

Equivalency of *In vitro* fertilization Success Rates in Elective Single Blastocyst Transfer and Elective Double Blastocyst Transfer: An Example of Equivalence Methodology in Clinical Reproductive Health

Marni B. Jacobs, Hillary Klonoff-Cohen¹, V. Gabriel Garzo²

Department of Biostatistics and Study Methodology, Children's Research Institute, Children's National Health System, Washington, DC 20010, ¹Department of Kinesiology and Community Health, College of Applied Health Sciences, University of Illinois at Urbana-Champaign, Champaign, IL 61820, ²Reproductive Partners Medical Group - UCSD Regional Fertility Center, La Jolla, CA, USA

ABSTRACT

Context: When comparing success rates between treatments, it is more appropriate to structure analyses in terms of equivalence rather than traditional analyses that assess differences. Unfortunately, no studies of elective single blastocyst transfer (eSBT) have been conducted in this manner. **Aims:** The objective of this study was to assess clinical equivalence of *in vitro* fertilization success rates among patients undergoing eSBT. **Settings and Design:** A historical prospective study was conducted at a private fertility center. **Methods:** Medical records were reviewed to identify patients eligible for eSBT. Equivalency of success rates, defined as no more than a 10% difference based on 95% confidence intervals (CIs), was compared between eSBT ($n = 125$) and eDBT ($n = 213$) groups. **Results:** Using traditional analysis techniques, no differences in pregnancy or live-birth rates were seen (eSBT: 84.6% vs. eDBT: 84.5%, $P = 0.99$; eSBT: 65.3% vs. eDBT: 72.3%, $P = 0.23$). The 95% CI around the difference in pregnancy rates ranged from -7.9 to 8.1 , suggesting clinically equivalent pregnancy rates. Clinical equivalence was not established for live-births (95% CI = $-18.5-4.5$). **Conclusions:** Findings suggest comparable pregnancy rates can be achieved in a clinical setting when utilizing eSBT in good-prognosis patients. Although live-birth rate equivalence was not demonstrated, it is thought the additional complications associated with multiple gestations outweigh the potentially higher live-birth rate. The present study highlights the importance of utilizing equivalence analyses when making statements regarding the similarity of two treatments in reproductive health, rather than relying on superiority analyses alone.

KEYWORDS: Clinical equivalence, elective double blastocyst transfer, elective single blastocyst transfer, *in vitro* fertilization success rates, superiority analyses

INTRODUCTION

In an effort to combat the high rate of multiple gestation pregnancies commonly seen in *in vitro* fertilization (IVF), elective single-embryo transfer (eSET) has been proposed. However, patients may be reluctant to undergo eSET due to the perceived risk of decreased success associated with the transfer of fewer embryos. Both the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproduction (SART) in the US and the Society of Obstetricians and Gynaecologists of Canada and the Canadian Fertility and Andrology Society

advise that eSET should be more readily applied with blastocyst, rather than cleavage, stage embryos, as higher success rates are generally seen.^[1-3] Despite this recommendation, studies of eSET at the blastocyst stage are limited.^[4-11] The only existing randomized trial found no differences in implantation or pregnancy rates between eSET and double-embryo transfer.^[6] Cohort

Address for correspondence: Dr. Marni B. Jacobs, 801 Roeder Road, Suite 600, Silver Spring, MD 20910, USA. E-mail: mjacobs@childrensnational.org

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Jacobs MB, Klonoff-Cohen H, Garzo VG. Equivalency of *in vitro* fertilization success rates in elective single blastocyst transfer and elective double blastocyst transfer: An example of equivalence methodology in clinical reproductive health. J Hum Reprod Sci 2018;11:45-51.

