

Trends in perinatal health after assisted reproduction: a Nordic study from the CoNARTaS group

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STUDY QUESTIONS: Has the perinatal outcome of children conceived after assisted reproductive technology (ART) improved over time?

SUMMARY ANSWER: The perinatal outcomes in children born after ART have improved over the last 20 years, mainly due to the reduction of multiple births.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: A Swedish study has shown a reduction in unwanted outcomes over time in children conceived after ART. Our analyses based on data from more than 92 000 ART children born in four Nordic countries confirm these findings.

STUDY DESIGN: Nordic population-based matched cohort study with ART outcome and health data from Denmark, Finland, Norway and Sweden.

PARTICIPANTS, SETTING AND METHODS: We analysed the perinatal outcome of 62 379 ART singletons and 29 758 ART twins, born from 1988 to 2007 in four Nordic countries. The ART singletons were compared with a control group of 362 215 spontaneously conceived singletons. Twins conceived after ART were compared with all spontaneously conceived twins ($n = 122\,763$) born in the Nordic countries during the study period. The rates of several adverse perinatal outcomes were stratified into the time periods: 1988–1992; 1993–1997; 1998–2002 and 2003–2007 and presented according to multiplicity.

MAIN RESULTS AND ROLE OF CHANCE: For singletons conceived after ART, a remarkable decline in the risk of being born preterm and very preterm was observed. The proportion of ART singletons born with a low and very low birthweight also decreased. Finally, the stillbirth and infant death rates have declined among both ART singletons and twins. Throughout the 20 year period, fewer ART twins were stillborn or died during the first year of life compared with spontaneously conceived twins, presumably due to the lower proportion of monozygotic twins among the ART twins.

LIMITATIONS, REASONS FOR CAUTION: We were not able to adjust for some potential confounders such as BMI, smoking, length or cause of infertility. The Nordic ART populations have changed over time, and in recent years, both less as well as severely reproductive ill couples are being treated. This may have affected the observed trends.

WIDER IMPLICATIONS OF THE FINDINGS: It is assuring that data from four countries confirm an overall improvement over time in the perinatal outcomes of children conceived after ART. Furthermore, data show the beneficial effect of single embryo transfer, not only in regard to lowering the rate of multiples but also concerning the health of singletons.

STUDY FUNDING/COMPETING INTEREST(S): The European Society for Human Reproduction and Embryology (ESHRE), the University of Copenhagen and the Danish Agency for Science, Technology and Innovation has supported the project. The CoNARTaS group has received travel and meeting funding from the Nordic Federation of Obstetrics and Gynecology (NFOG). None of the authors has any competing interests to declare.

Key words: trends / preterm birth / small for gestational age / assisted reproductive technology

Introduction

For more than 30 years, children have been born after assisted reproductive technology (ART) and in recent years infants born after ART contribute up to 5% of the national birth cohorts in some countries (Step toe and Edwards, 1978; Ferraretti *et al.*, 2012). It has been estimated that almost 5 million children have been born worldwide as the result of ART (Nygren *et al.*, 2011). Still concerns remain about infant health and perinatal outcome (Helmerhorst *et al.*, 2004; Pinborg *et al.*, 2013). ART procedures have developed and the demographic characteristics of those treated with ART have changed over time. Intracytoplasmic sperm injection (ICSI) was introduced in 1992 for treatment of male infertility. A few years later cryopreservation of surplus embryos followed by replacement of a thawed embryo in a natural cycle became an option. Together with a more frequent use of elective single embryo transfer, this resulted in a reduction of the rate of multiples (Thurin *et al.*, 2004; Sullivan *et al.*, 2012). Furthermore, the subfertile population undergoing assisted reproduction has changed over time, as ART is now offered to couples with a shorter duration of infertility thus being less reproductively ill. On the other hand, the *in vitro* techniques also make it possible to treat couples with severe co-morbidity or a long duration of infertility and often complicated reproductive issues (Kallen *et al.*, 2010). The aim of this study was to explore trends in perinatal health in a Nordic population of ART singletons and twins born over a period of 20 years, allowing us to investigate the consequences of ICSI, cryopreservation of embryos and elective single embryo transfer in regard to infant health.

