Title

Risk of hypertensive disorders in pregnancy after fresh and frozen embryo transfer in assisted reproduction: A population-based cohort study with within sibship analysis.

Short title/running title

Embryo freezing and hypertension in pregnancy.

Authors

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Abstract

Background:

Frozen embryo transfer (frozen-ET) is increasingly common because of improved cryopreservation methods, and elective freezing of all embryos. Frozen-ET is associated with higher risk of hypertensive disorders in pregnancy (HDP) than both natural conception and fresh embryo transfer (fresh-ET), but whether this is attributable to parental factors or treatment is unknown.

Methods:

Using the Medical Birth Registries of Denmark (1994–2014), Norway and Sweden (1988–2015), linked to data from national quality registries and databases on assisted reproduction, we designed a population-based cohort study with within sibship comparison. We included 4 426 691 naturally conceived, 78 300 fresh-ET and 18 037 frozen-ET singleton pregnancies, of which 33 209 sibships were conceived using different conception methods. Adjusted odds ratios (aOR) of HDP for fresh-ET and frozen-ET versus natural conception with 95% confidence intervals (CI) were estimated using multilevel logistic regression, where random effects provided conventional population level estimates and fixed effects gave within sibship estimates. Main models included adjustment for birth year, maternal age, parity, and country.

Results:

HDP risk was higher after frozen-ET compared to natural conception, both at population level (7.4% vs 4.3%, aOR 1.74, 95% CI 1.61 to 1.89) and within sibships (aOR 2.02, 95% CI

1.72 to 2.39). For fresh-ET, risk was similar to natural conception, both at population level (aOR 1.02, 95% CI 0.98 to 1.07) and within sibships (aOR 0.99, 95% CI 0.89 to 1.09).

Conclusions:

Frozen-ET was associated with substantially higher risk of HDP, even after accounting for shared parental factors within sibships.

Key words

Hypertension, Pregnancy-Induced.

Cryopreservation.

Embryo Transfer.

Siblings.

Pre-Eclampsia.

Fertilization in Vitro.

Reproductive Technologies, Assisted.

Non-standard Abbreviations and Acronyms

ART	Assisted reproductive technology
CoNARTaS	The Committee of Nordic ART and Safety
ESHRE	European Society of Human Reproduction and Embryology
Fresh-ET	Fresh embryo transfer
Frozen-ET	Frozen embryo transfer
HDP	Hypertensive disorders in pregnancy
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
NC	Natural conception
NPR	National Patient Registry
pp	Percentage points

Introduction

Worldwide, use of assisted reproductive technology (ART) has increased, and today, children born after ART constitute up to 7% of birth cohorts in several Western countries.[1] The conventional approach in ART involves either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), followed by fresh embryo transfer (fresh-ET). If there are surplus embryos, these are frozen and can be thawed and transferred in subsequent cycles. In the last decade, the number of pregnancies after frozen embryo transfer (frozen-ET) has increased substantially,[1] due to improved cryopreservation methods which facilitate single embryo transfer.[2,3] Moreover, initial reports showed better perinatal and obstetric outcomes after frozen-ET than fresh-ET.[4] Elective freezing, where ovarian stimulation is not followed by fresh transfer, but instead freezing of all embryos for transfer in later cycles, has been successful in preventing ovarian hyperstimulation, while achieving similar or higher live birth rate compared to fresh cycles.[5] Consequently, elective freezing of all embryos is increasingly used, and in many high-income countries, most embryo transfers are now from frozen cycles.[1,6,7] Despite the advantages of frozen-ET, observational studies raise concern about treatment safety due to higher risk of hypertensive disorders in pregnancy (HDP) after frozen-ET compared to both natural conception and fresh-ET.[8,9] Importantly, conventional observational studies are prone to residual confounding, particularly from factors associated with infertility.[10-12] An increased risk of HDP after frozen compared to fresh transfer is supported by a recent meta-analysis of three randomized trials comparing 1193 pregnancies after elective freezing to 1205 after fresh transfer.[13] However, comparison with natural conception, which is needed to understand the potential contribution from infertility, cannot be investigated through randomization.

