

Elective single embryo transfer in women aged 36–39 years

Zdravka Veleva¹, Sirpa Vilska², Christel Hydén-Granskog³, Aila Tiitinen³,
Juha S.Tapanainen¹ and Hannu Martikainen^{1,4}

¹Department of Obstetrics and Gynecology, University of Oulu, Oulu, ²Infertility Clinic, Family Federation of Finland and ³Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland

⁴To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, University of Oulu, P.O. Box 5000, FIN-90014 Oulu, Finland. E-mail: hmartika@cc.oulu.fi

BACKGROUND: The elective single embryo transfer policy is the only effective strategy known to minimize the risk of multiple pregnancy. However, little is known about its applicability to women older than 35 years. **METHODS:** Analysis was carried out on 1224 fresh IVF/ICSI cycles with embryo transfer and 828 frozen embryo transfer (FET) cycles of women aged 36–39 years. In the fresh cycles, 335 elective single top quality embryo (eSET), 110 elective single non top quality embryo (nt-eSET), 194 compulsory single embryo (cSET) and 585 double embryo transfers (DET) were carried out. **RESULTS:** Pregnancy rate/embryo transfer (33.1 versus 29.9%) and live birth rate (26.0 versus 21.9%) in fresh cycles did not differ significantly between the eSET and the DET groups. However, women in the eSET group had a higher cumulative pregnancy rate (54.0% versus 35.0%) and a higher cumulative live birth rate (41.8% versus 26.7%, $P < 0.0001$) compared with those in the DET group. The cumulative multiple birth rate in the eSET group was 1.7%, whereas in the DET group it was 16.6% ($P < 0.0001$). **CONCLUSIONS:** The eSET policy can be applied also to patients aged 36–39 years, reducing the risk of multiple birth and increasing the safety of assisted reproduction technique (ART) in this age group.

Key words: cryopreservation/elective single embryo transfer/female age/ICSI outcome/IVF

Introduction

One of the most serious complications of IVF treatment is the high multiple birth rate, which leads to increased perinatal mortality and morbidity, as well as maternal complications (The ESHRE Capri Workshop Group, 2000; Gerris *et al.*, 2004; van Montfoort *et al.*, 2005). After the establishment of reliable embryo selection criteria (Van Royen *et al.*, 1999), elective single embryo transfer (eSET), combined with an effective cryopreservation programme, has been shown to result in a high cumulative pregnancy rate of at least 50% per oocyte retrieval (Tiitinen *et al.*, 2003; Martikainen *et al.*, 2004).

eSET was first used in women with medical contraindications to multiple gestations (Vilska *et al.*, 1999). Randomized trials have shown the effectiveness of eSET in selected groups of subjects who are in their first IVF/ICSI cycle and aged <36 years (Gerris *et al.*, 1999; Martikainen *et al.*, 2001; Thurin *et al.*, 2004; Lukassen *et al.*, 2005). Very recently, van Montfoort *et al.* showed that the outcome of eSET remains good even in women under the age of 38 years (van Montfoort *et al.*, 2005).

Age is an important determinant of the success rate of infertility treatment. Although increasing age is associated with a lower chance of pregnancy after IVF/ICSI (Templeton *et al.*, 1996), the risk of multiple pregnancy seems to be elevated even in women of over 35 years of age. According to US national surveys, multiple births in women aged 35–39 years

constitute 12–21% of all live births in this age group if two embryos are transferred (Schieve *et al.*, 1999; Reynolds *et al.*, 2001; Kissin *et al.*, 2005). Multiple pregnancies represent an increased health risk in older women, because the frequency of complications such as gestational diabetes and pre-eclampsia is higher in these subjects (Saftlas *et al.*, 1990; Xiong *et al.*, 2001).

