Morphologically poor blastocysts could affect the implantation rate of a morphologically good blastocyst during a double-blastocyst transfer for patients who have experienced repeated implantation failures

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Abstract

Purpose: Increasing the number of transferred blastocysts sometimes is selected for patients with repeated implantation failure (RIF). To confirm this strategy, the pregnancy rates (PRs) were compared among the groups who had transferred either a single morphologically good blastocyst (MGB) group, double blastocysts with both a MGB and a morphologically poor blastocyst (MGB + MPB group), or a double-BT with 2 MGBs (two-MGB group).

Methods: This study was performed between April, 2009 and September, 2014, including 634 cycles for 354 patients with RIF. All the patients received cryopreserved blastocysts in either hormone replacement or natural ovulatory cycles. The included MGBs were at more than the Gardner grade 3BB stage. The PR and implantation rates (IRs) among the three groups were statistically evaluated by the chi-square test. Statistical significance was set at \( P < .01 \).

Results: Although the PRs were similar in these three groups, the IR in the MGB + MPB group was significantly lower than that of the MGB group. The rate in the two-MGB group also was significantly lower than that of the MGB group.

Conclusion: A double-BT with a MGB and a MPB does not increase the pregnancy rate, compared with a single-BT with a MGB among patients with RIF.

Keywords

double-blastocyst transfer, implantation rate, morphologically good blastocyst, morphologically poor blastocyst, repeated implantation failure

1 | INTRODUCTION

Implantation, which is a process whereby a blastocyst becomes implanted in the lining of the uterine endometrium, is a mysterious phenomenon and this mechanism still has been in the “black box.” Nevertheless, during implantation, the interaction between a blastocyst and the uterine endometrium occurs naturally. Recently, a study reported that the uterine endometrium might be able to distinguish good and bad signals from the transferred embryos or blastocyst, which was implanted or not. According to this report, a morphologically good blastocyst (MGB) can change the uterine endometrium to be an appropriate condition for implantation. This
phenomenon is satisfied with the fact that a MGB possesses a high implantation ability.

In the assisted reproductive technology field, clinicians sometimes struggle to deal with some patients who experience consecutive repeated in vitro fertilization (IVF) failures. It is now common knowledge that a good blastocyst possesses a high implantation ability; namely, the implantation ability is expected to increase when two blastocysts are transferred at once. Therefore, increasing the number of transferred embryos or blastocysts is sometimes selected as a strategy for patients with repeated implantation failure (RIF) because it seems logical that an increase in the number of transferred blastocysts will increase the pregnancy rate.2

A recent report indicated that poor-quality embryos might reduce the chance of the implantation of co-transferred embryos.3 It was concluded that poor-quality embryos might elicit a rejection response from the endometrium. The authors’ recent report also indicated that the implantation rate for a double-blastocyst transfer (BT) with morphologically good and poor blastocysts was significantly lower than that for a single good blastocyst and it was concluded that poor blastocysts interfere with the implantation of good blastocysts.4 Although the groups of patients in these reports varied between the first embryo transfer attempt and multiple implantation failures, it was clear that the double-BT might not be better than the single-BT. No evidence has shown that the double-BT contributes to an improvement in the pregnancy rate among patients with RIF.

In order to establish whether the double-BT truly contributes to an improvement in the pregnancy rate among patients with RIF, their pregnancy rates were compared between a single good blastocyst transfer and a double-BT with either a good and poor blastocyst or two good blastocysts.

2 | MATERIALS AND METHODS

2.1 | Patients

From March, 2009 to April, 2014, a total of 634 cycles for 354 patients who had failed more than twice with a single-BT at Yanaihara Women’s Clinic was recruited for the present study. Informed consent from all the participants was acquired and the Institutional Review Board of the Yanaihara Women’s Clinic approved this study. All the patients received either a vitrified-warmed single- or a double-BT. All the patients received either a vitrified-warmed single- or a double-BT with either a good and poor blastocyst or two good blastocysts.

