identified to decrease the number of embryos transferred. Preimplantation genetic screening (PGS) may be one of the choices [36]. However, PGS is a laborious and expensive procedure. The cost-effectiveness of PGS for advanced age women has to be determined by further investigation. Such parameters are not easily available, therefore, the ASRM guidelines stratifying women by age is a good reference for clinical practice. Our results further provide an individualized algorithm to determine the number of transferred blastocysts in our center, and it can be safely said that the results are in accordance with the ASRM guidelines.

In conclusion, patient age and blastocyst morphology score are important factors that predict pregnancy and multiple pregnancy in a BT program. The blastocyst score system can be relied upon to decrease the number of embryos transferred in younger women undergoing IVF. Other parameters need to be researched to determine the implantation potential to lower the number of transferred embryos in older women. Prior to the identification of these parameters, we suggest developing an effective strategy for BT programs making the best use of women’s age and blastocyst morphology to decrease the rate of multiple pregnancy, and meanwhile maintain a satisfactory rate of pregnancy.

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