Guidelines on the Number of Embryos Transferred

Jason Min, MD, FRCSC
Camille Sylvestre, MD, FRCSC

CFAS Clinical Practice Guideline Committee

William Buckett, MD
Jon Havelock, MD
Hananel Holzer, MD
Neal Mahutte, MD
Jason Min, MD (Chair)
Jeff Roberts, MD
Sony Sierra, MD
Camille Sylvestre, MD
Beth Taylor, MD

Introduction

It is universally agreed that high-order multiples (triplets or more) are associated with adverse outcomes and are considered an undesirable outcome of in vitro fertilization (IVF). In 2007, 45.3% of 4499 babies born following embryo transfer in Canada resulted from multi-fetal pregnancies, of which only 0.8% were from high-order multiples.[1] It is also well-established that IVF twin pregnancies are associated with a higher incidence of adverse maternal, fetal and neonatal outcomes when compared to singleton gestations, primarily due to increases in preterm birth[2] In 2007 in Canada, of all singleton births resulting from assisted reproductive technologies (ART), the total perinatal mortality rate was 1.3%, preterm delivery under 37 weeks was 14.3%, under 32 weeks was 2.3%, and birth weight less than 2500g was 10.3%. For twins, the perinatal mortality rate was 3.2%, delivery less than 37 weeks was 67.5%, under 32 weeks was 11.3%, and birth weight less than 2500g was 53.5%.[1] Given the increased morbidity and mortality associated with any multi-fetal pregnancy, the aim of IVF treatment should be the birth of a healthy singleton.[3]

In 2006, the Canadian Fertility and Andrology Society (CFAS) and Society of Obstetricians and Gynecologists (SOGC) published joint guidelines on embryo transfer with the aim of optimizing healthy live birth and minimizing multiple pregnancies.[4] In 2007, 95.6% of babies born from multi-fetal gestations were from twin pregnancies.[1] In that same year, 89% of embryo transfers were compliant with the recommendations of the 2006 joint guidelines.[5]

The overall purpose of this guideline is to provide guidance to Canadian IVF clinics and IVF practitioners regarding the number of embryos to transfer to minimize multiple pregnancy rates (including twins) while maintaining acceptable live birth rates. In 2009, Assisted Human Reproduction Canada sponsored a roundtable meeting of key stakeholders in order to develop a Canadian framework for the minimization of multiple pregnancies resulting from infertility treatments. Recognizing that twin gestations accounted for the majority of preventable morbidity and mortality associated with IVF, a consensus was reached targeting a decrease in the twin pregnancy rate to 25% by 2012 and to 15% by 2015[6]. These targets were thought to be realistic and achievable, and were recently reaffirmed by the IVF Medical Directors of the CFAS.[7]

Individual programs are encouraged to develop their own embryo transfer policies based upon their own data, with the aim of reducing multiple pregnancy rates to the consensus targets outlined previously while maintaining acceptable live birth rates. In the absence of individualized, clinic-specific policies, the recommendations in this guideline should be utilized in order to guide embryo transfer practices. These recommendations outline the maximum numbers of embryos to be transferred, and are based upon good-quality, published literature when available, outcome data from the Canadian Assisted Reproductive Technologies Register (CARTR) and the consensus targets for minimization of multiple pregnancy rates outlined above. When transferring more than the recommended maximum number of embryos outlined in this guideline, the rationale should be clearly documented. Irrespective of the embryo transfer policy followed, individual clinics should frequently review their multiple pregnancy rates in order to ensure that they are in keeping with the consensus targets.

The following recommendations are made separately for cleavage-stage embryos, those cultured for two or three days after fertilization, and blastocyst stage embryos, those cultured for five or six days. Recommendations are further stratified by patient prognosis. Characteristics associated with more favourable prognosis include but are not limited to: first or second IVF attempt, good embryo quality by morphology, surplus embryos of sufficient quality to warrant cryopreservation and a previously successful IVF cycle.[8-13]

Evidence is graded as outlined in the report of the Canadian Task force on Preventative Health Care (Table 1).[14, 15]

Table 1. Quality of evidence assessment and classification of recommendations as defined by the Canadian Task Force on Preventative Health Care