Materials and Methods

All singletons and twins conceived after *in vitro* fertilization (IVF), ICSI and frozen embryo transfer in Denmark, Finland, Norway and Sweden were included in a common Nordic database (Henningsen *et al.*, 2011). Supplementary data, Figure S1 provides an overview of the included infants from each of the four countries. Only newborns with a gestational age of 22 + 0 weeks or more were included. The ART singletons and twins were matched 1:4 with a control group of spontaneously conceived children from their own country regardless of multiplicity. The matching criteria were parity (0 versus ≥ 1) and year of birth. To allow reasonable analyses of twins, data on all spontaneously conceived twins born within the study period were further included in a second control group. Children born after ovulation induction and intrauterine insemination were not included in the ART registries until recently, thus children conceived after these techniques were included among the controls. Data on method of ART conception were available from all countries except Finland. In our data set, we were not able to differentiate between mono- and dizygotic twins. Low birthweight was defined as birthweight <2500 g and very low birthweight <1500 g. Preterm birth was defined as birth before 37 weeks of gestation and very preterm birth as birth before 32 weeks of gestation. Small for gestational

age was defined as less than two standard deviations and large for gestational age more than two standard deviations calculated using Marsal's formula (Marsal *et al.*, 1996). Owing to national differences in the registration of stillbirth, we restricted the analyses on stillbirth to $\geq 28 + 0$ gestational weeks. Infant death was defined as death of a live born child within the first year of life. In the analyses on perinatal outcomes, we excluded all stillbirths ($n = 3693$) and children with missing or outlying values of birthweight and gestational age (Supplementary data, Fig. S1). Data were available from Sweden from 1982 to 2007, but since only very few ART children ($n = 141$) were born during the first 6 years, Swedish data are only included from 1988. Norway contributed with data from 1988 to 2007; Finland from October 1990 to 2007 and Denmark from 1995 to 2007 (Henningsen *et al.*, 2011). All results presented are on combined Nordic data, except for the analysis on mode of ART conception, where Finnish data were excluded.

The analyses were stratified according to child year of birth into four periods: 1988–1992; 1993–1997; 1998–2002 and 2003–2007. The prevalence of several adverse perinatal outcomes was illustrated in graphs showing the development over time. The risk of being born with a birthweight of <2500 or 1500 g, with a gestational age of <37 or 32 weeks or small for gestational age were calculated in logistic regression models comparing ART with spontaneously conceived children. The analyses were adjusted for the matching criteria: mother's parity (0 versus ≥ 1), year of birth and country. They were then further adjusted for the confounding factors: maternal age (<30; 30–34; 35–39; ≥ 40) and child's sex. All of the analyses were carried out separately for singletons and twins. The regression analyses of twin data were adjusted for correlation between twin pairs using generalized estimating equations to fit the logistic regression models. The analyses were performed using SAS statistical software, version 9.1 (SAS Institute).

The study was approved by the Data Protection Agencies and register keeping authorities in each participating country. Positive statements from the regional ethical committees were required in Norway (REK 2010/1909-11) and Sweden (Dnr 023-09, T431-09), but not in Denmark and Finland for research based on register data only.

Results

The merged database included 62 379 singletons and 29 758 twins conceived after ART and a control group of 362 215 spontaneously conceived singletons and 122 763 twins (Supplementary data, Fig. S1).