The use of eSET in Finland has been constantly increasing to maximize the safety of IVF/ICSI. By employing an eSET policy, an overall multiple pregnancy rate of less than 10% has been achieved in many clinics (Soderstrom-Anttila *et al.*, 2003; Tiitinen *et al.*, 2003; Martikainen *et al.*, 2004). The purpose of this study was to further decrease the overall multiple pregnancy rate. To this end, we evaluated the outcome of eSET cycles in older women (aged 36–39 years at the time of oocyte retrieval) and compared the results with those previously observed in younger women after eSET.

Materials and methods

During the years 2000–2003, a total of 1224 fresh embryo transfers were carried out in women aged 36–39 years at the Infertility Clinics of Oulu and Helsinki University Hospitals and the Family Federation of Finland (Figure 1). In 639 cases, SET was performed, and in 445 cycles, extra embryos were frozen after elective transfer of a top quality (eSET, 335 cases) or non top quality embryo (nt-eSET, 110 cases). Only one embryo was available for transfer in 194 cycles (compulsory

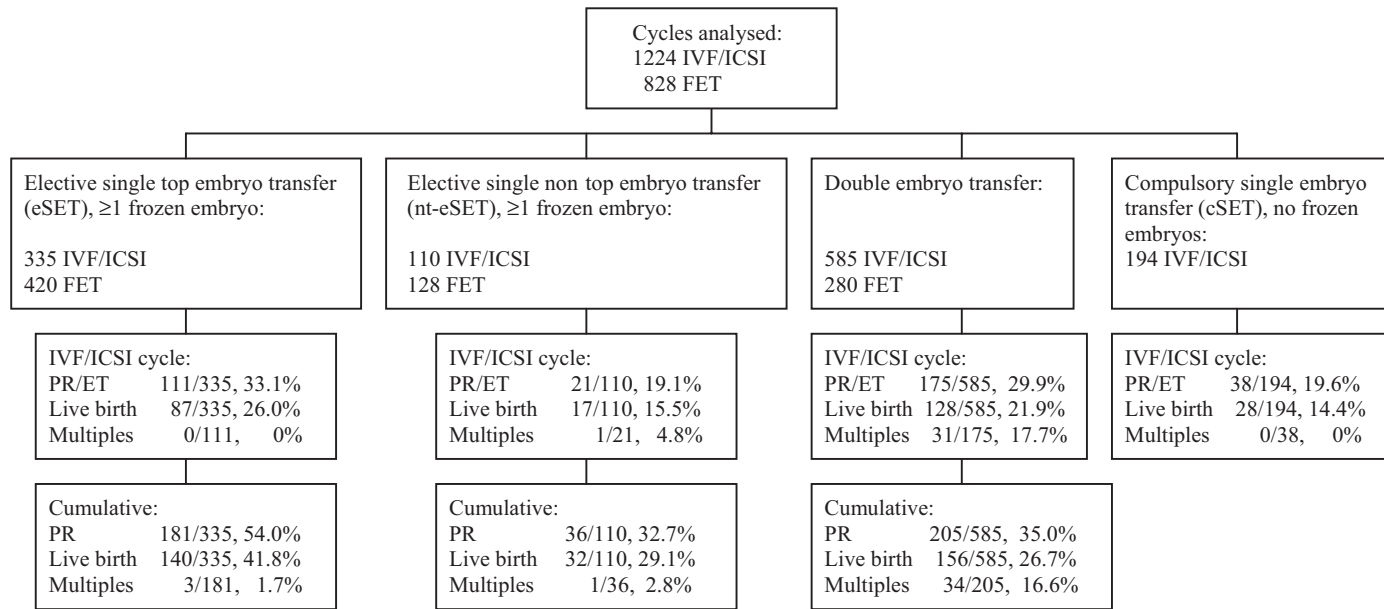


Figure 1. Study design and clinical outcome.

SET, cSET), in which a top quality embryo was transferred in 47 cycles and a non top quality one in 147 cycles. A top quality embryo was defined as having normal fertilization [2 pronuclei (PN)], four blastomeres on day 2 or ≥8 blastomeres for a transfer on day 3, less than 20% fragmentation and no multinuclear blastomeres. Double embryo transfers (DETs) were carried out in 585 cycles, and in 287 cases extra embryos were frozen.