Access this article online	
Quick Response Code: 	Website: www.jhrsonline.org
	DOI: 10.4103/jhrs.JHRS_136_17

study results are heterogeneous, with one study showing a significant reduction in pregnancy rates in eSET,^[7] four reporting no significant differences,^[4,5,9,11] and two reporting differences among select patients.^[8,10]

Although research at the blastocyst stage has increased, gaps exist. Only two blastocyst stage studies specifically stated that donor-recipient cycles were included,^[4,10] though these patients are an identified target group for eSET.^[1,3] Furthermore, not all single-embryo transfers in existing studies were elective, suggesting that the groups considered may not be truly comparable.^[11] Finally, even though these studies assess whether similar success rates can be obtained in eSET, all analyses utilize superiority techniques. To properly assess whether two procedures are similarly successful, analyses evaluating the similarity of success rates should be undertaken, rather than analyses that focus on their differences. This has been done previously in one cleavage stage study,^[12] however, no blastocyst stage studies have been evaluated in this manner. Here, we apply equivalence analysis methodology and demonstrate how differences in choice of analytic method can result in differing conclusions.

METHODS

The aim of the present study was to assess whether similar success rates are seen in good prognosis patients undergoing elective single blastocyst transfer (eSBT) compared to those opting for elective double blastocyst transfer (eDBT) and to contrast these findings with traditional superiority analyses.

The present investigation is a retrospective cohort study of medical records from a private fertility center. Consistent with SART/ASRM recommendations,^[1] center guidelines recommend eSET for: (a) autologous patients age <35 years or patients of any age undergoing a donor-recipient cycle, and (b) patients with at least three fair to good quality blastocysts. Patients meeting these criteria are counseled regarding possible complications of multiple gestation pregnancies, and the potential benefit of single-embryo transfer; however, the decision of whether to transfer one or two embryos is left up to the patient.

All fresh IVF cycles from January 1, 2006 to December 31, 2011 were reviewed to identify patients who were eligible for eSET. Patients were stratified according to the number of embryos they elected to transfer, and comparability of pregnancy and live-birth rates was assessed.

Controlled ovarian hyperstimulation consisted of standard gonadotropin-releasing hormone downregulation, followed by use of human

menopausal gonadotropin (75 IU) and recombinant follicle-stimulating hormone (75–300 IU). Human chorionic gonadotropin (hCG) was administered when two or more follicles reached a diameter of 18 mm. Luteal support consisted of 50 mg intramuscular (IM) progesterone in oil and 4 mg estradiol administered orally and continued through 10 weeks gestation in women who became pregnant.

For donor-recipients, the endometrium was prepared using 0.1 mg transdermal estrogen replacement patches, adjusted up to six to achieve an estradiol level 300–500 pg/ml. Luteal support consisted of 100 mg IM progesterone in oil, and estrogen and progesterone were continued through 10 weeks gestation.

Standard insemination versus intracytoplasmic sperm injection was performed as clinically appropriate. Blastocyst grading was done on the day of blastocyst transfer (day 5), based on criteria described by Gardner and Schoolcraft.^[13] Blastocysts were graded as good if the inner cell mass consisted of many, tightly packed cells, and the trophoblast was comprised of many cells forming a cohesive epithelium, or a few cells forming a loose epithelium. Blastocysts with very few cells either in the inner cell mass or the trophoblast, early blastocysts with no inner cells mass, and morula stage embryos were graded as poor.

Clinical pregnancy was defined as the presence of a fetal heartbeat on ultrasound examination. Patients whose beta-hCG levels following embryo transfer were indicative of pregnancy but did not progress to a clinical pregnancy were considered not pregnant. Implantation rate was calculated by dividing the number of fetal sacs observed on ultrasound by the number of embryos transferred. Pregnancy rate was expressed as the number of cycles with at least one fetal heartbeat divided by the number of embryo transfer procedures. Live-birth was defined as the birth of a living infant; live-birth rate was expressed as the number of cycles with at least one live-birth divided by the number of embryo transfer procedures. Patient characteristics were compared between eSBT and eDBT groups using Chi-square for categorical variables and *t*-test for continuous variables.