2.2 | Oocyte stimulation protocol

The ovarian stimulation protocol for assisted reproductive technology (ART) treatment was selected as either a mild stimulation protocol using gonadotropin-stimulating hormone (GnRH) antagonist or a GnRH-agonist short protocol.4 The combination with clomiphene citrate (CC) and gonadotropins was used as a mild stimulation protocol. Briefly, between days 3 and 7 of the menstrual cycle, 100 mg per day of CC (Clomid®; Shionogi, Osaka, Japan) were administrated, with 150 IU of human menopausal gonadotropin (HMG) (HMG-Fuji®; Fuji-Pharma, Tokyo, Japan; or HMG Ferring; Phering Pharma, Tokyo, Japan) on days 3, 5, and 7 of the menstrual cycle. When the follicles developed to ≥14 mm in diameter, 0.25 mg of GnRH-antagonist (Cetrotide®; Merk-Serono, Tokyo, Japan) was started and continued to the day of a maturation trigger. When the follicles developed to >17 mm in diameter, 10 000 IU of human choric gonadotropin (hCG) (Gonatropin; Mochida, Tokyo, Japan) or 600 μg of nasal spray of buserelin acetate (Buserequr®; Fuji-Pharma) was administered. Then, 35 hours later, an oocyte pick-up (OPU) was done. The HMG (150 IU/d) could have been added because of the inadequate growth of the follicle, which was assessed by vaginal ultrasound.

In the GnRH-agonist short protocol, 450 μg/d of buserelin acetate was started nasally from the second day of the menstrual cycle and it continued until the day of the maturation trigger using hCG. On the third day of the menstrual cycle, 225 IU of recombinant follicle-stimulating hormone (Gonal-F®; Merk Serono) was started and continued daily until a dominant follicle developed that was >17 mm in diameter. When the follicles developed >17 mm in diameter, 10 000 IU of hCG was injected as a maturation trigger and an OPU was planned 35 hours after the hCG injection.

2.3 | In vitro fertilization/intracytoplasmic sperm injection procedure

The procedures for insemination and oocyte and embryo culture for this study were reported previously.5 The oocytes were picked up via a needle that was able to be done by ultrasonography. It was attempted to retrieve follicles that were >15 mm in diameter one-by-one with a 20-gauge needle that was connected with a tube for aspiration. The semen was obtained by masturbation and washed by a culture medium to remove the seminal fluid and the swim-up procedure was performed for 30-60 minutes in order to obtain motile sperm. The conventional insemination method was used for one- to-four oocytes with 200 000-300 000 motile sperm within four hours after OPU and a commercial medium (Universal IVF Medium®; Origio, Måløv, Denmark) was used. These oocytes and sperm were incubated under a gas mixture of 6% CO2, 5% O2, and 89% N2.

All the inseminated oocytes were denuded and confirmed for an extrusion of a second polar body for 6 hours after conventional insemination, with an inverted Hoffman differential interference phase-contrast microscope (Leica M165-C; Leica, Wetzlar, Germany). The oocytes with a second polar body extraction were thought to be fertilized and incubation was continued. For male factor infertility, intracytoplasmic sperm injection (ICSI) was selected and the procedure has been described previously.5

2.4 | Blastocyst culture, grading, and cryopreservation and warming protocol

The fertilized oocytes were cultured in the same medium and conditions, as described above, for ≤72 hours after either the ICSI or conventional insemination. All the cleaved embryos were checked
TABLE 1 Background of the morphologically good blastocyst (MGB), morphologically good blastocyst + morphologically good blastocyst (two-MGBs), and morphologically good blastocyst + morphologically poor blastocyst (MGB + MPB) groups

| Variable                        | MGB        | MGB + MPB   | Two-MGBs   |
|---------------------------------|------------|-------------|------------|
| Age (years) [mean ± SD]         | 37.4 ± 3.9 | 37.5 ± 4.3  | 36.7 ± 4.4 |
| Previous BT attempts [mean ± SD]| 3.0 ± 1.3  | 2.9 ± 1.2   | 3.4 ± 1.6  |
| BT cycle (N)                    | 468        | 76          | 90         |
| Transferred blastocysts per cycle (N) | 1       | 2           | 2          |
| Total no. of transferred MGBs (N) | 468      | 76          | 180        |
| Total no. of transferred MPBs (N) | 0        | 76          | 0          |
| Clinical pregnancy (N)          | 177        | 30          | 41         |
| Gestational sac (N)             | 177        | 34          | 47         |
| Twin pregnancy (N)              | 0          | 4           | 6          |

BT, blastocyst transfer; SD, standard deviation.

and moved to a medium for blastocyst culture (global or total; Life Grobal, Guilford, CT, USA) and were cultured for more than 2 days. All the blastocysts could be cryopreserved by using the vitrification method.

