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Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006

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BACKGROUND: The number of children born after frozen embryo transfer (FET) is steadily rising. However, studies on obstetric and perinatal outcomes are limited. Our primary aim was to compare the perinatal health of children born after FET and fresh embryo transfer, and to use data from children born after spontaneous conception as a reference.

METHODS: In a register-based cohort study we evaluated the obstetric and perinatal outcomes of children born after FET ($n = 2293$), fresh embryo transfer ($n = 4151$) and those born after spontaneous pregnancy (reference group; $n = 31\,946$). Data were collected from the registers of two infertility outpatient clinics, two university hospitals and the Finnish Medical Birth Register (1995–2006).

RESULTS: After adjusting for confounding factors the FET group showed decreased risks of preterm birth [adjusted odd ratio (AOR) 0.83, 95% confidence interval (CI) 0.71–0.97], low birthweight (AOR 0.74; 0.62–0.88) and being small for gestational age (AOR 0.63; 0.49–0.83) compared with the fresh embryo transfer group. Mean birthweight was 134 g higher in the FET singletons versus the fresh embryo transfer singletons ($P < 0.0001$). When FET singletons were compared with the reference group, increased risks of preterm birth (AOR 1.45; 1.25–1.68) and low birthweight (AOR 1.22; 1.03–1.45) and a decreased risk of being small for gestational age (AOR 0.71; 0.54–0.92) were found. No excess of perinatal and infant mortality occurred between the groups.

CONCLUSIONS: Embryo freezing does not adversely affect perinatal outcome in terms of prematurity, low birthweight and being small for gestational age versus the fresh embryo transfer and the outcome is similar or even better, particularly regarding fetal growth. Our study, which is one of the largest on FET pregnancies, provides further evidence on the safety of FET.

Key words: frozen embryo transfer / IVF / ICSI / obstetric outcome / perinatal outcome

Introduction

The first live birth after frozen embryo transfer (FET) was in 1984 (Zeilmaker *et al*., 1984), and the number of FET cycles per annum was relatively constant in Europe during the 1990s. During the last few years the role of cryopreservation has increased, since embryo transfer policy currently favours single embryo transfer (SET). In Europe more than 79 000 FETs were performed in 2005 (Nyboe Andersen *et al*., 2009). Elective SET (eSET) is the only way to avoid complications associated with multiple pregnancies (Tiitinen *et al*., 2004). FET also increases the possibility of having more than one pregnancy after a single oocyte pick-up, without having additional ovarian stimulation and thus diminishing the maternal risk related to the procedures involved (Wennerholm *et al*., 1997). Furthermore, it seems to be more effective and less expensive than traditional treatment with double embryo transfer (Veleva *et al*., 2009).

A limited number of studies have been conducted to evaluate the obstetric and perinatal outcome of children born after FET (Wada *et al*., 1994; Sutcliffe *et al*., 1995; Wennerholm *et al*., 1997; Aytoz *et al*., 1999; Westergaard *et al*., 1999; Schieve *et al*., 2004; Källén *et al*., 2005; Wang *et al*., 2005; Belva *et al*., 2008; Shih *et al*., 2008; Pinborg *et al*., 2009). According to the systematic review

(Wennerholm *et al*., 2009) and recently published large Danish population-based cohort study (Pinborg *et al*., 2009) the health of children born after FET is comparable or even better than that of children born after fresh embryo transfer. Further, in population-based registry studies (Westergaard *et al*., 1999; Shih *et al*., 2008; Pinborg *et al*., 2009) major malformation rates did not show significant difference between FET and fresh embryo transfer children. However, a recently published prospective hospital-based cohort study from Belgium showed a higher major malformation rate in FET–ICSI children compared with fresh ICSI and FET–IVF children at 2 months of age (Belva *et al*., 2008).

Finland has adopted a policy of eSET combined with extensive use of FET (Tiitinen *et al*., 2004), the proportion of frozen versus fresh embryo transfer cycles is higher than in most other countries (Nyboe Andersen *et al*., 2009) and a substantial proportion (37%) of live births after embryo transfer originates from FET cycles (THL, 2009). For this reason we urgently need more solid scientific information on obstetric and perinatal outcome among children born after FET.

The aim of our study was to compare the perinatal health of children born after FET and fresh embryo transfer using children born after spontaneous conception as reference population. Our hypothesis was that FET is as safe as fresh embryo transfer.

Materials and Methods

Our study is a register-based cohort study. In 1995–2006, women who underwent assisted reproductive technology (ART) treatment with embryo transfer leading to birth were identified in registers of the infertility outpatient clinics of the Väestöliitto Fertility Clinics Ltd (Oulu and Helsinki) as well as in those of the University Hospitals of Oulu and Helsinki. By using the personal identification numbers of the women, the corresponding births were matched with data from the Finnish Medical Birth Register (FMBR). A random sample of 10% of mothers with spontaneous pregnancies from the FMBR served as a reference group which was matched to the study groups as regards area of residence and year of birth of the child. Births from the frozen and fresh embryo transfer groups were excluded if women had received donated eggs, or sperm, or needed preimplantation genetic examinations. The other exclusions are presented in the flow chart (Fig. 1). The final data concerned 6444 births after embryo transfer: 2293 births after FET and 4151 births after fresh embryo transfer. The cohort of spontaneous pregnancies (reference group) included 31 946 births (Fig. 1). During the 11-year follow-up period some women may have had several births, since combining IVF/ICSI and FET is currently a common practice in Finland. Therefore, the same women were kept in both groups. Their proportion is though $<$10%, which does not significantly affect study results.

