# Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis

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**Objective:** To perform a systematic review and meta-analysis of obstetric and perinatal complications in singleton pregnancies after the transfer of frozen thawed and fresh embryos generated through IVF.
**Design:** Systematic review.
**Setting:** Observational studies, comparing obstetric and perinatal outcomes in singleton pregnancies subsequent to frozen thawed ET versus fresh embryo transfer, were included from Medline, EMBASE, Cochrane Central Register of Clinical Trials, DARE, and CINAHL (1984–2012).
**Patient(s):** Women undergoing IVF/intracytoplasmic sperm injection (ICSI).
**Intervention(s):** Two independent reviewers extracted data and assessed the methodological quality of the relevant studies using critical appraisal skills program scoring. Risk ratios and risk differences were calculated in Rev Man 5.1. Subgroup analysis was performed on matched cohort studies.
**Main Outcome Measure(s):** Antepartum hemorrhage, very preterm birth, preterm birth, small for gestational age, low birth weight, very low birth weight, cesarean section, congenital anomalies, perinatal mortality, and admission to neonatal intensive care unit.
**Result(s):** Eleven studies met the inclusion criteria. Singleton pregnancies after the transfer of frozen thawed embryos were associated with better perinatal outcomes compared with those after fresh IVF embryos. The relative risks (RR) and 95% confidence intervals (CI) of antepartum hemorrhage (RR = 0.67, 95% CI 0.55–0.81), preterm birth (RR = 0.84, 95% CI 0.78–0.90), small for gestational age (RR = 0.45, 95% CI 0.30–0.66), low birth weight (RR = 0.69, 95% CI 0.62–0.76), and perinatal mortality (RR = 0.68, 95% CI 0.48–0.96) were lower in women who received frozen embryos.
**Conclusion(s):** Although fresh ET is the norm in IVF, results of this systematic review of observational studies suggest that pregnancies arising from the transfer of frozen thawed IVF embryos seem to have better obstetric and perinatal outcomes. (Fertil Steril® 2012;98:368–77. ©2012 by American Society for Reproductive Medicine.)
**Key Words:** IVF, ICSI, obstetric outcomes, perinatal outcomes, frozen replacement cycles

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The first live birth after the transfer of a thawed cryopreserved embryo was reported in 1984. With refinement of technology during the past few decades, the proportion of frozen ETs has increased (1) as have pregnancy rates (PR) that, according to some investigators are better than those after the transfer of fresh IVF embryos (2). Although there have been concerns about the impact of cryopreservation on the health of children born, outcome data after frozen ET have been generally reassuring (3). Results of a number of observational studies (4–6) indicate that obstetric and perinatal outcomes in pregnancies resulting from cryopreserved embryos are similar to those in fresh IVF/intracytoplasmic sperm injection (ICSI) cycles, whereas other investigators suggest that they are superior (7, 8). Currently, there are no randomized controlled trials that have compared obstetric and perinatal outcomes after elective cryopreservation of all embryos followed by delayed transfer versus fresh ET in an IVF/ICSI cycle, which is the current practice in almost all IVF units. Individual observational studies, although large, lack the power to demonstrate clinically meaningful differences in key perinatal outcomes as the background risk of complications is very low.

It is already known that obstetric and perinatal outcomes in singleton IVF/ICSI pregnancies are worse when compared with those after spontaneous conceptions (9). An early review (10) suggested that there was reduced incidence of preterm and low birth weight babies in pregnancies after frozen thawed ET. However, they did not attempt meta-analysis. Hence, it was not possible to quantify the obstetric and perinatal risks in singleton pregnancies after frozen thawed transfer.

During the past few years an increased proportion of treatments in IVF/ICSI are after frozen ET (1). Furthermore, there are new technologies for freezing with higher survival rate of embryos. More data on outcomes are now available (at least six publications since last review). Hence, there is a need to perform a meta-analysis to quantify the risks of obstetric and perinatal complications in singleton IVF/ICSI pregnancies after frozen thawed ET and compare them with pregnancies after fresh ET.

## MATERIALS AND METHODS

PRISMA guidelines for systematic reviews were followed (http://www.plosmedicine.org/article/info:doi%2F10.1371%2Fjournal.pmed.1000097).

### Data Sources and Searches

A literature search was performed (1984–2012) on Medline, EMBASE, Cochrane Central Register of Clinical Trials, CINAHL, and DARE. There were no language restrictions. Relevant journals in the specialty (*Human Reproduction, Human Reproduction Update, RBM online* and *Fertility and Sterility*) were searched electronically. Cross references from the included studies were hand searched. Two review authors (A.M., S.P.) independently conducted the searches and selected the studies to be included. Differences of opinion were resolved after team discussion. Contact with authors was attempted wherever additional information was needed. Data were extracted using predesigned tables.

### Study Selection

**Inclusion criteria.** An initial scoping exercise did not reveal any randomized controlled trials. Hence all observational studies (published) that compared obstetric and perinatal outcomes in singleton pregnancies after transfer of fresh and frozen embryos were included.

**Exclusion criteria.** Studies were excluded if there was no comparator group, obstetric and perinatal outcomes were not reported, or if it was not possible to differentiate the outcomes for singletons and twins. Case reports and case series were excluded. Data from pregnancies after GIFT were excluded.

### Outcome Measures

The following outcome measures were included: antepartum hemorrhage; hypertensive disorders of pregnancy (including pregnancy-induced hypertension, pre-eclampsia, and eclampsia), gestational diabetes, very preterm birth (delivery before 32 weeks); preterm birth (delivery before 37 weeks); small for gestational age, low birth weight (birth weight $<$2,500 g); very low birth weight (birth weight $<$1,500 g); induction of labor; cesarean section (both emergency and elective); congenital anomalies (major and minor); perinatal mortality; and admission to the neonatal intensive care unit.

### Statistical Analysis

For each outcome, data were extracted in $2 \times 2$ tables. If categorical data were not provided (to construct $2 \times 2$ tables) contact with investigators was attempted. Data were pooled if there were at least two studies with similar outcomes for the comparison groups. Meta-analysis was attempted wherever appropriate. Analysis was done using Rev Man 5.1 software (The Nordic Cochrane Centre, The Cochrane Collaboration 2011). For binary (or dichotomous) outcomes, the results for each study were expressed as risk ratios and risk differences with 95% confidence intervals (CI). Subgroup analysis was performed only on matched cohort studies.

