

Single Blastocyst Transfer: Contemporary Experience

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Abstract: Recent studies demonstrated an overwhelming success in single blastocyst transfer (SBT): implantation rates (IR) were 60.9%-70.5% and pregnancy rates (PR) were 60.9%-76% while the multiple pregnancy rates (MPR) were 0%-3.2%. Most of these studies involved good prognosis patients not more than 37 years of age. The results indicated that SBT decreased the number of multiple pregnancies while maintaining desirable pregnancy outcomes. However, SBT and cryopreserved single blastocyst transfer (cSBT) in the field of *in vitro* fertilization (IVF) are still in their infancy. Guidelines for the number of blastocysts being transferred and the techniques have not yet been standardized. The method to estimate the most viable blastocyst has not yet been proposed. The success of SBT also was found to be highly associated with the technique and patients' and clinicians' perceptions toward it.

Keywords: Single blastocyst transfer (SBT), embryo transfer, blastocyst grading, cryopreservation, *in vitro* fertilization (IVF), assisted reproductive technology (ART).

DEVELOPMENT OF EMBRYO TRANSFER

Since the first success of human birth after the implantation of human embryo in 1978 [1], IVF has become the most effective treatment for infertile patients regardless of the cause of infertility [2]. To achieve higher IR, the practice of transferring multiple embryos per transfer cycle has been widely employed [3]. The results of multiple embryo transfer have been shown to have an IR from 3% to 69% and a PR from 24% to 66% [4-10]. However, with the relatively satisfying success of pregnancy outcomes, multiple gestations have become a serious complication resulting from the transfer of multiple embryos at a time [3].

The MPR revealed by many studies ranged from 17% to 75% [6-10]. With this high MPR, studies have been advocating reducing the number of transferred embryos and defining the success of embryo transfer as the single live birth rate. Luke *et al.* analyzed 69,028 data entries regarding embryo transfer during 2004-2006 in the Society for Assisted Reproductive Technology (SART) Clinic Outcomes Reporting System (CORS) database. The single live birth rate was found to be 42.9%, 32.5%, 26.5%, and 23.2% for transferring of one, two, three, and more than four embryos at a time, respectively [11]. The results showed that the single live birth rate after single embryo transfer is significantly higher than those of multiple embryo transfer ($p < 0.0001$). However, despite the support and advocating efforts in the literature, single embryo transfer still represented a minority of practice in the United States—12% overall in 2001 [12], and 4.4% during 2004-2006 [11].

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DEVELOPMENT OF BLASTOCYST TRANSFER

Blastocyst Culture: Higher Performance as the Culture Technique Improves

Recent improvements in culture technology have enabled more robust embryo development to the blastocyst stage. Furthermore, a better understanding of the dynamic uterine and endometrial environment as the embryo develops from a zygote to blastocyst has aided specifically in improvement of the required nutritive contents in culture systems to more closely mimic the reproductive tract. The addition of amino acids, sugars, and other compounds permits early embryos to develop into blastocysts [13], the embryonic stage known to be associated with a higher IR, without compromising PR [14]. With regard to nutrition requirements, the preimplantation period should be considered in two phases for correct medium formulation, pre and post-compaction [15]. Prior to Day 3 of embryo development the first couple of cell cycles utilize reserves containing maternal transcripts and stored mRNA; in other words, its metabolism is regulated at the post-transcriptional level [16]. During post-compaction, the embryonic genome has taken over and the embryo becomes more susceptible to transcriptional inhibition from nutrient deficiencies [13].

Two types of culture systems are involved in blastocyst culture, monoculture media and sequential media. In a monoculture system, a single medium is supplemented with all the required components to sustain the embryo for growth to the blastocyst stage. A sequential system requires growing the early embryo until Day 3 in culture with amino acids and low levels of glucose and phosphate to allow the embryo to use its reserve (maternal transcripts and stored mRNA) and then transfer the embryo to a more complex medium with numerous metabolites (citrate, malate, various lipids, etc.) to support higher demands and avoid metabolic blocks

[17]. As noted before, the embryo depends on its reserves prior to compaction, and so it is reasonable for embryos to grow in a medium with lower glucose levels during this period.