The FMBR, active since 1987, is currently run by the National Institute for Health and Welfare (THL). Information for the FMBR is recorded by midwives at delivery hospitals, and submitted to the THL. The FMBR identifies the mother, and child by their unique personal identification numbers and it contains information on maternal background, and on the infant's outcome until the age of 7 days, including all live and stillbirths after the 22nd gestational week and birthweight of 500 g or more. After linking the data on live births from the Population Register Centre, and the data on perinatal deaths from the Cause-of-Death Register at Statistics Finland, the coverage of the FMBR data have been shown to be practically 100%. Furthermore, the content of most of its variables is highly reliable (Gissler *et al*., 1995). The following maternal variables in the FMBR were included in the study data: age, marital status, socioeconomic status (SES) based on occupation, area of residence, number and type of previous pregnancies including miscarriages, induced abortions (all legitimated terminations of pregnancy before the end of 20 and of 24 weeks of pregnancy in cases of confirmed severe, life-threatening congenital anomalies or birth defects) and ectopic pregnancies, smoking (yes or no), induction of labour, mode of delivery and placental disturbance (placental abruption, placenta praevia). The included perinatal variables included were: gestational age at birth (defined according to the best clinical estimate), preterm birth ($<$37 weeks), very preterm birth ($<$32 weeks), birthweight, low birthweight (LBW $<$ 2500 g), very low birthweight (VLBW $<$ 1500 g), small for gestational age (SGA, defined as birthweight $<$ mean $-$ 2 SD) and large for gestational age (LGA, defined as birthweight $>$ mean $+$ 2 SD) children [mean in the Finnish population according to sex (Pihkala *et al*., 1989)], ponderal index (measure of thinness, reflects intrauterine growth; kg/m^3), low Apgar scores at 1 min (0–6), newborn special care up to the age of 7 days including observation at a neonatal unit, neonatal intensive care, and transfer to higher level hospital, respirator treatment and perinatal death (stillbirths and early neonatal deaths during the first week of life). The infant mortality rate (deaths up to 364 days of life) was calculated from data in the Cause-of-Death Register at Statistics Finland.

In the four outpatient clinics the practice of ovarian stimulation was practically identical, involving the long GnRH agonist or the short GnRH antagonist protocol. Luteal support was provided via vaginal micronized progesterone. Embryos were cultured as previously described (Tomás *et al*., 1998; Hydén-Granskog *et al*., 2005). Embryo transfers were carried out on Days 2 or 3 after oocyte retrieval. Extra embryos were frozen on the day of embryo transfer, using a slow freezing protocol with 1, 2-propanediol as cryoprotectant (Hydén-Granskog *et al*., 2005). In the FET cycles the women having spontaneous ovulation measured their urinary LH surge with a home test kit. Embryo transfer was carried out 2–5 days after a positive ovulation test. In most of the spontaneous cycles luteal support with progesterone for 2 weeks was used. In cases of hormonally substituted FET cycles, estradiol valerate or 17β-estradiol was administered daily and the dose of the medication was adjusted according to the results of ultrasonography examination of the endometrium. Before embryo transfer, vaginal progesterone was added 2–4 days. Medication was continued until the pregnancy test and, if positive, medication was continued until 10th pregnancy week. In all pregnancies, transvaginal ultrasonography was performed before the end of the eighth week of gestation and thereafter the pregnancies were followed according to routine practice in municipal maternity welfare centres. In Finland, antenatal care is free of charge, well accepted (almost 100%) and commonly provided as part of primary health care, the recommendations for the number of visits being 11–15 for nulliparous women and 7–11 for parous women (Viisainen, 1999). Practically all deliveries take place in public hospitals.

The study plan and the use of sensitive health register information were approved by the National Research and Development Centre for Welfare and Health (STAKES), currently THL and Statistics Finland. For register linkages, the National Data Protection Authority was consulted and permission from the register keepers was obtained.

Statistical analyses

Differences in maternal backgrounds between the FET group, the fresh embryo transfer group and the reference group were tested by using Student's *t*-test and the χ^2-test. Statistical significance was defined as $P <$ 0.05. Comparisons among the three study groups adjusted for maternal age, parity, SES and the numbers of the fetuses were run using logistic regression. Smoking and SES are strongly correlated (Jaakkola *et al*.,