A vitrification protocol was selected for blastocysts with cryotop (Kitazato Company, Shizuoka, Japan), according to a report by Kuwayama et al., with slight modifications. Vitrification was used as a commercially available kit (Vitrification kit; Kitazato Company). Concisely, the blastocysts were vitrified via a two-step process with a cryoprotectant. Initially, the blastocysts were set in a solution-equilibration solution, which was the basic medium (TCM199 medium) with 7.5% (v/v) ethylene glycol (EG), 7.5% dimethylsulfoxide (DMSO), and 20% synthetic serum substitute (SSS) for between 5 and 15 minutes at room temperature. After that, the blastocysts were moved to the solution-vitrification solution (VS) that consisted of TCM 199 medium with 15% EG, 15% DMSO, and 0.5 mol sucrose. The period of the placement for the blastocysts depended on the conditions of dehydration. After one minute submerged in a solution-V5, the blastocysts were set on a cryotop® and were submerged into filtered liquid nitrogen as soon as possible.

The warming process of vitrified blastocysts used a two-step solution of different sucrose concentrations. A tube that contained a plastic blade was submerged under liquid nitrogen and the plastic blade with vitrified blastocysts was taken from the liquid nitrogen and submerged into a dish containing the basal medium with both 1.0 M sucrose and 20% SSS at 37°C. The blastocysts were moved from the cryotop® to the solution as soon as possible. After one minute, the blastocysts were moved to the basal medium with both 0.5 M sucrose and 20% SSS at room temperature for three minutes. As the last step, the warmed blastocysts were submerged into a basal medium containing 20% SSS twice for five minutes at room temperature and then they were transferred to a medium for blastocyst culture until the transfer of the warmed blastocyst.

The warmed blastocysts were evaluated by using a grading score that had been proposed by Gardner and Schoolcraft. The trophoderm (TE) was classified into one of the following grades: A, many cells organized in the epithelium; B, several cells organized in a loose epithelium; C, a few large cells. The inner cell mass (ICM) was classified into one of the following grades: A, numerous tightly packed cells; B, several loosely packed cells; C, very few cells. Blastocyst expansion was assigned one of the following descriptors: "early," the blastocele was less than half of the blastocyst (Gardner grade 2); "expanded," the blastocele filled the blastocyst with a thin zona pel lucida (Gardner grade 3-4); "hatched," the blastocyst had hatched or was hatching out of the zona pellucida (Gardner grade 5-6). A MGB showed an ICM and TE of grade A or B with a Gardner grade of between 3 and 6, but the early blastocysts (Gardner grade 2) were the exception. The remainder was considered to be morphologically poor blastocysts (MPBs). This evaluation was performed both before freezing and after thawing.

2.5 | Endometrial preparation and embryo transfer

Either the natural ovulatory cycle or the hormone replacement cycle (HRC) was used as the endometrial preparation for the warmed blastocyst transfer. In the natural ovulatory cycle, the warmed blastocyst transfer day could be scheduled on the fifth day after ovulation. Oral progesterone tablets were used as luteal support for 14 days. When using the HRC, the uterine endometrium was made for the blastocyst transfer mainly by using transdermal estradiol (Estrana TAPE 0.72 mg; Hisamitsu Pharmaceutical Company, Tokyo, Japan). The HRC was started from either the second or third day of the menstrual cycle or after withdrawal bleeding until the pregnancy test. The progesterone suppository (400 mg/d), which was provided courtesy of the International Institute of Medical Technology (Tochigi, Japan), was started after confirmation of an adequate level of endometrial thickness. On the fifth day after the start of the progesterone administration, the blastocysts were warmed and transferred. A progesterone suppository was used as the luteal support between the day of the blastocyst transfer and the pregnancy test.