Maternal and birth characteristics

The rate of primiparous ART mothers declined over the years from ~75% in 1990 to 60% at the end of the study period (Supplementary data, Fig. S2). The mean age of ART singleton mothers was stable around 33.3 years throughout the study period, but for mothers of singletons conceived spontaneously the mean maternal age increased from 26.5 years in the first study period to 29.1 years in the end of the study period. For ART twin mothers the mean age was 32.7 years and remained unchanged during the study period, whereas for twin mothers pregnant after spontaneous conception, the mean maternal

age increased from 28.8 years in the early years to 30.6 years in the last part of the study period. The rate of Caesarean sections was 30% and significantly higher among ART pregnant women compared with a rate of 20% among women who became pregnant after spontaneous conception. The rates of Caesarean section were relatively stable over time in both groups (Supplementary data, Fig. S2). When analysing singleton and twin pregnancies separately, the difference in the Caesarean section rates between ART versus spontaneously conceived was consistent over time within both singleton and twin pregnancies (data not shown).

Multiplicity and mode of conception

From 1989 to 2002, the proportion of twin deliveries after ART remained stable at ~23%, but hereafter it decreased gradually and in

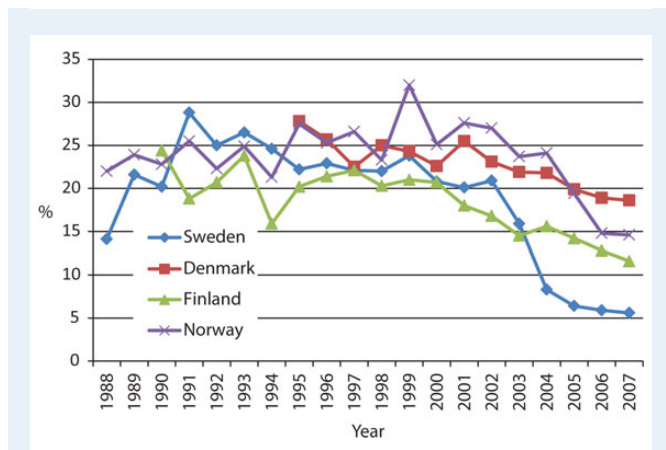


Figure 1 Proportion of twin deliveries among the births of children conceived by ART in four Nordic countries.

2007 the overall Nordic twin delivery rate after ART was only 11.6%. Until year 2002 when the twinning rates began to decline, these rates had been rather similar in the four Nordic countries. Thereafter, a marked difference in the slope of the decline was observed between the four Nordic countries and in 2007 the most notable difference was seen between Denmark and Sweden with twin rates of 18.6 and 5.6%, respectively (Fig. 1). In the early days of ART, the majority of treatment cycles were IVF, but since the introduction of ICSI and frozen embryo replacement both of these methods have been used increasingly (Fig. 2).

Adverse perinatal outcomes

The preterm birth rate among ART singletons declined throughout the study period with the steepest decrease in the early years (Figs 3 and 4). Similarly, the low birthweight rate declined over the years (Table 1). The rates of birthweight below 1500 g and birth before 32 weeks of gestation ART children also decreased during the early time period, but then remained stable and were only slightly higher than for spontaneously conceived children (Table 1). For ART twins the rate of preterm birth did not change over time (Fig. 4). Neither did the rate of very preterm birth (Table 1). The risk of being born small for gestational age decreased throughout the study period for both singletons and twins conceived after ART (Fig. 5). The stillbirth rate in singletons ≥ 28 weeks of gestation declined over the years and from 1990 and onwards no significant difference between ART and spontaneously conceived children was seen. The risk of death within the first year of life also decreased markedly but a difference between ART and spontaneously conceived singletons still remained in the late part of the study period. ART twins had lower rates of both stillbirth and infant death at all times (Fig. 6). In country specific analyses for both singletons and twins on the risk of all adverse perinatal outcomes, the same trends over time were found and no significant differences between the Nordic countries were observed (data not shown).