Quality assessment of included studies was performed independently by two authors (S.P. and A.M.). Any disagreement regarding type and quality of the study was resolved after discussion. Checklists from the critical appraisal skills program (CASP) (http://www.phru.nhs.uk/pages/phd/resources.htm) were used to assess and assign a quality score.

### Assessment of Heterogeneity

We assessed whether there was sufficient similarity between the eligible studies in terms of design and clinical characteristics to allow pooling of data. Statistical heterogeneity was assessed by using the $\chi^2$ test. A low $P$ value (or a large $\chi^2$ statistic relative to its degree of freedom) suggested heterogeneity (11). The $I^2$ statistic was used to assess the impact of the heterogeneity on the meta-analysis. In the event of moderate heterogeneity ($I^2$ >50%), a sensitivity analysis was performed by altering the fixed-to-random effect analysis. Further sensitivity analyses were performed by excluding studies with low CASP score (CASP $\leq 10$).

### Assessment of Reporting Biases

Funnel plots were constructed where a statistically significant difference was obtained in outcome measure, if at least five studies reported that outcome. This was to investigate whether the difference was due to publication or reporting bias.

## RESULTS

### Results of the Searches

The literature search yielded 1,929 citations. Of these, 1,913 were excluded after reading the title and the abstract. Full text versions of 16 articles were obtained, whereas another 3 were identified from a hand search of cross references. Of these 19 articles, 11 were included in the completed review (Fig. 1). Table 1 gives the details of all included studies. The excluded studies, along with the reasons for exclusion, are provided in Table 2. Most studies were removed at the title and abstract stage as they did not give any data on outcomes after frozen replacement cycles.

### Methodology of Included Studies

Characteristics of the 11 included studies are provided in Table 1. Of 11 included publications 4 were matched cohort studies. All except one (4) scored high ($\geq 10$) on the CASP scoring system. Data were obtained from databases and data linkage of routinely collected data except in two studies where clinical information was reported by clinicians using case notes (6, 8).

### Population in the Included Studies

Although pregnancies conceived through IVF/ICSI using fresh or frozen embryos were included, there was a variation in the gestation after which they were included in the studies: all clinical pregnancies (4, 6, 8); ongoing pregnancies beyond 20 weeks (12, 13); beyond 22 weeks (14); and beyond 28 weeks (5, 15).

In two studies women acted as their own reference group (13, 16). Fresh IVF/ICSI could have been first or second conception. Two studies had cohort group matched for age and parity (15, 17). There was no information on demographic profiles of women in two other studies (7, 12) as we used subgroup of fresh and frozen thawed treatment. Demographic information on these subgroups was not separate from the overall information. The characteristics in the two groups were similar in two unmatched cohort studies (5, 6). Maternal age was higher in the fresh ET group in Belva et al. (8), whereas it was lower in Pelkonen et al. (14) when compared with the frozen ET group. No details on other confounders such as parity, smoking, duration of infertility, and pre-existing medical diseases were available.

### Exposure in the Included Studies

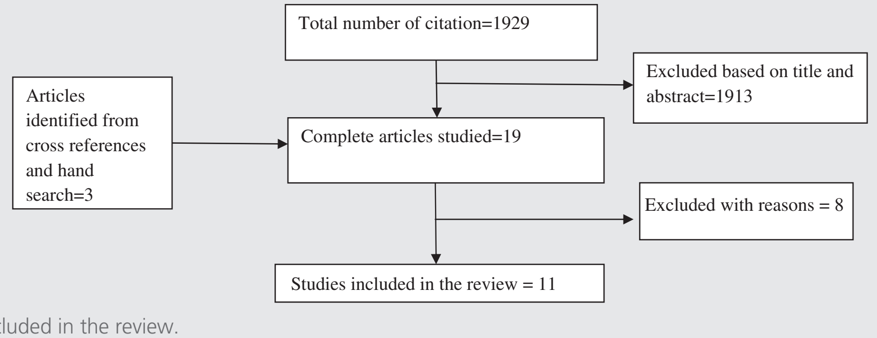
In the included studies, embryos were frozen on day 2/3 (cleavage stage) or day 5/6 (blastocyst stage) using either vitrification (newer method) or slow freezing (older method) techniques. Frozen embryos (after thawing) were transferred with or without giving additional hormones to women (to prepare endometrium) in a menstrual cycle (Fig. 1).

Six of 11 studies did not provide details of the method used for embryo cryopreservation, developmental stage of the embryo, and whether exogenous hormones were used to prepare the endometrium for frozen ET. Of those studies reporting these details, there was variation in the method of cryopreservation and the developmental stage of embryos at cryopreservation (Table 1). Most studies used slow freezing (4, 8, 14), whereas Wikland et al. (5) and Aflatoonian et al. (6) used vitrification. The embryos were frozen at cleavage stage (4, 6, 14), at blastocyst stage (5), and at blastocyst as well as cleavage stage (8). Embryos were transferred either in hormone replacement cycles (6) or natural, as well as hormone replacement cycles (5, 8, 14).

### Results of the Outcome Measures

**Antepartum hemorrhage.** Two unmatched cohort studies were included in the meta-analysis (n = 3,875 vs. 7,000 pregnancies after frozen vs. fresh ET) (12, 14). Antepartum hemorrhage was defined as bleeding of 15 mL or more that occurred from birth

**FIGURE 1**




Selection of studies included in the review.

