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IVF/ICSI twin pregnancies: risks and prevention

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Since the 1970s, the national twin birth rates have been increasing worldwide. Apart from the increasing childbearing age, the main cause is the use of assisted reproductive technologies (ART). To explore the overall consequences of dual embryo transfer (DET), the literature has been reviewed systematically regarding short- and long-term outcomes of IVF/ICSI twin pregnancies i.e. pregnancy complications, maternal risks, obstetric outcome and long-term morbidity including neurological sequelae, cognitive development and family implications. Another consequence of DET is vanishing twins, which seems to be a possible cause of adverse outcome in IVF singletons. The sparse literature on vanishing twins in IVF pregnancies and the influence on the surviving co-twin were also addressed. Finally, to determine the effects of implementing elective single embryo transfer (eSET), trials concerning eSET versus DET were analysed. In the light of the steadily increasing twin birth rates and the findings in this overview, where IVF/ICSI twins carry adverse outcome, it should be emphasized that the major obstacle in IVF remains the high twin birth rate. Furthermore vanishing twins account for another hazard of DET. These problems can be resolved by implementing eSET, diminishing the twin birth rate without affecting the overall goal of achieving a healthy infant.

Key words: assisted reproduction/obstetric outcome/single embryo transfer/twin pregnancies/vanishing twins

Introduction

During the last two centuries the twin birth rates have increased worldwide. The main contributors to this rise are the increasing childbearing age and the use of assisted reproductive technologies (ART) including IVF and other procedures such as ovulation induction and intrauterine insemination. In the Nordic countries dual embryo transfer (DET) has been the standard for several years. This practice made triplets almost disappear, although the twin birth rate remained stable (Nyboe Andersen *et al*., 2004). Hence, two questions have arisen: to change the current IVF policy to elective single embryo transfer (eSET) and if so, what should the indications for eSET be? In an attempt to answer these questions, we initiated a study in 2001 on the Danish national twin birth cohort between, 1995 and 2000, to assess the outcome of IVF/ICSI twin pregnancies including long-term consequences. In collaboration with the Danish National Board of Health, national data from The Danish IVF Registry, The Danish Hospital Registry, The Danish Registry of Causes of Deaths, The Danish Cancer Registry and The Danish Psychiatric Central Registry were cross-linked.

The aim of this article was to systematically review the literature on the consequences of DET, reporting short- and long-term outcome of IVF twin pregnancies discussed in relation to the results generated from the Danish national twin cohort. Secondly, studies on vanishing twins in IVF pregnancies, a hazard of DET, were assessed. Finally, the implications of eSET were evaluated.

Materials and methods

A computerized search in PubMed (1978–2004), EMBASE (1985–2003) and the Cochrane Central Register of Controlled Trials (Pandian *et al*., 2004) was conducted to identify relevant studies published in English. Searches were restricted to literature published from 1978 (since the first IVF child was born that year). In addition, reference lists of all identified studies were reviewed to search for cross-references, and abstracts from relevant meetings were checked. The latest search was done on November 2004. The most recent publications were selected when multiple publications reported data from the same study subjects.

Outcome measures

Outcomes included obstetric outcome [twin births, gestational age, birthweight, preterm birth, low birthweight (LBW), small for gestational age (SGA), perinatal mortality, congenital malformations and chromosomal aberrations] and long-term follow-up (growth and physical health, neurological sequelae, mental development, behaviour, socioemotional development and childhood cancer). In addition, the incidence of spontaneous and selective reductions in IVF/ICSI pregnancies and consequences for the outcome were dealt with. Finally, pregnancy/delivery rates after single embryo transfer and cumulative delivery rates after fresh and frozen-thawed SET were addressed.

Search strategies

Three different search strategies were used for the subheadings: (i) Short- and long-term outcomes, (ii) single embryo transfer and (iii) spontaneous (vanishing twins) and selective reduction of twin pregnancies. The following medical subheadings (MeSH terms) and all combinations of these words were used.

Short- and long-term outcomes

Twin$, in vitro fertil$, IVF, intracytoplasmic sperm injection, ICSI, assisted reprod$ techn$, infertility, subfertility, pregnancy outcome, obstetric outcome, congenital malformation$, chromosome aberration$, chromosomal abnormalit$, developmental disorder$, cerebral palsy, neurological sequelae, long-term follow-up, children follow-up, childhood cancer$, child$, infant$, child development, morbidity, mortality.

Single embryo transfer

Embryo transfer, multiple pregnancy, in vitro fertil$, IVF, intracytoplasmic sperm injection, ICSI, infertility, subfertility, assisted reprod$ techn$, ART, single/one embryo, two/double embryo, randomized controlled trial, clinical trial, cryopreservation.

Spontaneous and selective reduction

Twin$, in vitro fertil$, IVF, intracytoplasmic sperm injection, ICSI, assisted reprod techn, infertility, subfertility, spontaneous fetal reduction, selective fetal reduction, selective abortion, vanishing twin, pregnancy outcome, obstetric outcome, developmental disorder, cerebral palsy, neurological sequel, long-term follow-up, children follow-up, childhood cancer, child, infant, child development, morbidity, mortality.

Inclusion and exclusion criteria

Articles including singletons and twins conceived through conventional IVF or ICSI. All sperm sources, fresh and frozen-thawed sperm and frozen-thawed embryos were included, whereas articles concerning the outcome of children born after ovum donation and surrogate were excluded. Editorials and articles written in languages other than English were excluded.

Regarding obstetric outcome, this review was focused on matched studies with sample size above 400, which is sufficient to show a 10% difference in prematurely with 80% power and a significance level of 0.05. Because increasing maternal age and nulliparity is positively correlated to increased obstetric risks, matching on maternal age and parity is of outmost importance in IVF children, as IVF mothers are older and more frequently nulliparous as compared with mothers, who conceive spontaneously. To make an overview all studies including year of publication, sample size and matching criteria on obstetric outcome in IVF/ICSI twins are listed in Table I.

With respect to long-term consequences the review was focused on the only two register-based national cohort studies with sufficient data to show differences in the very rare outcomes such as developmental disorders including cerebral palsy; however, smaller studies were critically scrutinised.

All observational and randomized controlled clinical trials in set were referenced and discussed. All eSET studies including year of publication, sample size and inclusion criteria are listed in Table II. Finally, the limited studies on spontaneous and selective reduction were systematically reported and discussed.

Results

National twin birth rates

Secular changes in national twin birth rates

Vital statistics from 17 countries showed that the twin birth rates in Europe, United States of America, Canada and Asia remained nearly constant or gradually increased until the mid-1980s with a rapid increase from 1990 to 1996 (Imaizumi, 1998). The most pronounced rise in twin birth rates has been observed in Denmark with a 1.7-fold increase during 1980–1994 (Westergaard *et al*., 1997). As a further rise was observed from 17.8 to 22.0 twin deliveries per 1000 live-births between 1996 and 2003, the total increase in the Danish twin birth rate since the 1970s reaches 2.4-fold (The Danish Society of Gynecology and Obstetrics, 2003, http://www.DSOG.dk). This is a consequence of the liberal access to ART with Denmark having the highest number of IVF cycles per inhabitant in Europe (Nyboe Andersen *et al*., 2004). Though less pronounced the same pattern has been observed in the other Scandinavian countries, i.e. in Sweden the twin deliveries have increased 1.9-fold between 1973 and 2000 and in Norway the twin birth rate climbed 2.2-fold from 1974 to 2002 (The National Board of Health and Welfare, 2003, http://www.sos.se; University of Bergen, 2002, http://www.uib.no/mfr/html). Finland has been the first country to implement set with more than 30% single embryo transfers in Finland (Tiitinen *et al*., 2003). This has resulted in a twin birth rate reduction from 17.1 in 1998 to 14.9 per 1000 births in 2004 (Official statistics of Finland, 2004, http://stakes.info/files/pdf/Tilastotiedotteet/Tt15-04.pdf). A recent report from the United States of America showed that the twin birth rate exceeded 30 per 1000 in 2002 with an overall increase of 38% since 1990 and 65% since 1980 (Martin *et al*., 2003).

ART are not the sole contributor to the climbing twin birth rates increasing child-bearing age also plays a role. The average age at delivery increased between the late 1970s and mid-1990s: from 26 to 29 years old in Denmark, France, Finland and Sweden and to 30 years in the Netherlands (Blondel and Kaminski, 2002). This trend has resulted in a progressive shift of deliveries to the 30–39 years age group. A Swedish register study estimated that one third of the rise in the twin birth rate was explained by the increasing childbearing age, one third of ART procedures other than IVF and one third of IVF procedures (Bergh *et al*., 1999). In accordance, a US study stated that 20% of the increasing twin birth rates were attributable to the reproductively ageing female, 40% to ovulation induction and 40% to IVF (Jones, 2003). In Denmark the increase in the national multiple birth rate was almost exclusively observed in women aged ≥30 years and was limited to dizygotic (DZ) twinning (Westergaard *et al*., 1997). This is in compliance with the United States of America, where the increase in twin birth rates were most pronounced among women aged ≥30 years, i.e. between 1990 and 2001 the twin birth rate for women aged 40–44 was almost doubled (from 24.7 to 48.1 per 1000) (Martin and Park, 1999). Overall one fourth to one third of the increase in twin pregnancies is attributable to the increase in maternal age (Blondel and Kaminski, 2002). From 1978 when the first IVF child was born, the secular changes in twinning rates highlight the substantial effect the introduction of ART, performed in a relatively small group of women, has caused on the overall national twin birth rates.