Within sibship analyses may strengthen causal inference by controlling for unmeasured or unknown confounders at the parental level.[14] Using a sibship design, we have recently shown that singletons conceived by fresh-ET are smaller, and singletons conceived by frozen-ET are larger for gestational age than their naturally conceived siblings, whereas risk of preterm birth is higher after both ART treatments.[15] So far, only two small studies, both including births up to 2007, have investigated risk of HDP after ART using a sibship design. In a Nordic registry-based cohort, risk of HDP was higher after frozen-ET compared to fresh-ET in 100 double discordant pairs of singleton siblings.[16] In a Dutch cohort comparing risk of HDP after any ART vs natural conception within 1813 sibling pairs, no clear association was found.[17] HDP is associated with severe morbidity in mother [18,19] and child,[20,21] and identification of ART treatments that influence risk contributes to informed decision-making, but could also reveal valuable opportunities for prevention.

The objective of this study was to investigate whether the risk of HDP following fresh-ET and frozen-ET is higher compared to naturally conceived pregnancies. We used within sibship comparison to control for confounding from unmeasured and unknown parental factors, such as genetics, preconception lifestyle and health, as well as socioeconomic status.

Methods

The data cannot be shared publicly due to national data protection regulations but may be accessed from a server at Statistics Denmark, after approval by the relevant Ethics Committees and registry keeping authorities in each country.

Materials

The Committee of Nordic ART and Safety (CoNARTaS) cohort includes all deliveries registered in the Medical Birth Registries in four Nordic countries, described in detail elsewhere.[22] We included data from Denmark (1994–2014), Norway (1984–2015) and Sweden (1985–2015), but not from Finland, where details on ART treatment were not registered. Using the national identity number assigned to each resident in the Nordic countries,[23] we linked data from the Medical Birth Registries and national ART registries and databases, as well as the National Patient Registry in Denmark.

In Denmark, all ART cycles in public and private clinics have been recorded in the national ART quality registry since 1994. In Norway, public and private ART clinics have provided information to the Medical Birth Registry since 1984 on all ART cycles resulting in an ultrasound verified pregnancy (week 6–7). In Sweden, conception method was reported to the National Board of Health and Welfare between 1982 and 2006, and from 2007 all ART cycles have been registered in the national ART quality registry.

Exposures, outcome, and other factors

The exposures were frozen-ET or fresh-ET versus natural conception (reference group). Natural conception comprised all pregnancies with no registration of ART conception (including ovulation induction and insemination).

Diagnoses during pregnancy, delivery, and the puerperal period were registered in the Danish National Patient Registry and the Norwegian and Swedish Medical Birth Registries according to national adaptations of the International Statistical Classifications of Diseases and Related Health Problems (ICD), as outlined in Table S1. We defined HDP as a combined outcome, including gestational hypertension, preeclampsia, eclampsia, and chronic hypertension with superimposed preeclampsia. We also repeated the analyses after restricting the outcome to preeclampsia, superimposed preeclampsia, and eclampsia, i.e., pregnancies with isolated gestational hypertension were considered as not having the outcome.

In Denmark and Norway, gestational age was estimated from ultrasound scans in the first or second trimester. If unavailable, we used transfer date for ART pregnancies, and last menstrual period for naturally conceived pregnancies. For Sweden, gestational age was based on transfer date for ART pregnancies, and second trimester ultrasound scans for natural conception, and if these were unavailable, the date of the last menstrual period was used.

Smoking status was self-reported and registered throughout the study period in Denmark and Sweden and since 1999 in Norway and harmonized across the countries as no versus any smoking during pregnancy. Maternal height and weight were registered in 1988–1989 and 1992–2015 in Sweden, and since 2004 and 2007 in Denmark and Norway, respectively. In all countries, the proportion of observations with missing data on these variables was considerable during the first years of registration.