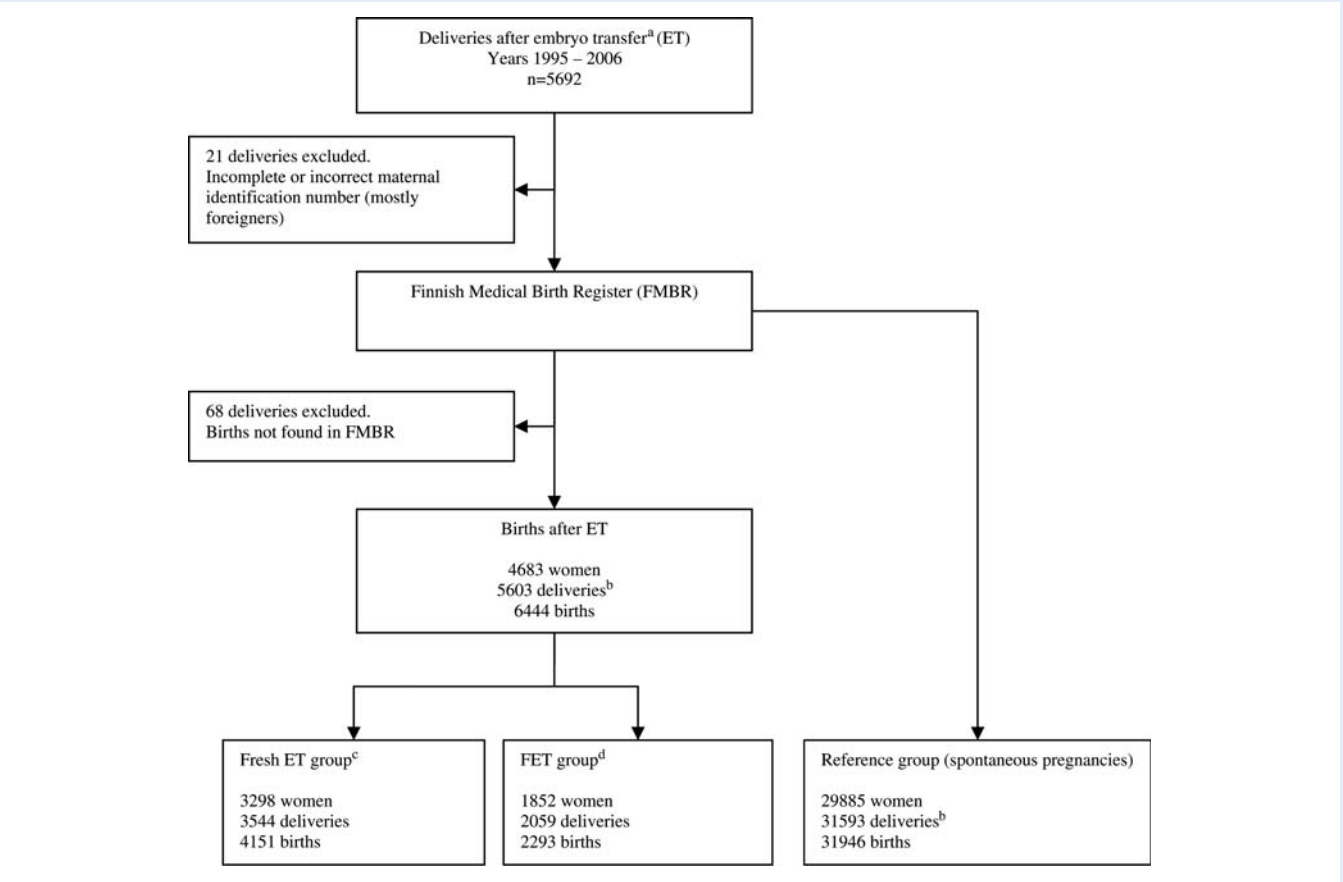

Figure 1 Flow chart of the study.

[a]Embryo transfer (ET) = women who underwent In-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) or frozenembryo transfer after in-vitro fertilization (FET-IVF) or frozen embryo transfer after intracytoplasmic sperm injection (FET-ICSI). [b]The same woman may have had several deliveries. [c]Fresh ET group includes fresh IVF (n = 2777) and fress ICSI (n = 1374) births. [d]FET group includes FET-IVF (n = 1625) and FET-ICSI (n = 668) births.

2001) therefore only SES was included in the logistic regression model. The results were displayed as unadjusted odds ratios (ORs) and adjusted ORs (AORs) with 95% confidence intervals (CIs). Twins and triplets were analysed as one group. Subanalysis were also run, comparing FET–IVF versus fresh IVF, FET–ICSI versus fresh ICSI, FET–IVF versus FET–ICSI and each subgroup versus the reference group.

Results

Maternal background

Age differences between the three study groups were statistically significant, mothers in the FET group being the oldest (Table I). The proportions of women having their first pregnancy were 35% in the FET group, 52% in the fresh embryo transfer group and 32% in the reference group ($P < 0.0001$). Higher rates of ectopic pregnancy were found in the FET and fresh embryo transfer groups compared with the reference group ($P < 0.0001$). The frequency of nulliparous women was highest (72%) in the fresh embryo transfer group (versus 56% in the FET group and 41% in the reference group). Mothers in the FET and fresh embryo transfer groups had a significantly higher SES and were more often married than the mothers in the reference group. Those in the FET and fresh embryo transfer groups smoked less frequently during pregnancy than those in the reference group (Table I).

Pregnancies and deliveries

As expected, there were significantly more multiple pregnancies in the FET and fresh embryo transfer groups than in the reference group (twins 10.9 and 16.8% versus 1.1%, triplets 0.2 and 0.1% versus 0.01%, respectively). Among singleton pregnancies the incidence of Caesarean section (CS) was highest in the FET group (28.4%; Table II), but after adjusting for background factors the difference disappeared between the FET and the fresh embryo transfer group (AOR 1.01; 95% CI 0.90–1.14). The risk of CS, however, was increased when comparing the FET (AOR 1.53; 1.38–1.70) and the fresh embryo transfer groups (AOR 1.49; 1.36–1.62) with the reference group. On the other hand, the reference group showed a higher CS rate before the 37th gestational week than the FET and the fresh embryo transfer groups (67.9, 51.2 and 57.7%, respectively; data not shown). The risk of placental disturbance did not differ significantly between the FET and fresh embryo transfer groups (AOR 0.75; 0.45–1.25). However, in comparison with the reference group, an increased

Table I Maternal background characteristics in frozen embryo transfer (FET), fresh embryo transfer and reference (spontaneously conceived) groups.