Table I. Meta-analyses and original controlled papers on neonatal outcome in IVF/ICSI twin pregnancies listed according to study design and sample size

Author	Country	Publication year	Number of infants		Outcome measures		Neonatal morbidity	Mortality	Matching criteria
			IVF twins	Control twins	BW GA	Birth defects			
Meta-analysis									
Helmerhorst	The Netherlands	2004	3437	3429	+	–	+	+	Various*
National register studies									
Schieve *et al.*	United States of America	2002	18399	NA	+	–	–	–	Age, parity, cause of infertility, number of fetal hearts, previous ART, ICSI
Pinborg *et al.*	Denmark	2004b	3438	10362	+	+	+	+	Age, parity, different sex
Bergh *et al.*	Sweden	1999	2136	NA	+	+	–	+	Age, parity, previous subfertility, year of birth
Lambalk and van Hooff	The Netherlands	2001	960†	1226	+	–	+	+	Age, zygosity
Westergaard *et al.*	Denmark	1999	854	854	+	+	–	+	Age, delivery year, parity
Agustsson *et al.*	Iceland	1997	138	906	+	–	+	+	–
Koivurova *et al.*	Finland	2002a	103	103	+	+	+	+	Age, parity, social class, area of residence, gender, year of birth
Case-control studies									
Dhont *et al.*	Belgium	1999	2482	2482	+	+	+	+	Age, parity, gender, date of delivery
Minakami *et al.*	Japan	1998	272	144	+	+	+	+	Chorionisity
Tan *et al.*	England	1992	250	42	+	+	–	+	Age, primiparous controls
Dhont *et al.*	Belgium	1997	230	230	+	+	+	+	Age, parity, date of delivery, zygosity
Bernasko *et al.*	United States of America	1997	210	558	+	–	+	+	–
Daniel *et al.*	Israel	2000	208	242	+	+	+	+	–
Koudstaal *et al.*	The Netherlands	2000	192	192	+	+	+	+	Age, parity, ethnicity, delivery date, zygosity, height, weight
Kozinszky *et al.*	Hungary	2003	150	150	+	+	+	+	Age, parity, preventive obstet outcome
Olivennes *et al.*	France	1996	144	328	+	–	–	+	–
Putterman *et al.*	United States of America	2003	120	202	+	–	+	–	Chorionisity
Fitzsimmons *et al.*	Canada	1998	112	216	+	–	+	+	Age, parity, fetal no, medical problems
Tallo *et al.*	United States of America	1995	72	72	+	–	+	–	Age, race, insurance type, gender, order in delivery, date of delivery
Isaksson *et al.*‡	Finland	2002	40	200	+	+	+	+	Age, parity, delivery year, area of residence
Moise *et al.*	Israel	1998	40	80	+	–	+	+	Age, parity, ethnicity, term infants
Zuppa *et al.*	Italy	2001	32	228	+		+	+	+
Petersen *et al.*	Denmark	1995	32	32	+	+	–	–	–
Questionnaires									
Saunders *et al.*	Australia	1996	94	34	+	+	+	–	Gestation, date of birth
Addor *et al.*	Switzerland	1998	26	154	+	+	+	+	Age, social class, BW, GA, gender

Age, maternal age; ART, assisted reproductive technologies; BW, birthweight; GA, gestational age; NA, crude numbers not available, control group was the general population stratified for multiplicity.
*This study included 17 studies (10 matched and 7 nonmatched).
†Including 608 children born after IVF and 352 children conceived after ovulation induction.
‡Only couples with *unexplained infertility* were enrolled in the IVF group.

Table II. Randomized studies comparing ongoing pregnancy rates in single with double embryo transfer

Author	Publication year	Country	Number of patients		Ongoing pregnancy/live birth rate (%)			Twin birth rates (%)		Inclusion criteria
			eSET	DET	eSET	DET	P-value/RR (95% CI)	eSET	DET	
Martikainen*†	2001	Finland	144	70	32.4	47.1	NS	4.5	39.0	<36 years, 1. IVF/ICSI cycle, >4 high quality embryos
Gerris*†	1999	Belgium	26	27	38.5	74.0	1.8 (1.1–2.9)	10	30	<34 years, 1. IVF/ICSI cycle, >2 high quality embryo
Gardner†	2004	United States of America	23	25	60.9‡	76.0	NS	0/14	47.4	FSH≤10IU/L, ≥10 follicles >12 mm at day of HCG
Thurin†	2004	Sweden	330	331	38.8§ (27.6)	42.9	0.3 <0.001	0.8	33.1	<36 years, 1. or 2. IVF/ICSI cycle, <0.001 ≥2 high quality embryo
Lukassen*	2002	The Netherlands	22	21	36.4¶ (27.3)	28.6	NS	0/21	2/21	<35 years, 1. IVF/ICSI cycle, ≥2 high quality embryo, FSH<10IU/L

DET, dual embryo transfer.
*Studies included in the Cochrane review by Pandian *et al*. (2004).
†Studies included in the review by Gerris *et al*. (2004).
‡Only blastocyst transfers.
§Ongoing Pregnancy Rate (OPR) in elective single embryo transfer (eSET) group with one fresh eSET and if no live birth then a *frozen* eSET, in parentheses OPR with only one fresh embryo transfer in the eSET group.
¶OPR in eSET group with one fresh eSET and if no live birth then a *fresh* eSET, in brackets OPR with only one fresh embryo transfer in the eSET group.

Twin birth rates after IVF techniques

After the introduction of DET in most European countries, twinning rates have remained fairly constant lingering around 25%, whereas the overall pregnancy rates for IVF patients have stayed constant (ESHRE Campus Course Report, 2001; Nyboe Andersen *et al*., 2004). In the World collaborative report on ART in 1998, twin pregnancy rates in Australia and Asia were quite similar to the European rates with the total twin birth rate being 27.3% (Adams *et al*., 1998). Data from the American Society for Reproductive Medicine gives a similar, albeit worse picture, as the IVF twin birth rate in 2000 was 30.8% (American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry, 2000).

In relation to the most important factor, child health, the incidence of twin deliveries is not the best outcome measure instead the number of live-born twin infants should be preferred. Overall, 56.7% of infants born after IVF in Europe in 2000 were singletons, 38.7% were twins and 4.6% were from high-order multiple births (Nyboe Andersen *et al*., 2004).

Twin pregnancies after other types of ART

A Danish national postal questionnaire survey showed that ART other than IVF accounted for 12.2% of the twin deliveries, whereas 29.4% were the result of in-vitro methods and 58.4% were spontaneously conceived (Pinborg *et al*., 2003b). Hence, ART accounts for more than 40% of the twin births in Denmark, which is similar to the Dutch speaking part of Belgium, where in-vitro techniques contributed to 26% of the twin deliveries and 14% were the result of ovarian stimulation without IVF in 1999 (Dhont, 2001). In a cohort of women from Colorado, who delivered between 1996 and 1999, ovarian stimulation without IVF attributed to 21% and IVF to 15% of the multiple pregnancies (Lynch *et al*., 2001). In an earlier Australian postal questionnaire study performed in 1991, 6.1% of twin confinements followed IVF procedures, 11.2% ovarian stimulation regimens alone and 82.7% followed spontaneous conception. It was concluded that nearly twice as many twin pregnancies were associated with ovarian stimulation treatment administered alone than with IVF (Kurinczuk *et al*., 1995). Hence, a shift from regimens dominated by ovarian stimulation treatment alone towards more IVF treatments has been observed during the last ten years.

In summary, ART attributes to more than 40% of the national twin births with close to 40% of children born after IVF being twins.

Zygocity and chorionicity

Twin gestations are divided into two major types: DZ and monozygotic (MZ), although MZ twins occur sporadically, DZ twins increases with advancing age and parity. The more frequent occurrence of poly-ovulation may explain the higher twin gestation rate seen with advancing maternal age. In some families DZ twinning is apparently inherited (Sperling and Tabor, 2001). In Caucasians about 30% of twin pregnancies are MZ and about 70% DZ. Zygocity denotes the type of conception, whereas chorionicity refers to the type of placentation. MZ twins result from the splitting of one fertilized ovum during the first 2 weeks of embryogenesis, whereas DZ twins origin from the fertilization of two ova by different spermatozoa. According to the number of layers in the septum between the amnion sacs, twin placentas are categorized into monochorionic (MC) and dichorionic (DC). DZ twins are always DC, whereas MZ twins can be either DC or MC. Apart from some defects that may result from intrauterine crowding including foot deformities, dislocation of the hip and skull asymmetry (Newman, 1998), the malformation rate per foetus in spontaneously conceived DZ twins is similar to singletons, whereas the rate is two- to three-fold higher in MZ twins (Källén, 1986). Also

mortality rates are higher amongst MZ twins (Rydhström, 1994). It has been shown that these higher risks are limited to MC MZ twins, whereas similar outcomes are seen in DZ and DC MZ twin pairs (Sebire *et al*., 1997; Loos *et al*., 1998; Minakami *et al*., 1998; Dubé *et al*., 2002).

Although the great majority of ART twinning appears to be DZ because of DET, MZ twinning from embryo splitting is also of concern in ART twin pregnancies. In 1987, a two-fold increase in the MZ rate among assisted reproductive births was noted (Derom *et al*., 1987). Subsequent reports documented higher rates of MZ twinning (1–5%) among ART births (Blickstein *et al*., 1999; Derom *et al*., 2001; Schachter *et al*., 2001; Alikani *et al*., 2003), than typically observed in the general population (0.4%) (Bulmer, 1970).

In the Danish twin birth cohort between 1995 and 2000, the rate of MZ twinning was estimated to be 1.6% in IVF twins versus 31% in controls by using Weinberg's differential method based on the number of opposite-sex twin-sets (Weinberg, 1902; Pinborg *et al*., 2004b). In comparison with previous studies the MZ rate in our study was relatively low. One explanation may be that assisted hatching and blastocyst culture, which may increase the rate of MZ twinning (Hershlag *et al*., 1999; Schieve *et al*., 2002; Milki *et al*., 2003), are performed very rarely in Denmark. The association between zona pellucida micromanipulation and MZ twinning is, however, still controversial. By dividing the MZ rate with the mean number of embryo transferred Sills and coworkers reached a result very close to the background MZ rate, further as they found no increased MZ rate by assisted hatching and ICSI, they claimed that the higher MZ rate in IVF should primarily be explained by the increased number of implantations due to multiple embryo transfer (Sills *et al*., 2000). The lower average number of embryos transferred in Denmark (1.9) versus the overall mean number in Europe (2.3) may be another explanation for the lower MZ rate found in our study compared with previous studies (Nyboe Andersen *et al*., 2004).

Twin-to-twin transfusion syndrome (TTTS) markedly increases the risk of an adverse fetal outcome occurring in 15–30% of MC twin pairs (Rausen *et al*., 1965; Patten *et al*., 1989). TTTS is very rare in twins with DC placentas (Robertson and Neer, 1983). Among naturally conceived twins the MC rate is 20%, whereas the rate is considerably lower in ART twins conceived (Derom *et al*., 1987; Wenstrom *et al*., 1993; Putterman *et al*., 2003). Hence, the incidence of TTTS is lower in IVF twinsets (Pinborg *et al*., 2004a). In a US study, 1.8% of 164 IVF twin pregnancies were MC with a tendency of higher MC rates in blastocyst transfers (Chow *et al*., 2001). Further the MC rate increased significantly by increasing number of gestations. The implications of the lower MC rate in IVF/ICSI twins is an expected better outcome than in naturally conceived twins; however, the overall much higher risk in twins may conceal this modest advantage of ART twins (Helmerhorst *et al*., 2004).