The blastocysts were transferred into the patients’ uterus through the use of a soft catheter (embryo transfer catheter; Kitazato Company) under transabdominal ultrasound. For all the patients, either one or two blastocysts was transferred.

2.6 | Assessment and data analysis

A pregnancy was defined as the presence of a gestational sac that was confirmed via transvaginal ultrasound on the 21st day after the day of the blastocyst transfer. The pregnancy and implantation rates among the groups that received either a single-BT with a MGB (MGB group), a single-BT with a MPB (MPB group), and a double-BT with both a MGB and a MPB (MGB + MPB group) were compared. The rate of twin births also was compared among the three groups.

| Variable                        | MGB        | MGB + MPB   | Two-MGBs   |
|---------------------------------|------------|-------------|------------|
| Twin pregnancy (N)              | 0          | 4           | 6          |
The statistical analyses regarding the pregnancy, implantation, and twin rates were performed by using a chi-square test. Statistical significance was set at $P < .01$, which was adjusted by Bonferroni correction.

3 | RESULTS

The backgrounds of the patients are summarized in Table 1. The average ages (mean ± standard deviation [SD]) in the MGB, MGB + MPB, and two-MGB groups were 37.4 ± 3.9, 37.5 ± 4.3, and 36.7 ± 4.4 years, respectively, and there was no significant difference among these three groups. The previous embryo transfer attempts (mean ± SD) in the MGB, MGB + MPB, and two-MGB groups were 3.0 ± 1.3, 2.9 ± 1.2, and 3.4 ± 1.6, respectively, and there was no significant difference among these three groups. In the MGB group, 468 cycles of a single-BT were performed and a total of 468 MGBs were transferred. In the MGB + MPB and two-MGB groups, 76 and 90 cycles of a double-BT were performed, respectively, and the numbers of transferred BTs were 152 (76 of the MGBs and 76 of the MPBs) and 180, respectively (Table 1).

In the MGB + MPB group, 30 clinical pregnancies were achieved, for a clinical pregnancy rate of 39.5% (30/76), which was comparable to the MGB group (37.8%, 177 clinical pregnancies; Figure 1). In the two-MGB group, 41 clinical pregnancies were seen, for a clinical pregnancy rate of 45.6%, but this also was not significantly higher than those of either the MGB and MGB + MPB groups (Figure 1). No twin birth was seen in the MGB group, while four and six twin births were seen in the MGB + MPB and two-MGB groups, respectively. Therefore, the twin birth rates for the two-MGB and MGB + MPB groups were 13.3% and 14.6%, respectively. These results showed that there was no significant difference among these three groups in terms of pregnancy rates (Figure 2). The implantation rate in the MGB + MPB group was 22.4% (34/152) and this was significantly lower than that of the MGB group (37.8%, $P < .01$). Moreover, the implantation rate in the two-MGB group was 26.1% (47/180) and it also was significantly lower than that of the MGB group ($P < .01$; Figure 3).

4 | DISCUSSION

Based on the results of the present study, the clinical pregnancy rates for a double-BT with a MGB and a MPB or 2 MGBs were similar
to that of the single-BT with a MGB. According to these results, the double-BT with a MGB and a MPB or 2 MGBs did not improve the pregnancy rate among the patients with RIFs. Furthermore, the implantation rates in the MGB + MPB group were significantly lower than that of the MGB group. In the two-MGB group also was significantly lower than that of the MGB group.