Quality of Evidence Assessment[15]		Classification of Recommendations[14]	
I	Evidence obtained from a least one properly randomized controlled trial.	A	There is good evidence to recommend the clinical preventative action.
II-1	Evidence from well-designed controlled trials without randomization.	B	There is fair evidence to recommend the clinical preventative action.
II-2	Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group.	C	The existing evidence is conflicting and does not allow making a recommendation for or against use of the clinical preventative action; however, other factors may influence decision-making.
II-3	Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category.	D	There is fair evidence to recommend against the clinical preventative action.
		E	There is good evidence recommend against the clinical preventative action.
III	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.	L	There is insufficient evidence (in quantity and/or quality) to make a recommendation; however, other factors may influence decision-making.

Women less than 35 years old

Seven randomized controlled trials (RCTs) have been published to date comparing elective single embryo transfer (eSET) with double embryo transfer (DET)[8-13, 16] A meta-analysis of the six RCTs comparing eSET and DET at the cleavage stage reported a 16% absolute reduction in the likelihood of live birth after eSET (26%) compared to DET (42%) (RR 0.62; 95%CI, 0.53, 0.72) and a 94% relative reduction in the likelihood of multiple birth from 30% after DET to 1% after eSET (RR 0.06; 95% CI, 0.02, 0.18)[17] Two other meta-analyses have reported similar differences in live birth rate.[18, 19]

In the largest RCT, cumulative live birth rates in the eSET group after transfer of a single frozen-thawed embryo in those who did not achieve a live birth following fresh transfer was similar to fresh DET (38.8% v 43.4%, NS, per-protocol analysis), but not equivalent as the confidence interval for the relative difference in live birth rate exceeded the a priori limit of 10%.[13] It should be noted that almost all of these transfers (97.6%) were at the cleavage stage. In another trial, the cumulative live birth rate following two fresh eSET cycles was similar to that after a single fresh DET cycle (41 v 36%, NS) but with a significantly lower multiple birth rate (0 v 37%, P <0.002).[10] Non-randomized observational studies have demonstrated similar cumulative live birth rates after fresh eSET compared to fresh DET when considering subsequent frozen embryo transfer cycles.[20, 21]

A single randomized controlled trial of 48 women compared eSET and DET at the blastocyst stage. The ongoing pregnancy rate at 6.5 weeks was lower after eSET compared to DET (60.9% v 76.0%, NS). While the difference was not statistically significant, the study was underpowered.

However, the multi-fetal pregnancy rate was significantly lower with eSET (0 v 47.4%).[8] Two meta-analyses have shown significantly higher live birth rates following blastocyst versus cleavage stage transfer with an absolute difference of 7%, and odds ratios of 1.35 (95% CI 1.05, 1.74) and 1.39 (95% CI 1.10, 1.76), respectively.[22, 23] Selective application of elective single blastocyst transfer in good-prognosis patients has resulted in large reductions in the multiple pregnancy rates of entire IVF programs.[24, 25]

One of the eSET trials was conducted in a population with a more heterogeneous prognosis, as 58% of participants did not have good-quality embryos available for cleavage stage transfer. The ongoing pregnancy rate after eSET was half of that following DET (21.4 v 40.2%, $p<0.05$) and lowest of the seven eSET trials. However, the multiple pregnancy rate was still significant after DET (21.0%).[16] A recent prospective, non-randomized study of women under 36 years of age without top-quality embryos available for transfer found identical delivery rates per retrieval (26.7%) following transfer of two day two embryos or a single blastocyst stage embryo, but with significantly lower multiple deliveries after single blastocyst transfer (3.3 v 23.3%, $p<0.01$).[26]

In women under 35 years of age, an analysis of data from The Society for Assisted Reproductive Technology (SART) in the United States from 2001, showed that transfer of more than two embryos (day three or five) did not result in improvement in live birth rate, except for cleavage stage transfer in women aged 30-34 years when surplus embryos were not cryopreserved (DET v TET, 31.9 v 39.9%, $p\leq0.01$). Accordingly, there was an accompanying increase in the multiple pregnancy rate (29.3 v 41.8%, $p\leq0.01$).[27] An analysis of the CARTR database from 2001-2007 in women less than 35 years found birth rates peaked with transfer of two embryos. Multiple birth rates continued to increase with transfer of additional embryos; however, the largest incremental increase occurred with the transfer of two instead of one embryo. In cycles with cryopreservation of surplus embryos, the birth rate after eSET and eDET were almost identical.[5]