Study population

We defined the study period from 1988 (the first year with a registered delivery after frozen-ET) to 2014 (Denmark) or 2015 (Norway and Sweden). The eligible cohort was defined as all singleton deliveries with mothers who were 20 years or older and had their first delivery within the study period, comprising 4 637 605 pregnancies in 2 392 505 women (Figure 1). We excluded observations with missing parity, maternal age, birthweight, or gestational age. Next, we excluded pregnancies with parity ≥4 (as there were very few ART conceptions among mothers with five or more deliveries) and/or maternal age ≥45 years, as well as observations with extreme values on birthweight (<300 or >6500 grams, >6 standard deviations above expected)[24] or gestational age (<22 or >44 weeks). Available data on the 114 577 excluded pregnancies are presented in Table S2. Our main sample comprised 4 523 028 deliveries from 2 379 130 mothers, including 78 300 after fresh-ET and 18 037 after frozen-ET. In total, 33 209 singleton sibships with the same mother and conceived from at least two of the three conception methods were defined from maternal identity codes.

Statistical analyses

We compared odds of HDP across conception methods in multilevel logistic models, with deliveries as one level and mothers as another (using Stata's xtlogit command with maternal identity codes defining the clusters).[25,26] We used random effects models to obtain conventional population level estimates and fixed effects models for within sibship estimates (i.e., comparison within mothers). Precision was estimated by 95% confidence intervals (CIs). To increase interpretability of odds ratios (OR), we used postestimation commands to obtain risk differences (RDs). To facilitate comparison with previous studies,[27,28] we also compared frozen-ET to fresh-ET.

We defined potential confounders as factors that could influence the need for ART and risk of developing HDP. In random effect models, we adjusted for birth year, maternal age, parity, and country as categorical variables. Fixed effects models were adjusted for the same covariates except for country (which is stable within mothers). Within sibship analyses control for unknown and unmeasured confounding under the assumption that most of these confounders are at the family-level, not at the individual-level.[25]

To investigate if associations were affected by which conception method occurred first, we restricted the random effect models to mothers with singletons in their first two consecutive deliveries, and added interaction terms between parity and conception method (bidirectional analysis).[25] This analysis included 1 579 190 sibships belonging to one of nine possible sibship combinations. Further, to investigate whether experiencing HDP influenced the selection into the population of double discordant sibships,[29] we categorized the first delivery by conception method and HDP occurrence (resulting in six subgroups). For these subgroups, we calculated the probability of having a second singleton with either conception method within five years following the first singleton and estimated OR of HDP in the second pregnancy for each subgroup.

We conducted several sensitivity analyses to evaluate the robustness of our findings (Figure 1). First, we adjusted for maternal smoking and body mass index (BMI) in the subsample with available information. Second, we repeated our main models for full siblings (same mother and father) to account for constant paternal factors, for siblings born within a three-year interval as their parents' health might be more constant than for singletons born further apart in time, and for each country separately. Finally, we restricted deliveries after ART to explore the impact of other treatment factors: fertilization by IVF (i.e., excluding

fertilization by ICSI, which is used mainly for male infertility in the Nordic countries),[30] single embryo transfers (to limit the potential impact of vanishing twins),[31,32] and blastocyst transfers (to account for prolonged culture media and in vitro exposure).[33] During the study period, most frozen blastocysts were vitrified whereas most cleavage stage embryos were slow-frozen.[33]

To explore whether the higher risk of preterm birth after ART reduced the probability of being diagnosed with HDP,[15] we repeated analyses using Cox regression with gestational duration as the time scale. We estimated hazard ratios (HR), adjusting for the same covariates as the main analyses. For population level estimates, we used robust standard errors to account for dependency of observations within mothers, and for within sibship estimates, we used stratified models with maternal identity in separate strata. The proportional hazards assumption was examined by inspection of log-log plots.

Statistical analyses were performed using Stata/MP for Windows, Version 17.0 (StataCorp LLC, College Station, TX).

This study is reported according to The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement guideline (Supporting document: RECORD checklist).

Results

Table 1 shows that women who gave birth after frozen-ET (mean age 34.3 years) or fresh-ET (33.8 years) were older than women with natural conception (29.6 years). Parity was lower after fresh-ET (75.3% primiparous) than frozen-ET (58.0%) and natural conception (51.2%). A lower proportion of ART conceiving mothers smoked during pregnancy, while mean BMI

was similar between all conception groups. Prevalence of chronic hypertension was low for all conception methods (0.6% to 0.9%).