Characteristics of the women	FET (n = 1852)	Fresh embryo transfer (n = 3298)	Reference group (n = 29 885)	P-value†,*
Maternal age (years)				a = 0.002, b, c < 0.0001
<30	327 (15.9)	708 (20.0)	15 963 (50.5)	
30–34	864 (42.0)	1452 (41.0)	9800 (31.0)	
35–39	705 (34.2)	1123 (31.7)	4789 (15.2)	
≥40	163 (7.9)	261 (7.4)	1042 (3.3)	
Mean (±SD)	34.2 (4.1)	33.7 (4.2)	30.0 (5.4)	a, b, c < 0.0001
Pregnancies				a, b, c < 0.0001
0	718 (34.9)	1849 (52.2)	10 021 (31.7)	
1	738 (35.8)	979 (27.6)	9293 (29.4)	
≥2	601 (29.2)	714 (20.1)	12 173 (38.5)	
Mean (±SD)	1.2 (1.2)	0.8 (1.2)	1.6 (1.9)	a, b, c <0.0001
History of non-viable pregnancies				
Miscarriage				a, b < 0.0001, c = 0.531
0	1494 (72.6)	2807 (79.2)	25 008 (79.2)	
1	403 (19.6)	537 (15.2)	4840 (15.3)	
≥2	157 (7.6)	190 (5.4)	1608 (5.1)	
Ectopic pregnancy				a = 0.075, b, c < 0.0001
0	1836 (89.2)	3198 (90.2)	30 233 (95.7)	
1	151 (7.3)	224 (6.3)	475 (1.5)	
≥2	64 (3.1)	93 (2.6)	40 (0.1)	
Induced abortion				a, b, c < 0.0001
0	1910 (92.8)	3273 (92.4)	27 531 (87.1)	
1	124 (6.0)	223 (6.3)	3081 (9.8)	
≥2	20 (1.0)	38 (1.1)	823 (2.6)	
Parity				a, b, c < 0.0001
0	1142 (55.5)	2557 (72.2)	13 033 (41.3)	
1	768 (37.3)	794 (22.4)	10 453 (33.1)	
≥2	144 (7.0)	185 (5.2)	7988 (25.3)	
Mean (±SD)	0.5 (0.5)	0.4 (0.7)	1.1 (1.5)	a, b, c < 0.0001
Marital status				a, b, c < 0.0001
Married	1624 (78.9)	2673 (75.4)	19 930 (63.1)	
Cohabiting	390 (18.9)	801 (22.6)	8986 (28.4)	
Single	43 (2.1)	67 (1.9)	2578 (8.2)	
Socioeconomic position				a = 0.249, b, c < 0.0001
Upper white-collar	653 (31.7)	1047 (29.5)	6559 (20.8)	
Lower white-collar	895 (43.5)	1545 (43.6)	12 023 (38.1)	
Blue collar	191 (9.3)	355 (10.0)	4026 (12.7)	
Other	320 (15.5)	597 (16.8)	8984 (28.4)	
Smoking during pregnancy	142 (6.9)	246 (6.9)	4775 (15.1)	a = 0.309, b, c < 0.0001

Data are presented as numbers (%) or mean (SD).
†P-value assessed by using χ^2 or Student's t-test.
*Interaction between a = FET versus fresh embryo transfer, b = FET versus reference group and c = fresh embryo transfer versus reference group.

risk of placental disturbance was found in the FET (AOR 2.44; 1.52–3.93) and the fresh embryo transfer (AOR 3.27; 2.28–4.70) groups. In multiple pregnancies the differences between groups were considerably smaller (Table II).

Perinatal outcome

Perinatal outcome and infant mortality up to the age of 1 year among singletons are presented in Table III. The mean gestational age was 2 days

Table II **Onset and mode of delivery, placental complications and breech presentation in frozen embryo transfer (FET), Fresh embryo transfer and Reference (spontaneously conceived) groups in singleton and multiple deliveries.**