Subsequently, an equivalence analysis was conducted to evaluate the similarity of pregnancy and live-birth rates between groups. Traditional statistical analyses focus on determining whether two treatments are different from one another, a concept known as superiority. Conversely, with equivalence analyses, the focus is on how similar two groups are. The appropriate response for a null finding in a superiority analysis is that we cannot say the two groups are different; however, this does not

necessarily mean they are the same or equal. Thus, researchers have pointed out that it is inappropriate to conclude that two treatments are similar when utilizing traditional superiority statistics, such as Chi-square, rather than techniques that evaluate whether success rates fall within a predetermined range of equivalence.^[14]

While we cannot expect treatments to be exactly the same, we can establish an acceptable difference between the two that might not be considered clinically relevant. Once this has been done, analysis focuses on whether confidence intervals (CIs) around any observed difference falls within this acceptable range. Therefore, in line with a previous study,^[12] the predetermined difference to establish clinical equivalence was set at 10%, indicating that the CIs around the difference in the pregnancy and live-birth rates between the two groups should not exceed this magnitude. It is thought that a 10% decrease would be viewed as an acceptable risk among couples undergoing IVF given the prospective benefit of a singleton pregnancy. Chi-square tests were also conducted to assess whether rates significantly differed between groups to facilitate comparison with previous literature. Analyses were subsequently stratified by cycle type. To confirm that performing eSBT was successful in reducing multiples, rates of multiple gestations were compared. For all superiority analyses, a two-sided $P = 0.05$ was considered statistically significant. Analyses were conducted using SPSS 20 for Windows (IBM Corp., Armonk, NY).

The study was granted a Health Insurance Portability and Accountability Act waiver and received ethics approval from the University of California, San Diego Human Research Protection Program.

RESULTS

Among 338 patients eligible for eSET, 125 opted to transfer a single blastocyst, while 213 (63.0%) chose to transfer two [Table 1]. Compared to the eDBT group, eSBT patients were slightly less likely to be undergoing an autologous cycle (61.6% vs. 71.4%, $P = 0.07$), and women using their own oocytes in the eSBT group were significantly younger (30.9 years vs. 31.7 years, $P = 0.03$). Women in the eSBT group had a lower body mass index (BMI) than those in the eDBT group (22.3 vs. 23.4, $P = 0.009$), more oocytes retrieved (17.2 vs. 14.8, $P < 0.001$), and a higher number of fertilized oocytes (11.1 vs. 9.6, $P = 0.004$).

Two patients in the eSBT group were missing cycle outcome information. Implantation rate was higher in the eSBT group compared to eDBT (82.4% vs. 64.8%, $P < 0.001$), though no difference in pregnancy rates were seen ($P = 0.99$) [Table 2]. Pregnancy rates reached 84.6% in the eSBT group and 84.5% in the eDBT group,

indicating a 0.1% higher rate in the eSBT group. The 95% CI around this difference ranged from -7.9 to 8.1 , suggesting the pregnancy rate in eSBT could be 7.9% lower than the eDBT group or 8.1% higher. Therefore, based on previously defined criteria, clinical equivalence was demonstrated overall as the 95% CI did not reach 10% in either direction (suggesting that neither group had more than a 10% increased chance of becoming pregnant). Among autologous cycles only, correspondingly high pregnancy rates were observed (eSBT: 86.8% and eDBT: 83.6%). Although these rates did not differ ($P = 0.52$), clinical equivalence was not established as the upper limit of the 95% CI crossed 10% (-6.4% – 12.8%). However, the 95% CI favored eSBT, suggesting that those who elected to have a single blastocyst transferred could have up to a 12.8% increased chance of becoming pregnant. Similarly, among donor cycles, no statistical differences were seen in pregnancy rates (80.9% vs. 86.9%, $P = 0.39$), while the 95% CI exceeded 10% (-20.1% – 8.1%), this time in favor of eDBT.

