Supernumerary Blastocyst Cryopreservation: A key Prognostic Indicator for Patients Opting for an Elective Single Blastocyst Transfer (eSBT)

Christine Mullin · Alan S. Berkeley · Jamie A. Grifo

Received: 15 July 2011 / Accepted: 25 April 2012 / Published online: 18 May 2012
© Springer Science+Business Media, LLC 2012

Abstract

Objective To determine if patients, less than 40 years of age with or without day 5 cryopreservation (d5 cryo), compromise their pregnancy rate (PR) by choosing an eSBT.

Design Retrospective analysis

Setting University IVF center

Patients 2,203 non-donor fresh IVF cycles in women <40 years of age from January 2004 to January 2010.

Interventions None

Main outcome measure(s) Eggs retrieved, Embryos cryopreserved, Implantation Rates, Clinical Pregnancy Rates, Live Birth Rates, Spontaneous Abortion Rates

Results Pregnancy outcomes in women <40 years with or without d5 cryo were compared according to whether patients underwent an eSBT versus a 2BT in non-donor fresh IVF cycles. Overall, eSBT was associated with elimination of twinning while maintaining a high clinical pregnancy rate in both groups with d5 cryo (75 % eSBT versus 72 % 2BT) and groups without d5 cryo (48 % eSBT versus 56 % 2BT).

Conclusions In this study, patients <40 years of age have eliminated twinning by electively choosing to transfer a single blastocyst without compromising their PR if embryos are available for d5 cryo, and suffer only a non-statistically significant drop in their PR if there are no embryos available

for d5 cryo in exchange for the benefit of eliminating the obstetrical risk of twinning.

Keywords Single blastocyst transfer · Blastocyst · Cryopreservation

Introduction

It has been more than 32 years since medical advancements by Steptoe and Edwards led to the birth of the first child through IVF. Today, ~3 % of babies born in developed countries are conceived as a result of ART [1]. Initially, it was not only commonplace, but acceptable to transfer multiple embryos to compensate for low IR. This practice led to a rise in multiples, which are associated with increased risks for adverse pregnancy outcomes [2–4]. Accordingly, multiple births have become the most common adverse outcome in ART challenging those who practice to focus on their reduction. Substantial advances in stimulation protocols and culture techniques, have led to higher IR and PR [5, 6], thus affording a more tangible opportunity for a live birth. Hence, the goals of ART have evolved from maximizing PR with the transfer of multiples to minimizing the incidence of multiples with the reduction in the number of embryos transferred (ET).

eSBT is becoming a more widely accepted strategy in reducing multiples since several studies have demonstrated that PR are not compromised by reducing the number of ET from 2 to 1 [7–11]. This is most prevalent in Europe, where IVF costs are covered by government agencies resulting in eSET making up 60 % of all transfers resulting in <10 % multiple PR and 0 % triplet rate [7, 12–14]. Most recently, Finland showed that eSET with cryopreservation is more effective and less expensive than 2ET, with 2-fold reduction

Capsule The elective transfer of single blastocysts in patients < 40 years of age resulted in elimination of twinning while maintaining high implantation and pregnancy rates.

C. Mullin (✉) · A. S. Berkeley · J. A. Grifo
NYU Fertility Center, New York University,
Langone Medical Center,
660 First Avenue,
New York, NY 10016, USA
e-mail: christine.mullin@gmail.com

in multiple births [15]. Conversely, in the US, where IVF costs are typically endured by patients, eSBT is relatively uncommon [16]. The burden of high expenses combined with years of infertility is enough to discourage the good prognostic patient from choosing eSBT. Such factors are often more influential in decision-making processes than ample evidence supporting the practice of eSBT. Consequently, multiple births after IVF remains consistently high (33 % twins, 1.9 % high order multiples (hom)), greater than the natural twinning rate (1.1 %) [17] and Europe's multiple rates from IVF: 26 % (24 % twins, 2 % hom) [14].