Sequential media systems were introduced in the late 1990s and became a controversial issue. How can a simple change in culture media have such a significant improvement in embryo development to ensure a quality embryo is cultured to the blastocyst stage? Opponents of blastocyst culture and transfer contend that the self-selection criteria, the main underpinning benefit for use of sequential media for blastocyst culture, is thwarted by the preimplantation embryo's high incidence of chromosomal abnormality, an increased risk for monozygotic twinning, and an increased risk for epigenetic mutation. A prospectively randomized control study by Staessen *et al.* evaluated the incidence of chromosomal abnormality of cleavage-stage versus blastocyst-stage embryos in women of advanced maternal age and noted a difference: a reduction of aneuploidy incidence from 59% on Day 3 to 35% on Day 5 [18]. Proponents argue that considerable evidence suggests a significant improvement in IR without compromising PR in blastocyst transfer as compared with Day 2 or 3 transfer [19]. Nonetheless, sequential media is widely accepted today for blastocyst culture and transfer.

Early studies evaluating sequential media culture systems show evidence of improved PR and significant IR (32.3%) following blastocyst transfer in a heterogeneous group of infertile patients [20]. However, both Sepulveda *et al.* and Reed *et al.* found it was unnecessary to transfer the embryo to a subsequent media after Day 3 [21, 22]. Reed *et al.* demonstrated a significantly higher mean number of optimal blastocysts on day five with the single medium compared to the sequential medium ($P < 0.05$) [21]. A total of 893 embryos from patients and donors were divided into three groups: (i) 232 patient embryos cultured for Day 3 transfer, (ii) 480 patient embryos for Day 5 transfer, and (iii) 181 donor embryos for Day 5 transfer. Each group of 232, 480, and 181 oocytes was split in half to be cultured in sequential and continuous media. Embryos in the sequential medium group were moved to the second sequential medium, while embryos in the single medium treatment remained in the original dish, without renewal of the microdrops. The mean number of embryos transferred for embryos cultured in sequential (1.3 embryos) versus continuous (1.6 embryos) for Day 3 transfer did not differ significantly ($P > 0.05$). Also, the mean quality score of Day 3 transfer was 2.5 and 2.4 ($P > 0.05$) for sequential and continuous media, respectively; and did not show a significant difference. On the other hand, the mean number of Day 5 blastocysts was higher in the continuous media than in the sequential media. The same results were found for the mean number of Day 5 blastocysts transferred. Surprisingly, the mean number of Day 5 blastocysts to be transferred from continuous media was two times that of sequential media ($P < 0.01$). Also, mean embryo quality scores did not differ between the media for Day 5 blastocysts [21].

Further support was demonstrated in a prospective, randomized, controlled study using donor oocytes cultured in single medium versus sequential media in which no significant difference was found in number or quality of blastocysts

on Day 5 when comparing embryo culture in either system, single or sequential [22]. A total of 287 and 322 embryos were cultured in continuous and sequential media, respectively. By Day 5, 24.7% of the 287 embryos in the continuous medium and 13.7% of 322 embryos from the sequential medium ($P = 0.001$) were in full to hatching status; thus, a greater portion of embryos developed to Day 5 blastocysts in continuous medium than in sequential [22]. In this study, embryos in both groups of single and sequential medium were moved to fresh droplets of single medium and the second sequential medium, respectively on Day 3. It is important to note that the single medium used in this study contained all 20 amino acids as opposed to the sequential media with significantly fewer amino acids (alanyl-glutamine only) [22]. To put it briefly, the single, continuous, uninterrupted culture system was just as good as or better than the sequential media system when it was properly formulated with all the required nutrients. If true, the monoculture system eliminates one step in handling the embryo - a more desirable option to reduce any unknown risks associated with moving the embryo to another medium. The extra step translates into more work, increased cost, and a theoretical chance for negative events to occur. A comparison of the efficacy of the variety of single and sequential medium for culture to the blastocyst stage also would be important.

Advance culture technology such as sequential media is not the only critical success factor for improving SBT and increasing IVF success. Prerequisites to successful blastocyst culture are dependent on (i) lab protocols, (ii) quality management system, and (iii) superior oocytes from a good ovarian response in good prognosis patients [13]. Other important features of the culture system also have an impact on IVF success per transfer cycle, including gas phase, embryo incubation volume and group size, and macromolecule supplementation [13]. The autocrine effect of volume and group size on embryo is only proven in mouse and remains controversial in humans. Quality control of procedures has long been declared as critical to protect embryos from potential toxins, especially during the move to sequential media [15]. For instance, lab conditions must be stable, and so close monitoring is required to protect against atmospheric fluctuations [21]. Unfortunately, comparisons of lab quality conditions and lab protocols among various IVF laboratories are not often studied and so only very limited reports are available; no standard laboratory requirements for performing single blastocyst culture and transfer are documented.