The type of embryo transfer (eSET, nt-eSET or DET) was determined in each participating clinic on the basis of the patient characteristics and response to stimulation. Women with a good response to stimulation, resulting in at least one top quality embryo and at least one embryo of freezeable quality, were eligible for eSET, especially if they were undergoing their first or second treatment cycle. An nt-eSET was performed in cases where ovarian response was good, and there were several non top quality embryos of freezeable quality.

Following the fresh embryo transfers, a total of 828 frozen embryo transfers (FETs) were performed in 2000–2004: 420 cycles in the eSET group, 128 in the nt-eSET group and 280 in the DET group. Characteristics of the study subjects are summarized in Table I. Data

were collected retrospectively as a randomized study was considered ethically difficult because of lower pregnancy rates in older women in general.

Ovarian stimulation

Ovarian stimulation was performed using the long protocol, as described previously (Vilksa *et al.*, 1999; Tiitinen *et al.*, 2001; Veleva *et al.*, 2005). In 11.2% (137/1224) of the stimulations, a protocol with GnRH antagonist was used. Oocytes/embryos were cultured as previously described (Tomás *et al.*, 1998; Hydén-Granskog *et al.*, 2005). One or two embryos were transferred to the uterine cavity on day 2 or day 3 after oocyte retrieval. Embryos were frozen on the day of embryo transfer using a slow-freezing protocol with 1,2-propanediol as cryoprotectant (Hydén-Granskog *et al.*, 2005).

FET cycles

After thawing, embryos were transferred during a natural cycle, 3 days after an LH surge measured by means of a home test kit (Clearplan; Unipath, Bedford, UK). In cases of anovulatory cycles, hormone

Table I. Characteristics of the study groups

	eSET	nt-eSET	DET	cSET	<i>P</i> value
<i>N</i>	335	110	585	194	
Age ^d (years)	37.5 ± 1.1	37.3 ± 1.0	37.6 ± 1.1	37.6 ± 1.1	0.07
BMI ^d (kg/m ²)	23.4 ± 3.9	23.4 ± 3.7	23.3 ± 3.7	23.9 ± 4.8	0.4
Primary infertility (%)	146 (43.6)	49 (44.5)	272 (46.5)	75 (38.7)	0.3
Diagnosis (%)					0.06
Endometriosis	36 (13.2)	5 (6.6)	56 (11.3)	19 (12.2)	
Anovulation	16 (4.9)	8 (10.5)	31 (6.2)	9 (5.8)	
Male factor	70 (25.6)	25 (32.9)	156 (31.4)	61 (39.1)	
Tubal facto	55 (20.2)	19 (25.0)	103 (20.7)	30 (19.2)	
Unexplained	93 (34.1)	19 (25.0)	135 (27.2)	36 (23.1)	
Other	3 (1.1)	0 (0)	16 (3.2)	1 (0.6)	
Rank of cycle ^d	2.5 ± 2.7 ^{a,b,c}	4.2 ± 4.0 ^a	3.0 ± 2.7 ^b	2.9 ± 2.4 ^c	<0.0001
ICSI cycles (%)	79 (23.6)	29 (26.4)	199 (34.0)	61 (31.4)	0.008

DET, double embryo transfers; SET, single top quality embryo.

^{a,b,c}Between groups, means with the same superscripts are significantly different (ANOVA, Tukey *post hoc* test, *P* < 0.05).

^dMean ± SD.

replacement with estradiol valerate and vaginal micronized progesterone was used.

Clinical pregnancies were confirmed by transvaginal ultrasonography at gestational week 6–7. Cumulative pregnancy rate was calculated after fresh and FET cycles performed until now by taking into account only the first pregnancy/oocyte retrieval.