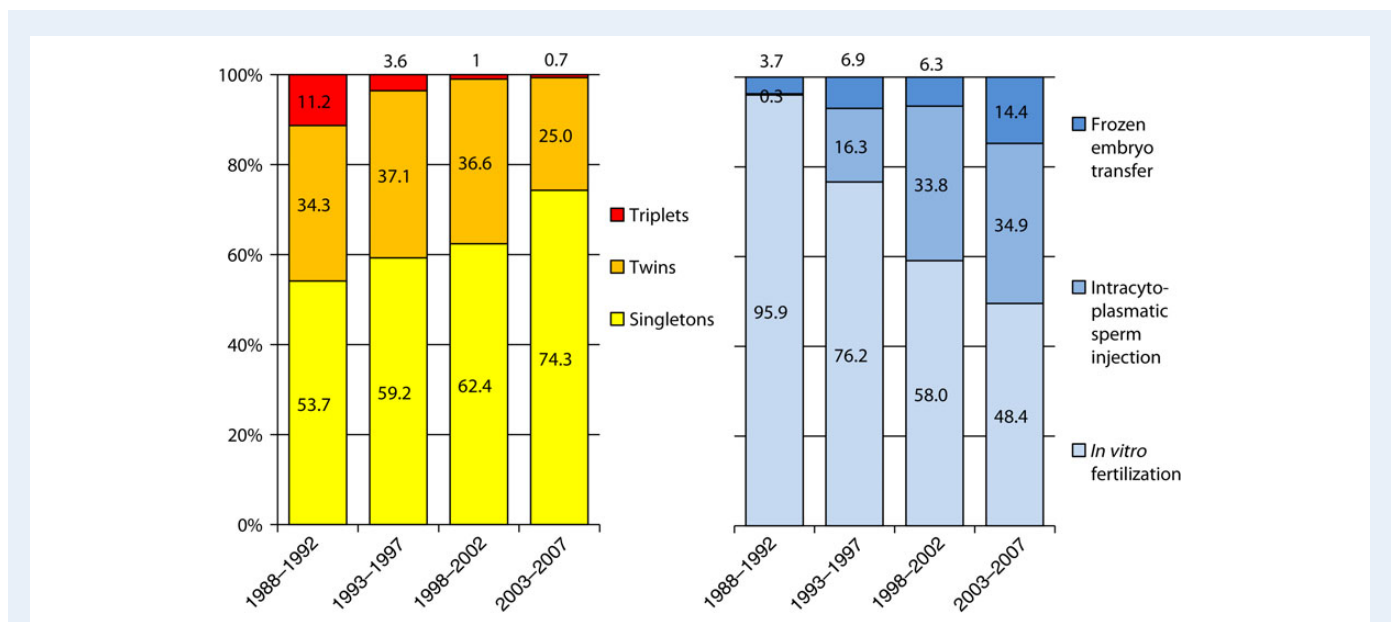


Figure 2 Plurality and mode of conception over two decades in children conceived by ART. Finland does not contribute with data on mode of conception.

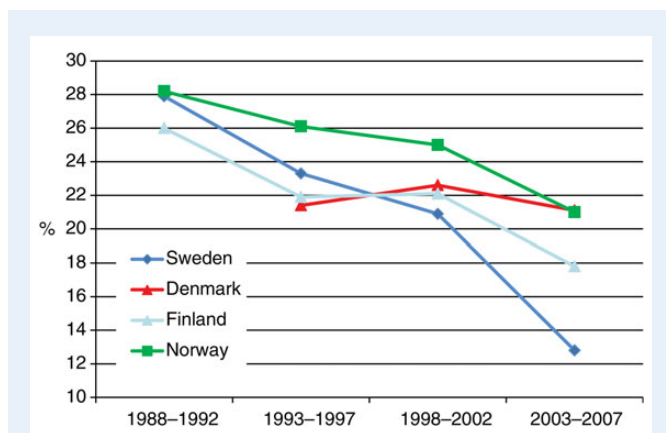


Figure 3 Preterm births among children conceived by ART in four Nordic countries (singletons + twins).