Pregnancy complications

Maternal risks

Most previous reports have demonstrated similar age- and parity-adjusted risk of pregnancy induced hypertension and gestational diabetes in IVF and control twin pregnancies (Olivennes *et al*., 1996; Bernasko *et al*., 1997; Fitzsimmons *et al*., 1998; Koudstaal *et al*., 2000; Isaksson *et al*., 2002; Koivurova *et al*., 2002b). Only one Dutch study restricted to DZ twins revealed a lower risk of diastolic blood pressure >90 mm Hg in IVF versus spontaneous pregnancies (Lambalk and van Hooff, 2001). Though IVF/ICSI twin mothers carry a similar age- and parity-adjusted risk of pregnancy-induced hypertension, pre-eclampsia and gestational diabetes as control twin mothers, they had a 2.5-fold adjusted risk of being on leave because of sickness in pregnancy and a 1.9-fold risk of being admitted to hospital in pregnancy (Pinborg *et al*., 2004a). This was in accordance with two Finnish register studies observing a significantly higher rate of maternal admissions in IVF multiple pregnancies in comparison with non-IVF multiple pregnancies, though none of these studies distinguished between twins and higher-order multiple pregnancies (Gissler *et al*., 1995; Klemetti *et al*., 2002).

The incidence of pre-eclampsia is higher in spontaneous twin versus singletons pregnancies (Coonrod *et al*., 1995; Santema *et al*., 1995; Campbell and MacGillivray, 1999). In compliance, the risk of pre-eclampsia was 2.4-fold increased for IVF/ICSI twin versus singletons pregnancies and the risk of sick leave and hospitalization was 6.8 and 3.5-fold higher, respectively (Pinborg *et al*., 2004a). This was confirmed in two studies showing higher morbidity in IVF twin than singletons pregnancies in pregnancy induced hypertension and intrahepatic cholestasis (Koivurova *et al*., 2002b) and higher maternal hospitalization rates (Klemetti *et al*., 2002).

It is obvious that more complications and maternal admissions were seen in twin than in singleton IVF pregnancies. However, though no higher risk was observed in IVF compared with control twin pregnancies, a higher rate of maternal sickness leave and admissions was seen in IVF twin pregnancies. This may be related to more precautions being taken by health care professionals and the mothers themselves in IVF twin pregnancies.

Obstetric outcome

General obstetric outcome

Previous studies have shown that the obstetric outcome in IVF pregnancies is poorer than in the general population (MRC Working Party on Children Conceived by In Vitro Fertilisation, 1990; Friedler *et al*., 1992; Rufat *et al*., 1994; Gissler *et al*., 1995; Bergh *et al*., 1999; Dhont *et al*., 1999; Westergaard *et al*., 1999; Schieve *et al*., 2002). The poorer outcome in IVF pregnancies is mainly explained by the higher IVF multiple birth rates. Albeit, higher order multiple births account for the most severe obstetric outcomes, the predominance of twin pregnancies in IVF make them by far the main contributor.

The content of the wide range of controlled studies on obstetric outcome in IVF/ICSI twin pregnancies were summarized in Table I. As indicated in the Materials and methods this review was focused on matched studies with sample sizes above 400, which is sufficient to show a 10% difference in prematurely.

Birthweight and gestational age

A various range of studies report the risk of prematurely and LBW in IVF/ICSI and control twins (Tables III and IV). Only five original papers with sufficient sample size are available, all reporting similar risks in IVF/ICSI and control twins (Dhont *et al*., 1999; Westergaard *et al*., 1999; Lambalk and van Hooff, 2001; Schieve

Table III. Preterm (<37 weeks) and very preterm birth (<32 weeks), studies in which IVF/ICSI twins are compared with spontaneously conceived twins

Author	Number of IVF twins	Gestational age [weeks (Mean ± SD)]			Children born <37 week (%)			Children born <32 week (%)			Matching
		IVF	Control	*P*-value	IVF	Control	OR	IVF	Control	OR	
Meta-analysis											
Helmerhorst	3437	–	–	–	–	–	1.07 (1.02–1.13)	–	–	0.95 (0.78–1.15)	Yes
Original papers											
Pinborg *et al.* (2004b)	3438	35.9 ± 3.0	36.1 ± 2.9	0.002	43.9%	41.5%	0.95 (0.87–1.04)	8.5%	7.8%	0.94 (0.80–1.10)	Yes + different sex
Dhont *et al.* (1999)*	2482	36.1 + 2.8	36.2 + 2.6	NS	49.4%	47.7%	1.04 (0.98–1.10)†	7.0%	7.2%	0.97 (0.79–1.19)†	Yes + different sex
Lambalk and van Hooff	960	34.9 + 4.0	35.4 + 3.8	<0.02	–	–	–	6%*	5%*	1.4 (1.0–2.0)	Yes + zygocity
Westergaard *et al.*	854	–	–	–	41.2%	43.1% ‘	NS	–	–	–	Yes
Minakami *et al.*	272	35.6 + 2.2	35.3 + 2.4	NS	–	–	–	–	–	–	Only chorionisity
Tan *et al.**	250	–	–	–	58%	52%	1.1 (0.7–1.7)†	–	–	–	Yes
Dhont *et al.* (1997)*	230	36.2	36.3	NS	52.2%	42.2%	1.22 (1.01–1.49)	7.0%	5.2%	1.33 (0.65–2.76)	Yes
Bernasko *et al.*	210	35.5	35.9	NS	22.9%	29.4%	0.8 (0.5–1.1)	–	–	–	No
Daniel *et al.*	208	35.4 + 2.5	36.2 + 3	<0.02	25.0%	20.7%	NS	–	–	–	No
Koudstaal *et al.**	192	35.9	36.6	NS	51.0%	41.7%	0.12	–	–	–	Yes + zygocity
Kozinszky *et al.*	150	35.5 ± 3.0	35.5 ± 3.0	NS	–	–	–	–	–	–	Yes
Olivennes *et al.* (1996)	144	–	–	–	–	–	1.3 (0.6–3.0)†	–	–	1.8 (0.5–5.9)†	No
Agustsson *et al.*	138	36.1 + 3.4	35.4 + 3.9	NS	–	–	–	–	–	–	No
Putterman *et al.*	120	35.9 + 3.7	35.7 + 3.0	NS	53.3%	60.4%	0.75 (0.37–1.50)	13.3%	11.9%	1.1 (0.4–3.3)	Chorionicity
Fitzsimmons *et al.*	112	35.4 + 3.3	34.6 + 4.5	NS	–	–	–	–	–	–	Yes
Koivurova *et al.* (2002a)*	103	–	–	–	43.7%	43.7%	1.0 (0.6–1.8)	1.9%	10.8%	S	Yes
Saunders *et al.*	94	35.0 + 0.3	33.7 + 0.5	NS	–	–	–	–	–	–	Yes
Tallo *et al.**	72	35 + 3	37 + 3	0.03	59%	37%	0.01	–	–	–	Yes
Moise *et al.**	40	36	37	NS	60%	40%	0.03	–	–	–	Yes
Isaksson *et al.**	40	36.5 + 2.8	36.4 + 3.0	NS	35.0%	41.0%	–	–	–	–	Yes
Petersen *et al.**	32	37.1	37.5	NS	18%	37%	NS	–	–	–	No
Zuppa *et al.*	32	33.9 + 4.3	35.7 + 3.6	<0.02	75%	52.6%	<0.05 (*P*-value)	31.3%	16.7%	<0.05	No
Addor *et al.*	26	–	–	–	46.2	44.8	NS	–	–	–	Yes

*Studies included in the meta-analysis by Helmerhorst *et al.*
†Relative risk.

Table IV. Low birthweight (LBW) (<2500 g) and very LBW (VLBW) (<1500 g), studies in which IVF/ICSI twins are compared with spontaneously conceived twins

Author	Number of IVF twins	Birthweight [g (mean ± SD)]			Number of children born LBW <2500 g (%)			Number of children born VLBW <1500 g (%)			Matching
		IVF	Control	*P*-value	IVF	Control	OR/*P*-value	IVF	Control	OR/*P*-value	
Meta-analysis											
Helmerhorst	3437	–	–	–	–	–	1.03 (0.99–1.08)	–	–	0.89 (0.74–1.07	Yes
Original papers											
Schieve *et al.*	18 399	–	–	–	–	–	1.0 (1.0–1.1)*	–	–	–	Yes
Pinborg *et al.* (2004b)	34 38	2508 ± 615	2540 ± 612	<0.01	42.3%	40.5%	0.9 (0.8–1.0)	7.5%	6.8%	0.9 (0.8–1.1)	Yes + different sex
Dhont *et al.* (1999)†	2482	2386 ± 542	2365 ± 562	NS	–	–	–	–	–	–	Yes + different sex
Lambalk and van Hooff	960	2250 ± 686	2319 ± 663	<0.02	–	–	–	–	–	–	Yes + Zygosity
Westergaard *et al.*	854	2516	2443	<0.05	42.2%	44.8%	NS	6.7%	10.2%	NS	Yes
Minakami *et al.*	272	2126‡	2143‡	NS	–	–	–	–	–	–	Only chorionicity
Tan *et al.*†	250	2388 ± 606	2408 ± 841	NS	53%	40%	1.3 (0.9–1.9)§	–	–	–	Yes
Dhont *et al* (1997)†	230	2360 + 557	2427 + 498	NS	55.3%	54.8%	–	6.1%	5.7%	–	Yes
Bernasko *et al.*	210	2388 ± 586	2360 ± 560	NS	71.8%	59.1%	1.7 (1.0–2.8)	–	–	–	No
Daniel *et al.*	208	2199‡	2393‡	<0.05	67.6%	52.3%	<0.05	12.4%	8.8%	<0.05	No
Koudstaal *et al.*†	192	2362‡	2318‡	NS	60.8%	44.4%	0.02	–	–	–	Yes + Zygosity
Kozinszky *et al.*	150	2362 ± 637	2297 ± 683	<0.001	–	–	–	–	–	–	Yes
Olivennes *et al.*	144	–	–	–	–	–	1.1 (0.7–1.6)	–	–	1.5 (0.6–3.5)	No
Agustsson *et al.*	138	2520 ± 658	2453 ± 730	NS	–	–	–	–	–	–	No
Putterman *et al.*	120	2414	2438	NS	29.2%	41.1%	0.6 (0.4–1.0)	14.2%	8.4%	1.8 (0.8–3.9)	Chorionicity
Fitzsimmons *et al.*	112	2374‡	2166‡	NS	–	–	–	–	–	–	Yes
Koivurova *et al.* (2002a)†	103	2594 ± 528	2547 ± 602	NS	45.7%	45.7%	1.1 (0.6–1.9)	1%	4.9%	0.2 (0.02–1.8)	Yes
Saunders *et al.*	94	2297 ± 63	2053 ± 98	S	–	–	–	–	–	–	Yes
Tallo *et al.*†	72	2212¶	2571¶		71%	43%	0.001	–	–	–	Yes
Moise *et al.*†	40	2074 ± 590	2361 ± 468	<0.01	70%	59%	<0.003	25%	4%	<0.003	Yes
Isaksson *et al.*†	40	2540 ± 662	2449 ± 658	NS	45.0%	46.0%	NS	5.0%	7.5%	NS	Yes
Petersen *et al.*†	32	2650¶	2512¶	NS	38%	47%	NS	–	–	–	No
Zuppa *et al.*	32	2015 ± 763	2288 + 659	NS	75%	53.9%	<0.05	25%	12.3%	<0.05	No
Addor *et al.*	26	–	–	–	50.0%	53.9%	NS	–	–	–	Yes