Overall, 48 pregnancies were ongoing (28 eSBT, 20 eDBT) and pregnancy outcome was unknown in two additional eDBT pregnancies. Live-birth rates were slightly lower among those opting for eSBT compared to those choosing eDBT (65.3% vs. 72.3%, $P = 0.23$) [Table 3]. Referring to the 95% CI around the difference in live-birth rates, clinical equivalence was not established, as the interval ranged from -18.5 to 4.5 , indicating that compared to the eDBT group, patients in the eSBT group could have as much as an 18.5% lower chance or up to a 4.5% higher chance of achieving a live-birth. Similar outcomes were seen among autologous and donor-recipient cycles individually, where slightly lower, nonsignificant differences in live-birth rates were seen in the eSBT group (64.4% vs. 73.7%, $P = 0.19$ and 66.7% vs. 69.0%, $P = 0.82$, respectively), with an inability to establish clinical equivalence (-23.6% – 5.0% and -21.8% – 17.2% , respectively).

To assess whether pregnancy and live-birth rates were influenced by significant differences between eSBT and eDBT groups, rates adjusted for these characteristics were calculated. Among all patients, after controlling for BMI, number of oocytes and number of fertilized oocytes, pregnancy, and live-birth rates were slightly lower in the eSBT group compared to the eDBT group, but were not significantly different (84.3% vs. 84.6%, $P = 0.95$, and 63.9% vs. 73.3%, $P = 0.11$, respectively). The 95% CI around the difference in pregnancy rates shifted slightly, ranging from -8.4% to 7.8% , again demonstrating clinical equivalence. The 95% CI around the difference in live-birth rates was comparable to that of the crude rates (-20.9% – 2.1%), with an inability to

Table 1: Patient and cycle characteristics of elective single blastocyst transfer (eSBT) versus elective double blastocyst transfer (eDBT) groups

Characteristic	eSBT (n=125)	eDBT (n=213)	P*
Autologous cycles (%)	61.6	71.4	0.07
Age [†] (mean, years)	30.9	31.7	0.03
BMI (mean, kg/m ²)	22.3	23.4	0.009
Cause of infertility (%)			0.58
Female factor	58.7	52.9	
Male factor	20.2	27.0	
Both female and male factor	12.8	11.1	
Unexplained	8.3	9.0	
History of previous pregnancies (%)			0.20
Yes	96.8	99.1	
No	3.2	0.9	
History of previous live birth (%)			0.12
Yes	68.8	77.0	
No	31.2	23.0	
Previous treatment with IVF (%)			0.09
Yes	14.4	22.5	
No	85.6	77.5	
Number of oocytes retrieved (mean)	17.2	14.8	<0.001
Percent (%) ICSI	97.2	97.6	0.78
Number of fertilized oocytes (mean)	11.1	9.6	0.004
Peak E ₂ level (mean, pg/ml)	1999.4	2143.4	0.39

*Chi-square or Fisher's Exact Test (categorical), *t*-test (continuous). [†]Autologous only; Oocyte donor age by cycle is not recorded-donors are typically under age 25 and mean age of oocyte donors was 24.5 years

Table 2: Pregnancy rates in eSBT compared to eDBT

Cycle type	eSBT pregnancy rate (%) (n=123)	eDBT pregnancy rate (%) (n=213)	Difference in rates	95% CI*	P [†]
All [‡]	84.6	84.5	0.1	-7.9-8.1	0.99
Autologous [§]	86.8	83.6	3.2	-6.4-12.8	0.52
Donor [‡]	80.9	86.9	-6.0	-20.1-8.1	0.39

*95% confidence interval around the difference in rates. [†]P calculated for Chi-square statistic. [‡]Implantation rate: eSBT, 82.4%; eDBT, 64.8%, *P*<0.001. [§]eSBT: *n*=76; eDBT: *n*=152. [‡]eSBT: *n*=47; eDBT: *n*=61

Table 3: Livebirth rates in eSBT compared to eDBT

Cycle type	eSBT livebirth rate (%) (n=95)	eDBT livebirth rate (%) (n=191)	Difference in rates	95% CI*	P [†]
All	65.3	72.3	-7.0	-18.5-4.5	0.23
Autologous [‡]	64.4	73.7	-9.3	-23.6-5.0	0.19
Donor [§]	66.7	69.0	-2.3	-21.8-17.2	0.82