From 2007 to 2009, 43.7% of embryo transfers in Canada occurred in women under 35 years old and 63.1% of multiple births occurred in this cohort. The transfer of more than a single embryo, irrespective of day of transfer or cryopreservation of surplus embryos, was associated with multiple birth rates that ranged from 29 to 67%. In order to substantially reduce the incidence of multiples, single embryo transfer should be routinely practiced in this age group.

Recommendations (<35 years old)

For cleavage stage embryos, a single embryo should be transferred. (IA)

For women with poor prognosis, transfer of up to two embryos is reasonable. (II-2B)

For blastocyst stage embryos, a single embryo should be transferred. (IA)

For women with poor prognosis, transfer of up to two embryos might be considered. (IIIC)

Women 35 to 37 years old

A limited number of observational studies have reported good outcomes with eSET in patients between 35 and 40 years of age. The largest study found similar live birth rates in 335 women with a mean age of 37.5 years receiving eSET of a top-quality, cleavage-stage embryo compared to 585 women receiving DET largely due to absence of top-quality embryos (26.0 v 22.4%).[28] Three other observational studies of eSET at the blastocyst stage in this age group reported pregnancy and live birth rates comparable to DET in patients with favourable prognosis.[29, 30]

In an analysis of SART data from 2001, in women aged 35 to 37 years, transfer of more than two embryos, either on day three or five, did not result in improvement in live birth rate except for cleavage stage transfer when supernumerary embryos were

not cryopreserved (DET v 3ET, 25.9 v 34.2%, p≤0.01). In this sub-group, transfer of three day three embryos also resulted in a significant increase in the multiple pregnancy rate (18.3 v 35.7%, p≤0.01). For day five, DET did not result in a significant increase in live birth rate compared to SET (p>0.05).[27]

An analysis of the CARTR database from 2001-2007 in women 35 to 39 years found birth rates peaked with transfer of two and three embryos in cases with and without cryopreservation of surplus embryos, respectively. The multiple pregnancy rate continued to increase with transfer of additional embryos, irrespective of cryopreservation.[5] CARTR data from 2007 to 2009 in women aged 35 to 37 years showed similar findings. However, the transfer of more than a single embryo, irrespective of day of transfer or cryopreservation of surplus embryos, was associated with multiple birth rates that ranged from 23 to 45%. This age group represented 24.5% of fresh autologous transfers resulting in 23.8% of all multiple births.

Recommendations (35 to 37 years old)

For cleavage stage embryos, transfer of a single embryo may be reasonable. (IIIL)

For women with poor prognosis, transfer of up to two embryos is reasonable. (II-2B)

For blastocyst stage embryos, transfer of a single embryo is reasonable. (II-2B)

For women with poor prognosis, transfer of up to two embryos may be reasonable. (II-2L)

Women 38 to 39 years old

In an analysis of SART data from 2001, in women aged 38 to 40 years, transfer of more than two embryos on either day three or five, did not result in an improvement in live birth rate except for cleavage stage transfer when additional embryos were not cryopreserved. In this group, live birth rate was significantly higher with the transfer of additional embryos up to four (p<0.05), as was the multiple pregnancy rate (3ET v 4ET, 25.0 v 32.7%, p<0.05).[27] In a more recent analysis of 36103 first IVF cycles from 2000-2004 from SART with day three transfers, in women aged 38 years, clinical pregnancy and live birth rates peaked with 3ET while multiple rates increased with transfer of additional embryos. In women aged 39 years, clinical pregnancy and live birth rates increased with up to 4 embryos transferred.[31]

A small retrospective cohort study found similar pregnancy rates following transfer of two or three blastocysts; however, a significantly higher multiple pregnancy rate was seen with 3ET (39 v 79%, p<0.025).[32] In an analysis of 5569 first IVF cycles from 2000-2004 in the USA with blastocyst stage transfer, in women aged 38 to 39 years, clinical pregnancy and live birth rates peaked with DET. Multiple pregnancy rates increased with transfer of additional embryos.[33]