*Rate ratio.
†Studies included in the Helmerhorst study.
‡Mean birthweight of twin A and B.
§Relative risk.
¶Median.

et al., 2002; Pinborg *et al*., 2004b). In accordance, the only meta-analysis on matched studies stated that the relative risks of prematurely and LBW were similar in IVF versus spontaneously conceived twins (Helmerhorst *et al*., 2004). This was also the case for infants being SGA (OR = 1.27, 95% CI = 0.97–1.65). Risk estimates on preterm and very preterm birth in this meta-analysis were based on 3437 and 2815 IVF twins respectively, predominated by a large matched survey (Dhont *et al*., 1999), which contributed to 72% of the twin cases on preterm birth and 88% of the cases on very preterm birth (Helmenhorst *et al*., 2004).

Our national cohort study showed that even in opposite-sex twins, no difference between IVF/ICSI and controls was observed, OR of preterm birth 1.05 (95% CI = 0.92–1.21) and very preterm birth 1.11 (95% CI = 0.88 – 1.41) (Pinborg *et al*., 2004b). Correspondingly, the risks of LBW and very LBW (VLBW) in opposite-sex IVF/ICSI twins were OR = 1.03 (95% CI = 0.90–1.18) and 0.98 (95% CI = 0.76–1.26), respectively.

As a rough estimate, IVF twins are born with an average gestational age 3 weeks lower than IVF singletons and with a mean birthweight ranging between 800 and 1000 g lower (Rizk *et al*., 1992; Rufat *et al*., 1994; Pinborg *et al*., 2004c). In the Danish cohort study, the risk of preterm labour was 10-fold and the risk of very preterm labour was seven-fold increased in IVF twins versus singletons. Similar results were obtained for LBW and VLBW (Pinborg *et al*., 2004c).

To summarize, IVF/ICSI twins have adjusted risks of prematurely and LBW similar to control twins even after exclusion of MZ twins. However, in comparison with IVF singletons, they do noticeably worse.

Caesarean section

Most studies state that crude caesarean section (CS) rates including the frequency of emergency sections and the rate of vacuum extractions are higher in IVF/ICSI than in control twin pregnancies (Dhont *et al*., 1999; Westergaard *et al*., 1999; Koivurova *et al*., 2002b; Pinborg *et al*., 2004a,b). However, when corrected for maternal age and parity, these differences disappear.

In line with the finding in the Australian review, where the relative risk of CS rate in IVF twins versus control twins was 1.2 (1.1–1.3) (Helmenhorst *et al*., 2004), the age and parity adjusted risk was OR = 1.1 (95% CI = 1.0–1.2) in the Danish National twin cohort (Pinborg *et al*., 2004b). The crude percentages of CS in our study were 52.9% in IVF/ICSI versus 42.7% in spontaneously conceived twin pregnancies (Pinborg *et al*., 2004b).

It is well known that Caesarean section rates are considerably higher in IVF twin as in IVF singleton pregnancies with relative risks lingering around two to three (Dhont *et al*., 1999; Westergaard *et al*., 1999; Klemetti *et al*., 2002; Koivurova *et al*., 2002b; Pinborg *et al*., 2004c).

Congenital malformations

Malformations in IVF and spontaneously conceived twins. The vast majority of studies on malformations in IVF twins are based on a limited number of cases with varying definitions of malformations and different or no matching criteria, resulting in a wide range of malformation rates from 25 to 115 per thousand in IVF twin infants (Table V). Only four studies met our criteria for a sample size of >400 IVF twins (Bergh *et al*., 1999; Dhont *et al*., 1999; Lambalk and van Hooff, 2001; Pinborg *et al*., 2004b), of which only two were adjusted according to maternal age (Dhont *et al*., 1999; Lambalk and van Hooff, 2001), and only one also according to parity (Lambalk and van Hooff, 2001). Furthermore, the Swedish study calculated OR for multiples, but not for twins separately (Bergh *et al*., 1999). Based on the two age-matched studies with sufficient sample size the risk of major malformations in IVF versus control twins ranged from OR = 1.2–1.5 with 95%

Table V. Major malformations in IVF/ICSI twins versus spontaneously conceived twins listed after sample size

Author	Publication year	Per 1000 (number of infants)		OR (95% CI)	*P*-value	Matching criteria
		IVF	Control			
Matched studies						
Dhont *et al.*	1999	35 (86/2482)*	29 (73/2482)*	1.18 (0.86–1.63)	0.3	Maternal age, parity, date of delivery, sex
Lambalk and van Hooff†	2001	32 (31/960)	20 (24/1226)	1.54 (0.88–2.69)	NS	Maternal age, zygosity
Koudstaal *et al*.	2000	37 (7/192)*	26 (5/192)*	1.42 (0.44–4.54)	0.6	Maternal age, parity, date of delivery, height and weight, ethnic origin, smoking, zygosity
Kozinszky *et al*.	2003	33 (5/150)	13 (2/150)	2.55 (0.49–13.4)	0.2	Maternal age, parity, previous obstetric outcome
Dhont *et al.*	1997	35 (4/115)*	17 (2/115)*	2.04 (0.37–11.3)	0.4	Maternal age, parity, date of delivery
Koivurova *et al*.	2002a	78 (8/103)*	87 (9/103)*	0.88 (0.33–2.38)	0.8	Maternal age, parity, year of birth, sex, social class, residence
Isaksson *et al*.	2002	25 (1/40)	35 (7/200)	0.71 (0.09–5.91)	0.8	Maternal age, parity, year of birth, residence, nonmatched studies
Pinborg *et al.*	2004b	41 (139/3393)	47 (488/10239)	0.85 (0.70–1.04)	0.1	No adjustment
Zuppa *et al*.	2001	62 (2/32)*	13 (3/228)*	5.00 (0.80–31.2)	0.1	No adjustment
Addor *et al*.	1998	115 (3/26)‡	39 (6/154)‡	3.22 (0.75–13.8)	0.1	No adjustment
Daniel *et al*.	2000	26	22	–	NS	No adjustment
Bergh *et al*.§	1999	–	–	1.08 (0.93–1.25)	NS	No adjustment

NS, non significant.
*No distinction between major and minor malformations was made.
†Restricted to dizygotic twins.
‡All malformations including genetic and metabolic diseases.
§Multiple births, no separate analysis on twins.

CI of 0.9–2.7 (Dhont *et al*., 1999; Lambalk and van Hooff, 2001). In the study by Lambalk, they found a very close to a significant increased risk of malformations in IVF versus control twins OR = 1.7 (95% CI = 1.0–2.9), which disappeared after correction for maternal age (Lambalk and van Hooff, 2001).

Based on the existing literature with only very few matched studies with sufficient sample size, there is no significant increased risk of major malformations in IVF versus control twins.

Malformations in IVF twins and singletons. In a recent Australian review by Hansen and coworkers, the pooled odds ratio of major birth defects in IVF versus spontaneously conceived children was 2.01 (1.49–2.69) in seven reviewer-selected studies, for singletons only, OR was 1.35 (95% CI = 1.20–1.51) (Hansen *et al*., 2004). The difference of these odds ratios indicates that twinning does have some influence on the overall increased risk of malformations in IVF infants. In accordance a Swedish register study on 736 ICSI singletons and 400 twins found that the stratified OR = 1.8 (95% CI = 1.2–2.6) of malformations in ICSI versus spontaneous children dropped to 1.2 (0.8–1.8) after adjustment for twins (Wennerholm *et al*., 2000a). As indicated by the authors a possible reason for the excess malformation risk in ICSI children could, to a large extent, be explained by conditions associated with multiples and preterm birth, i.e. patent ductus arteriosis (PDA) and undescended testicle.

This is in agreement with our study, where an increased total malformation rate in IVF/ICSI twins (74/1000) versus singletons (55/1000) was found (P = 0.001). By excluding PDA, which is strongly associated with preterm birth, the malformation rate in twins decreased considerably to 57/1000 and in singletons to 52/1000. Furthermore, the difference between twins and singletons disappeared after exclusion of PDA (P = 0.3) (Pinborg *et al*., 2004c). In a large Belgian study, major malformations were found in IVF in 3.2% (49/1556) of the singleton children, in 4.5% (63/1556) of the children from *multiple* pregnancies and in 4.4% (55/1250) of the twins (Bonduelle *et al*., 2002). For ICSI the corresponding numbers were for singletons 3.1% (46/1499), for multiples 3.7% (50/1341) and for twins 3.5% (45/1288), respectively (Bonduelle *et al*., 2002). Malformation rates were significantly higher in multiples as in singletons for both IVF and ICSI (P = 0.046). Though, triplets accounted for 8% of the ICSI and 10% of the IVF multiples, analyses were not carried out for twins separately (Bonduelle *et al*., 2002).

Even though the Swedish rate of anencephaly in spontaneous twins are twice as high as in singletons (Källén *et al*., 1994), and the rate of twins in IVF children is above 40%, the expected number of IVF children with anencephaly will only be marginally increased (Ericson and Källén, 2001). In spontaneous twins the risk of alimentary tract defects appears to be three times increased, but this seems to be mainly associated with MZ twinning and maybe caused by the MZ twinning process (Källén, 1986; Harris *et al*., 1995). It has been claimed that the rate of MZ twinning is increased in IVF (Sills *et al*., 2000), but this cannot in itself explain the excess risk of alimentary tract defects assessed in some studies (Ericson and Källén, 2001).