Statistical analysis

Variables in the study groups were compared by using chi-square tests and two-tailed *t*-tests, with $P < 0.05$ as the limit of significance. ANOVA was used for comparison of continuous variables in more than two study groups, followed by the Tukey multiple comparisons test as a *post hoc* analysis to determine where significant effects occurred. The primary outcome measures were the clinical pregnancy rate per fresh embryo transfer (pregnancy rate/embryo transfer) and the cumulative pregnancy rate after fresh embryo transfers and FETs. The multiple pregnancy rate was chosen as a secondary outcome measure. Statistical analysis was performed with SPSS 12.0.1 software (SPSS, Chicago, IL, USA).

Results

Compared with the DET and cSET groups, women in the eSET and nt-eSET groups needed a smaller dose of gonadotropin for stimulation (Table II). Furthermore, more oocytes were collected and fertilized in the eSET and nt-eSET groups. The pregnancy rate/embryo transfer (111/335, 33.1% versus 175/585, 29.9%, $P = 0.3$) and the live birth rate (87/335, 26.0% versus 131/585, 22.4%, $P = 0.2$) were similar in the eSET and DET groups, respectively. These values were higher than the pregnancy rate/embryo transfer (21/110, 19.1%, $P = 0.02$) and live birth rate (17/110, 15.5%, $P = 0.06$) in the nt-eSET group, although the difference in live birth rate did not reach statistical significance. In the cSET group, the pregnancy rate/embryo transfer (38/194, 19.6%) and the live birth rate (28/194, 14.4%) were also lower than in the eSET and DET groups ($P < 0.01$) but similar to those in the nt-eSET group ($P > 0.9$). In all participating clinics, pregnancy rate/embryo transfer were similar (26.5–32.0%, $P = 0.4$). The fertilization mode was not found to affect the pregnancy rate (IVF: 247/856, 28.9% versus ICSI: 98/368, 26.6%, $P = 0.5$).

After the transfer of fresh embryos, there were 31 twin deliveries in the DET group (17.7%), none in the eSET group, one in the nt-eSET group (4.8%) and none in the cSET group ($P < 0.0001$). The number of miscarriages was similar in the four groups: 21 in the eSET group (18.9%), 38 in the DET group (21.7%), three in the nt-eSET group (14.3%) and 10 in the cSET group (26.3%, $P = 0.7$). There were three cases of

ectopic pregnancy in the eSET group, six in the DET group and one in the nt-eSET group.

In the cSET group, women who had a top quality embryo tended to have a higher pregnancy rate/embryo transfer (14/47, 29.8% versus 24/147, 16.3%, $P = 0.06$) and live birth rate (9/47, 19.1% versus 19/147, 12.9%, $P = 0.3$) than those with a non top quality embryo transferred. The pregnancy and live birth rates after cSET with a top quality embryo were similar to those after eSET with a top quality embryo ($P > 0.6$).

In the FET cycles, more embryos were transferred in women who had had DET in the fresh cycle (1.6 ± 0.6) than in those who had had eSET (1.4 ± 0.5 , $P < 0.0001$) or nt-eSET group (1.3 ± 0.5 , $P < 0.0001$). The pregnancy rate/FET did not differ significantly in the eSET and DET groups (eSET: 98/420, 23.3% versus DET: 52/280, 18.6%, $P = 0.7$) but was lower in the nt-eSET group (16/128, 12.5%, $P = 0.02$). Live birth rates were comparable in the three groups (eSET: 14.8%, DET: 11.4%, nt-eSET: 11.7%, $P = 0.4$). Three pairs of twins were born in the eSET group (3.1%). In the DET group, there were two deliveries of twins and one of triplets (multiple birth rate of 5.9%). Fourteen pregnant women were lost to follow-up: 11 in the eSET group, one in the nt-eSET group and two in the DET group.

Until now, the cumulative pregnancy rate in the eSET group is 54.0% (181/335) and 35.0% (205/585) in the DET group ($P < 0.0001$). Similarly, the live birth rate/oocyte retrieval is 41.5% (139/335) in the eSET group and 26.7% (156/585) in the DET group ($P < 0.0001$). In the DET group, there have been 34 multiple births (16.6%); three twin births have occurred in the eSET group (1.7%) and one in the nt-eSET group (2.8%, $P < 0.0001$).