In conclusion, we noted that sequential media has had a positive effect on the outcome of single blastocyst culture and transfer for a select patient population. Yet, the majority of the studies regarding blastocyst culture and transfer have eliminated patients with advanced maternal age. This suggests that extending studies to determine whether SBT would be just as beneficial for older patients with good prognosis, good response to ovarian stimulation, an optimal number (10 or more) of high quality embryos, and no other known indications would be worthwhile. Comparing the efficacy of the variety of single and sequential medium for culture to the blastocyst stage in the future may also be beneficial, especially in media with undefined contents and/or concentrations to standardize the components in complex media.

Multiple Blastocyst Transfer: Widely Employed to Yield High IR

In the not too distant past, an IR above 10% was an acceptable milestone for an IVF clinic. Given this low IR, transferring two or more embryos was considered necessary to achieve acceptable IR or PR. Milki *et al.* found that the IR was 47% and the PR was 58% with 24 cycles of three-blastocyst transfer [23]. This result demonstrated the success of multiple blastocyst transfer. Furthermore, Jain *et al.* reported the results of 75 cycles of blastocyst transfer with a mean number of 2.0 ± 2 blastocysts being transferred per cycle. The IR was found to be 45.3%, and the clinical PR was found to be 50.7% [24]. In addition, Yamamoto *et al.* reported the results of 290 cycles of multiple blastocyst transfer with a mean number of 1.44 ± 0.50 blastocysts being transferred per cycle. The IR was found to be 35.2%, and the clinical PR 42.4% [25]. These studies all indicated the success of utilizing multiple blastocysts in blastocyst transfer.

SBT: a Way to Curb High MPR in ART

Although great success was achieved with multiple blastocyst transfer, a high MPR also occurred. The high MPR has been a problem for the Assisted Reproductive Technology (ART) industry over the past decade. Ryan *et al.* reported that the MPR approached 40% after double blastocyst transfers (DBT) in their center [26]. Recently, IR above 50% for transfers of top-quality cleavage-stage embryos have been reported, while IR above 60% for transfer of selected blastocysts have been reported. Stillman *et al.* demonstrated that IR for double blastocyst transfers were almost 50% for all patients regardless of age or blastocyst quality [27]. IRs as high as these put patients at risk of multiple pregnancies if more than one embryo or blastocyst is transferred. Stillman *et al.* demonstrated that MPR were 44% among 4083 cycles of fresh double blastocyst transfers [27].

Multiple pregnancy is associated with well-documented increases in maternal morbidity and mortality from gestational diabetes, hypertension, cesarean delivery, pulmonary emboli, and postpartum hemorrhage. It also increases the risk of premature birth, resulting in fetal, neonatal, and childhood complications from neurologic insults, ocular and pulmonary damage, learning disabilities and retardation, and congenital malformations [28, 29].

Reducing the MPR has become the crucial public health requirement for the further development of effective IVF practices. The only truly effective means by which to avoid multiple pregnancies (aside from monozygotic twinning) is to transfer a single embryo [27]. Recent studies demonstrated an overwhelming success in SBT: IRs were 60.9%-70.5%, and PRs were 60.9%-76%, while the MPRs were 0%-3.2% [27, 30-35]. Most of these studies required good prognosis patients not more than 37 years of age. While other studies did not specify the diagnosis of the patients, Styer *et al.* indicated that patients with infertility diagnoses of idiopathic, male factor, and other female factors are all included in their study. The IR was 70.5% with an MPR of 3.1%. The results suggested that SBT has the promising potential to significantly reduce the risk of multiple births while maintaining the success of pregnancy outcomes.