Taken together some types of malformations are known to appear at a higher rate in spontaneous twins than in singletons such as neural tube defects, hydrocephaly, PDA and alimentary tract defects (Källén, 1986; Doyle *et al*., 1990). Some of these excess risks have also been confirmed in IVF twins, but the risk increase is moderate and requires a large database to be demonstrated. The changed risk for certain types of malformations may at least partly be secondary to preterm birth; in other case (notably minor defects) it may be due to a changed ascertainment. As expected defects specifically related to MZ twinning are not increased in IVF/ICSI twins.

Neonatal morbidity

The existing literature on neonatal admissions in IVF versus control twins have shown no, or slightly excess rates in IVF twins (Table VI). In the meta-analysis the relative risk of admittance to a neonatal intensive care unit (NICU) in the matched studies was 1.05 (1.01–1.09) and in the nonmatched studies 1.26 (1.16–1.36) (Helmerhorst *et al*., 2004). This was in accordance with our national cohort study, where the risk of NICU admittance in IVF/ICSI twins was OR = 1.18 (95% CI = 1.09–1.27) (Pinborg *et al*., 2004b). When restricted to opposite-sex twin pairs, the risk was even higher OR = 1.34 (95% CI = 1.19–1.51). Also the frequencies of infants admitted for >7 and >28 days were significantly higher in IVF/ICSI twins (Pinborg *et al*., 2004b).

There is general consensus that IVF/ICSI twins are more likely to be admitted to NICU than IVF/ICSI singletons. In our national cohort study, IVF/ICSI twins had a 3.8-fold increased risk of admittance to NICU compared with IVF/ICSI singletons diminishing to 1.8 after stratification for prematurity. IVF twins spent on average 9 days more in NICU than singletons and the frequency of admittance >7 days was 75% versus 45% in IVF singletons, the corresponding frequency of admittance >28 days was 28% and 10%, respectively (P < 0.001) (Pinborg *et al*., 2004c).

To summarize, it is likely that the slightly higher risk of NICU admittance in IVF twins is due to more precautions being taken in the highly valued IVF twin pregnancies; however, a genuine increased neonatal morbidity cannot be excluded. Not surprisingly, neonatal morbidity is considerably higher in IVF/ICSI twins than in singletons.

Mortality

Perinatal mortality rates are summarized in Table VII. In the meta-analysis, the relative risk of perinatal mortality in IVF versus control twins was 0.58 (0.44–0.77) in six matched studies and in three nonmatched studies 0.84 (0.53–1.32) (Helmerhorst *et al*., 2004). Matched studies were dominated by the Flanders study (Dhont *et al*., 1999), which accounted for 78% of the cases and by a study with extraordinarily high mortality among controls (Fitzsimmons *et al*., 1998). In the latter a lower morbidity in IVF (0/112) versus control twins (12/216) was observed (P = 0.01) even after exclusion of MC twins. A large Swedish register study on 2136 IVF twins versus twins in the general population reported no difference in mortality rates, although the definition of mortality was not given and crude numbers not available (Bergh *et al*., 1999).

Our national cohort study revealed no significant difference in perinatal mortality between IVF and control twins, however, the rate of live-born, who died within the first year of life, was significantly lower among IVF/ICSI twins (10/1000) compared with control twins (15/1000) (P = 0.04) (Pinborg *et al*., 2004b). This difference disappeared, when restricted to unlike-sexed twins, which was in agreement with a study on DZ twins, where the risk of perinatal mortality in IVF versus natural twins was OR = 1.47 (1.01–2.15) (Lambalk and van Hooff, 2001).

Table VI. Admission to neonatal intensive care unit (NICU) in IVF/ICSI twins versus control twins

Author	Number of infants		NICU [days (mean ± SD)]			Admission (%)			Matching
	IVF	Controls	IVF	Controls	*P*-value	IVF	Controls	OR/RR (95% CI)	
Meta-analysis									
Helmerhorst	NA	NA	–	–	–	–	–	1.05 (1.01–1.09)	Various
Original papers									
Pinborg *et al.* (2004b)	3438	10362	19.8	18.4	0.003	56.3	52.4 (S)	1.2 (1.1–1.3)*	Yes
Dhont *et al.* (1999)†	2482	2482	–	–	–	69.6	67.8 (NS)	–	Yes
Dhont *et al.* (1997)†	230	230	–	–	–	46.9	26.1 (<0.05)	–	Yes
Bernasko *et al.*†	210	558	–	–	–	45.7	35.8 (NS)	1.3 (1.0–1.7)	No
Daniel *et al.*†	192	192	10.0 ± 11.0‡	10.8 ± 20.2‡	S	44.3	30.2 (S)	–	No
Kozinszky *et al.*	150	150	–	–	–	24.0	23.3 (NS)	–	Yes
Agustsson *et al.*†	138	906	–	–	–	45.7	40.6 (NS)	0.9 (0.6–1.3)	No
Putterman *et al.*	120	202	–	–	–	39.2	39.6 (NS)	1.0 (0.6–1.6)	Yes
Koivurova *et al.* (2002a)†	103	103				38.2	44.7 (NS)	0.8 (0.4–1.4)	Yes
Saunders *et al.*	94	34	19.4 ± 2.7	30.2 ± 10.6	S	–	–	–	Yes§
Tallo *et al.*	72	72	17 ± 20	9 ± 10	0.002	–	–	–	Yes
Moise *et al.*†	40	80	11.5 ± 14.2	12.3 ± 10.8	–	40	29 (NS)	–	Yes
Isaksson *et al.*‡	40	200	–	–	–	10.0	7.5 (NS)	–	Yes
Addor *et al.*†	26	154	–	–	–	26.9	12.3 (NS)	–	Yes

NA, Crude numbers not available; NS, non significant; S, significant.
*Matched for gestational age.
†Studies included in the meta-analysis by Helmerhorst *et al.*
‡Mean duration of hospitalization of first twin.
§Matched for birthweight.

Table VII. Perinatal mortality in IVF/ICSI twins versus spontaneously conceived twins

Author	Publication year	Per 1000 (number of infants)		RR (95% CI)	*P*-value
		IVF	Control		
Meta-analysis					
Helmerhorst	2004	*N* = 3128	*N* = 3392	0.58 (0.44–0.77)	–
Original papers					
Pinborg *et al.*	2004b	20.7 (71/3438)	23.4 (242/10362)	0.88 (0.68–1.15)*	NS
Dhont *et al.*†	1999	24.6 (61/2482)	33.0 (82/2482)	0.74 (0.54–1.03)	NS
Lambalk and van Hooff‡§¶	2001	65.6 (63/960)	47.3 (58/1226)	1.47 (1.01–2.15)*	S
Westergaard *et al.*	1999	22.2 (19/854)	31.6 (27/854)	0.70 (0.39–1.26)	NS
Dhont *et al.*†	1997	0 (0/230)	26.1 (6/230)	0.08 (0.00–1.36)	NS
Koudstaal *et al.*†¶	2000	31.1 (6/192)	10.4 (2/192)	3.07 (0.61–15.38)*	NS
Kozinszky *et al.*	2003	0 (0/150)	13.3 (2/150)	Not relevant	NS
Olivennes *et al.*‡	1996	69.4 (10/144)	85.4 (28/328)	0.81 (0.41–1.63)	NS
Agustsson *et al.*‡	1997	14.5 (2/138)	17.6 (16/906)	0.82 (0.19–3.53)	NS
Fitzsimmons *et al.*†	1998	17.9 (2/112)	111.1 (24/216)	0.14 (0.03–0.63)*	0.003
Tallo *et al.*†	1995	55.6 (4/72)	55.6 (4/72)	1.00 (0.26–3.85)	NS
Isaksson *et al.*†	2002	0 (0/40)	30.0 (6/200)	0.38 (0.02–6.56)	NS
Tan *et al.*	1992	38.2 (NA)	NA	–	NS

NA, not available; NS, non significant; S, significant.
*Odds ratio.
†Matched studies included in the meta-analysis by Helmerhorst *et al.*
‡Nonmatched studies included in the meta-analysis by Helmerhorst *et al.*
§Only dizygotic twins.

In the Danish twin cohort, perinatal mortality in IVF twins was almost twice as high as in IVF singletons; 20.7 versus 11.0 per 1000 ($P < 0.001$) (Pinborg *et al.*, 2004c). Two earlier studies found perinatal mortality rates ranging between 34.4 and 38.2 in IVF twins to 13.5–18.6 per thousand in IVF singletons including 288 and 1164 IVF twins and 592 and 916 IVF singletons, respectively (Rizk *et al.*, 1992; Rufat *et al.*, 1994).

To summarize, based on the existing literature predominated by smaller studies, perinatal mortality is similar to or perhaps slightly lower in IVF than in control twins. If perinatal mortality in IVF

twins is reduced, it seems to be related to the lower frequency of MZ twinning. As expected perinatal mortality in IVF twins was twice as high as in IVF singletons.

Obstetric outcome in ICSI twins

The largest prospective, controlled study on ICSI children with 1228 eligible ICSI and 1250 IVF twins, revealed no differences in neonatal measurements and total malformation rates between IVF and ICSI twins (Bonduelle *et al*., 2002). In accordance, malformation rates in IVF and ICSI twins were similar in the Danish cohort study (Pinborg *et al*., 2004b). Further the risk of LBW and prematurely was similar in ICSI and IVF children in our Danish cohort study (unpublished data). On the contrary three earlier studies yielded better obstetric outcome in ICSI twins; however, these reports were either very small or lacked control groups (Govaerts *et al*., 1998; Loft *et al*., 1999; Wennerholm *et al*., 2000b).

In conclusion, as in singletons, the ICSI procedure compared to conventional IVF does not seem to have any negative influence on obstetric outcome in twins.

Summary on obstetric outcome

The general consensus with few exceptions is that IVF twins have neonatal outcomes that are similar to those conceived spontaneously.

Evidence has accumulated that there are higher obstetric risks in assisted reproductive than in spontaneously conceived singletons (Verlaenen *et al*., 1995; Dhont *et al*., 1999; Westergaard *et al*., 1999; Koudstaal *et al*., 2000; Schieve *et al*., 2002). This was recently documented in two reviews, where odds ratios of LBW in IVF singletons versus spontaneously conceived singletons ranged from 1.4 to 1.8 and of VLBW 1.8–2.7 (Helmerhorst *et al*., 2004; Jackson *et al*., 2004). It still remains unclear whether the increased risk of adverse obstetric outcome in IVF singletons is a direct effect of the procedure involving such technology (Olivennes *et al*., 1993; Sundstrom *et al*., 1997) or reflects some other factor related to the underlying infertility of the couples (Williams *et al*., 1991; Henriksen *et al*., 1997; McElrath and Wise, 1997). Recent studies have shown that infertility *per se*, unrelated to treatment, is associated with an increased risk of adverse obstetric outcome (Pandian *et al*., 2001; Basso and Baird, 2003). If it is the IVF technology itself, we should expect a poorer outcome also in IVF twins, however, as previously stated IVF twins have an advantage in terms of a lower rate of MC twins. Further, the overall much higher risk in twin pregnancies might conceal a limited risk of adverse outcome in IVF twins.