Since SART/ASRM made the recommendation in 2004 to consider eSBT in women <35yo with a favorable prognosis and to transfer ≤ 2 embryos, the average number of ET has declined from 2.5 in 2004 to 2.2 in 2008. A corresponding decline in $\geq$ triplets from 4.9 % to 1.9 % reflects such practice. Despite the increase in cycles receiving an eSBT (1.2 % to 5.2 %), the twinning rate remains fixed (33 %). Hence, in the US, it is evident that both patients and physicians are still hesitant to embrace eSBT in appropriate patients and continue to opt for multiple ET in the hopes of increasing the chance of a live birth. Therefore, eSBT continues to remain a serious clinical dilemma for the self-paying American woman despite previous studies showing its efficacy, as patients fear the potential of compromising PR for the benefit of reducing multiples.

The aim of our study was to identify a subset of patients who would most benefit from eSBT, thus providing substantial evidence to afford patients the ability to confidently choose eSBT.

Materials and methods

Patient selection

This is a retrospective review of all fresh IVF cycles with either eSBT or 2BT from 2004 to 2010. Patients included in our study were women <40yo who had eSBT or 2BT. Exclusion criteria included donor egg recipients, and patients who had a SET for medical indication. Moreover, patients who had only one blastocyst available on day of transfer were excluded given that the SBT was considered non-elective.

Follicular phase/IVF stimulation

Ovarian stimulation was individualized. Patients underwent either down-regulation with GnRH agonist or antagonist with combinations of recombinant human FSH and/or human menopausal gonadotropins. Patients were followed with periodic ultrasounds and estradiol assessment. When lead follicles reached a mean diameter of 17 mm, hCG

intramuscularly was given and ~35 h later, oocytes were collected by ultrasound guided, transvaginal aspiration.

Laboratory procedures

Oocytes were fertilized with routine insemination or ICSI. Fertilization was assessed by visualization of two pronuclei. Embryos were monitored daily. Good quality embryos on d3 were defined as >5 cells with a grade >2.5 . Embryos that were still dividing were cultured to d5. The decision to culture to d5 was based on the quantity and quality of embryos on d3. If an excess number of good quality embryos are available for transfer, then embryo culture would be continued [18]. Embryos were graded on d5 using the Gardner and Lane criteria [19]. The decision on the number of blastocysts to transfer was in accordance to ASRM/SART guidelines [20]. On day of transfer, all patients with a good cohort of blastocysts were counseled and offered eSBT and counseled, with patients ultimately making the final decision. Transcervical ET was performed under ultrasound guidance. The remaining good quality ($\geq$ grade 3Bb per Gardner's criteria) blastocysts were cryopreserved on d5/6 [21].

Luteal phase

Luteal support consisted of IM progesterone daily. An initial pregnancy test was performed on d28. Monitoring with β -hCG levels and ultrasounds were performed to diagnose a viable intrauterine pregnancy, miscarriage, or ectopic pregnancy.

Statistical analyses

Using SigmaStat, variables amongst study groups were compared by χ^2 analyses and Fisher's exact test. Values of $p < 0.05$ were of statistical significance. The data were analyzed according to whether patients <40yo, with or without d5 cryo, underwent either eSBT or 2BT.

Results

Cycles of all women <40yo undergoing fresh IVF cycle were reviewed and subsequently divided into two groups depending on whether or not d5 cryo of blastocysts occurred, and then further subdivided into eSBT or 2BT. Of note, patients grouped into no d5 cryo had no embryos frozen on day 5; however, they may have had embryo cryopreservation on day 6; thus, embryo cryopreservation (Table 3) for patients grouped in the no day 5 cryo represents day 6 embryos only. Patient/cycle characteristics and pregnancy outcomes of patients who underwent eSBT were then compared to those who underwent 2BT in each

cryopreservation group. 61 % (59/96) of patients who chose eSBT were <35yo (Fig. 1).