CARTR data from 2007 to 2009 in women aged 38 to 39 years found birth rates peaked with cleavage-stage transfer of three embryos irrespective of cryopreservation of surplus embryos. Multiple pregnancy rates continued to increase with transfer of additional embryos, and were substantially higher in cycles with cryopreservation. Blastocyst-stage transfer without cryopreservation resulted in increasing live birth and multiple birth rates with transfer of additional embryos. The increase in live birth rate with transfer of three or more embryos was modest, being 2.8% higher than DET; however, the associated rise in multiple birth rate was substantial (14.5% higher that DET). With cryopreservation, live birth rate peaked with the transfer of two blastocysts.

Recommendations (38 to 39 years old)

For cleavage stage embryos, transfer of up to two embryos is reasonable. (II-2B)

For women with poor prognosis, transfer of up to three embryos is reasonable. (II-2B)

For blastocyst stage embryos, transfer of one to two embryos is reasonable. (II-2B)

For women with poor prognosis, transfer of up to two embryos may be reasonable. (II-2B)

Women 40 to 42 years old

Women over the age of 40 years generally have poor success rates overall, and a low incidence of multiple pregnancy and birth in spite of higher numbers of embryos per transfer.[34, 35] Stern et al. reported an analysis of 36103 first IVF cycles from 2000-2004 in the USA with day three transfers. In women aged 40 years, live birth and twin rates increased with increasing number of embryos transferred, while in women aged 41 to 42 years, similar trends were seen with transfer of up to five embryos. In this group, the twin rate was only 5% with transfer of up to 5 embryos.[31] In a similar analysis of 5569 blastocyst stage transfers in women aged 40 years, clinical pregnancy and live birth rates peaked with 3ET, while in women aged 41 to 42 years, clinical pregnancy and live birth rates peaked with DET. Multiple pregnancy rates increased with transfer of additional embryos.[33]

An analysis of the CARTR database from 2001-2007 in women 40 years and older found birth rates peaked with transfer of five or more and four embryos in cases with and without cryopreservation of surplus embryos, respectively. Multiple pregnancy rates peaked with the transfer of four embryos without cryopreservation and two embryos with.[5] More recent data from CARTR (2007-2010) showed increasing live birth and multiple pregnancy rates with increasing numbers of cleavage-stage embryo transferred, irrespective of cryopreservation. However, with cryopreservation, the increase in live birth rate was minimal with transfer of four or more embryos (2.3%) with a slightly higher increase in multiple pregnancy rate (4.9%). For blastocyst-stage transfer, omitting eSET as only 10 such transfers occurred in the period analyzed, live birth rate increased with increasing numbers of embryos transferred without cryopreservation, while multiple pregnancy rates peaked at 3ET. In cycles with cryopreservation of surplus embryos, live birth rates peaked with 3ET while multiple pregnancy rates continued to rise with increasing numbers of embryos transferred.

Recommendations (40 to 42 years old)

For cleavage stage embryos, transfer of up to three embryos may be considered. (II-2C)

For women with poor prognosis, transfer of up to four embryos may be considered. (II-2C)

For blastocyst stage embryos, transfer of up to two embryos may be considered. (IIIL)

For women with poor prognosis, transfer of up to three embryos may be considered. (IIIL)

Women more than 42 years old

A retrospective cohort of day three embryo transfers from 1998-2003 in women over 40 years of age with a mean age of 42 years demonstrated improved live birth rate with the transfer of five embryos. The reported live birth rate per transfer was 22.6% with a 36.7% twin delivery rate. No high-order multiple were reported in this cohort.[36] Through analyses of embryo transfers from 2000-2004 in the USA, Stern et al. recommends a maximum of five cleavage-stage and three blastocyst-stage embryos be transferred in women aged 43 to 44 years, although data upon which these recommendations are made was not presented.[31, 33] From 2007-2010, the CARTR database reports 3.5% of all embryo transfers occurred in women over 42 years old.