Pregnancies after frozen-ET and fresh-ET were more frequently preterm (6.6% and 8.1%, respectively) compared to naturally conceived pregnancies (5.0%) and more frequently induced and/or delivered by cesarean section. Among frozen-ET pregnancies, 36.7% were fertilized by ICSI, 64.3% were single embryo transfers, and 20.8% were blastocyst transfers. Fresh-ET pregnancies had similar proportions of ICSI and single embryo transfers, but only 5.7% were blastocyst transfers.

Main analyses

The unadjusted risk of HDP was 7.4% after frozen-ET, 5.9% after fresh-ET, and 4.3% after natural conception (Table 2). In population level analysis, frozen-ET was associated with higher odds of HDP compared to naturally conceived pregnancies, adjusted odds ratio 1.74 (95% CI 1.61 to 1.89), with a corresponding adjusted RD of 1.95 percentage points (95% CI 1.61 to 2.28). There was little difference in adjusted odds of HDP between fresh-ET and naturally conceived pregnancies. Within sibships, the odds of HDP after frozen-ET were twice as high as for their naturally conceived siblings. Pregnancies after fresh-ET had similar odds of HDP as their naturally conceived siblings. When the more restricted outcome definition was used (preeclampsia, superimposed preeclampsia, and eclampsia), associations remained similar or were slightly strengthened. Frozen-ET pregnancies had higher odds of HDP compared to fresh-ET pregnancies, both at population level and within sibships (Table S3).

Among women with two consecutive singletons, risk of HDP declined from the first to the second pregnancy for all combinations of conception methods (Figure 2, and Table S4). The

highest risk in first pregnancy and the largest decline was seen for frozen-ET/natural conception, whereas the highest risk in second pregnancy and the smallest decline was seen for natural conception/frozen-ET, indicating that a single subgroup did not drive the within sibship estimates.

For all conception methods, women with HDP in their first pregnancy were less likely to have a second pregnancy than women without HDP (Figure S1). Among women with frozen-ET in their first pregnancy, continuation with natural conception was more common if they had experienced HDP, whereas those with fresh-ET in their first pregnancy were less likely to have natural conception in their second pregnancy, than women without HDP. When accounting for this selection by estimating risk in the second pregnancy for women with similar experience in their first pregnancy, frozen-ET showed higher odds of HDP compared to natural conception in most subgroups, while fresh-ET showed no or weak positive associations. However, power was limited in some of these subgroups.

Sensitivity analyses

Subgroup analyses with adjustment for BMI and smoking, restriction to full siblings and siblings born within a three-year interval, yielded results consistent with our main findings (Figure 3, Table S5 and Table S6). Results were also similar when restricting the ART pregnancies to IVF fertilization (i.e., excluding ICSI), single embryo transfer, and blastocyst transfers. Both population level and within sibship estimates consistently indicated higher odds of HDP among frozen-ET compared to natural conception. For fresh-ET, population level estimates overall indicated similar odds of HDP as in naturally conceived pregnancies, while most within sibship estimates tended towards lower odds of HDP compared to naturally conceived siblings. Results were also similar between countries (Table S7).

Cox regression gave broadly similar results as our main analyses (Table S8), with a higher risk of HDP after frozen-ET in both population level and sibship analyses and no strong associations for fresh-ET. No clear violations of the proportional hazard assumption were found, indicating that associations were similar throughout pregnancy.

Discussion

In this population-based cohort study with nationwide data from three countries over almost three decades, the risk of HDP following frozen-ET was substantially higher than after natural conception, even when controlling for constant parental characteristics within sibships. In contrast, pregnancies following fresh-ET were at a similar or lower risk than naturally conceived. Considering the robustness of these findings across subgroups and with different regression models, our study provides strong indications that treatment factors may contribute to the higher risk of HDP observed in frozen-ET pregnancies.