There was no significant differences in day 2 FSH level, mean number of previous cycles, diagnosis, amount of gonadotropins used, %GnRH agonist cycles, number oocytes retrieved and fertilized, total number of blastocysts available for transfer, or number blastocysts cryopreserved in patients who had d5 cryo and chose eSBT, despite being younger (33 ± 4 vs. 34 ± 4 , $p=0.0147$) as compared to those choosing 2BT (Table 1). Despite higher IR (79 vs. 61, $p=0.0084$) amongst those patients choosing eSBT rather than 2BT, there was no difference in PR (75 % vs. 72 %, $p=0.7699$), LBR (67 % vs. 68 %, $p=0.8889$), or SAB (8 % vs. 4 %, $p=0.2063$) (Table 2). Singleton PR was significantly higher in patients who underwent eSBT with d5 cryo in comparison to those who underwent 2BT with rates of 67 % (45/67) and 34 % (152/445), respectively while completely eliminating twins. Patients choosing 2BT had a twinning rate of 34 % (151/445) or 50 % (151/303) of those who went on to have a live birth. Moreover, there was no statistically significant difference with regards to cumulative pregnancy rates between women choosing a eSBT over a 2BT (90 % (60/67) vs. 82 % (363/445)). Cumulative pregnancy was defined as the sum of clinical pregnancies resulting from the fresh cycle and if not pregnant, then any subsequent clinical pregnancies from subsequent frozen-thaw cycles (Table 2).

In those patients who did not have d5 cryo, there were no significant differences between those patients who chose eSBT versus 2BT in respect to their age, day 2 FSH level, mean number of previous IVF cycles, diagnosis, %GnRH agonist cycles, or gonadotropins used (Table 3). In addition, despite the significantly higher number of oocytes retrieved (19.3 vs. 15.1, $p=0.0030$), fertilized (11.9 vs. 9.0, $p=0.0011$) and embryos cryopreserved (1.9 vs. 1.1, $p=0.0174$) amongst those patients who chose eSBT versus 2BT, the total number of blastocysts available (2.9 vs. 3.1, $p=0.6061$) did not differ (Table 3). Moreover, there was no difference in IR (50 % vs. 43 %, $p=0.3521$), PR (48 % vs.

Fig. 1 Study groups

Table 1 Patient and cycle characteristics in patients with day 5 cryopreservation

	eSBT (n=67)	2BT (n=445)	P value
Mean age	33±4	34±4	0.0147
FSH	2.7	2.8	NS
Mean # previous IVF cycles	0.2	0.6	NS
Diagnosis			
Tubal	5	43	NS
Ovulatory dysfunction	7	26	NS
Diminished ovarian reserve	0	4	NS
Endometriosis	1	10	NS
Uterine	1	9	NS
Male	17	152	NS
Unexplained	36	201	NS
% of GnRH agonist cycles	52	56	NS
Mean Gonadotropins used (IU)	2,205±123	2,309±324	NS
Mean # oocytes retrieved/cycle	21±9	20±9	NS
Mean # oocytes fertilized/cycle	13.9±6	13.6±6	NS
Mean # blastocysts frozen/cycle	6.5±3	5.4±3	0.0050
Total # blastocysts/cycle	7.5±3	7.4±3	NS

56 %, $p=0.4530$), LBR (48 % vs. 51 %, $p=0.8522$), or SAB (0 % vs. 5 %, $p=0.3966$) in patients choosing eSBT rather than 2BT (Table 4). The singleton PR was significantly higher in patients who underwent eSBT in comparison to 2BT with rates of 48 % (14/29) and 34 % (578/1662), respectively ($p<0.0014$) while completely eliminating twins. Those choosing a 2BT had a twinning rate of 16 % (273/1662) or 32 % (273/847) of those who went on to have a live birth. Moreover, there was no statistically significant difference with regards to cumulative pregnancy rates between women choosing a eSBT over a 2BT (52 % (15/29) vs. 59 % (974/1662)) (Table 4).