Discussion

The present results demonstrate for the first time that the eSET policy is applicable to women in the age group of 36–39 years. In this age group, a clinical pregnancy rate of 33% was achieved after eSET, which is similar to that (30.8–34.5%) found in previous studies on eSET in younger women (Martikainen *et al.*, 2001; Tiitinen *et al.*, 2003; Kissin *et al.*, 2005). During the study period, the proportion of women who underwent eSET was 27.4%, whereas during the same time period eSET was performed in 52.8% of all transfers in subjects aged <36 years. The proportion of eSET cycles has increased in all four clinics, and during the years 2004–2005, it was about 40% in the age group 36–39 years. The high pregnancy rate

Table II. Characteristics of the fresh treatment cycles

	eSET	nt-eSET	DET	cSET	<i>P</i> value
Gonadotropin dose, amp	26.4 ± 9.1 ^{a,b}	27.9 ± 14.3 ^{c,d}	31.6 ± 13.7 ^{a,c,e}	37.0 ± 17.5 ^{b,d,e}	<0.0001
Collected oocytes	12.4 ± 6.8 ^{a,b}	11.9 ± 6.7 ^{c,d}	10.2 ± 5.8 ^{a,c,e}	5.5 ± 4.6 ^{b,d,e}	<0.0001
Fertilized oocytes	7.6 ± 4.2 ^{a,b}	7.2 ± 3.5 ^c	6.2 ± 3.8 ^{a,d}	2.2 ± 1.9 ^{b,c,d}	<0.0001
Embryos frozen	5.3 ± 3.4 ^{a,b,c}	4.2 ± 2.9 ^{c,d,e}	2.1 ± 2.8 ^{b,d,f}	0.0 ± 0.0 ^{c,e,f}	<0.0001

DET, double embryo transfers; SET, single top quality embryo.

^{a,b,c,d,e,f}Between groups, means with the same superscripts are significantly different (ANOVA, Tukey *post hoc* test, $P < 0.05$).

Data are mean ± SD.

observed in women with good quality embryos, even in this age group, suggests that embryo morphology, rather than calendar age, determines the chance of pregnancy. Therefore, selection for eSET should be on the basis of embryo quality rather than age.

Our eSET population had a live birth rate (26%) similar to that reported after eSET in younger women (range 27.2–29.7%) (Martikainen *et al.*, 2001, 2004; Tiitinen *et al.*, 2003; Thurin *et al.*, 2004). Thus, although the chance of a birth in unselected IVF/ICSI cycles among women over 35 years is reduced compared with younger women (Engmann *et al.*, 2001), the selection of patients for eSET on individual basis ensures acceptable outcome. In this study, the miscarriage rate after eSET was 18.9%, which is slightly higher than that previously reported in younger women (15.3–16.8%) (Martikainen *et al.*, 2004; Thurin *et al.*, 2004). These results show that in women selected for eSET, the risk of miscarriage is lower than that (25%) in the general population as regards maternal age of 35–39 years (Nybo Andersen *et al.*, 2000). On the contrary, the miscarriage rate may increase by age even if a top embryo has been transferred, as has been shown by comparing women over and under 30 years of age (De Neubourg *et al.*, 2004).

The cumulative pregnancy rate achieved after FETs is the best indicator of the efficacy of assisted reproduction technique (ART). In this study, after one or two FET cycles, the cumulative pregnancy rate was 54%, with a live birth rate of over 40%. The pregnancy rate in this selected group of older women is very satisfactory, although in younger women, we have reported even higher pregnancy rates, up to 60–70% (Tiitinen *et al.*, 2001; Martikainen *et al.*, 2004).