Recommendations (>42 years old)

For cleavage stage embryos, transfer of up to five embryos may be considered. (IIIL),

For blastocyst stage embryos, transfer of up to three embryos may be considered. (IIIL)

Women receiving donor eggs

Several studies have reported high implantation rates in donor egg IVF cycles that are determined by the prognosis of the oocyte donor, and largely independent of the age of the recipient.[37-39]

Recommendation

In donor egg cycles, the number of embryos transferred should be guided by the age and prognosis of the egg donor. (II-2A)

Women with medical contraindications to multi-fetal gestation.

Recommendation

In women with absolute or significant relative contraindication to mutli-fetal gestation, transfer of a single embryo, either cleavage or blastocyst-stage, is recommended. (IIIL)

Frozen-thawed cycles

In 2007, CARTR reported a multiple delivery rate of 24.1% in frozen-thawed embryo transfer (FET) cycles.[1] FET has been shown to contribute significantly to multiple pregnancies if multiple embryos are transferred[40-43] High implantation rates after cryopreservation has been reported,[44, 45] and many studies have reported similar implantation rates after cryopreservation compared with fresh embryos.[41, 42, 46]

Recommendation

The age-related recommendations for fresh embryo transfer should be used for frozen embryo transfers, using the woman's age at the time of embryo cryopreservation. (II-2B)

Cryopreserved oocytes

Although embryos derived from slow-frozen and thawed oocytes result in poor implantation rates, vitrified and warm oocytes provide similar implantation rates to embryos derived from fresh oocytes.[47-51]

Recommendation

The age-related recommendations for fresh embryo transfer should be used for embryos derived from vitrified-warmed oocytes (IA)

Summary

From 2007 to 2009 in Canada, 68% of all fresh transfers of embryos derived from autologous oocytes occurred in women up to 37 years of age, resulting in 87% of all multiple births. This younger cohort is the obvious target for reduction of multiple pregnancies. In order to meet the multiple pregnancy targets set at the 2009 roundtable meeting and reaffirmed by the CFAS, reductions in the numbers of embryos transferred must be made. All IVF practitioners are encouraged to develop embryo transfer policies that work towards these targets. In the absence of such policies, the recommendations within this guideline should guide embryo transfer practices.

Table 2. Summary of embryo transfer recommendations

Table 2. Recommended maximum numbers of embryos to transfer.					
	Age (years)				
	<35	35-37	38-39	40-42	>42
Cleavage Stage	1	1	2	3	5
Poor Prognosis	2	2	3	4	5
Blastocyst Stage	1	1	1-2	2	3
Poor Prognosis	2	2	2	3	3

1. *Gunby J, Bissonnette F, Librach C, Cowan L. Assisted reproductive technologies (ART) in Canada: 2007 results from the Canadian ART Register. Fertil Steril 95:542, 2011.*
2. *Pinborg A, Loft A, Nyboe Andersen A. Neonatal outcome in a Danish national cohort of 8602 children born after in vitro fertilization or intracytoplasmic sperm injection: the role of twin pregnancy. Acta Obstet Gynecol Scand 83:1071, 2004.*
3. *Ethical guidelines in the prevention of iatrogenic multiple pregnancy. Eur J Obstet Gynecol Reprod Biol 96:209, 2001.*
4. *Min JK, Claman P, Hughes E. Guidelines for the number of embryos to transfer following in vitro fertilization. J Obstet Gynaecol Can 28:799, 2006.*
5. *Gunby J. Multiple births from assisted reproductive technologies (ART) in Canada. A report from the Canadian ART Register (CARTR). Prevention of Multiple Births Associated with Infertility Treatments in Canada: A Scientific Roundtable 2009.*
6. *Min J, Yuzpe A, Greene C, Leveille MC, Bissonnette F, Collins J, Hughes E, Cook JL. Prevention of Multiple Births Associated with Infertility Treatments: A Canadian Framework. 2010.*
7. *Reduction of multiple pregnancy risk associated with IVF/ICSI. IVF Medical Directors of Canada position statement 2012. Canadian Fertility and Andrology Society 2012.*
8. *Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. Fertil Steril 81:551, 2004.*
9. *Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. Hum Reprod 14:2581, 1999.*
10. *Lukassen HG, Braat DD, Wetzels AM, Zielhuis GA, Adang EM, Scheenjes E, Kremer JA. Two cycles with single embryo transfer versus one cycle with double embryo transfer: a randomized controlled trial. Hum Reprod 20:702, 2005.*
11. *Martikainen H, Tiitinen A, Tomas C, Tapanainen J, Orava M, Tuomivaara L, Vilska S, Hyden-Granskog C, Hovatta O. One versus two embryo transfer after IVF and ICSI: a randomized study. Hum Reprod 16:1900, 2001.*
12. *Moustafa MK, Sheded SA, El Aziz Mousta MA. Elective single embryo transfer versus double embryo transfer in assisted reproduction. Reprod Biomed Online 17:82, 2008.*
13. *Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, Bergh C. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med 351:2392, 2004.*
14. *New grades for recommendations from the Canadian Task Force on Preventive Health Care. CMAJ 169:207, 2003.*