Comparison with other studies and interpretation of findings

Our study is in agreement with earlier population level studies showing a higher risk of HDP after frozen-ET transfer.[9,16,28] On the population level, it has been demonstrated that in our study cohort, the association between frozen-ET and HDP is similar for blastocyst transfers.[33,34] However, our study's lack of a clear association between fresh-ET and HDP differs from other studies.[16,28]

We are not aware of other studies that could separate fresh and frozen transfers and compare risk of HDP to naturally conceived siblings. However, the higher risk of HDP after frozen-ET is in agreement with an earlier CoNARTaS study comparing siblings born after fresh-ET and frozen-ET between 1988 and 2007.[16] In contrast, a Dutch study comparing siblings conceived after any ART versus natural conception between 1999 and 2007, found higher crude risk of HDP within sibships and no clear association after adjustments.[17] However, the results from that study may have been unintentionally biased by adjustment for level of care, which could be a common consequence of ART and HDP.[35]

Several recent cohort studies found that odds of HDP or preeclampsia in frozen cycles were from 43% to 173% higher for transfer in programmed cycles (substituted with estrogen and progesterone, but no ovulation) than transfer in natural, ovulatory cycles.[8,36-38] It has been suggested that these observations could be attributed to the absence of a corpus luteum in programmed cycles.[27,38] Unfortunately, we did not have information on type of cycle in our data, but previous studies from the Nordic countries indicate that only 15-30% of frozen-ET were programmed cycles during our study period.[8,36] This suggests that the strong association between frozen-ET and HDP in our study was not driven by cycle programming alone. Additional explanations that have been proposed include embryo selection,[39] and epigenetic or other changes associated with freezing and thawing,[40-42] possibly affecting trophoblast invasion, in turn leading to abnormal placentation.[43] Lastly, differential obstetric management seems unlikely to play a role, as we found similar results when accounting for preterm birth using survival analysis.

Strengths and limitations

A strength of our study is the sibship design which allowed control for confounding shared by siblings (observed and non-observed), such as genetics, preconception parental health, and socioeconomic status.[25] The use of nationwide, prospectively collected registry data of high quality from three countries,[44-46] ensured a large and unselected study population with opportunities for a range of sensitivity analyses that supported the main findings.

Despite the extra control for shared confounders provided by the within sibship analyses, we cannot exclude residual confounding from non-shared confounders, such as smoking and BMI where confounder control was limited by a large proportion of missingness, and causes of infertility, which were largely unknown. Although couples who conceive after

fresh and frozen cycles may be expected to be more similar than couples who conceive naturally and after ART, causes and severity of infertility are likely to influence the couple's probability of having embryos for freezing. Unfortunately, we did not have data on number of embryos obtained from the stimulation cycle, and we could therefore not determine whether couples with fresh-ET conception had surplus embryos eligible for freezing. Nor could we determine if the frozen-ET pregnancies were after an initial, unsuccessful fresh-ET or from an elective freezing approach. However, during our study period, elective freezing was still relatively uncommon and most frozen-ET conceptions would have been preceded by a fresh transfer. Results from randomized controlled trials show that the chances of a successful pregnancy are similar or slightly higher after elective freezing compared to fresh transfer.[47-50] This suggests that for couples with surplus embryos eligible for freezing in our cohort, the chances of pregnancy after either transfer type might be comparable.

Another limitation is that most pregnancies conceived after ART treatment abroad would be misclassified as naturally conceived in our data, but these would be greatly outnumbered by the correctly classified naturally conceived pregnancies. Further, it is possible that increased parental awareness and a lower threshold for seeking medical attention could increase detection of HDP after ART conception. However, in the Nordic countries, ART conceived pregnancies are followed in the same antenatal program as the background population. It also seems unlikely that a potentially increased detection of HDP after ART would differ for fresh-ET versus frozen-ET.

We found some evidence of selection into the within sibship population (double discordant sibships), due to lower probability of continued reproduction among women with HDP in first pregnancy and differential probability of a second, naturally conceived singleton for

women with HDP and fresh-ET or frozen-ET in their first pregnancy. Although this may have biased the within sibship estimates, overall conclusions appeared robust in our attempts to control for this selection.

Although we consider pooling of data from three Nordic countries justifiable because all are high-income countries with publicly financed, accessible health care systems and similar ART policies and antenatal care programs,[22] these characteristics of our societies may also limit generalizability to other populations.