In this study, eSET was also performed in a smaller group of subjects with only non top quality embryos. As expected, the pregnancy rate in fresh cycles was similarly low as reported previously (18–19%) (Tiitinen *et al.*, 2001; Saldeen and Sundstrom, 2005). However, through several FET cycles, this group reached an acceptable cumulative pregnancy rate of 32.7%. At the same time, the multiple birth rate was only 2.8%, which is much lower than that (18.6%) in the DET group with two non top quality embryos transferred. Hence, even if no top quality embryo is available, eSET seems to be beneficial to women who are at increased risk of complications related to multiple pregnancy.

Although cases with a poorer prognosis were selected for DET, the multiple birth rate was still above the target rate of 10% recommended by ESHRE (ESHRE Campus Course Report, 2001). Increasing age aggravates the health risks presented by pregnancy, and this is even more so in cases of multiple pregnancy. In singleton pregnancies, the risk of pre-eclampsia increases by 30% for every additional year of age past 34 years (Saftlas *et al.*, 1990) and is three times higher in the presence of a twin pregnancy (Duckitt and Harrington, 2005). Gestational diabetes is also more frequent in women aged >35 years (Xiong *et al.*, 2001), and the incidence increases further in cases of multiple pregnancy (Walker *et al.*, 2004).

Women in whom cSET of a top quality embryo was performed had a live birth rate similar to that after eSET. These findings further extend the results of our earlier studies on women with cSET, who had a pregnancy rate between 14 and

20% (Vilksa *et al.*, 1999; Tiitinen *et al.*, 2001; Martikainen *et al.*, 2001). Even if the ovarian response is significantly diminished and only one embryo is available, good embryo quality ensures satisfactory treatment outcome.

In conclusion, this study demonstrates that the eSET policy is applicable to women older than 35 years to increase the safety of ART and minimize the health risks faced by these women. The results support the view that embryo morphology is a more important determinant of outcome than age, at least until the age of 40 years.

Acknowledgements

This work was supported by grants from the Sigrid Jusélius Foundation and the Oulu University Scholarship Foundation.

References

- The ESHRE Capri Workshop Group (2000) Multiple gestation pregnancy. *Hum Reprod* 15,1856–1864.
- ESHRE Campus Course Report (2001) Prevention of twin pregnancies after IVF/ICSI by single embryo transfer. *Hum Reprod* 16,790–800.
- De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M and Elseviers M (2004) Single top quality embryo transfer as a model for prediction of early pregnancy outcome. *Hum Reprod* 19,1476–1479 [Epub 29 April 2004].
- Duckitt K and Harrington D (2005) Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *BMJ* 330,565 [Epub 2 March 2005].
- Engmann L, Maconochie N, Tan SL and Bekir J (2001) Trends in the incidence of births and multiple births and the factors that determine the probability of multiple birth after IVF treatment. *Hum Reprod* 16,2598–2605.
- Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M and Valkenburg M (1999) Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. *Hum Reprod* 14,2581–2587.
- Gerris J, De Sutter P, De Neubourg D, Van Royen E, Vander Elst J, Mangelschots K, Vercruyssen M, Kok P, Elseviers M and Annemans L *et al.* (2004) A real-life prospective health economic study of elective single embryo transfer versus two-embryo transfer in first IVF/ICSI cycles. *Hum Reprod* 19,917–923 [Epub 27 February 2004].
- Hyden-Granskog C, Unkila-Kallio L, Halttunen M and Tiitinen A (2005) Single embryo transfer is an option in frozen embryo transfer. *Hum Reprod* 24,24.
- Kissin DM, Schieve LA and Reynolds MA (2005) Multiple-birth risk associated with IVF and extended embryo culture: USA, 2001. *Hum Reprod* 20,2215–2223 [Epub 14 April 2005].
- Lukassen HG, Braat DD, Wetzels AM, Zielhuis GA, Adang EM, Scheenjes E and Kremer JA (2005) Two cycles with single embryo transfer versus one cycle with double embryo transfer: a randomized controlled trial. *Hum Reprod* 20,702–708 [Epub 23 December 2004].
- Martikainen H, Tiitinen A, Tomas G, Tapanainen J, Orava M, Tuomivaara L, Vilksa S, Hyden-Granskog C and Hovatta O (2001) One versus two embryo transfer after IVF and ICSI: a randomized study. *Hum Reprod* 16,1900–1903.
- Martikainen H, Orava M, Lakkakorpi J and Tuomivaara L (2004) Day 2 elective single embryo transfer in clinical practice: better outcome in ICSI cycles. *Hum Reprod* 19,1364–1366 [Epub 22 April 2004].
- Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J and Melbye M (2000) Maternal age and fetal loss: population based register linkage study. *BMJ* 320,1708–1712.
- Reynolds MA, Schieve LA, Jeng G, Peterson HB and Wilcox LS (2001) Risk of multiple birth associated with in vitro fertilization using donor eggs. *Am J Epidemiol* 154,1043–1050.
- Saftlas AF, Olson DR, Franks AL, Atrash HK and Pokras R (1990) Epidemiology of preeclampsia and eclampsia in the United States, 1979–86. *Am J Obstet Gynecol* 163,460–465.
- Saldeen P and Sundstrom P (2005) Would legislation imposing single embryo transfer be a feasible way to reduce the rate of multiple pregnancies after IVF treatment? *Hum Reprod* 20,4–8 [Epub 24 November 2004].
- Schieve LA, Peterson HB, Meikle SF, Jeng G, Danel I, Burnett NM and Wilcox LS (1999) Live-birth rates and multiple-birth risk using in vitro fertilization. *JAMA* 282,1832–1838.