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**TABLE 1**

**Included studies.**

| Study ID | Design of study | Population | Method of data collection | Details of cryopreservation | Risk of bias | Scoring |
|---|---|---|---|---|---|---|
| Aflatoonian et al., 2010 (6) | Un matched cohort study | Pregnancies after fresh ET vs. frozen ET | Questionnaires filled by gynecologists, pediatricians, and women regarding perinatal and obstetric outcomes | Vitrification was done at cleavage stage. Embryos were transferred in hormone replacement cycles | The characteristics of two groups are similar. All pregnancies were included | 10/12 |
| Belva et al., 2008 (8) | Unmatched cohort study | IVF or ICSI cryo vs. fresh IVF or ICSI. Exposed cohort: all pregnancies irrespective of cryo procedure used were consecutively included. Unexposed cohort: fresh IVF/ ICSI cycles | Data on pregnancies, deliveries, and neonatal history were obtained by gynecologists, pediatricians, and double-checked with parents, when child was 2 months old | Embryos were frozen at cleavage as well as blastocyst stage by slow freezing. Frozen embryos were transferred using natural cycles mainly but some were hormone replacement cycle | Pregnancies after mixed IVF/ICSI were not included. Cycles with PGD were excluded. Fresh cycles had maternal age—higher than frozen transfer. All pregnancies were included | 10/12 |
| Healy et al., 2010 (12) | Retrospective unmatched cohort study Jan 1991–Dec 2004 | Only fresh vs. frozen comparison was a subgroup analysis | Data were collected using record linkage | No details of frozen ET given natural/hormone replacement; cleavage/ blastocyst; slow freezing/ vitrification | Includes first singleton birth only.<br>Only ongoing pregnancies beyond 20 weeks of gestation were included. No data on demographic profile of the women in frozen ET vs. fresh group as this was subgroup analysis | 12/12 |
| Henningsen et al., 2011 (16) | Matched cohort with women acting as their own reference | Data collection from national population-based registry from 1994–2008. Comparison groups: frozen vs. fresh IVF/ICSI | The data were collected from Danish Medical Birth Register | No details of embryo freezing protocol; stage of freezing and protocols for replacement | Women were their own reference.<br>Fresh IVF/ICSI conception could be first/second one. Minimum gestation of included ongoing pregnancy was not mentioned | 11/12 |
| Pelkonen et al., 2010 (14) | Unmatched cohort study (1995–2006) | Exposed cohort: frozen ET resulting in singleton pregnancy.<br>Controls = fresh IVF/ICSI treatment. Some women may have had both fresh and frozen births; however, their proportion was $<10\%$ | Data taken from Finnish Medical Birth Register | Both natural and hormone replacement frozen replacement cycles were included. Exact proportion not mentioned. Only slow freezing at cleavage stage was done | All live births and stillbirths after 22 weeks of gestation and birth weight of $\geq 500$ g were included. Mothers in frozen ET group were slightly older.<br>Proportion of women having first pregnancy was 35% in frozen ET group compared with 52% in fresh ET group. The data on variables of pregnancy complications are incomplete in Finnish Medical Birth Register before 2004 | 11.5/12 |

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**TABLE 1**

**Continued.**

| Study ID | Design of study | Population | Method of data collection | Details of cryopreservation | Risk of bias | Scoring |
|---|---|---|---|---|---|---|
| Pinborg et al., 2010 (17) | Matched cohort study | Exposed cohort: singletons born after frozen ET (Jan 1995–Dec 2006). Unexposed cohort: singletons born after fresh IVF/ICSI within the same time frame 5 times the size of cryo group | Danish IVF and Danish Birth Register | No details of embryo freezing protocol; stage of freezing and protocols for replacement | Age and parity showed statistically significant difference in the groups; data adjusted for age, parity, gender, and year of birth. Minimum gestation of included ongoing pregnancy was not mentioned | 11/12 |
| Shih et al., 2008 (13) | Matched cohort with women acting as their own reference | Comparison groups: frozen vs. fresh IVF/ICSI | Neonatal perinatal statistics unit Australia | No details of embryo freezing protocol; embryos frozen at cleavage stage protocols for replacement cycles were not specified | All pregnancies after 20 weeks were recorded. Fresh IVF/ICSI conception could be first/ second one | 11/12 |
| Wada et al., 1994 (4) | Unmatched cohort | 232 consecutive deliveries after embryo cryopreservation between 1985 and 1991. Fresh IVF data: 763, gestational age, birth weight, congenital abnormalities | Not mentioned how data were collected | Embryos frozen at early cleavage stage using slow freezing | All deliveries were included | 8/12 |
| Wang et al., 2005 (7) | Unmatched cohort study | Infants conceived through ART procedures and born in Australia during 1996–2000. Only the comparison between fresh and frozen singleton was used for this study (subgroup analysis from the main study) | The study used data from two national collections. Assisted conception data collection & Australian national perinatal data collection | No details of frozen ET given; natural/hormone replacement; cleavage/ blastocyst; slow freezing/ vitrification | Fresh and frozen pregnancies were subgroup analysis, and hence not matched for the confounders. Viable pregnancy was defined as pregnancy of at least 20 weeks' gestation and or 400 g birth weight | 10/12 |
| Wennerholm et al., 1997 (15) | Matched cohort | Unexposed cohort: IVF conception with fresh embryos between 1990 and 1995 with frozen embryos. Exposed cohort: births between 1990 and 1995 with frozen embryos | Data were collected after medical records review | No details of embryo freezing protocol; stage of freezing and protocols for replacement | IVF conception and spontaneous pregnancies, both were control groups for pregnancies after frozen ET during that time period. Controls were matched for age and parity. All births were >28 weeks of gestation | 10.5/11 |

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**TABLE 1**

**Continued.**

| Study ID | Design of study | Population | Method of data collection | Details of cryopreservation | Risk of bias | Scoring |
|---|---|---|---|---|---|---|
| Wikland et al., 2010 (5) | Unmatched cohort study | Unexposed cohort: fresh blastocyst transfer. Exposed cohort: pregnancies after transfer of vitrified, blastocyst | Data for obstetric and perinatal complications were collected from maternity records | Both natural and hormone replacement frozen cycles were included; majority blastocyst cycles were hormonally supplemented | Fresh vs. frozen blastocysts only, although no matching was done but the characteristics were similar in the two groups. For the study period, deliveries in Sweden included all live born babies and stillbirths after 28 weeks of gestation. Maternal characteristics were comparable in vitrified and fresh blastocysts group concerning maternal age, BMI, parity, smoking, and education level | 11/12 |

*Note:* BMI = body mass index; ICSI = intracytoplasmic sperm injection; PGD = preimplantation genetic diagnosis.