The considerable higher risk of adverse obstetric outcome in IVF twins than in singletons and the 20-fold higher ART twin birth rate is still one of, if not, the most serious adverse effect of ART

Long-term consequences

Interpretation of most published reports on long-term outcome in ART twins is constrained by numerous methodological limitations especially small sample size ensuing limited statistical power. Many studies are vitiated by selection bias, because often only a small proportion of the total number of ART births from the target clinic population, appears to have been enrolled. Additionally, in some studies no or an inadequate comparison group is included, and loss to follow-up is high. In addition, the follow-up period is often too short to identify neurodevelopmental sequelae and several studies lack adjustment for plurality. This review will mainly be focused on the few existing controlled population-based studies; however, smaller studies have been critically assessed.

Neurological sequelae

Only two population-based controlled studies on neurological sequel in IVF/ICSI twins have been published; a Swedish and a Danish both with stratification for gender and year of birth enrolling 2060 and 3393 IVF twins, respectively (Strömberg *et al*., 2002; Pinborg *et al*., 2004d). Similar adjusted risks of cerebral palsy in IVF/ICSI versus control twins were provided in both studies, although the only predictive factors of cerebral palsy were male sex and prematurity or LBW, whereas maternal age had no influence (Strömberg *et al*., 2002; Pinborg *et al*., 2004d).

The Danish study was based on the national twin birth cohort between 1995 and 2000 (Pinborg *et al*., 2004d). Children were followed until 2–7 years of age with an average age at follow-up of 4.2 years. IVF children were identified through the compulsory Danish IVF Registry (Nyboe Andersen *et al*., 1999), and diagnosis codes through the Danish Hospital Registry and the Danish Psychiatric Central Registry with coverage rates close to 100% (Munk-Jorgensen and Mortensen, 1997; Andersen *et al*., 1999). The Danish study yielded similar prevalence rates of neurological sequel (8.8; 9.6; 8.2) and cerebral palsy (3.2; 4.0; 2.5) per 1000 children in IVF/ICSI twins, control twins and IVF/ICSI singletons, respectively (Pinborg *et al*., 2004d). Similar results were found, when restricting the analysis to different-sex twins. The crude prevalence of cerebral palsy in eastern Denmark in 1987–1990 was 2.4 per 1000 (Topp *et al*., 2001), which was very close to the 2.5 per 1000 in IVF/ICSI singletons observed in our study.

The Swedish study was based on an earlier cohort including all IVF children born between 1982 and 1995 (Strömberg *et al*., 2002). The mean follow-up period in the Swedish study was shorter with 25% of the children being aged 3 years or more at time of follow-up in contrast to 62% of the Danish children. The prevalence rates of cerebral palsy in the Swedish study were (7.4; 6.9; 3.8 per 1000) in IVF/ICSI twins, spontaneously conceived twins and IVF/ICSI singletons, respectively. These rates are considerably higher than those found in the Danish study and also higher than the prevalence rate of cerebral palsy in the western health care region of Sweden in 1991–1994 (2.3 per 1000) (Hagberg *et al*., 2001). However, an under-ascertainment in the Danish study is more likely than an over-ascertainment in the Swedish study, as the Swedish diagnoses were obtained from medical records in habilitation centres, whereas diagnoses in the Danish study were discharge diagnosis codes from somatic and psychiatric hospitals. Thus only children with cerebral palsy diagnosed in a hospital setting were eligible, whereas children treated by health care professionals out of a hospital setting were never encountered. This is also supported by the fact that the prevalence rate in spontaneously conceived twins (4.0/1000) in the Danish study was lower than in five populations in Australia and the United States of America (3.1–9.5 per 1000) with a total prevalence of 5.9 per 1000 reported in a recent paper (Scher *et al*., 2002), whereas the Swedish prevalence was higher (6.9/1000).

In a Danish register study on singletons born between 1995 and 2001, rate ratio of cerebral palsy in IVF versus non-IVF singletons was 1.8 (1.2–2.8) ($P < 0.01$) (Lidegaard *et al*., 2005). Thus, it

seems that the same prevalence rate of cerebral palsy observed in twins and singletons after IVF/ICSI is attributable to a higher rate of cerebral palsy in IVF/ICSI singletons than spontaneously conceived singletons. This is in agreement with the Swedish study, where IVF singletons carried an increased risk of cerebral palsy 2.8 (1.3–5.8) as compared with singletons from the general population (Strömberg *et al*., 2002).

Two other controlled studies have assessed neurological sequel in IVF twins with sample size between 94 and 272, which is too small to draw valid conclusions (Saunders *et al*., 1996; Minakami *et al*., 1998).

Motor and cognitive development

Most of the reports on cognitive development in ART children are too small to make separate analyses on twins or had a lack of control groups (Mushin *et al*., 1985; Yovich *et al*., 1986; Morin *et al*., 1989; Brandes *et al*., 1992; Ron-El *et al*., 1994; Cederblad *et al*., 1996; Olivennes *et al*., 1997; Levy-Shiff, 1998).

A population-based study on 100 Finnish IVF and control twins followed up to 3 years of age with matching for sex, year of birth, area of residence, parity, maternal age and social class reported similar psychomotor development in the two groups (Koivurova *et al*., 2003). Our national postal survey on 472 Danish, 4-year-old IVF/ICSI twins yielded that special needs were present in (9.9; 10.7; 6.1%) and speech therapy was provided to (6.4; 7.8; 3.2%) of the IVF/ICSI twins, control twins and IVF/ICSI singletons, respectively (Pinborg *et al*., 2003b). Special needs included ergo therapy, physiotherapy or requirement of speech therapy or a special remedial teacher. In only one outcome measure, maternal-rated speech development, IVF/ICSI twins did better than control twins, OR = 2.5 (95%CI = 1.7–3.3).

The risk of having special needs were similar in IVF/ICSI twins and IVF/ICSI singletons after adjustment for LBW, but IVF/ICSI twins were still more likely to receive speech therapy than IVF/ICSI singletons after birthweight adjustment (OR = 2.0, 95% CI = 1.1–5.0) (Pinborg *et al*., 2003b). In line with this, maternal rating of speech development in their children was significantly poorer in IVF/ICSI twins than singletons even after adjustment for LBW. The differential speech development in IVF twins observed in our study is a well-described problem in twins in general, where twins tend to lag behind singletons in their speech development (Rutter and Redshaw, 1991). Three main risk factors for this have been suggested: (i) pre- and perinatal environment, (ii) features that apply only to twins such as monozygosity, minor congenital anomalies and 'inter-twin language' and (iii) postnatal experiences that differ between twins and singletons including additional family stress, dyadic interaction with their mothers and more interaction with a child at the same developmental level (Rutter *et al*., 2003). A recent study found that obstetric/perinatal features in children born after 33 weeks of gestation did not account for the slower language development in twins compared with singletons (Rutter *et al*., 2003). The language delay in twins was found in healthy nonhandicapped children and was not a function of diagnosable brain damage, further it persisted after adjustment for gestational age in twins born after 33 weeks of gestation (Rutter *et al*., 2003). In a companion paper the same authors concluded that patterns of parent–child interaction and communication within the normal range have environmental mediated effects on language and account for twin-singleton differences in language development (Thorpe *et al*., 2003).

Neurological sequelae and cognitive development in ICSI twins

Results from the Danish twin birth cohort revealed similar risk of neurological sequelae in ICSI versus twins after conventional IVF (Pinborg *et al*., 2004d). Only one study on psychomotor development in ICSI children has specifically addressed twins, the group of Bonduelle examined 69 2-year-old ICSI and 61 IVF twins. In this limited number of twins, no differences were found in cognitive development between IVF and ICSI twins using a Bayley Scale of Infant development (Bonduelle *et al*., 2003); however, both IVF and ICSI twins had significantly lower scores than IVF and ICSI singletons.

Growth and chronic diseases

In the previously mentioned Finnish study similar growth features were observed in IVF and control twins, except for a difference in mean height at 1 year in favour of IVF twins (Koivurova *et al*., 2003). A smaller study found postnatal growth features up to 18 months similar in 97 twins conceived with cryopreserved embryos, 95 IVF twins from fresh embryos and 96 spontaneously conceived twins (Wennerholm *et al*., 1998). In addition, an Australian study yielded that mean weight percentiles for twins up to 2 years were not significantly different than those from singletons, when age was corrected for prematurely, indicating catch-up growth from birth (Saunders *et al*., 1996).

Although, the cumulative incidence of chronic diseases was significantly higher among IVF children in the full sample (singleton + twins) and in IVF singletons particularly regarding respiratory diseases and diarrhoea, no differences were observed in the cumulative incidence of chronic diseases at different age levels in the twin comparisons (Koivurova *et al*., 2003), which was in accordance with our national postal survey (Pinborg *et al*., 2003b).

Hospital care utilization and surgery

Based on data from the Danish twin birth cohort followed until 2–7 years of child-age, the risks of hospitalization and operations were comparable in IVF/ICSI and control twins also when analysing different-sex twins only (Pinborg *et al*., 2004e). However, IVF/ICSI twins were more likely to be admitted in hospital OR = 2.4 (95% CI = 2.2–2.6) and to have undergone surgery OR = 1.3 (95% CI = 1.1–1.5) than IVF/ICSI singletons with adjustment for year of birth, maternal age and parity. Although the risk of admission was maintained, the risk of operation disappeared when restricted to term children. In all three groups the vast majority of admissions occurred within the first year of life. In accordance a Swedish population-based study found increased risk of hospitalization in IVF twins as compared with singletons and no excess risk, when compared with control twins (Ericson *et al*., 2002).

The average number of days spent in hospital in the Danish study was for IVF/ICSI twins, control twins and IVF/ICSI singletons (14.5; 13.8; 5.3) showing a difference of 9.2 days of admission between IVF/ICSI twins and singletons, which was very similar to 7.4 days in the Swedish study. Similar results were provided in a small Australian study (Leslie *et al*., 1998). However these data may be skewed with a small number of babies with very long admissions. Because long admissions will have disproportionate influence on the mean number of stay, the appropriate comparison of central tendency would have been the median.