- Soderstrom-Anttila V, Vilksa S, Makinen S, Foudila T and Suikkari AM (2003) Elective single embryo transfer yields good delivery rates in oocyte donation. *Hum Reprod* 18,1858–1863.
- Templeton A, Morris JK and Parslow W (1996) Factors that affect outcome of in-vitro fertilisation treatment. *Lancet* 348,1402–1406.
- Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A and Bergh C (2004) Elective single embryo transfer versus double embryo transfer in in vitro fertilization. *N Engl J Med* 351,2392–2402.
- Tiitinen A, Halttunen M, Harkki P, Vuoristo P and Hyden-Granskog C (2001) Elective single embryo transfer: the value of cryopreservation. *Hum Reprod* 16,1140–1144.
- Tiitinen A, Unkila-Kallio L, Halttunen M and Hyden-Granskog C (2003) Impact of elective single embryo transfer on the twin pregnancy rate. *Hum Reprod* 18,1449–1453.
- Tomás C, Orava M, Tuomivaara L and Martikainen H (1998) Low pregnancy rate is achieved in patients treated with intracytoplasmic sperm injection due to previous low or failed fertilization in in-vitro fertilization. *Hum Reprod* 13,65–70.
- van Montfoort AP, Dumoulin JC, Land JA, Coonen E, Derhaag JG and Evers JL (2005) Elective single embryo transfer (eSET) policy in the first three IVF/ICSI treatment cycles. *Hum Reprod* 20,433–436 [Epub 18 November 2004].
- Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W and Gerris J (1999) Characterization of a top quality embryo, a step towards single embryo transfer. *Hum Reprod* 14,2345–2349.
- Veleva Z, Jarvela IY, Nuojuu-Huttunen S, Martikainen H and Tapanainen JS (2005) An initial low response predicts poor outcome in in vitro fertilization/intracytoplasmic sperm injection despite improved ovarian response in consecutive cycles. *Fertil Steril* 83,1384–1390.
- Vilksa S, Tiitinen A, Hyden-Granskog C and Hovatta O (1999) Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. *Hum Reprod* 14,2392–2395.
- Walker MC, Murphy KE, Pan S, Yang Q and Wen SW (2004) Adverse maternal outcomes in multifetal pregnancies. *BJOG* 111,1294–1296.
- Xiong X, Saunders LD, Wang FL and Demianczuk NN (2001) Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes. *Int J Gynaecol Obstet* 75,221–228.

Submitted on December 8, 2005; resubmitted on March 28, 2006; accepted on April 5, 2006