15. *Woolf SH, Battista RN, Angerson GM, Logan AG, Eel W. Canadian Task Force on the Periodic Health Exam. Ottawa: Canada Communication Group p.xxxvii, 1994.*
16. *van Montfoort AP, Fiddelers AA, Janssen JM, Derhaag JG, Dirksen CD, Dunselman GA, Land JA, Geraedts JP, Evers JL, Dumoulin JC. In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. Hum Reprod 21:338, 2006.*
17. *Gelbaya TA, Tsoumpou I, Nardo LG. The likelihood of live birth and multiple birth after single versus double embryo transfer at the cleavage stage: a systematic review and meta-analysis. Fertil Steril 94:936, 2010.*
18. *Baruffi RL, Mauri AL, Petersen CG, Nicoletti A, Pontes A, Oliveira JB, Franco JGJ. 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 7:36, 2009.*
19. *McLernon DJ, Harrild K, Bergh C, Davies MJ, de Neubourg D, Dumoulin JC, Gerris J, Kremer JA, Martikainen H, Mol BW, Norman RJ, Thurin-Kjellberg A, Tiitinen A, van Montfoort AP, van Peperstraten AM, Van Royen E, Bhattacharya S. Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. BMJ 341:c6945, 2010.*
20. *Fauque P, Jouannet P, Davy C, Guibert J, Viallon V, Epelboin S, Kunstmann JM, Patrat C. Cumulative results including obstetrical and neonatal outcome of fresh and frozen-thawed cycles in elective single versus double fresh embryo transfers. Fertil Steril 94:927, 2010.*
21. *Henman M, Catt JW, Wood T, Bowman MC, de Boer KA, Jansen RP. 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 84:1620, 2005.*
22. *Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev CD002118, 2007.*
23. *Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, Devroey P. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. Hum Reprod 23:91, 2008.*
24. *Khalaf Y, El-Toukhy T, Coomarasamy A, Kamal A, Bolton V, Braude P. Selective single blastocyst transfer reduces the multiple pregnancy rate and increases pregnancy rates: a pre- and postintervention study. BJOG 115:385, 2008.*
25. *Ryan GL, Sparks AE, 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 88:354, 2007.*
26. *Guerif F, Frapsauce C, Chavez C, Cadoret V, Royere D. Treating women under 36 years old without top-quality embryos on day 2: a prospective study comparing double embryo transfer with single blastocyst transfer. Hum Reprod 26:775, 2011.*
27. *Kissin DM, Schieve LA, Reynolds MA. Multiple-birth risk associated with IVF and extended embryo culture: USA, 2001. Hum Reprod 20:2215, 2005.*
28. *Veleva Z, Vilska S, Hyden-Granskog C, Tiitinen A, Tapanainen JS, Martikainen H. Elective single embryo transfer in women aged 36-39 years. Hum Reprod 21:2098, 2006.*
29. *Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA. Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age. Fertil Steril 93:1837, 2010.*
30. *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 92:1895, 2009.*
31. *Stern JE, Goldman MB, Hatasaka H, MacKenzie TA, Surrey ES, Racowsky C. Optimizing the number of cleavage stage embryos to transfer on day 3 in women 38 years of age and older: a Society for Assisted Reproductive Technology database study. Fertil Steril 91:767, 2009.*
32. *Milki AA, Fisch JD, Behr B. Two-blastocyst transfer has similar pregnancy rates and a decreased multiple gestation rate compared with three-blastocyst transfer. Fertil Steril 72:225, 1999.*
33. *Stern JE, Goldman MB, Hatasaka H, MacKenzie TA, Racowsky C, Surrey ES. Optimizing the number of blastocyst stage embryos to transfer on day 5 or 6 in women 38 years of age and older: a Society for Assisted Reproductive Technology database study. Fertil Steril 91:157, 2009.*
34. *Bromer JG, Sakkas D, Siano LJ, Benadiva CA, Patrizio P. Reproductive efficiency of women over the age of 40 and the low risk of multiple pregnancies. Reprod Biomed Online 19 Suppl 4:4316, 2009.*