Perspectives

While cryopreservation has facilitated elective single embryo transfer, thereby reducing risk of HDP after ART through reduction of multiple pregnancies,[2,51,52] careful consideration of all benefits and harms is needed before freezing all embryos as routine, rather than for couples with clinical indications, such as high risk of ovarian hyperstimulation syndrome.[53] HDP is relatively common and can have severe maternal and fetal consequences,[18-21] suggesting that identifying subgroups at higher risk could provide opportunities for more targeted monitoring and interventions. The need for preventive measures is further emphasized by the fact that associations in our study were not driven by isolated gestational hypertension. Further, the previously reported increase in birthweight and risk of being born large for gestational age,[15] should also be included in the balance sheet. Future research should investigate which treatment factors associated with frozen-ET that might be involved in the development of HDP.

Statements

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Disclosure

None.

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Novelty and Relevance

What is New

- This study is the largest sibship design study to date to explore the risk of hypertensive disorders in pregnancies after assisted reproduction.

What is Relevant?

- In this Nordic population-based cohort study, frozen embryo transfer was associated with a substantially higher risk of hypertensive disorders compared to natural conception, even after accounting for shared parental factors within sibships.

Clinical/Pathophysiological Implications?

- The high risk of hypertensive disorders following frozen embryo transfer raises concerns about the increasingly popular elective freezing of all embryos.

Supplemental material

The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

Tables S1–S8.

Figure S1.

Figure titles and captions. Supporting information.

"Figure 1: Flowchart of the study population"

The subgroup with only blastocyst transfers was restricted to birth years 2005–2015.

Abbreviations: Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

"Figure 2: Risk of hypertensive disorders in pregnancy in consecutive sibling pairs according to birth order and conception method"

Absolute risks are obtained using random effects logistic models with post-estimation commands. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44), and country (Denmark, Norway, or Sweden).

Table S4 presents the full results from Figure 2.

Abbreviations: Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy.

"Figure 3: Risk of HDP by conception method: Population level estimates and within sibship comparisons in subgroups"

Adjusted odds ratios with 95% confidence intervals for fresh embryo transfer (fresh-ET) versus natural conception and frozen embryo transfer (frozen-ET) versus natural conception in our subgroups. All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden). Estimates for the body mass index (BMI) and smoking subgroup are additionally adjusted for BMI as a categorical variable (underweight <18.5 kg/m^2, normal 18.5–24.99 kg/m^2, overweight 25–29.99 kg/m^2, or obese ≥30 kg/m^2) and smoking status as a dichotomous variable (yes/no). Blastocyst analyses were restricted to 2005–2015.

Table S5 presents the full results from Figure 3.

Abbreviations: BMI, body mass index; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

"Figure S1: Risk of hypertensive disorders in pregnancy by conception method when accounting for selection"

Adjusted odds ratios with 95% confidence intervals for fresh embryo transfer (fresh-ET) and frozen embryo transfer (frozen-ET) versus natural conception (NC). All estimates are adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and country (Denmark, Norway, or Sweden).

Dashed blue lines indicate subgroups that contribute to within sibship estimates (fixed effects).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; Ref, reference group.

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Table 1. Baseline characteristics of the study population (main sample) by conception method.*