	Singleton delivery				Twins Triplets	Multiple deliveries‡			
	FET (*n* = 1830)	Fresh embryo transfer (*n* = 2942)	Reference group (*n* = 31 243)	*P*-value†,*		FET (*n* = 224) (*n* = 5)	Fresh embryo transfer (*n* = 597) (*n* = 5)	Reference group (*n* = 347) (*n* = 3)	*P*-value†,*
Induction of labour	360 (19.7)	504 (17.1)	4730 (15.2)	a = 0.027, b < 0.001, c = 0.004		56 (23.5)	148 (24.1)	85 (24.2)	a = 1.000, b = 1.000, c = 0.938
Mode of delivery				a = 0.075, b, c < 0.0001					a = 0.745, b = 0.011, c = 0.012
Vaginal	1112 (60.8)	1857 (63.2)	24 318 (77.8)			89 (38.9)	243 (40.4)	173 (49.3)	
Instrumental	199 (10.9)	265 (9.0)	1755 (5.6)			13 (5.6)	27 (4.4)	8 (2.3)	
Section	519 (28.4)	818 (27.8)	5087 (16.3)			127 (55.5)	331 (55.1)	170 (48.4)	
Caesarean section				a = 0.691, b, c < 0.0001					a = 0.938, b = 0.098, c = 0.048
Planned	195 (10.7)	354 (12.0)	2352 (7.5)			66 (28.8)	158 (26.2)	87 (24.8)	
Other	324 (17.7)	464 (15.8)	2735 (8·8)			61 (26.7)	173(28.4)	83 (23.6)	
Placental disturbance									
Placental abruption	3 (0.2)	6 (0.2)	55 (0.2)	a = 1.000, b = 0.800, c = 0.648		2 (0.9)	5 (0.8)	2 (0.6)	a = 1.000, b = 0.649, c = 1.000
Placenta praevia	16 (0.9)	38 (1.3)	57 (0.2)	a = 0.207, b, c < 0.0001		1 (0.4)	1 (0.2)	3 (0.9)	a = 0.474, b = 1.000, c = 0.144
Breech presentation	74 (4.0)	154 (5.2)	919 (2.9)	a = 0.061, b = 0.007, c < 0.0001		33 (14.7)	112 (18.8)	48 (14.0)	a = 0.155, b = 0.803, c = 0.050

Data are presented as numbers (%).

‡Twins and triplets were analysed as one group. **P*-value assessed by using χ^2 or Student's *t*-test.

†Interaction between a = FET versus fresh embryo transfer, b = FET versus reference group and c = fresh embryo transfer versus reference group.

longer in the FET group [277 ± 14 (SD) days] compared with the fresh embryo transfer group (275 ± 15 days; $P < 0.0001$), but 1 day shorter than in the reference group singletons (278 ± 13 days; $P < 0.0001$). The mean birthweight in FET singletons was 3550 ± 585 g, in fresh embryo transfer singletons 3416 ± 605 g and in reference group singletons 3538 ± 556 g ($P < 0.0001$ between FET and fresh embryo transfer). Mean birthweight in the FET and reference groups did not differ significantly. The proportions of all preterm and LBW births were lower in the FET singletons than in the fresh embryo transfer singletons (6.5 versus 8.8% and 4.2 versus 6.0%, respectively). No significant differences were observed as regards VLBW and very preterm births comparing the FET singletons with the fresh embryo transfer and the reference group singletons (data not shown), but after adjusting for background factors, the FET group showed decreased risk for VLBW (AOR 0.64; 95% CI 0.44–0.93) and very preterm birth (AOR 0.63; 0.47–0.84) in comparison with the fresh embryo transfer group. Furthermore, a low ponderal index (the lowest quartile <25 kg/m^3) was significantly more common in the fresh embryo transfer singletons than in the FET ($P = 0.036$) or reference group singletons ($P < 0.0001$). The perinatal and infant mortality rates of singletons did not differ between the three study groups (Table III).

In multiple pregnancies the frequency of preterm delivery and LBW was comparable between FET and fresh embryo transfer groups (47.8 versus 49.4% and 39.5 versus 44.3%, respectively; Supplementary

Table III Perinatal outcomes and infant mortality rate of singletons in frozen embryo transfer (FET), fresh embryo transfer and reference (spontaneously conceived) groups.

Perinatal outcomes of singletons	FET (n = 1830)	Fresh embryo transfer (n = 2942)	Reference group (n = 31 243)	P-value†*
Gender				a = 0.768, b = 0.804, c = 0.867
Girls	902 (49.3)	1437 (48.8)	15 242 (48.8)	
Boys	928 (50.7)	1505 (51.2)	15 998 (51.2)	
Gestational age (days)				
Mean (±SD)	276.7 (13.8)	275.0 (15.0)	278.4 (12.8)	a, b, c < 0.0001
Gestational weeks at birth				a = 0.036, b, c < 0.0001
≤31	17 (0.9)	42 (1.4)	236 (0.8)	
32–36	103 (5.6)	216 (7.4)	1177 (3.8)	
≥37	1703 (93.1)	2677 (91.8)	29 656 (94.9)	
Birthweight (g)				a = 0.020, b = 0.025, c < 0.0001
≤1499	16 (0.9)	36 (1.2)	203 (0.6)	
1500–2499 g	60 (3.3)	141 (4.8)	788 (2.5)	
≥2500 g	1754 (95.9)	2765 (94.8)	30 182 (96.6)	
Mean (±SD)	3550.6 (585.2)	3416.6 (604.5)	3538.5 (555.7)	a, c < 0.0001, b = 0.389
Birth length (cm)				
Mean (±SD)	50.3 (2.7)	49.8 (2.8)	50.2 (2.5)	a < 0.0001, b = 0.122, c = 0.020
Small for gestational age (SGA)	28 (1.5)	91 (3.1)	661 (2.1)	a, c < 0.0001, b = 0.092
Large for gestational age (LGA)	66 (3.6)	60 (2.1)	891 (2.9)	a, c <0.0001, b = 0.072
Ponderal index (kg/m^3)				
Mean (±SD)	27.7 (2.6)	27.5 (2.9)	27.9 (4.5)	a = 0.036, b = 0.002, c < 0.0001
Low Apgar scores at 1 min (0–6)	111 (0.9)	163 (0.8)	1299 (0.6)	a = 0.448, b, c < 0.0001
Need of Special care‡	386 (21.1)	640 (21.9)	4015 (12.8)	a = 0.559, b, c < 0.0001
Respirator treatment	28 (1.5)	64 (2.2)	317 (1.0)	a = 0.115, b = 0.035, c < 0.0001
Hospital care > 7 days	148 (8.1)	248 (8.6)	1510 (4.8)	a = 0.677, b, c < 0.0001
Perinatal mortality rate/1000	8 (4.4)	16 (5.5)	169 (5.4)	a = 0.679, b = 0.740, c = 1.000
Stillbirths	5 (2.7)	9 (3.1)	105 (3.4)	a = 1.000, b = 0.835, c = 1.000
Early neonatal deaths (deaths 0–6 days from live birth)	3 (1.6)	7 (2.4)	64 (2.0)	a = 0.750, b = 1.000, c = 0.671
Infant mortality rate/1000 (deaths 0–364 days from live birth)	4 (2.2)	14 (4.8)	107 (3.4)	a = 1.000, b = 0.458, c = 0.484