35. *Grimbizis G, Vandervorst M, Camus M, Tournaye H, Van Steirteghem A, Devroey P. Intracytoplasmic sperm injection, results in women older than 39, according to age and the number of embryos replaced in selective or non-selective transfers. Hum Reprod 13:884, 1998.*
36. *Combelles CM, Orasanu B, Ginsburg ES, Racowsky C. Optimum number of embryos to transfer in women more than 40 years of age undergoing treatment with assisted reproductive technologies. Fertil Steril 84:1637, 2005.*
37. *Check JH, Jamison T, Check D, Choe JK, Brasile D, Cohen R. Live delivery and implantation rates of donor oocyte recipients in their late forties are similar to younger recipients. J Reprod Med 56:149, 2011.*
38. *Clua E, Tur R, Coroleu B, Boada M, Barri PN, Veiga A. Analysis of factors associated with multiple pregnancy in an oocyte donation programme. Reprod Biomed Online 21:694, 2010.*
39. *Soderstrom-Anttila V, Vilska S, Makinen S, Foudila T, Suikkari AM. Elective single embryo transfer yields good delivery rates in oocyte donation. Hum Reprod 18:1858, 2003.*
40. *Berin I, Engmann LL, Benadiva CA, Schmidt DW, Nulsen JC, Maier DB. Transfer of two versus three embryos in women less than 40 years old undergoing frozen transfer cycles. Fertil Steril 93:355, 2010.*
41. *Berin I, McLellan ST, Macklin EA, Toth TL, Wright DL. Frozen-thawed embryo transfer cycles: clinical outcomes of single and double blastocyst transfers. J Assist Reprod Genet 2011.*
42. *Edgar DH, Bourne H, Speirs AL, McBain JC. A quantitative analysis of the impact of cryopreservation on the implantation potential of human early cleavage stage embryos. Hum Reprod 15:175, 2000.*
43. *Hyden-Granskog C, Unkila-Kallio L, Halttunen M, Tiitinen A. Single embryo transfer is an option in frozen embryo transfer. Hum Reprod 20:2935, 2005.*
44. *Ebner T, Vanderzwalmen P, Shebl O, Urdl W, Moser M, Zech NH, Tews G. Morphology of vitrified/warmed day-5 embryos predicts rates of implantation, pregnancy and live birth. Reprod Biomed Online 19:72, 2009.*
45. *Goto S, Kadowaki T, Tanaka S, Hashimoto H, Kokeguchi S, Shiotani M. Prediction of pregnancy rate by blastocyst morphological score and age, based on 1,488 single frozen-thawed blastocyst transfer cycles. Fertil Steril 95:948, 2011.*
46. *Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M, Ling X, Duan T, Tong GQ. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles--time for a new embryo transfer strategy? Fertil Steril 95:1691, 2011.*
47. *Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod 25:2239, 2010.*
48. *Garcia JI, Noriega-Portella L, Noriega-Hoces L. Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program. Hum Reprod 26:782, 2011.*
49. *Levi Setti PE, Albani E, Novara PV, Cesana A, Morreale G. Cryopreservation of supernumerary oocytes in IVF/ICSI cycles. Hum Reprod 21:370, 2006.*
50. *Smith GD, Serafini PC, Fioravanti J, Yadid I, Coslovsky M, Hassun P, Alegretti JR, Motta EL. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vitrification. Fertil Steril 94:2088, 2010.*
51. *Trokoudes KM, Pavlides C, Zhang X. Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. Fertil Steril 95:1996, 2011.*