Monozygotic Twinning Caused by SBT

The only possible mechanism for SBT to result in twins is through monozygotic twinning (MZT). MZT is a rare phenomenon in humans, occurring in 0.42% of spontaneous pregnancies. Guerif *et al.* demonstrated that MZT in 218 cycles of SBT is higher, although not statistically significant, compared with 243 cycles of single cleavage-stage embryo transfer (3.8% vs. 1.6%) [36]. da Costa *et al.* suggested that one probable reason for the higher incidence of MZT in blastocyst transfer could be the hardening of the zona pellucida resulting from prolonged exposure to embryo culture [37]. As the blastocyst herniates through the abnormally hardened zona pellucida, it might facilitate the blastocyst's division.

Moreover, Papanikolaou *et al.* demonstrated that MZT is not increased after SBT ($n=271$) compared with single cleavage-stage embryo transfer ($n=308$) (1.8% vs. 2.6%, $p>0.50$) [38]. It is important to note that assisted hatching and blastocyst coculture have not been performed in this study. Several studies reported monozygotic twins and triplets in association with SBT [39, 40]. Lee *et al.* reported three healthy boys each weighing 1.78 kg were born to a 28-year-old woman after a SBT [40].

To summarize, although current studies revealed that the incidence of MZT is increased following IVF compared with spontaneous conception, evidence is insufficient to support this observation. Further studies are needed to clarify the association between extended culture to the blastocyst stage and MZT.

CRYOPRESERVED SINGLE BLASTOCYST TRANSFER (cSBT)

With recent improvements in culture media and cryopreservation techniques, frozen embryo transfer has evolved. Blastocyst cryopreservation can be accomplished by slow-freezing or vitrification. Slow-freezing utilizes cryoprotectants to minimize intercellular ice crystal formation. Vitrification was first reported in 1985 utilizing high concentrations of cryoprotectants to achieve an ultra-rapid freeze to eliminate ice crystal formation [41, 42]. These two methods have been readily employed at the blastocyst stage [43, 44].

Although the results of transfer for more than one cryopreserved blastocysts are well-documented [25, 32, 36, 45], few studies have been published regarding the pregnancy outcomes of cryopreserved SBT. Desai *et al.* examined 56 slow-freezing cycles of cSBT [46]. A two-step glycerol freeze protocol was used in this study. The result demonstrated a 27% clinical PR and an 18% live birth rate.

Mukaida *et al.* showed that PR increased as the number of transferred blastocysts increased [47] when a Cryoloop technique was used to vitrify the blastocysts produced from 223 cycles. PR were 26% in cryopreserved single blastocyst transfer, 34% in cryopreserved double blastocyst transfer, and approaching 60% when four to five cryopreserved blastocysts were transferred. This study included blastocysts that were either re-expanded or not re-expanded before embryo transfer. In addition, Yanaiharu *et al.* reported the pregnancy outcomes of 412 cycles of vitrified single blastocyst transfer

in their center [48]. The PR and MPR were 40.7% and 2.3%, respectively. This study demonstrated that vitrification is a practical method for cSBT.

CRITERIA FOR SBT

Current Guidelines

Together, the American Society of Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) have published several versions of guidelines on the number of embryos to be transferred to maintain pace with changing technology. In the most current version, the number of blastocysts to transfer for ages <35, 35-37, 38-40, and 41-42 were 1, 2, 2, and 3 blastocysts, respectively, for patients with a favorable prognosis [49]. These include patients in their first cycle of IVF and/or those with high quality embryos, high number of embryos available, or have had previous successful IVF cycles. Alternatively, all other patients within the same age ranges should transfer the following (number of blastocysts): ages <35 (2), 35-37 (2), 38-40 (3), and 41-42 (3) [49].

A second set of guidelines published jointly by the Society of Obstetricians and Gynecologists of Canada and the Board of the Canadian Fertility and Andrology Society also includes recommendations for the number of blastocysts for transfer, but it is important to note that their guidelines are based mainly on studies of cleavage-stage embryos and not of blastocyst-stage embryos [50]. In accordance with these guidelines, good prognosis patients should transfer the following (number of blastocysts) or less: ages <35 (1), 35-37 (2), 38-39 (2), 39+ (3). In all other cases, patients with a less than desirable prognosis should transfer the following (number of blastocysts) or less: ages <35 (2), 35-37 (3), 38-39 (3), 39+ (4) [50].