A recent report indicated that the uterus possesses the ability to select either good- or poor-quality embryos.1 In that study, when the good-quality embryos were transferred, the uterine endometrium effectively accepted them and rejected the poor-quality ones. In the present study, although the clinical pregnancy rate for the double-BT with MGB and a MPB was similar to that of the single-BT, the implantation rate in the MGB + MPB group was significantly lower than that of the MGB group. In a similar study, a competent embryo enhanced the implantation chance of a co-transferred embryo and the data agreed with the theory that poor-quality embryos might reduce the implantation chance of co-transferred embryos.2 The poor-quality embryos might have elicited a rejection response from the endometrium. The authors suggested that such a mechanism might have allowed the endometrium to select embryos according to their quality. The decidual endometrial cells have a biosensor that could react with the embryo quality. In contrast, another study reported that human decidual endometrial stromal cells respond selectively to low-quality human embryos by inhibiting the secretion of key implantation factors, including interleukin-1 beta, heparin-binding epidermal growth factor-like growth factor, and leukemia inhibitory factor by using a co-culture system of decidual cells with or without a MGB or arrest embryo.9 Also, one study reported that human developmentally impaired embryos elicit an endoplasmic stress response in human decidual cells, leading to the suppression of the implantation response (found by using a DNA microarray of the co-culture of decidual cells with the condition media of a MGB that resulted in a live birth or developmentally impaired embryo, leading to not performing an embryo transfer.1) Therefore, the embryo selection process for transfer should allow for such an interaction and MPBs, but not MGBs, might impact on implantation adversely. The results of that report agreed with those found in the present study. The uterine embryo selection theory might be one of the reasons why the double-BT decreased the implantation rate, compared with a single-BT.

All MGBs have the potential to implant into the uterine endometrium and the potential of a MGB that is transferred as a single blastocyst was considered to be similar to that of the double-BT that included at least 1 MGB. Therefore, the transfer of a double blastocyst with 2 MGBs would result in at least an implantation rate that is at least equal to that of a single-BT. However, the implantation rate in the two-MGB group was significantly lower than that of the single-MGB group. The hypothesis as to why the two-MGB transfer showed a lower implantation rate was that the two blastocysts could not be implanted closely because of the embryo-spacing mechanism.

During the early pregnancy process, an embryo first attaches to the surface of the uterine endometrial epithelium; consequently, this endometrial epithelium begins to trigger apoptosis and decidualization of the endometrium occurs.10 In the uterus of a multibirth mammal, such as a pig or a mouse, the implanted embryos are located at a regular interval. In general, these multibirth embryos are located at a regular interval. In general, these multibirth mammals have an embryo-spacing mechanism.11 This mechanism maintains a specific interval between the implanted embryos in order to avoid interference with each other or an overlapping of placentas during the implantation period. Although this mechanism is not clearly understood, this mechanism is quite important in order to avoid all the implanted embryos also being lost from the other early pregnancy losses in these mammals. The double-BT in the present study indicated that two blastocysts were transferred simultaneously in the same place and that these two transferred blastocysts might be located closely to one another. There was no method to confirm the real location or distance of the two transferred blastocysts. At least one recent report has shown that ~80% of transferred embryos implanted in the areas to which they initially were transferred.12 Based on that report, it was easy to infer that when two blastocysts are transferred together, they might be located closely. The embryo-spacing mechanism might have prevented the two transferred blastocysts from being implanted nearby one another, and consequently, only one blastocyst could be implanted.

In the present study, the double-BTs that included at least 1 MGB did not contribute to an improved pregnancy rate among the patients with RIF. According to the embryo-spacing mechanism, a double-BT of two blastocysts might induce effects that are negative to their implantation. In further strategies to improve the implantation rate, either a single-BT should be performed or a single-BT should be performed twice during the same procedure. However, according to the uterine embryo selection theory, poor-quality embryos, or blastocysts, should never be selected for embryo transfer.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. Human Rights Statement and Informed Consent: This study was approved by the ethical committee of Yanaihara Women's clinic, Kamakura, Japan. Written informed consent was obtained from each participant couple. Animal studies: This article does not contain any study with animal participants that have been performed by any of the authors.

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How to cite this article: Ohgi S, Taga Y, Anakubo H, Kurata Y, Hatakeyama S, Yanaihara A. Morphologically poor blastocysts could affect the implantation rate of a morphologically good blastocyst during a double-blastocyst transfer for patients who have experienced repeated implantation failures. *Reprod Med Biol*. 2018;17:249–254. 
[https://doi.org/10.1002/rmb2.12097](https://doi.org/10.1002/rmb2.12097)