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canal after 20 weeks of gestation and before the birth of the baby (12). Placenta previa and placenta abruptio are reported separately by both studies. Singleton pregnancies after frozen thawed ET were at decreased risk of antepartum hemorrhage when compared with those after fresh embryos (relative risk [RR] = 0.67, 95% CI 0.55–0.81) with an absolute decrease in the risk of 2% (1%–2%). There was no heterogeneity among the studies.

There was a lower risk of placenta previa (RR = 0.71, 95% CI 0.53–0.95, $I^2 = 0$) and placental abruptio (RR = 0.44, 95% CI 0.24–0.83, $I^2 = 0$) in pregnancies after frozen thawed ETs (Supplemental Fig. 1, available online).

**Very preterm birth (delivery at < 32 weeks).** Of four studies (n = 3,050 vs. 13,630 pregnancies after frozen vs. fresh ET) reporting proportion of deliveries at <32 weeks, two were case control studies. All studies had high score on CASP scoring. The relative risk (95% CI) of having delivery at <32 weeks was 0.73 (0.50–1.08) in singleton pregnancies after frozen embryo thawed transfer when compared with those after fresh embryo transfer. There was minimal heterogeneity ($I^2 = 11\%$) among the studies.

**Preterm delivery (delivery at < 37 weeks).** Of nine studies (n = 10,017 vs. 27,686 pregnancies after frozen vs. fresh cycles) reporting proportion of deliveries at <37 weeks, three were matched cohort. All were good quality studies as per CASP scoring. Definition of preterm labor/delivery was delivery before 37 weeks in all studies. There were no data on how many of them were spontaneous or induced preterm labor.

The RR of having a delivery at <37 weeks was 0.84 (95% CI 0.78–0.90) in singleton pregnancies after frozen thawed ET, when compared with those after fresh ETs (Supplemental Fig. 2, available online) with an absolute decrease in risk (95% CI) of 2% (1%–3%). There was marked heterogeneity ($I^2 = 74\%$) among the studies. The reduction in the risk persisted when subgroup analysis was done using only matched cohort and good quality studies, but not when the analysis was repeated with random effect. Funnel plot did not reveal any publication bias (Supplemental Fig. 3, available online).

**Small for gestational age.** Two studies (n = 1,933 vs. 3,141 pregnancies after frozen vs. fresh cycles) have reported on outcome of small for gestational age. This was defined as birth weight <22% of the expected mean birth weight according to gestational age in a Swedish reference population (5) or birth weight <2 SD of the mean for that gestation (14).

The RR of having a small for gestational age baby was 0.45 (95% CI 0.30–0.66) in singleton pregnancies subsequent to frozen thawed ET compared with those with fresh ET (Supplemental Fig. 4, available online) with an absolute decrease in risk (95% CI) of 2% (1%–2%). There was minimal heterogeneity among the studies ($I^2 = 22\%$).

**Low birth weight (birth weight < 2,500 g).** Of nine studies (n = 8,536 vs. 25,800 pregnancies after frozen vs. fresh cycles) reporting this outcome measure three were matched cohort (Supplemental Fig. 5, available online). Eight studies had high CASP scores. The RR of having a baby with birth weight

**TABLE 2**

Excluded studies.

| Study | Reason for exclusion |
|---|---|
| Aytoz et al., 1999 (22) | Data from singleton and twins could not be separated |
| Aflatoonian et al., 2010 (20) | No data on obstetric and perinatal outcomes |
| Frydman et al., 1989 (23) | There is no control group |
| Kallen et al., 2005 (24) | 2 × 2 table cannot be made |
| Kallen et al., 2005 (25) | Data for singleton cannot be separated |
| Shapiro et al., 2011 (18) | No data on obstetric and perinatal outcomes |
| Wennerholm, 2000 (26) | Overlapping data from Wennerholm et al., 1997 |
| Wennerholm et al., 2009 (10) | Systematic review |

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<2,500 g was 0.69 (95% CI 0.62–0.76) in singleton pregnancies after frozen thawed embryos when compared with those after fresh embryos with an absolute decrease in risk (95% CI) of 3% (2%–3%). There was minimal heterogeneity ($I^2$ = 28%) among the studies. The increased risk persisted when data were only pooled from matched cohort and good quality studies (Table 3). Funnel plot did not reveal any publication bias (Supplemental Fig. 6, available online).

**Very low birth weight (birth weight < 1,500 g).** Of four studies (n = 3,552 vs. 16,469 pregnancies after frozen vs. fresh ET) reporting proportion of deliveries with birth weight <1,500 g, one was a matched cohort. All studies had high CASP scores.

The RR of having a baby with birth weight <1,500 g was 0.72 (95% CI 0.50–1.04) after singleton pregnancies subsequent to frozen thawed ET compared with those after fresh ET. There was no heterogeneity ($I^2$ = 0) among the studies.

**Cesarean section.** Five studies (3 matched cohort) were included in the meta-analysis (n = 5,435 vs. 16,740 pregnancies after fresh vs. frozen ET). Only two studies (14, 15) gave separate data on elective and emergency cesarean section. Elective and emergency cesarean sections were pooled together (proportion of elective and emergency cesarean section was equal in both groups). Pregnancies after frozen thawed embryos had an increased risk of cesarean section when compared with those after fresh embryos (RR = 1.10, 95% CI 1.05–1.15) with an absolute risk increase of 3% (1%–5%). There was low heterogeneity ($I^2$ = 18%) among the studies (Supplemental Fig. 7, available online). Subgroup analysis did not alter the results (Table 3). Funnel plot did not reveal any publication bias (Supplemental Fig. 8).

**Congenital anomalies.** Only three studies (n = 3,152 vs. 6,308 pregnancies after frozen vs. fresh ET) reported congenital anomalies (one matched cohort study). Major and minor anomalies were pooled together. The RR of having a congenital anomaly was 1.05 (95% CI 0.81–1.35) in pregnancies after frozen thawed embryos compared with fresh embryos. There was moderate heterogeneity ($I^2$ = 47%) among the studies.

**Perinatal mortality.** Of six studies (n = 5,546 vs. 17,424 pregnancies after frozen vs. fresh ET) reporting perinatal mortality, three were matched cohort. There was a slight variation in the definition of perinatal mortality: death of child with a gestational age of >20 weeks of gestation or up to day 28 of birth (6, 8); deaths occurring after the 24th week of gestation and during the first week of life (4); after 22 weeks of gestation and first 7 days of life (14); stillbirth after 28 weeks of gestation and first 7 days of life (5); stillbirth after 20 weeks, later terminations and all neonatal deaths (13).