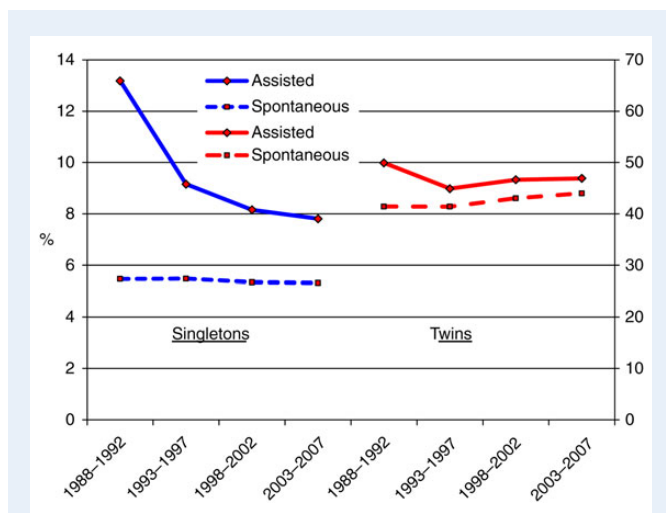


Figure 4 Preterm births according to plurality in children conceived by ART and spontaneously on combined Nordic data.

Singletons born after IVF had a small although statistically significantly increased risk of low birthweight compared with ICSI singletons, odds ratio (OR) 1.12 [95% CI 1.03–1.23]. They also had an increased risk of being born preterm in the last 10 years of the study period, OR 1.21 [95% CI 1.12–1.30]. Singletons conceived after IVF had no significantly increased risk of birthweight below 1500 g, birth before 32 weeks of gestation or being born small for gestational age compared with singletons conceived after ICSI (data not shown). For IVF and ICSI twins the risk of these adverse outcomes did not differ during the study period. When comparing children born after fresh embryo transfer versus frozen embryo transfer the risk of being born with birthweight below 2500 g or small for gestational age was increased for singletons conceived after fresh embryo transfer in the last part of the study period from 2003 to 2007, OR 1.35 [95% CI 1.13–1.60] and OR 1.36 [95% CI 1.09–1.69], respectively. In the same period singletons born after transfer of a fresh embryo had a significantly lower risk of being born large for gestational age, OR 0.63 [95% CI 0.53–0.74] than singletons conceived after

transfer of a frozen–thawed embryo. The risks of being born with a birthweight below 1500 g or before 37 or 32 weeks of gestation were similar in singletons born after transfer of a fresh versus a frozen–thawed embryo (data not shown). When comparing twins conceived after fresh versus frozen embryo transfer only the risk of birthweight below 2500 g, OR 1.07 [95% CI 1.03–1.12] and the risk of being born small for gestational age, OR 1.04 [95% CI 1.02–1.07] were increased and only significantly during the last part of the study period from 2003 to 2007.

Discussion

This Nordic study with more than 92 000 ART children convincingly shows that the considerable increase in the number of ART cycles has been followed by improved perinatal outcomes in ART children over the last 20 years. Especially for ART singletons, we found a marked decrease in the rates of adverse perinatal outcomes such as preterm birth and low birthweight. The causes for the positive development are unknown, but a change in patient characteristics in those treated with ART, as well as refinement of clinical and laboratory skills have probably had a positive impact on the outcomes of the children. The overall improved perinatal outcomes over the past two decades have several explanations. The most important reason is the dramatic decline in multiple births due to elective single embryo transfer. During the study period the rate of twin deliveries was reduced by one third. Double embryo transfer has also in some studies, even when the transfer of two embryos result in a single gestation, been associated with an increased risk of preterm birth and perinatal mortality in ART children (Pinborg *et al.*, 2005; Luke *et al.*, 2012; Sullivan *et al.*, 2012). In a large Swedish study, however, the risk of low birthweight and preterm birth were similar for singletons conceived after single versus double embryo transfer (Sazonova *et al.*, 2011). With less double embryo transfer, we must also expect the rate of vanishing twins to decline and therefore an overall improvement in the health of the surviving twins (Pinborg *et al.*, 2005).