*95% confidence interval around the difference in rates. [†]P calculated for Chi-square statistic. [‡]eSBT: *n*=59; eDBT: *n*=133, [§]eSBT: *n*=36; eDBT: *n*=58

Table 4: Multiple gestation pregnancies in eSBT compared to eDBT

ET Group	Rate of multiple gestation pregnancies (%)	P*
eSBT (n=94)	8.5	<0.001
eDBT (n=165)	53.9	

*P calculated for Chi-square statistic

establish clinical equivalence. Examining autologous cycles only, pregnancy rates remained slightly higher in the eSBT group compared to the eDBT group (86.7% vs. 83.6%, *P* = 0.56), while live-birth rates

were slightly lower (62.3% vs. 74.6%, *P* = 0.10), after adjusting for autologous patient age, BMI, number of oocyte retrieved, and number of fertilized oocytes. Clinical equivalence could not be demonstrated for either pregnancy (95% CI = -6.5-12.7%) or live-birth rates (-26.7%-2.1%) among autologous cycles based on 95% CIs around the difference in adjusted rates.

As expected, pregnancies in the eDBT group were significantly more likely to be multiple gestations than those in the eSBT group (53.9% vs. 8.5%, *P* < 0.001) [Table 4].

DISCUSSION

Among all patients eligible for eSBT, no significant differences were seen in pregnancy or live-birth rates between patients opting to transfer a single embryo and those who transferred two. However, we were only able to demonstrate “clinical equivalence” in pregnancy rates overall between the eSBT and eDBT groups. The 95% CI around the difference in overall pregnancy rates ranged from -7.9% to 8.1% , meaning that neither group had more than a 10% increase over the other. We were unable to show clinical equivalence for live-birth rates or among patients utilizing donor oocytes. Estimates were not materially different controlling for baseline differences between groups. Since this study took place over a short time span among a highly select group of patients, it is possible that the small number of eligible women may have impacted our ability to establish clinical equivalence as variability in CIs is influenced by population size. Nevertheless, eSBT did accomplish the intended task of reducing multiple gestation pregnancies, as the proportion of multiples in the eSBT group was significantly lower than the eDBT group. These findings highlight the importance of considering not only traditional superiority analyses in the assessment of clinical success rates, but also equivalence studies, as the conclusions reached differ based on the methodology selected. This is especially important in a field like reproductive medicine, where patients and their doctors need to make decisions that weigh potential decreases in success over the increased risks associated with alternative procedures.

Baseline differences between the eSBT and eDBT groups in the present study suggest that although clinic guidelines exist, there may remain inherent biases and reservations among patients and physicians regarding the procedure. Although eSBT is supposed to be promoted among select patients, more than half opted for eDBT, and discrepancies in patient characteristics raise the possibility that physicians may be more likely to encourage the procedure to certain types of patients. Thus, it is thought the present study demonstrates the utility of an eSET policy on pregnancy and live-birth rates in current clinical practice, providing an evaluation of current performance rather than procedural efficacy. While the high pregnancy and live-birth rates in the current study reflect the strength of the clinical program at this fertility center,^[4-11] they also suggest that eSET policies could be strengthened to further reduce the extremely high rate of multiples seen (54% among eDBT patients and an unexpectedly high rate of monozygotic twinning [8%] overall).

As this is the first study to consider clinical equivalency at the blastocyst stage, we are unable to directly

compare our findings to other studies. While previous researchers may have stated that success rates observed were similar between groups, the accuracy of these conclusions are questionable because equivalency analyses were not performed.^[15] Had we not performed equivalence analyses, we also would have concluded pregnancy and live-birth rates did not differ between groups, consistent with existing literature.^[4-6,8,9,11] This is important as the true intention of these studies should be to establish whether two procedures result in similar outcomes to facilitate use in a clinical setting. Results from the present study are in accord with results from the one cleavage stage study that utilized equivalency analysis. Thurin *et al.* were also unable to establish that live-birth rates were similar as the 95% CI around the difference exceeded 10%, ranging from -11.6% to 3.4% ;^[12] pregnancy rates were not assessed. Notably, this cleavage-stage study was a randomized controlled trial among autologous patients only, and intent-to-treat analyses were based on cumulative live-birth rates following the transfer of one fresh plus one frozen embryo in the eSET group. In comparison, our study focused on the original fresh transfer cycle, and both autologous and donor-recipient cycles were included. Even with this additional frozen transfer cycle considered, the live-birth rates in our study were higher than what was observed by Thurin *et al.* at the cleavage stage (eSBT = 65.3% and eDBT = 72.3% vs. 27.6% and 42.9%, respectively).