Data are presented as numbers (%) or mean (±SD).
†P-value assessed by using χ^2 or Student's t-test.
*Interaction between a = FET versus fresh embryo transfer, b = FET versus reference group and c = fresh embryo transfer versus reference group.
‡Observation at neonatal unit, neonatal intensive care and transfer to higher level hospital calculated as one group.

data, Table S1). Perinatal outcomes and infant mortality rates up to the age of 1 year among multiples are shown in Supplementary data, Table S1.

In logistic regression analyses before and after adjusting for background factors, the FET group revealed significantly decreased risks of preterm birth (OR 0.67; 95% CI 0.59–0.77, AOR 0.83; 0.71–0.97), LBW (OR 0.61; 0.53–0.71, AOR 0.74; 0.62–0.88), being SGA (OR 0.55; 0.43–0.71, AOR 0.63; 0.49–0.83) and newborn's need of respirator treatment (OR 0.55; 0.41–0.75, AOR 0.66; 0.48–0.90), but an increased risk of being LGA (OR 1.86; 1.33–2.61, AOR 1.70; 1.21–2.40) in comparison with the fresh embryo transfer group (Table IV). In comparison with the reference group, significantly increased risks of preterm birth (OR 3.11; 2.74–3.52, AOR 1.45; 1.25–1.68), LBW (OR 3.11; 2.70–3.58, AOR 1.22; 1.03–1.45), low Apgar scores (OR 1.72; 1.45–2.03, AOR 1.43; 1.19–1.71) and newborn's need of special care (OR 2.34; 2.12–2.58, AOR 1.60; 1.44–1.78) were found in FET group. In the adjusted analyses, the risk of being born SGA was significantly decreased in the FET group in comparison with the reference group (AOR 0.71; 0.54–0.92; Table V).

In a subanalysis, perinatal outcomes in the FET–IVF and FET–ICSI groups were compared with those in the fresh IVF and fresh ICSI groups are shown in Supplementary data, Table S2. In the adjusted analyses, the risks of LBW (0.70; 95% CI 0.57–0.86) and being born SGA (AOR 0.53; 0.39–0.73) was significantly lower, but the risk of being born LGA (1.71; 1.12–2.59) was higher in the FET–IVF versus fresh IVF groups (Supplementary data, Table S2). However, the FET–ICSI versus fresh ICSI groups the significant differences remained only for the newborn's need of respirator treatment (AOR 0.53; 0.30–0.94; Supplementary data, Table S2). Comparing the FET–IVF with the FET–ICSI groups, in adjusted analyses, no differences were observed as regards the perinatal outcomes of those two study groups (data not shown).

Table IV Risk of adverse perinatal outcome in the frozen embryo transfer (FET) group versus the fresh embryo transfer group expressed as unadjusted and adjusted odds ratios with 95% CIs.

Perinatal outcome	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)
Preterm birth <37 weeks	0.67 (0.59–0.77)	0.83 (0.71–0.97)
Low birthweight <2500 g	0.61 (0.53–0.71)	0.74 (0.62–0.88)
Small for gestational age	0.55 (0.43–0.71)	0.63 (0.49–0.83)
Large for gestational age	1.86 (1.33–2.61)	1.70 (1.21–2.40)
Low Apgar scores at 1 min (0–6)	0.90 (0.74–1.09)	0.98 (0.81–1.20)
Newborn special care[b]	0.86 (0.76–0.96)	0.95 (0.84–1.07)
Respirator treatment	0.55 (0.41–0.75)	0.66 (0.48–0.90)
Perinatal mortality	0.90 (0.50–1.65)	1.07 (0.58–1.97)
Infant mortality	0.56 (0.28–1.15)	0.72 (0.42–1.23)

[a]Adjusted for maternal age, parity, SES and number of fetuses.
[b]Observation at neonatal unit, neonatal intensive care and transfer to higher level hospital calculated as one group.

Discussion

In the present study, we analysed the perinatal outcome of children born after FET compared with those born after fresh embryo transfer and spontaneous pregnancies. On the basis of our results, children born after FET have a similar perinatal outcome as children born after fresh embryo transfer or even an improved outcome as regards preterm birth, LBW and SGA. When compared with spontaneously conceived pregnancies, increased risks of preterm birth, LBW, low Apgar scores and need for special care following FET were observed.