Discussion

Since its inception, IVF has rapidly become one of the most successful and widely used techniques in infertility treatment,

Table 2 Pregnancy outcomes in patients with day 5 cryopreservation

	eSBT (n=67)	2BT (n=445)	P value
Implantation rate (%)	79	61	0.0084
Pregnancy rate (%)	75	72	NS
Singleton live birth (%)	67	34	0.0001
Twin live birth (%)	0	34	0.0001
Spontaneous abortion (%)	8	4	NS
Cumulative pregnancy rate (%)	90	82	NS

Table 3 Patient and cycle characteristics in patients without day 5 cryopreservation

	eSBT (n=29)	2BT (n=1,662)	P value
Mean age	34±4	34±4	NS
FSH	2.6	3.6	NS
Mean # previous IVF cycles	0.6	0.8	NS
Diagnosis			
Tubal	2	103	NS
Ovulatory dysfunction	1	61	NS
Diminished ovarian reserve	0	45	NS
Endometriosis	3	43	NS
Uterine	0	33	NS
Male	11	609	NS
Unexplained	12	668	NS
% of GnRH agonist cycles	62	50	NS
Mean Gonadotropins used (IU)	2,549±237	2,831±36	NS
Mean # oocytes retrieved/cycle	19.3±10	15.1±7	0.0030
Mean # oocytes fertilized/cycle	11.9±8	9.0±5	0.0011
Mean # blastocysts frozen/cycle	1.9±3	1.1±2 ^a	0.0174
Total # blastocysts/cycle	2.9±3	3.1±2	NS

^a Indicates d6 cryo

resulting in 1 % of newborns born in the US [22] and 3 % of those born in developed countries [1]. Initially, eSET only occurred in women who had just one available embryo, thus, there were “non-elective” SETs in poor responding patients which inevitably resulted in poor pregnancy outcomes. Advances in the field quickly led to the production of multiple oocytes/cycle and ultimately the transfer of >1 embryo/transfer. Such improvements eventually led to higher IR and LBR, but at the expense of an increasing multiples, which are associated with increased morbidity and mortality for both mother and fetus [2–4]. Accordingly, multiple births have been deemed the most unfavorable outcome associated with ART, and have been referred to as an epidemic with some advocating for the quality of an IVF program to be measured on its ability to maximize the singletons rather than by its overall PR [23]. Moreover, it is believed by some that twins

Table 4 Pregnancy outcomes in patients without day 5 cryopreservation

	eSBT (n=29)	2BT (n=1,662)	P value
Implantation rate (%)	50	43	NS
Pregnancy rate (%)	48	56 ^a	NS
Singleton live birth (%)	48	35	0.0014
Twin live birth (%)	0	16	0.0142
Spontaneous abortion (%)	0	5	NS
Cumulative pregnancy rate (%)	52	59	NS

^a Includes eight elective terminations secondary to chromosomal abnormalities and one triplet pregnancy

should be regarded as a complication [24]. Such beliefs are driving physicians to find an acceptable solution.

Currently, the only solution available to physicians is to limit the number of ET in tandem with educating patients on the risks of multiples. There has been a trend amongst IVF programs to reduce the number of ET, with some implementing the practice of mandatory eSET [25]. Europe is at the forefront of such practice as a result of findings that PR in good prognostic patients are not compromised regardless of whether a patient receives one versus two embryos, while dramatically reducing the incidence of multiples [7]. Belgian law instituted the policy of funding and mandating the first two IVF cycles if SET occurred in a woman <36yo, allowing for 2ET only if the first two cycles failed [26].

In 2009, SART/ASRM offered guidelines on the number of blastocysts to transfer. They recommended that programs consider eSBT to good prognostic patients <35yo (1st IVF cycle or previous successful IVF cycle, good embryo quality, excess embryos for cryopreservation). These guidelines implied that fewer blastocysts could be transferred without adversely affecting the likelihood for success [20]. Despite recommendations and documented success [27–29], the US continues to lag behind our European counterparts, hindered by the combination of exorbitant costs endured by patients and years of frustration.

Our program offers eSBT and our experience encourages its use in good prognostic patients, but especially if patients have excess blastocysts to cryopreserve on d5 as the overall IR, PR and LBRs were significantly higher for patients choosing eSBT with d5 cryo (79 %, 75 %, and 67 %, respectively) as compared to those without d5 cryo (50 %, 48 %, and 48 %, respectively). Our age criterion is less stringent than the SART/ASRM guidelines, with the age of our study population including patients up to 39 yo. In addition, our blastocyst criterion (>2Bc) was less stringent than implemented in previous studies in which patients had to have ≥3 blastocysts available on transfer day with one blastocyst of Aa quality [28].