To assess why pregnancy rates were more similar than live-birth rates, outcome of all pregnancies were assessed. Excluding ongoing pregnancies, rates of spontaneous abortions were slightly higher in the eSBT group compared to eDBT group (17.1% vs. 10.8%), though this difference was not significant ($P = 0.15$). Subsequently, we looked at any the loss of any fetus, either by spontaneous abortion or stillbirth, meaning that if a woman was pregnant with twins and ultimately gave birth to only one live infant, she would be included as having a fetal loss although she had a live birth. When considering spontaneous abortion in this manner, rates of fetal loss were more similar between eSBT and eDBT groups (13.7% vs. 12.8%, $P = 0.86$), suggesting that having an additional embryo implant may increase the chances of at least one live birth. A similar phenomenon has previously been described among a wider range of patients, whereby the “take-home” baby rate appears to be higher in twin gestation pregnancies compared to singleton pregnancies,^[16-20] although rates of partial embryonic loss are similar.^[17,20] Within the eDBT group alone, 10.4% of known singleton pregnancies resulted in complete spontaneous abortion, compared to 2.6% of the multiple gestation pregnancies ($P = 0.12$), and

12.5% of singleton pregnancies in the eSBT group. Since the overall pregnancy rates in the eSBT and eDBT groups were similar, it may be important to examine risk for spontaneous abortion when evaluating patients best suited for eSBT, in addition to the clinical factors already taken into consideration.

Due to the retrospective nature of the study, we cannot be certain that all patients in our study sample were counseled regarding eSET; however, we know that they were eligible for eSET based on predefined inclusion guidelines and that all physicians at this center are aware that they should be discussing eSET with appropriate patients. Although the goal was to evaluate efficacy of the procedure overall, we endeavored to address baseline differences between the eSBT and eDBT groups by providing adjusted equivalence estimates. Nevertheless, this study demonstrates the use of eSBT in a practice-based clinical setting and illustrates its impact on IVF success rates in a predefined clinical population.

This study shows that comparable pregnancy rates can be achieved in a clinical setting when utilizing eSET at the blastocyst stage in a defined group of good prognosis patients. Although clinical equivalency could not be demonstrated, similarly high live-birth rates were seen among those undergoing elective single- and double-blastocyst transfer. It is thought that the additional complications associated with multiple gestation pregnancies outweighs the slightly higher live-birth rate seen in eDBT. The differences in eligible patients opting for and out of eSET and the unreasonably high rate of multiple gestations among those opting out suggests that clinics should consider stronger policies defining when eSET should be performed.

CONCLUSION

While no differences were seen in pregnancy and live-birth rates using traditional superiority analyses, comparable success rates were demonstrated for pregnancy rates only using equivalence methodology.