Our register-based cohort study is the largest research of perinatal data including 2293 FET children comparing to both fresh embryo transfer and spontaneously conceived pregnancies. Further strengths of our study are consistent, homogeneous and population-based data (coverage 98.4%), highly reliable registers (Gissler *et al*., 1995) and long experience with cryopreservation programmes (Tiitinen *et al*., 2004). In addition, the information on all three study groups is similarly extracted from the FMBR. To avoid the bias arising from a long recruitment time and different residential districts, the mothers were matched as regards year of delivery and district of residence. Furthermore, we were able to control for such potential confounding factors as maternal age, parity, SES and number of fetuses, because it is known that women who conceive after ART are often of advanced age, higher SES, primiparous and likely to deliver multiple infants, as was shown in the present study. The effect of parity is that there is an increase in mean birthweight from the first to the second pregnancy and as regards maternal age, older mothers have higher birthweight newborns (Goldstein, 1981). The mechanisms behind these well-known effects of parity and age are uncertain. Low SES is unlikely to contribute to the association between LBW and ART because women who become pregnant after ART are usually of higher SES than those conceiving naturally (Shih *et al*., 2008). In Finland, however, socioeconomic position has only a minor role in the use of IVF services (Klemetti *et al*., 2005), and socioeconomic differences among mothers of low-birthweight newborns are relatively small (Gissler *et al*., 2009). It is generally considered that the main cause of adverse fetal outcome in ART pregnancies arises from multiple pregnancies (Koivurova *et al*., 2002a), problems which could be partially solved through an eSET policy combined with FET.

Our study, like the great majority of this kind of population based register studies, has limitations concerning important variables of pregnancy complications and maternal characteristics. The data on variables of pregnancy complication are incomplete in FMBR before 2004. We are aware that information on birth defects belong to this kind of study, however, not all defects are detected during the perinatal period. Our ongoing study on malformations will deal with not only birth defects (including stillbirths and induced abortions due to malformations) but also morbidity until the age of 3 years in these groups. In our opinion, inclusion of all this information together would be too extensive and beyond the scope of this article.

In our study, there were fewer preterm and LBW infants in the FET singleton group versus those in the fresh embryo transfer group. These findings are in accordance with the results of some (Källén *et al*., 2005; Wang *et al*., 2005; Shih *et al*., 2008; Pinborg *et al*., 2009), but not all controlled studies on FET (Wada *et al*.,1994; Belva *et al*., 2008). A recently published large population-based

Table V Risk for adverse perinatal outcome in the frozen embryo transfer (FET) and Fresh embryo transfer groups versus reference (spontaneously conceived) group expressed as unadjusted and adjusted odds ratios with 95% CIs.

Perinatal outcome	FET versus reference group		Fresh embryo transfer versus reference group	
	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)
Preterm birth < 37 weeks	3.11 (2.74–3.52)	1.45 (1.25–1.68)	4.61 (4.22–5.04)	1.76 (1.56–1.97)
Low birthweight <2500 g	3.11 (2.70–3.58)	1.22 (1.03–1.45)	5.07 (4.59–5.59)	1.65 (1.44–1.87)
Small for gestational age	1.47 (1.16–1.85)	0.71 (0.54–0.92)	2.66 (2.30–3.08)	1.12 (0.93–1.35)
Large for gestational age	1.08 (0.84–1.39)	1.08 (0.84–1.40)	0.58 (0.45–0.74)	0.64 (0.50–0.83)
Low Apgar scores at 1 min (0–6)	1.72 (1.45–2.03)	1.43 (1.19–1.71)	1.91 (1.69–2.17)	1.46 (1.26–1.68)
Newborn special care[b]	2.34 (2.12–2.58)	1.60 (1.44–1.78)	2.73 (2.53–2.94)	1.68 (1.55–1.83)
Respirator treatment	2.20 (1.66–2.93)	1.33 (0.97–1.82)	3.99 (3.32–4.78)	2.11 (1.69–2.64)
Perinatal mortality	1.22 (0.73–2.04)	0.94 (0.54–1.62)	1.35 (0.92–1.97)	0.93 (0.61–1.44)
Infant mortality	1.20 (0.63–2.30)	0.86 (0.52–1.44)	2.13 (1.44–3.16)	1.11 (0.77–1.60)

[a]Adjusted for maternal age, parity, SES and number of fetuses.
[b]Observation at neonatal unit, neonatal intensive care and transfer to higher level hospital calculated as one group.

register study from Denmark (Pinborg *et al*., 2009) including 957 FET singletons and a systematic review (Wennerholm *et al*., 2009) concerning perinatal outcomes of children born after FET revealed that the data concerning FET children seem reassuring, with even higher birthweights and lower rates of preterm birth and LBW than among children born after fresh embryo transfer. In one of the largest population-based register studies from Australia (Shih *et al*., 2008), involving 2387 FET singletons, similar results after FET, ovulation induction and artificial insemination were reported. Belva *et al*. (2008) evaluated the neonatal outcome of 937 children born after FET in a prospective hospital-based cohort study. However, their results concerning preterm birth did not show any significant difference between the FET and the fresh embryo transfer group, although the LBW rate was significantly lower in the FET group than in the fresh embryo transfer group, probably as a result of different twinning rates in the populations, as discussed by the authors. Furthermore, the time periods of recruitment were different among their study groups.