Our study shows higher IR in patients who chose eSBT versus 2BT. This is probably due to the finding that patients who chose eSBT as compared to 2BT had slightly higher quality blastocysts on day of transfer, with the poorest blastocyst grading 2Bc versus 1, respectively. Moreover, the higher IR amongst eSBT probably led to higher singleton PR for those patients choosing an eSBT in comparison to those patients who underwent 2BT.

Our study supports eSBT use in patients <40 yo with a good cohort of blastocysts; especially if they have blastocysts available for d5 cryo. We showed that overall, the eSBT was associated with elimination of twinning while maintaining a high PR in both groups with d5 cryo (75 % eSBT versus 72 % 2BT) and without d5 cryo (48 % eSBT versus 56 % 2BT). No compromise in PR was seen if

embryos were available for d5 cryo; however, patients did suffer a non-statistically significant drop in their PR (48 % vs. 56 % $p=0.4530$) if there were no embryos available for d5 cryo in exchange for eliminating the obstetrical risk of twinning.

Most European studies have looked at PR with respect to d3 SET demonstrating that PR is not compromised by transferring SET. More recently studies showing that extending the embryo culture to d5 increases IR and PR [6, 8, 29–35]. A Cochrane Review demonstrated significant differences in PR and LBR in favor of blastocyst transfer in good prognosis patients and suggested that in selected patients, blastocyst culture may be applicable for eSBT [36–38]. Our study is one of the few studies to assess ART outcomes for eSBT.

Although there was no incidence of monozygotic twinning in our study, previous reports suggest monozygotic twinning of ~2 % [39]. The absence of such twinning may be a result of small sample size. Although low, monozygotic twinning is a potential risk to blastocyst transfer and is tenfold higher than the general population [40, 41] with a significantly higher perinatal morbidity and mortality.

One limitation of our study is that it is a retrospective analysis. Our program has neither formal inclusion criteria nor a mandatory SET policy for offering patients an eSBT. Patient self-selection for eSBT versus 2BT represents a possible confounding factor. In addition, there may be variables not appreciated by review of the database that caused physicians to counsel the better prognosis patient more aggressively about eSBT. This may explain why the overall IR was higher with eSBT (79 % and 61 %) in comparison to 2BT (50 % and 43 %) with or without d5 cryo, respectively.

Small sample size in the eSBT group was another weakness of the study which emphasizes both the reluctance of physicians to offer such practice and in turn, the reluctance of patients to accept eSBT. Although differences in PR with and without d5 cryo were not statistically significant, future studies using larger number of patients are needed to determine if PR are indeed higher with eSBT. However, randomizing patients to either eSBT or 2BT may be difficult to achieve when most patients pay for ART and as a result have strong preferences for the number of blastocysts transferred.

All patients were counseled by their physician during their initial consultation and for a second time regarding their blastocyst quality and options by the covering physician, who was not always the same physician at the initial visit. Therefore, one strength of our study was that two separate counseling sessions, usually by two different physicians, assisted in decreasing the confounding factor of non-uniform counseling between our physicians.

We believe that the best approach to reducing the multiple PR in IVF is multifactorial. Offering eSBT along with cryopreservation of the remaining blastocysts can help to

achieve high cumulative PR [25]. In addition, uniform counseling by physicians at both initial visit and on day of transfer is desirable. Several studies have shown that a woman's initial preference for 2BT was related to beliefs that PR was higher with 2BT versus eSBT, not a specific desire for twins. Educating patients on the risks associated with multiples resulted in a shift towards eSBT and a decreased desire for multiples as long as there was not a substantially lower PR [25, 42]. It is paramount that we not only communicate to our patients the risks associated with multiples, but also understand patients' desires and reservations which motivate them to choose >1 blastocyst.

The focus of implementing the practice of eSBT to the appropriate patient population should provide a more cost-effective, safer approach to patients undergoing ART with singletons at the pinnacle endpoint of IVF success.