Acknowledgment

The authors would like to express gratitude to Drs. Sanjay Agarwal, Donna Kritz-Silverstein, Suzanne Lindsay, and Hector Lemus for their input and guidance, as well as Susan Strachan, R.N., B.S.N, for her assistance with database management and clinical clarification, and Lisa Yeo, CLS, ELD (ABB), for her assistance with data retrieval and laboratory procedures.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. The Practice Committee of the Society for Assisted Reproductive Technology and The Practice Committee of the American Society for Reproductive Medicine. Elective single-embryo transfer. *Fertil Steril* 2012;97:835-42.
2. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, and Society for Assisted Reproductive Technology, 2012 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports, U.S. Department of Health and Human Services, Editor. Atlanta: CDC; 2014.
3. Min JK, Hughes E, Young D, Joint Socg-Cfas Clinical Practice Guidelines Committee, Reproductive Endocrinology and Infertility Committee. Elective single embryo transfer following *in vitro* fertilization. *J Obstet Gynaecol Can* 2010;32:363-77.
4. Criniti A, Thyer A, Chow G, Lin P, Klein N, Soules M, *et al.* Elective single blastocyst transfer reduces twin rates without compromising pregnancy rates. *Fertil Steril* 2005;84:1613-9.
5. Friedman BE, Davis LB, Lathi RB, Westphal LM, Baker VL, Milki AA, *et al.* Age-related success with elective single versus double blastocyst transfer. *ISRN Obstet Gynecol* 2011;2011:656204.
6. Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB, *et al.* Single blastocyst transfer: A prospective randomized trial. *Fertil Steril* 2004;81:551-5.
7. Henman M, Catt JW, Wood T, Bowman MC, de Boer KA, Jansen RP, *et al.* Elective transfer of single fresh blastocysts and later transfer of cryostored blastocysts reduces the twin pregnancy rate and can improve the *in vitro* fertilization live birth rate in younger women. *Fertil Steril* 2005;84:1620-7.
8. Kalu E, Thum MY, Abdalla H. Reducing multiple pregnancy in assisted reproduction technology: Towards a policy of single blastocyst transfer in younger women. *BJOG* 2008;115:1143-50.
9. Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA, *et al.* Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age. *Fertil Steril* 2010;93:1837-43.
10. Stillman RJ, Richter KS, Banks NK, Graham JR. Elective single embryo transfer: A 6-year progressive implementation of 784 single blastocyst transfers and the influence of payment method on patient choice. *Fertil Steril* 2009;92:1895-906.
11. Styer AK, Wright DL, Wolkovich AM, Veiga C, Toth TL. Single-blastocyst transfer decreases twin gestation without affecting pregnancy outcome. *Fertil Steril* 2008;89:1702-8.
12. 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;351:2392-402.
13. Gardner DK, Schoolcraft WB. *In vitro* culture of human blastocyst. In: Jansen R, Mortimer D, editors. *Towards Reproductive Certainty: Fertility and Genetics Beyond 1999*. Carnforth: Parthenon Press; 1999. p. 378-88.
14. D'Agostino RB Sr., Massaro JM, Sullivan LM. Non-inferiority trials: Design concepts and issues – The encounters of academic consultants in statistics. *Stat Med* 2003;22:169-86.
15. Baruffi RL, Mauri AL, Petersen CG, Nicoletti A, Pontes A, Oliveira JB, *et al.* Single-embryo transfer reduces clinical pregnancy rates and live births in fresh IVF and Intracytoplasmic Sperm Injection (ICSI) cycles: A meta-analysis. *Reprod Biol Endocrinol* 2009;7:36.
16. La Sala GB, Nucera G, Gallinelli A, Nicoli A, Villani MT, Blickstein I, *et al.* Spontaneous embryonic loss following *in vitro*

- fertilization: Incidence and effect on outcomes. *Am J Obstet Gynecol* 2004;191:741-6.
17. Matias A, La Sala GB, Blickstein I. Early loss rates of entire pregnancies after assisted reproduction are lower in twin than in singleton pregnancies. *Fertil Steril* 2007;88:1452-4.
 18. Matias A, Oliveira C, da Silva JT, Silva J, Barros A, Blickstein I, *et al.* The effect of ICSI, maternal age, and embryonic stage on early clinical loss rate of twin versus singleton pregnancies. *Eur J Obstet Gynecol Reprod Biol* 2007;130:212-5.
 19. Tummers P, De Sutter P, Dhont M. Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. *Hum Reprod* 2003;18:1720-3.
 20. Zegers-Hochschild F, Bravo M, Fernández E, Fabres C, Balmaceda JP, Mackenna A, *et al.* Multiple gestation as a marker of reproductive efficacy: Learning from assisted reproductive technologies. *Reprod Biomed Online* 2004;8:125-9.