The use of cryopreservation of embryos with succeeding frozen embryo transfer could be another explanation for the overall improved perinatal outcome in ART children over time, as singletons born after frozen embryo transfer have a lower risk of preterm birth and a higher mean birthweight than singletons conceived after fresh embryo transfer (Tiitinen *et al.*, 2004; Wennerholm *et al.*, 2009, 2013; Pelkonen *et al.*, 2010;). The explanation for this is yet not fully understood, although part of it may be explained by the patient and embryo selection. Women with surplus embryos for cryopreservation are good prognosis patients with high ovarian reserve, and their embryos good-quality embryos that have undergone a positive selection by surviving the freezing and thawing procedure. A third factor which may explain the improvements is the introduction of ICSI. A recent meta-analysis demonstrated a lower preterm birth rate after ICSI than IVF (Pinborg *et al.*, 2013). Our analyses confirmed this. In general, women conceiving after ICSI are more reproductively healthy than women conceiving after IVF, as the main indication for ICSI, especially in the Nordic countries, is still severe male infertility (Nyboe *et al.*, 2008). Fewer ART twins were stillborn or died during the first year of life compared with spontaneously conceived twins. We used Weinberg's differential rule based on the number of opposite-sex twin-sets and estimated the rate of monozygotic twins in our ART population to be ~3.5 versus 32% in our twin control

Table 1 Risk of adverse perinatal outcomes over time for children conceived after ART compared with children conceived after spontaneous conception.

	1988–1992		1993–1997		1998–2002		2003–2007	
	aOR*	[95% CI]	aOR*	[95% CI]	aOR*	[95% CI]	aOR*	[95% CI]
Birthweight <2500 g								
Singletons	2.94	[2.44–3.54]	1.70	[1.55–1.87]	1.49	[1.38–1.60]	1.49	[1.40–1.58]
Twins	0.97	[0.84–1.12]	0.94	[0.87–1.01]	0.87	[0.82–0.93]	0.86	[0.81–0.92]
Birthweight <1500 g								
Singletons	4.77	[3.25–7.01]	1.96	[1.59–2.40]	1.73	[1.48–2.02]	2.11	[1.85–2.41]
Twins	1.12	[0.85–1.48]	0.90	[0.77–1.05]	0.84	[0.74–0.96]	0.82	[0.72–0.94]
Birth <37 weeks								
Singletons	2.47	[2.09–2.92]	1.60	[1.48–1.74]	1.50	[1.41–1.60]	1.50	[1.43–1.58]
Twins	1.00	[0.64–1.55]	1.50	[1.04–2.15]	1.00	[0.75–1.33]	0.98	[0.85–1.13]
Birth <32 weeks								
Singletons	3.80	[2.62–5.52]	2.15	[1.77–2.60]	1.79	[1.55–2.07]	2.06	[1.82–2.33]
Twins	1.28	[0.97–1.69]	1.11	[0.91–1.35]	1.00	[0.89–1.12]	1.12	[0.86–1.46]
Small for gestational age								
Singletons	1.83	[1.48–2.26]	1.22	[1.09–1.37]	1.08	[0.99–1.18]	1.13	[1.05–1.22]
Twins	0.77	[0.65–0.91]	0.82	[0.75–0.90]	0.80	[0.74–0.86]	0.76	[0.71–0.82]

*aOR, odds ratio adjusted for maternal age, parity (0 versus ≥ 1), child's sex and country.

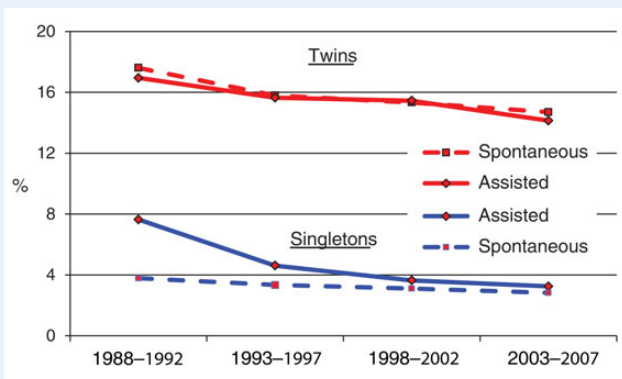


Figure 5 Rates of small for gestational age in singletons and twins by time.

population (Weinberg, 1902). This difference may partly explain the overall beneficial outcome found for the ART twins.