Acknowledgments The authors wish to thank the embryology staff and physicians at the NYU Fertility Center who have contributed to the art of IVF.

References

1. Kirby T. Robert Edwards: Nobel Prize for father of in-vitro fertilization. *Lancet*. 2010;376(9749):1293.
2. Pinborg A, Loft A, Schmidt L, Andersen AN. Morbidity in a Danish national cohort of 472 IVF/ICSI twins, 1132 nonIVF/ICSI twins and 634 IVF/ICSI singletons: health related and social implications for the children and their families. *Hum Reprod*. 2003;18:1234–43.
3. Kogan MD, Alexander GR, Kotelchuck M, MacDorman MF, Buekens P, Martin JA, et al. Trends in twin birth outcomes and prenatal care utilization in the United States, 1981–1997. *JAMA*. 2000;19:335–41.
4. Scher AI, Petterson B, Blair E, Ellenberg JH, Grether JK, Haan E, et al. The risk of mortality or cerebral palsy in twins: a collaborative population based study. *Pediatr Res*. 2002;52:671–81.
5. Cramer DW, Liberman RF, Powers D, Hornstein MD, McShane P, Barbieri RL. Recent trends in assisted reproductive techniques and associated outcomes. *Obstet Gynecol*. 2000;95(1):61–6.
6. Reh A, Fino E, Krey L, Berkeley A, Noyes N, Grifo J. Optimizing embryo selection with day 5 transfer. *Fertil Steril*. 2010;93(2):609–15.
7. Gerris J, DeNeubourg D, Margelschots K, Van Royen E, Vercruyssen M, Barudy-Vasquez J, et al. Elective single day 3 embryo transfer halves the twinning rate without a decrease in the ongoing pregnancy rate of an IVF/ICSI programme. *Hum Reprod*. 2002;17(10):2626–31.
8. Veleza Z, Vilksa S, Hyden-Granskog C, Tiitinen A, Tapanainen JS, Martikainen H. Elective Single Embryo transfer in women aged 36–39 years. *Hum Reprod*. 2006;21(8):2098–102.
9. Thurin A, Hausken J, Hillensjö T, Jablonowska B, Pinborg A, Strandell A, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med*. 2004;351(23):2392–402.
10. Mullin CM, Fino ME, Talebian S, Krey L, Licciardi F, Grifo J. Comparison of pregnancy outcomes in elective single blastocyst versus double blastocyst transfer stratified by age. *Fertil Steril*. 2010;93(6):1837–43.