The RR of perinatal mortality was 0.68 (95% CI 0.48–0.96) in singleton pregnancies after frozen thawed ETs when compared with those after fresh embryos (Supplemental Fig. 9, available online). The difference persisted in subgroup analysis of only matched cohort studies (RR = 0.64, 95% CI 0.43–0.97). There was no heterogeneity among the studies ($I^2$ = 0).

**TABLE 3**

Overall table for effect and sensitivity analysis (frozen vs. fresh IVF/ICSI pregnancies).

| Outcome | No. of frozen vs. fresh IVF/ICSI conceptions | Overall effect (RR, 95% CI) fixed effect | Heterogeneity ($I^2$) | Subgroup analysis (matched cohort) (calculated only if there was a statistical difference) | Risk difference (calculated only if there was a statistical difference) | Sensitivity analysis[a] Good quality studies only (CASP > 10) |
|---|---|---|---|---|---|---|
| Small for gestational age | 1,933 vs. 3,141 | 0.45 (0.30–0.66) | 22% | NA | −0.02 (−0.03, −0.01) | |
| Birth weight <2,500 g | 8,536 vs. 25,800 | 0.69 (0.62–0.76) | 28% | 0.59 (0.45–0.78) | −0.03 (−0.03, −0.02) | 0.69 (0.63, −0.76) |
| Birth weight <1,500 g | 3,552 vs. 16,469 | 0.72 (0.50–1.04) | 0 | NA | NA | |
| Delivery at <37 weeks | 10,017 vs. 27,686 | 0.84 (0.78–0.90)[b] | 74% | 0.72 (0.63–0.82) | −0.02 (−0.03, −0.01) | |
| Delivery at <32 weeks | 3,050 vs. 13,630 | 0.73 (0.50–1.08) | 11% | 0.76 (0.44–1.33) | −0.00 (−0.01–0.00) | |
| APH | 3,875 vs. 7,000 | 0.67 (0.55–0.81) | 0 | NA | −0.02 (−0.02, −0.01) | |
| Congenital anomalies | 3,152 vs. 6,308 | 1.05 (0.81–1.35) | 47% | 0.88 (0.63–1.24) | NA | |
| Cesarean section | 5,435 vs. 16,740 | 1.10 (1.05–1.15) | 18% | 1.12 (1.06–1.18) | 0.03 (0.01, −0.05) | |
| Transfer to NICU | 3,552 vs. 16,469 | 1.00 (0.92–1.08) | 69% | 0.86 (0.72–1.03) | NA | |
| Perinatal mortality | 5,546 vs. 17,424 | 0.68 (0.48–0.96) | 0 | 0.64 (0.43–0.97) | −0.00 (−0.01, −0.00) | 0.64 (0.43, −0.97) |

*Note:* APH = antepartum hemorrhage; CASP = critical appraisal skills program; CI = confidence interval; NA = not available; NICU = neonatal intensive care unit; RR = risk ratio.
[a] Sensitivity analysis performed only if there was a mixture of good and poor quality studies.
[b] Random effect model −0.86 (0.72–1.03).

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**Admission to neonatal intensive care unit.** Three unmatched and one matched cohort study reported the outcome of admission to the neonatal intensive care unit (n = 3,552 vs. 16,469 pregnancies after frozen vs. fresh ET). The length and the reasons for neonatal intensive care unit admission were not specified. There was no increase in the risk of admission to the neonatal intensive care unit (RR = 1.00, 95% CI 0.92–1.08) in pregnancies after frozen embryos. There was marked heterogeneity among the studies ($I^2 = 69\%$).

Hypertensive disorders of pregnancy, gestational diabetes, preterm premature rupture of membranes, and induction of labor was not reported by any of the included studies. These can only be answered by individual patient data meta-analysis.

# DISCUSSION

## Main Findings

Singleton pregnancies after transfer of frozen thawed embryos were associated with a lower risk of perinatal mortality, small for gestational age baby, preterm birth (<37 weeks), low birth weight baby (<2,500 g), and antepartum hemorrhage when compared with those after fresh ETs. However, the risk of a baby being born by cesarean section was lower in pregnancies after fresh ET.

## Strengths

This is the first systematic review and first meta-analysis on large data to quantify the obstetric and perinatal risks for singleton pregnancies after frozen ET compared with those after fresh ETs. Absolute risk differences were calculated in two groups to give a clearer idea to clinicians managing these women as opposed to RRs alone. Sensitivity analyses were performed to evaluate the robustness of the existing data.

## Limitations

Because there are no randomized controlled trials, this review is limited to data from observational studies. Individual methodological differences, variation in design, inclusion exclusion criteria, definition, and ascertainment of outcomes are inherent in systematic reviews of observational studies. Patients who have had fresh cycle may be different from those who had frozen replacement cycles. It is likely that the fresh cycle population are made up of both good and bad prognosis patients, whereas the frozen embryo population are more likely composed of the better prognosis patients and hence the difference in the outcomes. We were unable to adjust for confounders such as age, smoking, parity, duration of infertility, and pre-existing medical illness due to varied design of the studies. There is clinical heterogeneity in terms of the population sampled, design of studies, method of freezing, and regimens in replacement cycles (Table 1). Without individual patient data, we are unable to adjust for confounders and determine whether the risks are different for embryos frozen by slow freezing and vitrification; embryos frozen at cleavage and blastocyst stages. When it comes to thawing the embryos, there is uncertainty as to whether method of thawing and protocol used (natural or hormonally mediated cycle) for replacement has any bearing on different obstetric and perinatal outcomes.

Although data from this review suggest that both preterm labor and low birth weight are decreased in pregnancies subsequent to frozen ET, these two outcomes are related. The two studies reporting gestation-adjusted birth weights, however, report a decrease in small for gestational age babies in pregnancies subsequent to frozen ET. We could not differentiate between spontaneous and iatrogenic preterm delivery. There was no evidence of difference in neonatal intensive care unit admissions in both groups, which is not in keeping with the decreased risks of preterm and low birth weight reported with frozen ET.