Childhood cancer

Three large studies have demonstrated no increased incidence of cancer in ART versus spontaneously conceived children though none of the studies specifically address twins (Bruinsma *et al*., 2000; Klip *et al*., 2001; Ericson *et al*., 2002). With an average follow-up time of 4.2 years, none of the 3393 IVF/ICSI twins in the Danish twin cohort study developed cancer versus eleven of the control twins and nine of the IVF/ICSI singletons, the differences being of no statistical significance (Pinborg *et al.*, 2004b,c). Due to the rarity of infant malignancies, it is difficult to reach any conclusions (Doyle *et al*., 1998). However, studies on unselected twin populations suggest twinning *per se* is not a risk factor of cancer and there is no reason to believe that IVF/ICSI twins should be at increased risk compared with their singleton counterparts (Hemminki and Xinjun, 2002).

Socioemotional development and family implications

In our national questionnaire study, couples with twins were more likely to report increased marital strain and less marital benefit compared with singleton parents (Pinborg *et al*., 2003b). However, the divorce/separation rate 4 years after delivery in couples with IVF twins (7.3%) and IVF singletons (6.9%) was similar, whereas the rate in control twins (13.3%) was almost twice as high. Hence, despite twinning causing increased marital stress, IVF twin parents had a low risk of divorce/separation, which suggests strong marital relationship in these parents. Presumably IVF parents cope better with the increased strain caused by twins, thus avoiding divorce/separation. As expected twinning caused more influence on the professional and social life of their mothers than singletons (Pinborg *et al*., 2003b). Further, first time mothers were more likely to report that their offspring had had high impact on all aspects of maternal lives, as previously shown (Colpin *et al*., 1999). Hundred and three families with 1-year-old twins, who were either conceived after IVF, ovulation induction or naturally were compared and no differences regarding parenting stress or parental psychosocial wellbeing were found. When only first time mothers were studied, those with a history of infertility had higher stress scores and experienced less psychosocial wellbeing (Colpin *et al*., 1999). In agreement, another study on 12 families with 4- to 8-year-old IVF twins and 14 with naturally conceived twin pairs using interviews and questionnaires found parenting quality and child behaviour similar in the two groups (Cook *et al*., 1998). However, IVF parents reported greater stress associated with parenting than couples with naturally conceived twins, presumably because of more IVF parents being first-time parents (Cook *et al*., 1998). A recent study, assessing 121 families with twins conceived by IVF or ovulation induction compared with naturally conceived twins, revealed no significant differences between the two groups in any parent-related or teacher-related measure of child behaviour (Tully *et al*., 2003).

In conclusion, parents of twins conceived by ART may find parenting more demanding than those with ART singletons, which may in turn lead to poorer behavioural outcomes of the children. However, the strain on parenting does apparently not lead to a higher rate of divorce/separation. Because the literature on socioemotional development in ART is scarce, the behavioural outcome of IVF twins still needs further exploration.

Summary on long-term consequences

One of the most important clues concerning long-term outcome in IVF twins is that the three- to four-fold increased risk of cerebral palsy in spontaneously conceived twins versus singletons is not recovered in IVF twins versus IVF singletons (Scher *et al*., 2002; Pinborg *et al*., 2004d). However, this may in turn be due to a higher risk of cerebral palsy in IVF singletons (Strömberg *et al*., 2002; Lidegaard *et al*., 2004). After all, this must not lead us to conceal the fact that regarding other childhood consequences i.e. special needs, speech therapy, hospital admissions, surgery and mortality, IVF twins carry a higher risk than singletons some of this being related to the higher prematurity rate.

Vanishing twins

It appears that twins are more often conceived than born. The process of disappearance of gestational sacs or embryos after documented foetal activity is known as the vanishing twin phenomenon, which occurs not only in relation to foeti papyracei, as twin material can also be reabsorbed without leaving any trace (Landy and Keith, 1998).

As early as in 1993, Petterson and coworkers showed that in spontaneously conceived twins born in the 1980s identified from the Western Australian cerebral palsy register, the prevalence of cerebral palsy was 96.2 per 1000 in twins, who survived to 1 year after the death of a cotwin, 15 times higher than for twins, who were both live born (6.4/1000), and 60 times higher than for live born singletons (1.6/1000) (Petterson *et al*., 1993). Moreover, they found that pregnancies in which intrauterine death of a cotwin occurred were associated with a 10% greater risk of cerebral palsy. The question on vanishing twin in IVF/ICSI pregnancies arose as the literature, documented in two recent meta-analysis, has agreed that IVF singletons have slightly adverse obstetric outcome compared with spontaneously conceived singletons (Helmerhorst *et al*., 2004; Jackson *et al*., 2004). Further, two national cohort surveys have shown that IVF singletons are at a higher risk of developing cerebral palsy (Strömberg *et al*., 2002; Lidegaard *et al*., 2005). In addition, incidence rates of neurological sequelae were similar in IVF singletons and twins in the Danish twin cohort (Pinborg *et al*., 2004d), albeit spontaneously conceived twins carry a three- to four-fold higher risk of cerebral palsy than singletons (Scher *et al*., 2002; Topp *et al*., 2004).

Some researchers have argued that the reason for this excess risk is the underlying infertility of the couples seeking treatment rather than the treatment themselves (Pandian *et al*., 2001; Basso and Baird, 2003). Another plausible explanation is a higher rate of vanishing twins caused by multiple embryo transfer in IVF/ICSI treatment. In spontaneously conceived twin pregnancies, late intrauterine death of one twin has considerable influence on the risk of morbidity and mortality in the surviving cotwin (Pharoah and Adi, 2000; Scher *et al*., 2002). Two studies on IVF pregnancies have shown that after sonographic diagnosis of a twin pregnancy in gestational week 6–7, 12–30% will give birth to a singleton, 60–83% will have a twin delivery and 5–10% ends up with a spontaneous abortion of both foetuses (Landy and Keith, 1998; Tummers *et al*., 2003).

A Danish multicentre cohort study on 8542 clinical pregnancies detected by early ultrasonography between 1995 and 2001, reported that 10.4% of born IVF/ICSI singletons was a twin gestation

in early pregnancy (Pinborg *et al*., 2005). These survivors of a vanished cotwin carried a 2.3-fold increased risk of very preterm birth (<32 weeks) and a 2.1-fold increased risk of VLBW (<1500 g) and a mortality rate that was three-fold increased. Birthweight and gestational age was dependent on the onset of spontaneous reduction in pregnancy, the later onset the worse outcome (Table VIII). In accordance two recent papers revealed that birth occurred significantly earlier in singleton pregnancies with two gestational sacs than in those with one (Dickey *et al*., 2002; Lancaster, 2004). Moreover, a large US register study found that the risk of LBW was higher the higher the number of foetal hearts on early ultrasonography for both singletons and twins (Schieve *et al*., 2002).

The knowledge on the risk of long-term consequences for the singleton survivor of a vanished co-twin is sparse. The Danish multicentre survey revealed a nearly two-fold increased risk of cerebral palsy in singleton survivors of a vanished cotwin (OR = 1.9, 95% CI = 0.7–5.2), but presumably because of the limited sample size it was not statistically significant. We found, however, a significant correlation between the gestational age at onset of spontaneous reduction and the development of neurological sequelae (Table VIII) (Pinborg *et al*., 2005). Though, the study comprised 72% of all IVF/ICSI cycles performed in Denmark in 1995–2001 and the outcome on more than 8000 clinical pregnancies was detected, the final cohort comprised only 642 singleton survivors. As this is the first follow-up study on IVF/ICSI singleton survivors of a vanished cotwin, more studies are needed to draw firm conclusions on the long-term consequences. What we can conclude is, however, that one of the major causes for the slightly adverse obstetric outcome in IVF/ICSI singletons is the higher rate of singletons from vanishing twin gestations, which is another argument for eSET.

Selective termination

Selective termination/reduction is the directed reduction of a foetus in a multiple gestation due to an abnormality detected in that foetus (Chescheir, 2004). This is opposed to 'multifetal reduction' or elective reduction, which is a reduction procedure performed because of an excess number of foetuses in utero to maximize the chances of a delivery of at least one healthy child. To many people, any deliberate termination of a foetus is inherently controversial and according to the Danish law elective reduction of twin pregnancies is not permitted, thus only performed very rarely for maternal medical conditions and will not be discussed further in this review.

Accurate determination of chorionicity is crucial before performing ST as conventional termination techniques including percutaneously injected intracardiac potassium chloride which can only be performed in a DC foetus (Rochon and Stone, 2003). In MC twins any substance given to Twin A will transverse the common placenta and enter Twin B. Potential options for ST in MC twins include cord ligation, cord coagulation or laser cord occlusion; however, the aim of future studies will be to determine the optimal technique for this situation (Quintero *et al*., 1996; Rochon and Stone, 2003).

In 1997, Berkowitz and coworkers reported that selective termination in a series of 100 consecutive cases performed at a single centre was associated with a spontaneous pregnancy loss rate of 3% (Berkowitz *et al*., 1997). In international collaborative series at eight centres worldwide, outcomes of 345 selective terminations for fetal anomalies in DZ twins were evaluated (Evans *et al*., 1999). The spontaneous pregnancy loss rate was 7% among twin gestations, but exceeded 12%, when women started with three or more foetuses. There was no correlation between loss or prematurely and gestational age at procedure except that first-trimester cases showed a trend towards lower loss rates than those at ≥13 weeks (Evans *et al*., 1999). Of the pregnancies after selective reduction from 2 → 1, 4.6% was born in 25–28 gestational weeks, 7.8% in gestational week 29–32, 15.7% between 33 and 36 weeks and 64.1% ≥37 weeks.