A recent study was done to evaluate the prevalence of U.S. IVF clinics willing to act in accordance with the guidelines and led to additional interesting findings [51]. First, a majority of U.S. IVF clinics (94% of those responding to the survey) would follow the guidelines. Second, certain situations were likely to result in deviation from the guidelines; those identified from the study include patient request, IVF procedures involving the transfer of frozen embryos, and IVF cycles preceded by a failed previous IVF attempt. Third, a higher than expected number of patients (who had optimal conditions for single embryo transfer) with insurance coverage had deviated from the guidelines and had a double embryo transfer. Fourth, clinicians are not likely to communicate the single embryo transfer policy to their patients, only the risks involved with multiple pregnancies. These points indicate a potential for improvement of the guidelines to take patients' and clinicians' concerns into consideration in response to social, economic, and financial pressures. After the publication of the ASRM-SART guidelines, the number of higher order multiple deliveries in the United States declined during subsequent years indicating a voluntary adoption of single (or double) blastocyst transfers by IVF clinicians, embryologists, and patients [52]. The data reveals a decrease in the mean number of embryos transferred in women <35 years of age decreased from 3.12 in 1996 to 2.18 in 2003. During the same period, IR and delivery rate increased from

20.7% to 34.1 % and from 30% to 40%, respectively [52]. This is good news for the future of IVF children who have no voice to deny a future of complications and the movement toward a single, healthy live birth for all patients.

Embryo Grading and Blastocyst Grading

IVF technique has long included embryo grading to aid in selection of the most excellent quality embryos for implantation for the best results. Good embryo quality is associated with a high IVF success rate [53]. Embryos can be graded at various stages (e.g. pronuclear, zygote, cleavage, and blastocyst) by taking into account various markers, including blastomere morphology, developmental rate, fragmentation, metabolic markers, genetic markers, and epigenetic markers [54]. Here we will focus on blastocyst grading.

The two most popular blastocyst embryo grading systems are the Dokras and Gardner grading system, both based on morphology [14, 55-57]. Dokras grading is based on the blastocoele's rate of development and characteristics of the blastocoele cavity; whereas Gardner's system focuses on blastocoele size and developmental characteristics of the inner cell mass and trophectoderm. In the Dokras system, blastocysts are graded as BG1, BG2, or BG3. BG1 and BG2 have been shown to have higher IRs and have been recommended for SBT [53, 58].

According to the Gardner grading system, the blastocoele size, degree of expansion, and hatching status is initially examined and graded from 1-6. Next, the blastocysts graded 3 through 6 are identified and their inner cell mass and trophectoderm are further graded.

Studies have shown that transfer one blastocyst with grades $\geq 3AA$ using the Gardner system has had a significantly higher IR (54.3%) and higher clinical PR (69.6%) than blastocysts with $\leq 3AA$ [56]. A more recent study by Urman *et al.* had similar results when two blastocysts graded as 3AA were transferred [54]. Finally, a randomized study comparing the two grading systems illustrates a superior Gardner system for predicting blastocysts with a higher chance of implantation (37.6% vs. 25%, $P=0.01$) and clinical pregnancy (66.7 % vs. 53.0%, $P=0.11$) than the Dokras system. According to this study, both are valuable predictors for selecting blastocysts with high implantation potential and to implement elective SBT strategy to avoid multiple pregnancies [59].

In some countries where blastocyst selection is illegal (Germany), scoring is most effective when completed as close to latest stage permitted for selection. Zygote scoring for obtaining good quality blastocysts has not been promising [60-64]. The scoring system by Zollner *et al.* included an examination of the pronuclear zygote for the following: (i) number and size of pronuclei, (ii) juxtaposition of pronuclei, (iii) halo effect, (iv) alignment and number of pronuclei (evaluated separately) and nucleoli, (v) appearance of vacuoles, (vi) and appearance of ooplasm. Each criteria was evaluated and assigned a score with 10 being the best and 31 the worst. A pronuclear score of >15 correlated with poor blastocyst development and a decrease in PR [62]. Even

though the study showed a correlation between pronuclear morphology and blastocyst development, it was not strong enough to identify the highest quality embryos with high IRs.