	Fresh-ET (n = 78 300)	Frozen-ET (n = 18 037)	Natural conception (n = 4 426 691)
Country, No. (%)			
Denmark	25 056 (32.0)	3347 (18.6)	978 208 (22.1)
Norway	16 636 (21.3)	3300 (13.8)	1 198 150 (27.1)
Sweden	36 608 (46.8)	11 390 (63.2)	2 250 333 (50.8)
Birth year, No. (%)			
1988–1996	5802 (7.4)	498 (2.8)	1 024 162 (23.1)
1997–2001	11 208 (14.3)	1101 (6.1)	808 644 (18.3)
2002–2006	17 766 (22.7)	2545 (14.1)	911 965 (20.6)
2007–2011	24 405 (31.2)	6517 (36.1)	967 295 (21.9)
2012–2015	19 119 (24.4)	7376 (40.9)	714 625 (16.1)
Parity, No. (%)			
0	58 919 (75.3)	10 454 (58.0)	2 265 209 (51.2)
1	16 999 (21.7)	6545 (36.3)	1 589 045 (35.9)
2	2041 (2.6)	920 (5.1)	478 062 (10.8)
3	341 (0.4)	118 0.4)	94 375 (2.1)
Maternal age, mean (SD), years	33.8 (4.2)	34.3 (4.1)	29.6 (4.8)
Chronic hypertension, No. (%)	707 (0.9)	159 (0.9)	26 936 (0.6)
Maternal BMI, mean (SD), kg/m^2	24.2 (4.1)	24.2 (4.0)	24.2 (4.5)
Missing outside registration period, No. (%)	15 183 (19.4)	1196 (6.6)	1 314 826 (29.7)
Missing during registration period, No. (%)	8950 (11.4)	2290 (12.7)	502 475 (11.4)
Maternal smoking in pregnancy, No. (%)	4055 (5.2)	540 (3.0)	449 538 (10.2)
Missing outside registration period, No. (%)	2006 (2.6)	115 (0.6)	378 584 (8.6)
Missing during registration period, No. (%)	5357 (6.8)	996 (5.5)	305 443 (6.9)
Caesarean section, No. (%)	19 910 (25.4)	5133 (28.5)	670 949 (15.2)
Induction of labor, No. (%)	14 720 (18.8)	4500 (25.0)	567 072 (12.8)
Sex, No. (%)			
Male	40 019 (51.1)	9215 (51.1)	2 275 150 (51.4)
Female	38 257 (48.9)	8822 (48.9)	2 151 084 (48.6)
Birthweight, mean (SD), grams	3406.4 (620.7)	3578.0 (614.6)	3537.2 (565.2)
Gestational age, mean (SD), days	276.5 (15.8)	278.0 (14.9)	279.0 (13.0)
Preterm birth†, No. (%)	6351 (8.1)	1198 (6.6)	219 461 (5.0)
ART fertilization method, No. (%)			
IVF	44 602 (57.0)	9846 (54.6)	–

ICSI	32 239 (41.2)	6616 (36.7)	–
Unknown	1459 (1.9)	1575 (8.7)	–
Embryos transferred, No. (%)			
1	37 082 (47.4)	11 605 (64.3)	–
2	29 987 (38.3)	4209 (23.3)	–
3	1891 (2.4)	131 (0.7)	–
Unknown	9340 (11.9)	2092 (11.6)	–
Embryo culture, No. (%)			
2–3 days (Cleavage)	61 772 (78.6)	11 707 (64.9)	–
5–6 days (Blastocyst)	4450 (5.7)	3756 (20.8)	–
Unknown	12 178 (15.6)	2574 (14.3)	–

[*]Percentages may not total to 100% on account of rounding.
[†]Preterm birth was defined as birth before 37 weeks of gestation.
Abbreviations: ART, assisted reproductive technology; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); ET, embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; SD, standard deviation.

Table 2. Risk of hypertensive disorders in pregnancy by conception method: population level estimates and within sibship comparison.

	Population level estimates (Random effects)					Within sibship estimates (Fixed effects)		
	Cases/Deliveries (%)	RD*, *pp*	RD, *pp* (95% CI)†	OR*	Adjusted OR (95% CI)†	Cases/Deliveries‡ (%)	OR*	Adjusted OR (95% CI)†
HDP								
Natural conception	191 287/4 426 691 (4.32)	0	0 (Ref.)	1	1 (Ref.)	1364/34 151 (3.99)	1	1 (Ref.)
Fresh-ET	4600/78 300 (5.87)	1.55	0.06 (-0.07 to 0.19)	1.54	1.02 (0.98 to 1.07)	1539/30 333 (5.11)	1.46	0.99 (0.89 to 1.09)
Frozen-ET	1326/18 037 (7.35)	3.09	1.95 (1.61 to 2.28)	2.18	1.74 (1.61 to 1.89)	590/9651 (6.11)	1.91	2.02 (1.72 to 2.39)
Preeclampsia§								
Natural conception	142 195/4 426 691 (3.21)	0	0 (Ref.)	1	1 (Ref.)	933/34 151 (2.73)	1	1 (Ref.)
Fresh-ET	3371/78 300 (4.31)	1.11	0.18 (0.01 to 0.30)	1.48	1.08 (1.03 to 1.13)	1133/30 333 (3.74)	1.56	1.06 (0.94 to 1.19)
Frozen-ET	991/18 037 (5.49)	2.38	1.91 (1.59 to 2.22)	2.11	1.93 (1.77 to 2.12)	440/9651 (4.56)	2.11	2.45 (2.02 to 2.96)