The changes in perinatal outcomes observed during the past 20 years may also reflect the ART population. Nowadays both less as well as severely reproductively ill couples undergo ART treatment. The accessibility to ART has increased hence couples with shorter time-to-pregnancy undergo assisted reproduction. Since the ART professionals have become more and more skilled and aware of avoiding the potential complications related to ART, also patients with different kind of relatively severe co-morbidity may now undergo ART. One intriguing observation is that for both singleton and twin ART mothers, a remarkable decline

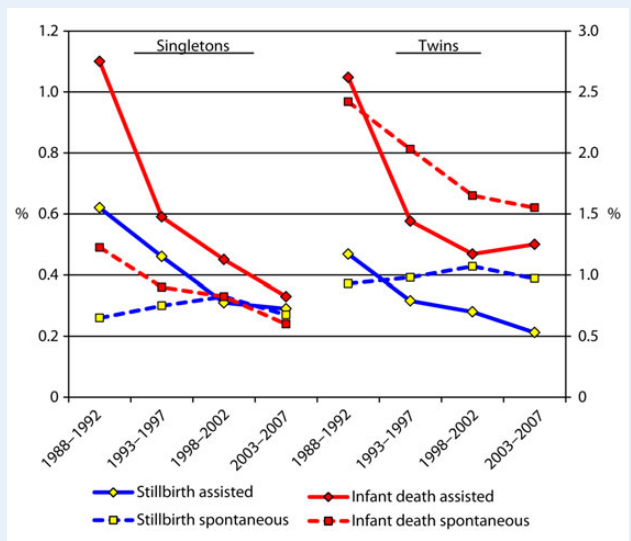


Figure 6 Rates of stillbirth and infant death in singletons and twins after assisted versus spontaneous conception.

occurred in the proportion of nulliparous, although the mean age for all ART mothers remained stable during the study period. In contrast, the mean maternal age in the control group increased significantly over time. The rising proportion of multiparous ART women may be an indicator of both an increased accessibility to ART, but also an increased use of cryopreservation of embryos helping the subfertile couples to achieve

a second child. Finally, there has been a development in terms of embryo culture techniques, both the hardware and the culture media. Our data do not allow any conclusions regarding this point, but currently there is an ongoing discussion concerning the possible effect of these factors on obstetric outcome (Dumoulin *et al.*, 2010; Lemmen *et al.*, 2014).

One of the strengths of our study is the large sample size of children born after ART in four countries over a period of 20 years. This enables us to evaluate the trends over time for both singletons and twins born after ART and the finding of overall considerably improved perinatal outcomes among ART children is very reassuring. The national treatment strategies differ in the Nordic countries, which are reflected in the uneven twin rates. Our data underline the importance of lowering the national twin rates, also in regard to the health advantages in ART singletons. A large randomized trial has shown, that the cumulative live birth rate in elective single embryo transfer followed by frozen embryo transfer is comparable to that after fresh double embryo transfer and therefore there is valid reason to lower the rate of multiples conceived after ART even further without lowering the live birth rates (Thurin *et al.*, 2004; McLernon *et al.*, 2010).

As new ART methods are continuously introduced e.g. vitrification and blastocyst culture, we strongly encourage a continuous surveillance of ART children. Moreover we recommend that countries without an established ART register consider to start building up one to ensure safety and quality of ART in the future (Wikland *et al.*, 2010; Kawachiya *et al.*, 2011; Shi *et al.*, 2012).

Supplementary data

Supplementary data are available at <http://humrep.oxfordjournals.org/>.

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Authors' roles

All authors planned the study and discussed the data and the results. A.A.H. merged the data, performed the analyses and drafted the manuscript. J.L.F. contributed with the data analyses. All authors were involved in finalizing the manuscript and have approved the final version. The authors have agreed on the order of authorship.

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Conflict of interest

None of the authors has any competing interests to declare.

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