There is inconsistency in definition of outcomes, such as antepartum hemorrhage, congenital anomalies, and perinatal mortality, as has been highlighted. In addition, not all outcomes have been reported by all studies.

## Explanation of Results

The reasons for better outcomes for frozen ET cycles compared with fresh ET are not known. It has been suggested that replacing embryos on the second or third day after fertilization in a fresh IVF cycle involves a degree of asynchrony between the phase of development of the embryo and endometrial receptivity (due to excessive estrogen [E] and P as a result of ovarian stimulation), which can affect implantation (18) in fresh IVF/ICSI treatment cycles. It has been suggested that a more natural uterine environment that occurs in a frozen replacement cycle is favorable for early placentation and embryogenesis, whereas ovarian stimulation in fresh cycles alter endometrial angiogenesis and implantation (3, 12, 19). This argument is further strengthened by the findings of Healy et al. (12), who showed that the increase in antepartum hemorrhage after fresh ETs increased as the number of oocytes increased (increased numbers of oocytes increases the concentration of E in circulation, hence exposing the endometrium to excess E).

The studies included in this review did not differentiate between frozen ETs done in natural or hormone replacement cycles. However, it has been argued that even when hormone replacement cycles have been used in frozen ETs, E and P are given in physiological doses to mimic natural cycles, compared with stimulation of fresh cycles where supraphysiological doses of gonadotropins are given (18, 20).

Another explanation put forward for better results in pregnancies subsequent to frozen ET is that the physical effects of freezing and thawing embryos may filter out weaker embryos and allow only good quality ones to survive, resulting in better fetal growth (13).

The high rate of cesarean section in pregnancies subsequent to frozen ET could be because these women may have had previous cesarean sections. In the subgroup analysis of studies where women were their own reference group, hence had previous IVF conception (which is more likely to have cesarean section), high rate of cesarean section was observed (Supplemental Fig. 7).

## Clinical Implications of the Study

Although obstetric and perinatal risks are less in pregnancies subsequent to frozen thawed embryos, the absolute reduction in some of these risks, including perinatal mortality, which fortunately are rare, are likely to be small. However, data from this review provide reassurance for cryopreservation programs.

In current practice, the best embryo is selected for transfer in the fresh cycle and spare ones are frozen (if they are deemed suitable to survive freezing thawing process). As our data suggest that pregnancies after frozen replacement cycles may have better obstetric and perinatal outcomes compared with those after fresh embryos, intuitively one would think that we should freeze all embryos and transfer them subsequently in what are considered as optimal conditions of endometrium for implantation. However, the better obstetric and perinatal outcomes presented in this review are per ongoing pregnancy. Despite improvement in cryopreservation, most places have better PRs with fresh treatment compared with frozen thawed replacement. Hence outcomes per started cycle may not be different and cost effective, especially when the costs of freeze and thaw cycles are added.

There are suggestions that the endometrial development in frozen thawed cycles can be controlled more precisely than in the cycles of ovarian stimulation with gonadotropins (18, 20). The segmentation (separation of ovarian stimulation and ET in different menstrual cycles) of IVF/ICSI treatment has also been suggested to reduce the risks of ovarian hyperstimulation syndrome (OHSS) (21).

Two recent small randomized trials have suggested that PRs after elective cryopreservation and later ET are at least similar to those after fresh ET in women who are predicted hyperresponders (i.e., those women who are at high risk of hyperstimulation) (20), as well as those women who are normal responders (18). As cryopreservation facilities and techniques improve further, it might be possible to introduce the practice of elective cryopreservation for later use.

## Future Research

Randomized controlled trials are needed to evaluate clinical and cost effectiveness, as well as acceptability of the practice of elective cryopreservation versus current practice of fresh ET.

In conclusion, singleton pregnancies after frozen thawed ET seem to have better outcomes than those after fresh ETs (which are the norm in IVF treatment). There is lower risk of perinatal mortality, small for gestational age, preterm birth, low birth weight, and antepartum hemorrhage. Further evidence is needed before elective cryopreservation can be routinely recommended in preference to current practice of fresh ET.

## REFERENCES


1. De Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, et al. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. Hum Reprod 2010;25:1851–62.
2. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles—time for a new embryo transfer strategy? Fertil Steril 2011;95:1691–5.
3. Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. Fertil Steril 2011;95:548–53.
4. Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR. Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. Hum Reprod 1994;9:543–6.
5. Wikland M, Hardarson T, Hillensjo T, Westin C, Westlander G, Wood M, et al. Obstetric outcomes after transfer of vitrified blastocysts. Hum Reprod 2010;25:1699–707.
6. Aflatoonian A, Mansoori Moghaddam F, Mashayekhy M, Mohamadian F. Comparison of early pregnancy and neonatal outcomes after frozen and fresh embryo transfer in ART cycles. J Assist Reprod Genet 2010;27: 695–700.
7. Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 2005;83: 1650–8.
8. Belva F, Henriet S, van den Abbeel E, Camus M, Devroey P, van der Elst J, et al. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod 2008;10:2227–38.
9. Helmerhorst FM, Perquin DAM, Donker D, Keirse MJNC. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 2004;328:261.
10. Wennerholm UB, SoderstromAnttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod 2009;24: 2158–72.
11. Higgins JPT GS, ed. Cochrane handbook for systematic reviews of interventions. 5.1st ed. Chichester, UK: John Wiley and Sons, 2011.
12. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, et al. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria, Australia. Hum Reprod 2010;25:265–74.
13. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birth weight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod 2008;23: 1644–53.
14. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari A, Hyden-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. Hum Reprod 2010; 25:914–23.
15. Wennerholm U, Hamberger L, Nilsson L, Wennergren M, Wikland M, Bergh C. Obstetric and perinatal outcome of children conceived from cryopreserved embryos. Hum Reprod 1997;12:1819–25.
16. Henningsen A-A, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling cohort study. Fertil Steril 2011;95:959–63.
17. Pinborg A, Loft A, Henningsen A-A, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril 2010;94:1320–7.
18. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: A prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011; 96:344–8.
19. Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL, Breheny S, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. Hum Reprod 2009;24:1330–8.