Because of the discrepancies of the above two studies the group by Berkowitz initiated an expanded study on 200 selective terminations

Table VIII. Neonatal and long-term outcome in survivor cohort according to time of vanish of cotwin

Time of vanish	Early	*P*-value	Intermediate	*P*-value	Late
Neonatal outcome					
Number of live-born children	424	*	187	†	31
Birthweight (mean ± SD)	3365 ± 695	<0.001	3185 ± 867	<0.001	2178 ± 940
Gestational age, weeks (mean ± SD)	39.4 ± 2.6	<0.001	38.5 ± 4.1	<0.001	34.3 ± 4.8
Neonatal intensive care unit (NICU)					
NICU admissions (%)	29.0%	0.6	31.0%	<0.001	51.6%
>7 days in NICU	41.7%	0.5	47.1%	<0.001	75.0%
>28 days in NICU	8.7%	0.2	15.7%	<0.001	43.8%

Time of vanish	Early	Intermediate	Late	Spearman correlation (*r*) ‡	*P*-value
Long-term outcome					
Number of live-born children	424	187	31		
Mortality <1year, n (per 1000)	1. (2.4)	8 (42.8)	1 (32.3)	–0.145	<0.001
Number of children with (per 1000)					
Cerebral palsy	3 (7.1)	2 (10.7)	0	–0.008	0.85
Neurological sequelae	4 (9.4)	5 (26.7)	2 (64.5)	–0.09	0.022
Neurodevelopmental diseases	14 (33.0)	15 (80.2)	3 (96.8)	–0.109	0.006

Early vanish, before 8 weeks of gestation; intermediate vanish, after 8 weeks of gestation and late vanish, cotwin recorded as stillbirth.
*Comparison of early versus intermediate vanish of cotwin. Proportions by Pearson's chi-square test and mean values by Student's *t*-test.
†Comparison of intermediate versus late vanish of cotwin. Proportions by Pearson's chi-square test and mean values by Student's *t*-test.
‡Spearman correlation coefficient (*r*) for ordinal data.

at their centre, of which 164 women started as a twin gestation (Eddleman *et al*., 2002). The loss rate for those carrying twins was 2.4%, but almost five-fold higher in patients carrying three or more foetuses (11.1%) (Eddleman *et al*., 2002). Gestational age at delivery after ST was 3.7% <28 weeks, 12.1% week 28.0–31.9, 16.3% week 32.0–35.9 and 67.9% >36.0 weeks, which was very close to the findings in the international collaborative series (Evans *et al*., 1999). Eddleman and coworkers claimed that the lower loss rates in their studies reflected the benefit of having a small number of operators adhere to a common protocol. The only factors significantly associated with a higher risk of pregnancy loss in these series were the starting number of foetuses (3 or more) and having more than one foetus selectively terminated (Eddleman *et al*., 2002). In contrast to the findings of previous studies (Lynch *et al*., 1996; Evans *et al*., 1999), there was no advantage of performing selective termination before 20 weeks of gestation (Eddleman *et al*., 2002). This is reassuring to patients whose fetal abnormality is not diagnosed until after 20 weeks of gestation.

Based on the existing literature selective termination for an abnormal DC twin in experienced hands appears to be safe and effective with pregnancy loss rates between 2 and 7%, when it is performed by intracardiac injection of potassium chloride. However, the benefits expected from selective termination should always be weighed against the potential risk of the procedure concerning the unaffected twin. In MC twins the ST procedures still remains to be properly evaluated.

eSET

Trials comparing elective single embryo transfer (eSET) and DET

Several studies have been conducted to identify patients suitable for eSET (Coetsier and Dhont, 1998; Strandell *et al*., 2000; Hunault *et al*., 2002). A large retrospective study on 2107 IVF cycles stated that the only variables predictive of multiple birth were female age and number of good quality embryos transferred (Strandell *et al*., 2000). Based on these findings the largest randomized-controlled trial on eSET versus DET was initiated in Sweden (Thurin *et al*., 2004b). In this Scandinavian multicentre study 331 women <36 years, undergoing their first or second IVF/ICSI cycle with at least two embryos of high quality, were randomized to DET transfer and 330 women to eSET. As to give women in both groups the opportunity of achieving two embryos, a new approach was used, i.e. women in the set group received a single embryo transfer and if no live birth, subsequently a frozen SET, whereas women in the DET group received a double embryo transfer at one occasion. This was to compare 1 + 1 with 2 embryos. In the eSET and DET group 38.8 and 42.9% ($P = 0.31$) achieved an ongoing pregnancy/live birth, respectively, with only one twin birth in the eSET group and a multiple pregnancy rate of 33.1% in the DET group (Table II). The ongoing pregnancy/live birth rate was 27.6% in the fresh set cycle, which was significantly lower than 42.9% in the DET ($P < 0.001$). In the subsequent cycle, involving transfer of a frozen-and-thawed embryo, the rate of live births was 16.4%. The results of the Scandinavian study are in accordance with a recent Cochrane review yielding a clinical pregnancy rate in two versus one embryo transfer of OR = 2.08 (95% CI = 1.24; 3.50), live birth rate OR = 1.90 (95% CI = 1.12–3.22) and multiple pregnancy rate 9.97 (95% CI = 2.61–38.19) (Pandian *et al*., 2004). Only three randomized trials fulfilled the selection criteria for inclusion in the Cochrane review, all being limited by their small sample size, further none included subsequent single frozen embryo transfers (Table II) (Gerris *et al*., 1999; Martikainen *et al*., 2001; Lukassen *et al*., 2002).

In a recent Belgian review (Gerris, 2004), four truly prospective randomized trials were referred to including an abstract with preliminary data from the Scandinavian multicentre study (Table II) (Gerris *et al*., 1999; Martikainen *et al*., 2001; Gardner *et al*., 2004; Thurin *et al*., 2004a). Taken together, the mean fresh pregnancy rate after SET in the four studies was 30.7% with 2.2% twins and 47.6% after DET with 33.8% twins.

In a single fertility clinic in Helsinki 1871 IVF/ICSI cycles carried out between 1997 and 2001 were retrospectively examined (Tiitinen *et al*., 2003). In this period the number of set increased from 11 to 56%, whereas the multiple delivery rate dropped markedly from 25 to 5% maintaining a relatively stable overall pregnancy rate, mean 34.0% (Tiitinen *et al*., 2003). The same experience was reported from a single entity in Belgium, where the proportion of eSET increased from 1.5 to 17.5% from 1997 to 2002, although the overall pregnancy rate was fairly constant on 34% and the twinning rate dropped to 14% (De Sutter *et al*., 2003).

It is evident that introducing eSET diminishes the twin birth rate considerably, while maintaining ongoing pregnancy rates of 30–40% per transfer. However, the transfer of two good embryos always yields more pregnancies than the transfer of one good embryo, therefore, a well-functioning freezing programme is of paramount importance (Tiitinen *et al*., 2001; Gerris *et al*., 2003; Tiitinen *et al*., 2004). In conclusion, eSET can be implemented, while maintaining good pregnancy rates, if the twin prone patients are carefully selected and if a high-standard freezing programme is present. Specific indications for eSET should be prepared on a European level.

Attitudes

Attitudes towards multiple pregnancy and various pregnancy complications differ between infertile and fertile women. Hence, not surprisingly, infertile women consider multiple pregnancy and the associated risks are more acceptable than for fertile women (Leiblum *et al*., 1990; Gleicher *et al*., 1995; Goldfarb *et al*., 1996; Grobman *et al*., 2001; Pinborg *et al*., 2003a). In a Danish population of mothers of 3- to 4-year-old IVF/ICSI children, acceptance of SET was expressed only by one fifth (Pinborg *et al*., 2003a). Another fourth could be convinced of SET, if more than the normally three reimbursed IVF cycles were offered. Predictive factors of agreement to SET were short duration of infertility and delivery of a child with birthweight less than 1500 g prone to a potentially higher morbidity risk.

In couples well aware of eSET pregnancy rates and the potential risks associated with twin pregnancy, additional information, involving an extra information leaflet and face-to-face discussion, did not change their attitudes towards SET (Murray *et al*., 2004). One study showed that women are being less risk averse than their partners and further that a sizeable proportion of couples do not agree in their preference to twin gestation (Kalra *et al*., 2003).

Consistently, all the above reports have noted that infertile women looked favourably upon the conception of twins, demonstrating that most of the infertile women seem willing to accept

eventual risks together with the social and physical strain. However, it has also been illustrated that being confronted with actual probabilities of specified perinatal complications associated with a twin pregnancy, women were less keen on having a twin pregnancy (Grobman *et al*., 2001; Child *et al*., 2004).

The concurrent view is that implementation of set requires profound counselling including exact risk estimates. In this process information on set pregnancy rates and improved embryo freezing programme play an essential role.

Health-economic costs

Medical costs from induction of IVF pregnancy until end of the neonatal period per twin pregnancy are more than five times higher than per singleton pregnancy (Lukassen *et al*., 2004). However, performing more SET will increase the number of cycles needed to obtain the same number of children, when compared with a standard policy of DET. From theoretical extrapolations and decision-analytical calculations it has been shown that the cost per child born in a programme judiciously applying eSET is comparable with the cost per child in a programme with a standard DET policy (Wølner-Hanssen and Ryedstroem, 1998; De Sutter *et al*., 2002; Garceau *et al*., 2002; Lukassen *et al*., 2004). This is explained by the fact that the higher pre- and neonatal cost due to the twin pregnancies arising after DET is counter balanced by the higher cost for more SET cycles needed to obtain the same number of children. The only prospective health economic study with follow-up of the children until 3 months of age has shown that transfer of a single top quality embryo is equally effective as, but substantially cheaper than, double embryo transfer in women <38 years of age in their first IVF/ICSI cycle (Gerris *et al*., 2004). This in itself is an argument in favour of applying eSET, because twins are prone to higher postnatal long-term morbidity comprising a long-lasting supplementary financial burden.

In Belgium, it has been calculated that that the money saved by avoiding half of the multiple pregnancies suffices to finance all IVF/ICSI in a year, which is the basis for the introduction of a new policy endorsed from July 1, 2003. In the 'Belgian project', six cycles of IVF/ICSI (in a lifetime) will be refunded per patient, provided all participants comply with a restrictive embryo replacement policy implying eSET in first and second IVF/ICSI cycle, if certain criteria are fullfilled. The consequences of this new regulation are yet to be evaluated (Gerris *et al*., 2004; Ombelet *et al*., 2004).

In summary, the higher cost with respect to twin pregnancies can counterbalance the surplus fresh/frozen cycles needed to obtain a pregnancy in eSET. However, prospective health-economic studies assessing the number of surplus cycles needed in eSET to achieve a pregnancy are still warranted.

Conclusions

Although IVF twins have similar outcomes as spontaneously conceived twins and though similar rates of neurological sequelae in IVF twins and singletons are reassuring, IVF twins do carry a considerably higher risk than IVF singletons regarding most other short- and long-term outcome measures. Thus the high twin birth rate and the adverse outcome carried in twins are still one of our main concerns in ART. According to recent reports, ART twin birth rates are still much too high and of even more concern steadily rising worldwide. Hence, a general change in the embryo transfer policy implying eSET to twin prone patients is highly recommended. The Finnish experience provides evidence that implementation of eSET, provided specific indications and a well functioning freezing program, can reduce the ART twin birth rate to less than 10%, while maintaining satisfactory pregnancy rates. The main intervention to cease the epidemic of twin births is set, which can be implemented without affecting the overall goal of achieving a healthy child.

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