There is some evidence that factors which influence on gestational age at birth also influence weight (Keirse, 2000), and ART may belong to the factors that have influence on both fetal growth and length of gestation (Helmerhorst *et al*., 2004). Pinborg *et al*. (2007) have shown that IVF singletons from vanishing twin gestations have a higher risk of being SGA than singletons from a single gestation in early pregnancy. On the other hand, if an SGA fetus is detected, this may prompt intervention, leading to earlier birth and thereby contributing to both preterm and LBW rates (Helmerhorst *et al*., 2004). Although preterm birth and LBW in our study occurred significantly more often in the FET and fresh embryo transfer groups compared with the reference group, conclusions concerning birthweight adjusted for gestational age and thinness of the newborns were different. In the FET group, the proportion of SGA newborns was significantly lower than in the fresh embryo transfer group and equal to that in the reference group, whereas belonging to the lowest quartile of the ponderal index was significantly less frequent than in the fresh embryo transfer group but higher than in the reference group. Hence we can suppose that FET newborns grow according to their gestational age and LBW originates mainly from preterm births in this group. It is known that pre-eclampsia is one of the main risk factors associated with being SGA and women with time to pregnancy of more than 1 year are at a higher risk of pre-eclampsia independent of treatment (Basso *et al*., 2003). Unfortunately, we did not have data on the whole study group concerning other pregnancy complications shown in the results. However, we had data on maternal hospital treated hypertension from year 2004 onwards including FET ($n = 698$), fresh embryo transfer ($n = 837$) and reference population ($n=7435$) pregnancies. No significant differences were seen between the ART groups. When comparing the ART groups with reference population, they had hypertension significantly more often (data not shown). The findings in this subpopulation are in accordance with earlier studies (Wennerholm *et al*., 1997, Koivurova *et al*., 2002b) and support ours.

An interesting finding concerning fetal growth in the present study was the significantly increased risk of LGA newborns in the FET group compared with the fresh embryo transfer group, the risk being the same as in the reference group. As shown in animal studies, reproductive technology might induce phenotypic effects. For example, *in vitro* fertilization and *in vitro* culture and cryopreservation tend to result in large calf syndrome in ruminants (Young *et al*., 1998; Romundstad *et al*., 2008). The mechanism for this effect is unknown. We had the opportunity to compare the study groups only in the subpopulation mentioned before in terms of maternal body mass index (BMI) and abnormal oral glucose tolerance test (OGTT). We could not find any significant differences between the ART groups in BMI and abnormal OGTT. When comparing both ART groups with the reference group, the mothers in the latter group were significantly leaner and had fewer disturbances in glucose metabolism. Our whole data included the information only on insulin-treated diabetes during pregnancy, and the prevalences were comparable between the FET and the fresh embryo transfer groups.

The perinatal and infant mortality data did not show any significant differences between the three study groups. Between the FET and fresh embryo transfer groups the neonatal morbidity data were comparable, except that in the FET group less need of respirator treatment was observed, which is most likely associated with the lower preterm birth rate in this group. However, the neonatal morbidity rate was clearly higher among FET newborns than in the reference group when defined as low Apgar scores and need for special hospital care. Only the need for respirator treatment in the FET group was comparable with that in the reference group.

The more favourable outcome in the FET group compared with the fresh embryo transfer group may be associated with a patient effect, because women who produce more and higher quality embryos are less likely to have preterm and LBW delivery (Källén *et al*., 2005; Wang *et al*., 2005). Another explanation might be associated with the use of medication during fresh embryo transfer cycles, when hormone levels are supraphysiologically high. In FET cycles, hormone supplementation can be given but at doses that mimic those in natural cycles. This may influence endometrial receptivity, early implantation and placental development (Shih *et al*., 2008). Alternatively, the physical effects of freezing and thawing embryos may filter out 'weak' embryos and allow only good quality ones to survive, resulting in better fetal growth (Shih *et al*., 2008). However, the aetiology of infertility and treatment type do not seem to play important roles in neonatal outcome according to the majority of studies (Wennerholm *et al*., 1997; Schieve *et al*., 2004; Källén *et al*., 2005; Wang *et al*., 2005; Poikkeus *et al*., 2006). Romundstad *et al*. (2008) concluded that the adverse outcomes of ART compared with those in the general population could be attributable to the factors leading to infertility, rather than to factors related to technology.

The present study revealed worse neonatal outcomes in both ART groups compared with spontaneously conceived group. Cryopreservation is nowadays an essential part of cost-effective ART programmes and our study provides further evidence of the safety of FET in comparison with fresh embryo transfer. Our results confirm previous findings that embryo freezing does not adversely affect perinatal outcome in terms of prematurity, LBW and being SGA compared with fresh embryo transfer and the outcome is similar or even better, particularly regarding fetal growth. This information should further promote clinicians to implement eSET combined with cryopreservation in their IVF programmes. However, large population-based studies are still needed to assess infrequent outcomes such as congenital anomalies and possible disturbances in development of children.

Authors' Roles

S.P. and R.K. initiated and designed the study, constructed the population, interpreted the data and writing of the manuscript. M.G. initiated and designed the study, participated the writing of the manuscript, responsible for the Finnish Medical Birth Registry and the statistical analysis. S.N.-H., H.M. and A.T. initiated and designed the study, interpreted the data and participated the writing of the manuscript. A.-M.S. initiated and designed the study, constructed the population and participated the writing of the manuscript. C.H.-G. initiated and designed the study and constructed the population. A.-L.H. initiated and designed the study, interpreted the data and responsible for the final version of the manuscript.

Supplementary Data

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

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