11. Martikainen H, Tiitinen A, Tomas C, Tapanainen J, Orava M, Tuomivaara L, et al. One versus two embryo transfer after IVF and ICSI: a randomized study. *Hum Reprod*. 2001;16(9):1900–3.
12. Debrock S, et al. New Belgian legislation regarding the limitation of transferable embryos in in vitro fertilization cycles does not significantly influence the pregnancy rate but reduces the multiple pregnancy rate in a threefold way in the Leuven University Fertility Center. *Fertil Steril*. 2005;83:1572–4.
13. Karlstrom PO, Bergh C. Reducing the number of embryos transferred in Sweden-impact on delivery and multiple birth rates. *Hum Reprod*. 2007;22:2202–7.
14. Nyboe A, et al. Assisted reproductive technology in Europe, 2000. Results generated from European registers by ESHRE. *Hum Reprod*. 2004;19:490.
15. Veleza Z, Karinen P, Tomas C, Tapanainen JS, Martikainen H. Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. *Hum Reprod*. 2009;24(7):1632–9.
16. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2003 assisted reproductive technology success rates. Atlanta: U. S. Department of Health and Human Services; 2005.
17. Crowther CA. Multiple pregnancies. In: James DK, Steer PJ, Weinger CP, Gonik B, editors. *High risk pregnancy: management options*. 2nd ed. Philadelphia: Saunders; 2002. p. 129–52.
18. Grifo JA, Flisser E, Adler A, McCaffrey C, Krey LC, Licciardi F, et al. Programmatic implementation of blastocyst transfer in IVF, maximizing pregnancy rates and minimizing triplet rates. *Fertil Steril*. 2006;88(2):294–300.
19. Gardner DK, Lane M. Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Hum Reprod Update*. 1997;3:367–82.
20. ASRM. Guidelines on the number of embryos transferred. *Fertil Steril*. 2009;92(5):1518–9.
21. Menezo Y, Nicollet B, Herbaut N, Andre D. Freezing co-cultured human blastocyst. *Fertil Steril*. 1992;59:977–80.
22. National Center for Chronic Disease Prevention and Health Promotion, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2001 Assisted reproductive technology success rates: national summary and fertility clinic reports. Atlanta: Centers for Disease Control and Prevention, December 2003.
23. Adashi EY, Barri PN, Berkowitz R, Braude P, Bryan E, Carr J, et al. Infertility therapy-associated multiple pregnancies (births): an ongoing epidemic. *Reprod Biomed Online*. 2003;7:515–42.
24. Land JA, Evers JL. Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. *Hum Reprod*. 2003;18:455–7.
25. Ryan GL, Sparks AET, Sipe CS, Syrop CH, Dokras A, Van Voorhis BJ. A mandatory single blastocyst transfer policy with educational campaign in a United States IVF program reduces multiple gestation rates without sacrificing pregnancy rates. *Fertil Steril*. 2007;88:354–60.
26. Hamberger L, Hardarson T, Nygren KG. Avoidance of multiple pregnancy by use of single embryo transfer. *Minerva Ginecol*. 2005;57(1):15–9.
27. Coskun S, Hollanders J, Al Hassan S, Al Sufyan H, Al Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. *Hum Reprod*. 2000;15:1947–52.
28. Karaki RZ, Samarraie SS, Younis NA, Lahloub TM, Ibrahim MH. Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. *Fertil Steril*. 2002;77:114–8.
29. Papanikolaou EG, Camus D, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with single blastocyst-stage versus cleavage-stage embryos. *N Engl J Med*. 2006;354:1139–46.
30. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril*. 2000;73:1155–8.
31. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod*. 1998;12:3434–40.
32. Levron J, Schulman A, Bider D, Seidman D, Levin T, Dor J. A prospective randomized study comparing day 3 with blastocyst-stage embryo transfer. *Fertil Steril*. 2002;77:1300–1.
33. Criniti A, Thyer A, Chow G, Lin P, Klein N, Soules M. Elective single blastocyst transfer reduces twin rates without compromising pregnancy rates. *Fertil Steril*. 2005;84:1613–9.
34. Utsunomiyama T, Naitou T, Nagaki M. A prospective trial of blastocyst culture and transfer. *Hum Reprod*. 2002;17:1846–51.
35. Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F, et al. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compare favorably with day 5 blastocyst transfer. *Hum Reprod*. 2002;17:1507–12.
36. Fraattarelli JL, Leondires M, McKeeby JL, Miller BT, Segars JH. Blastocyst transfer decreases multiple pregnancy rates in in vitro fertilization cycles: a randomized controlled trial. *Fertil Steril*. 2003;79:228–30.
37. Margreiter M, Weghofer A, Kogosowski A, Mahmoud KZ, Feichtinger W. A prospective randomized multicenter study to evaluate the best day for embryo transfer: does the outcome justify prolonged embryo culture? *J Assist Reprod Genet*. 2003;20:91–3.
38. Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev*. 2007;17(4):CD002118.
39. Milki AA, Jun SH, Hinckley MD, Behr B, Giudice LC, Westphal LM. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. *Fertil Steril*. 2003;79:503–6.
40. Behr B, Fisch JD, Racowsky C, Miller K, Pool TB, Milki AA. Blastocyst-ET and monozygotic twinning. *J Assist Reprod Genet*. 2000;17:349–51.
41. The practice committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology. Blastocyst culture and transfer in clinical assisted reproduction. *Fertil Steril*. 2008;90(3):S174–7.
42. Newton CR, McBride J, Feyles V, Tekpetey F, Power S. Factors affecting patients' attitudes toward single- and multiple-embryo transfer. *Fertil Steril*. 2007;87(2):269–78.