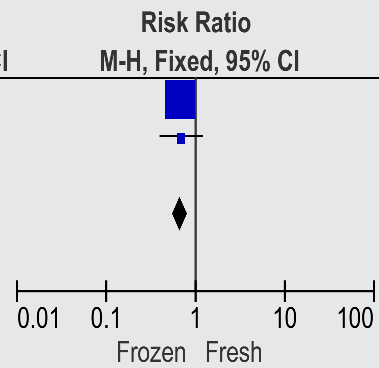
20. Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet 2010;27:357–63.
21. Devroey P, Polyzos NP, Blockeel C. An OHSS free clinic by segmentation of IVF treatment. Hum Reprod 2011;10:2593–7.
22. Aytoz A, van Den Abbeel E, Bonduelle M, Camus M, Joris H, van Steirteghem A, et al. Obstetric outcome of pregnancies after the transfer of cryopreserved and fresh embryos obtained by conventional in-vitro fertilization and intracytoplasmic sperm injection. Hum Reprod 1999;14: 2619–24.
23. Frydman R, Forman RG, Belaisch-Allart J, Hazout A, Fernandez H, Testart J. An obstetric analysis of fifty consecutive pregnancies after transfer of cryopreserved human embryos. Am J Obstet Gynecol;160:209–13.
24. Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. Fertil Steril 2005;84:611–7.
25. Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. Birth Defects Res 2005;73:162–9.
26. Wennerholm WB. Cryopreservation of embryos and oocytes: obstetric outcome and health in children. Hum Reprod 2000;15:18–25.

## SUPPLEMENTAL FIGURE 1

| Study or Subgroup | Frozen Events | Frozen Total | Fresh Events | Fresh Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Healy 2010 | 105 | 2,045 | 314 | 4,058 | 86.2% | 0.66 [0.54, 0.82] |
| Pelkonen 2010 | 19 | 1,830 | 44 | 2,942 | 13.8% | 0.69 [0.41, 1.19] |
| **Total (95% CI)** | | **3,875** | | **7,000** | **100.0%** | **0.67 [0.55, 0.81]** |
| Total events | 124 | | 358 | | | |

Heterogeneity: $\chi^2 = 0.02$, df = 1 ($P = .88$); $I^2 = 0\%$

Test for overall effect: Z = 3.98 ($P < .0001$)

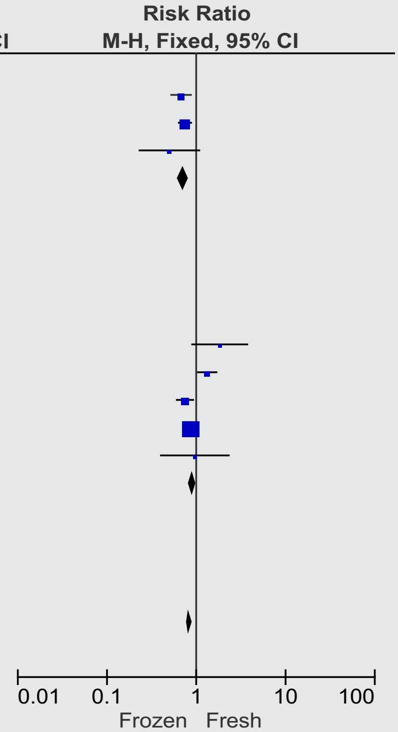



Antepartum hemorrhage. CI = confidence interval; M-H = Mantel-Haenszel.

*Maheshwari. Frozen versus fresh ET. Fertil Steril 2012.*

## SUPPLEMENTAL FIGURE 2

| Study or Subgroup | Frozen Events | Frozen Total | Fresh Events | Fresh Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| **5.8.1 matched cohort** | | | | | | |
| Pinborg 2010 | 59 | 957 | 940 | 10,329 | 10.7% | 0.68 [0.53, 0.87] |
| Shih 2008 | 220 | 2,387 | 383 | 3110 | 22.3% | 0.75 [0.64, 0.88] |
| Wennerholm 1997 | 9 | 160 | 18 | 160 | 1.2% | 0.50 [0.23, 1.08] |
| **Subtotal (95% CI)** | | **3,504** | | **13,599** | **34.2%** | **0.72 [0.63, 0.82]** |
| Total events | 288 | | 1,341 | | | |
| Heterogeneity: $\chi^2$ = 1.32, df = 2 (*P* = .52); $I^2$ = 0% | | | | | | |
| Test for overall effect: Z = 4.93 (*P* < .00001) | | | | | | |
| **5.8.2 unmatched cohort** | | | | | | |
| Aflatoonian2010 | 11 | 84 | 18 | 252 | 0.6% | 1.83 [0.90, 3.72] |
| Belva 2008 | 78 | 662 | 266 | 2,999 | 6.4% | 1.33 [1.05, 1.69] |
| Pelkonen 2010 | 120 | 1,830 | 258 | 2,942 | 13.3% | 0.75 [0.61, 0.92] |
| Wang 2005 | 437 | 3,834 | 1,008 | 7,695 | 44.9% | 0.87 [0.78, 0.97] |
| Wikland 2010 | 7 | 103 | 14 | 199 | 0.6% | 0.97 [0.40, 2.32] |
| **Subtotal (95% CI)** | | **6,513** | | **14,087** | **65.8%** | **0.90 [0.83, 0.98]** |
| Total events | 653 | | 1,564 | | | |
| Heterogeneity: $\chi^2$ = 17.62, df = 4 (*P* = .001); $I^2$ = 77% | | | | | | |
| Test for overall effect: Z = 2.40 (*P* = .02) | | | | | | |
| **Total (95% CI)** | | **10,017** | | **27,686** | **100.0%** | **0.84 [0.78, 0.90]** |
| Total events | 941 | | 2,905 | | | |
| Heterogeneity: $\chi^2$ = 27.25, df = 7 (*P* = .0003); $I^2$ = 74% | | | | | | |
| Test for overall effect: Z = 4.82 (*P* < .00001) | | | | | | |
| Test for subgroup differences: $\chi^2$ = 7.95, df = 1 (*P* = .005), $I^2$ = 87.4% | | | | | | |




Preterm labor. CI = confidence interval; M-H = Mantel-Haenszel.