The pronuclear scoring system by Scott and Smith (1998) and Tesarik and Greco (1999) continue to be the most widely used methods. Scott and Smith's is based on the combination of two scores, one for position of the pronuclei and the other score for cytoplasmic appearance when two pronuclei and two polar bodies are present [61]. Tesarik and Greco's is based on the appearance and distribution of the nucleolar precursor bodies within the pronuclei and scored between 0 and 5 [63]. A score of 0 is associated with normal development, and all other patterns are considered irregular. The use of scoring systems with blastocyst culture and transfer is a valuable tool for the selection of an embryo with a higher probability to be normal and result in a live birth, but is not always reliable and should be pursued with caution. Patients should be informed of the circumstances prior to their commitment.

Patients' Clinical Conditions

Most studies practiced SBT in patients not more than 37 years old [27, 30, 34]. However, a couple of studies practiced SBT in patients with ages up to 43 years [31, 35]. Kalu *et al.* compared PR after SBT in patients age 25-37 years and 38-43 years: 73.8% and 47.1%, respectively [31]. This study suggested that SBT could result in better pregnancy outcomes in young women. Furthermore, most studies are restricted to patients who had a good prognosis. In addition to young age, the good prognosis criteria often included no previous history of failed IVF cycles, no moderate or severe endometriosis, at least three (some studies at least five [34]), blastocysts available for transfer, and normal uterine cavity, etc [27, 30, 32]. However, Trout *et al.* also reported results in patients at risk of ovarian hyperstimulation syndrome (OHSS). Seven cases of SBT in women at risk of OHSS had an ongoing PR of 57% [65].

PATIENTS' PERCEPTIONS TOWARDS SBT

Patient Perceptions Differ from Clinicians

The patient's perception of blastocyst culture and transfer of one, two, or other multiples of embryos is important to consider. It aids the clinician's communication with couples and improves patient satisfaction. In one study, men and women were interviewed separately to remove influence from their partners, and each was asked about their decision-making process in deciding on single or double blastocyst transfer [66]. Several interesting points resulted from the study. First, patients who chose a double transfer perceived a higher chance of pregnancy. Second, patients who chose a single transfer say it was a much more difficult decision than deciding on a double transfer. Those that tended to select the single transfer had similar characteristics, including: (i) having experienced a prior childbirth, (ii) being young, (iii) having no previous failed IVF treatment, and (iv) having spare embryos to freeze. Thirdly, patients who were more likely to accept a double transfer and perceive it as more desirable were older, had no spare embryos to freeze, or perceived a single embryo transfer might lower chances of pregnancy per

transfer based on evidence either from a study or a previously failed IVF attempt [66].

In another study, de Lacey *et al.* identified three factors influencing the decision for a SBT, including repeated treatment, advanced age, and urgency to become pregnant [67]. This may be in relation to patients' perception that the risk of maternal and neonatal complications is low to moderate in association with single or double transfers, despite being fully informed of the risks [67]. Several studies also support findings that patients are willing to accept a double transfer along with the maternal and neonatal risks involved to have children [66-68]. Thus, awareness of complications is not a deterring factor in the decision for SBT, and so identifying the points that influence patient decisions is important. Perhaps patients are more willing to accept SBT if there are no financial pressures in case of a failed cycle of SBT [27]. For this reason, determining what factors will motivate patients to accept SBT over DBT in particular circumstances will be important.

Business interests do not always coincide with patients' best interests, and so clinicians may hesitate to decrease the number of blastocysts to transfer despite the risk for unhealthy babies being born [69]. However, a recent study by Stern *et al.* assessed the impact of embryo transfer guidelines designed to minimize multiple births by lowering the number of embryos transferred in IVF clinics. It reported a decrease in the number of embryo transfers and proved the effectiveness of voluntary reduction of blastocyst transfer [52]. Not surprisingly, this also has led to a decrease in the number of higher order multiple births.

Patient Education is Essential for SBT

Ryan *et al.* reported the results of an educational campaign with a two-fold process: First, a one-page description of the comparative risks of twins versus singletons to maternal, fetal, and neonatal health was given; second, this page was then discussed between the couples and physicians [26]. Pre- and post- educational campaign questionnaires (n=110) were collected and analyzed. Results showed that significantly more subjects ranked singletons as their most-desired treatment outcome post-educational campaign compared with pre-educational campaign (86% vs. 69%, $p<0.001$). This observation suggests that an improved understanding of twin risks appeared to affect patients' declared desire for numbers of embryos to transfer and for twin pregnancies.