*Unadjusted.
†Adjusted for birth year (1988–1996, 1997–2001, 2002–2006, 2007–2012 or 2013–2015), maternal age (20–24, 25–29, 30–34, 35–39 or 40–44) and parity (0, 1, 2 or 3). Random effects are additionally adjusted for country (Denmark, Norway, or Sweden).
‡Refers to deliveries that are part of a sibling group with at least two different conceptions methods within the group.
§Preeclampsia, chronic hypertension with superimposed preeclampsia, and eclampsia.
Abbreviations: CI, confidence interval; Fresh-ET, fresh embryo transfer; Frozen-ET, frozen embryo transfer; HDP, hypertensive disorders in pregnancy; OR, odds ratio; pp, percentage points; RD, risk difference; Ref, reference group.

Eligible Cohort
Singletons n=4 637 605, Mothers n=2 392 505

Exclusions n= 114 577 (2.5%)
Missing maternal age or parity n=5333
Missing conception method n=2537
Missing gestational age n=54 181
Missing birthweight n=12 786

Parity ≥4 n=28 859
Maternal age >45 years n=2045
Gestational age <22 weeks or >44 weeks n=4496
Birthweight >6500g, <300g or >6SD n=4340

Main Sample
Total singletons n=4 523 028 (97.5%);
Total mothers n=2 379 130 (99.4%)

Naturally conceived singletons n=4 426 691
Fresh-ET singletons n=78 300
Frozen-ET singletons n=18 037

Sibling groups (any order) n=33 209
Naturally conceived & Fresh-ET n=23 811
Naturally conceived & Frozen-ET n=4052
Naturally conceived & Fresh-ET & Frozen-ET n=681
Fresh-ET & Frozen-ET n=4665

Denmark
Naturally conceived singletons n=978 208
Fresh-ET singletons n=25 056
Frozen-ET singletons n=3347
Sibling groups n=8781

Norway
Naturally conceived singletons n=1 198 150
Fresh-ET singletons n=16 636
Frozen-ET singletons n=3300
Sibling groups n=6991

Sweden
Naturally conceived singletons n=2 250 333
Fresh-ET singletons n=36 608
Frozen-ET singletons n=11 390
Sibling groups n=17 437

Subgroup Analysis (BMI & smoking)
Naturally conceived singletons n=2 554 111
Fresh-ET singletons n=53 165
Frozen-ET singletons n=14 355
Sibling groups n=19 823

Subgroup Analysis (full siblings)
Naturally conceived singletons n=3 394 887
Fresh-ET singletons n=40 841
Frozen-ET singletons n=10 718
Sibling groups n=30 663

Subgroup Analysis (<3 years birth interval)
Naturally conceived singletons n=1 994 313
Fresh-ET singletons n=23 500
Frozen-ET singletons n=6747
Sibling groups n=19 532

Subgroup Analysis (IVF, ÷ICSI)
Naturally conceived singletons n=4 426 691
Fresh-ET singletons n=42 602
Frozen-ET singletons n=9846
Sibling groups n=21 866

Subgroup Analysis (single embryo transfers)
Naturally conceived singletons n=4 426 691
Fresh-ET singletons n=37 082
Frozen-ET singletons n=11 605
Sibling groups n=19 713

Subgroup Analysis (blastocyst transfers)
Naturally conceived singletons n=1 534 108
Fresh-ET singletons n=4214
Frozen-ET singletons n=3729
Sibling groups n=1967