Stillman *et al.* demonstrated the effect of a policy recommending SBT to patients identified as high risks for twins [27]. In this study, an educational program for physician, staff, and patient also was implemented to increase the program-wide proportion of SBT. This education program for patients constitutes laminated sheets for each physician office outlining the recommendation criteria, statistics, and risks regarding SBT and DBT. In addition to online education and in-person consulting with patients, the sheets also were hung in each physician office. The education program for physicians and staff took place periodically at physician and staff seminars. Results have shown that the program-wide number of embryos transferred was decreased significantly post-policy (n=2923) compared to pre-policy (n=1556) (2.16 ± 0.84 vs. 2.45 ± 0.84 , $p<0.003$). This observa-

tion suggested that patient education is an important factor that could pave the future for SBT among good-prognosis patients.

EXPERT COMMENTARY

The purpose of this article was to discuss various aspects regarding the current practice of single blastocyst transfer. Human blastocyst transfer has been evolving in the recent decade since the successful development of blastocyst culture and transfer techniques. Given the unwanted high multiple pregnancies resulting from multiple embryo transfer and multiple blastocyst transfer, single blastocyst transfer was proposed to be a prospective method to resolve this problem. Single blastocyst transfer was found to significantly decrease the number of multiple pregnancies while maintaining desirable pregnancy outcomes. A number of recent studies have demonstrated the potential role of single blastocyst transfer in young and good prognosis women.

Single blastocyst transfer and cryopreserved single blastocyst transfer in the IVF are still in their infancy. The guidelines for number of blastocysts being transferred and the techniques have not yet been standardized, and a method to estimate the most viable blastocyst has not yet been proposed. The success of single blastocyst transfer is highly dependent on the technique and patients' and clinicians' perceptions toward it. Further research is required to provide more data on single blastocyst transfer in different patient groups to standardize the practice based on different patient variables.

FIVE-YEAR VIEW

The practice of single embryo transfer is less common in the United States than in Europe. One reason for this has been the concern that transferring two or more embryos would lead to higher birth rates [12]. However, with more robust studies proving the effectiveness and the advantage of single blastocyst transfer [3], the unresolved problem of the optimal number of embryos to transfer is about to be debunked. Although the success of single blastocyst transfer has been overwhelming in controlled studies, it will require more mature blastocyst culture, grading, and cryopreservation techniques before gaining wide clinical acceptance. Clinics need to assess their own available techniques, laboratory conditions, and patients' willingness to participate in single blastocyst transfer.

Traditionally, the success of blastocyst transfer was determined by comparing implantation rates and pregnancy rates. However, there has been a shift in focus to a single live birth rate as the variable to be compared. In addition to patient characteristics, including age, BMI, and clinical conditions that need to be reported, the single live birth rate also should be discussed in future studies to aid comparisons across studies. Given more and more studies discussing the effects of single blastocyst transfer in a variety of patient populations, generating a standardized guideline for practitioners may be possible in the near future. It also is hoped that many proposed blastocyst assessment methods can be improved to more accurately predict the most viable blastocyst to transfer.

KEY ISSUES

- Multiple embryo transfer was successful in terms of pregnancy rates; however, it resulted in unwanted high multiple pregnancy rates.
- Increased number of studies revealed that single embryo transfer would be beneficial for young patients with good prognosis.
- Two types of culture systems have been developed and are widely available for culturing blastocysts: single media and sequential media. The single culture system was just as good as or better than the sequential media system when it is properly formulated with all the required nutrients.
- Multiple blastocyst transfer was able to generate satisfying implantation rates and pregnancy rates; however, it resulted in the same problem encountered in single embryo transfer—high multiple pregnancy rates.
- The only truly effective means by which to avoid multiple pregnancies is believed to be transfer of a single embryo or blastocyst.
- Frozen single blastocyst transfer has been developed as a practical method to optimize pregnancy outcomes following the possible failed initial transfer or to be employed for patients with inappropriate initial transfer conditions.
- An improvement of current guidelines for blastocyst transfer is possible when taking patients' and clinicians' concerns into consideration in response to social, economic, and financial pressures.
- Determining for certain which blastocyst will implant is not possible; however, many grading methods, including morphology assessment could provide possible information on the blastocyst' potential to implant.
- The patient's perception of blastocyst culture and transfer of one, two, or other multiples of blastocysts is important to consider. Physicians should communicate with patients to educate them on the pros and cons of various blastocyst transfer choices.

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