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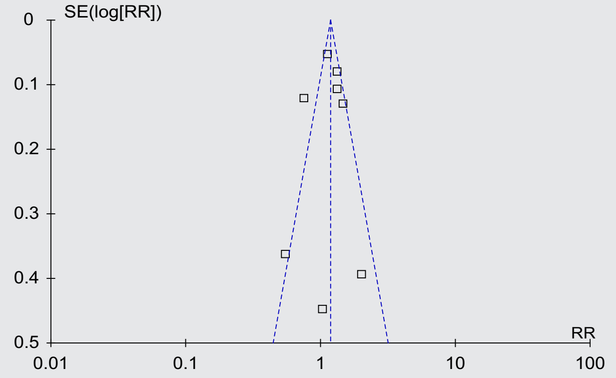
## SUPPLEMENTAL FIGURE 3




Funnel plot for studies reporting preterm labor.

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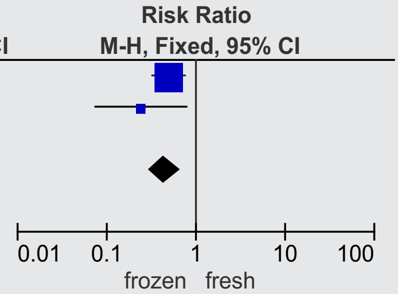
## SUPPLEMENTAL FIGURE 4

| | Frozen | | Fresh | | | Risk Ratio |
|---|---|---|---|---|---|---|
| Study or Subgroup | Events | Total | Events | Total | Weight | M-H, Fixed, 95% CI |
| Pelkonen 2010 | 28 | 1,830 | 91 | 2,942 | 81.0% | 0.49 [0.33, 0.75] |
| Wikland 2010 | 3 | 103 | 24 | 199 | 19.0% | 0.24 [0.07, 0.78] |
| **Total (95% CI)** | | **1,933** | | **3,141** | **100.0%** | **0.45 [0.30, 0.66]** |
| Total events | 31 | | 115 | | | |

Heterogeneity: $\chi^2 = 1.28$, df = 1 ($P = .26$); $I^2 = 22\%$

Test for overall effect: Z = 4.01 ($P < .0001$)




Small for gestational age. CI = confidence interval; M-H = Mantel-Haenszel.

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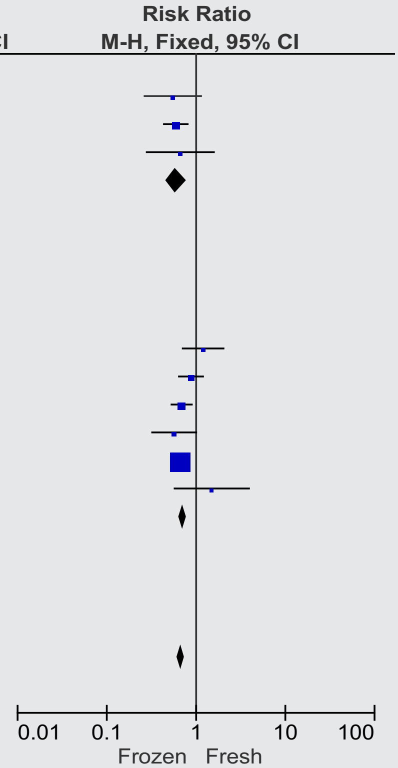
## SUPPLEMENTAL FIGURE 5

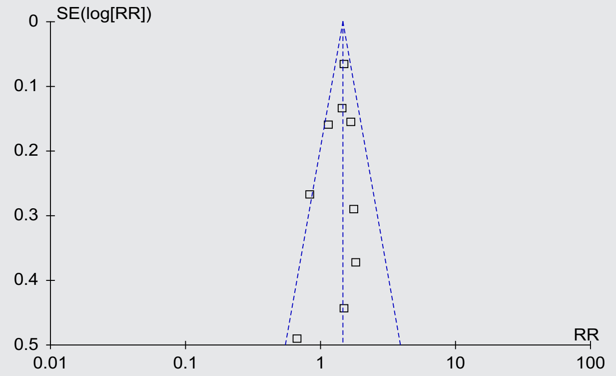
| | Frozen | | Fresh | | | Risk Ratio |
|---|---|---|---|---|---|---|
| Study or Subgroup | Events | Total | Events | Total | Weight | M-H, Fixed, 95% CI |
| **5.6.1 matched cohort** | | | | | | |
| Henningsen 2011 | 11 | 716 | 20 | 716 | 2.0% | 0.55 [0.27, 1.14] |
| Pinborg 2010 | 42 | 957 | 764 | 10,329 | 13.0% | 0.59 [0.44, 0.80] |
| Wennerholm 1997 | 8 | 160 | 12 | 160 | 1.2% | 0.67 [0.28, 1.59] |
| **Subtotal (95% CI)** | | **1,833** | | **11,205** | **16.3%** | **0.59 [0.45, 0.78]** |
| Total events | 61 | | 796 | | | |
| Heterogeneity: $\chi^2$ = 0.11, df = 2 (*P* = .95); $I^2$ = 0% | | | | | | |
| Test for overall effect: Z = 3.83 (*P* = .001) | | | | | | |
| **5.6.2 unmatched cohort** | | | | | | |
| Aflatoonian2010 | 16 | 84 | 40 | 252 | 2.0% | 1.20 [0.71, 2.03] |
| Belva 2008 | 44 | 662 | 227 | 2,999 | 8.3% | 0.88 [0.64, 1.20] |
| Pelkonen 2010 | 76 | 1,830 | 177 | 2,942 | 13.7% | 0.69 [0.53, 0.90] |
| Wada et al., 1994 | 13 | 177 | 68 | 527 | 3.4% | 0.57 [0.32, 1.00] |
| Wang 2005 | 277 | 3,847 | 829 | 7,676 | 55.7% | 0.67 [0.59, 0.76] |
| Wikland 2010 | 7 | 103 | 9 | 199 | 0.6% | 1.50 [0.58, 3.92] |
| **Subtotal (95% CI)** | | **6,703** | | **14,595** | **83.7%** | **0.71 [0.64, 0.78]** |
| Total events | 433 | | 1,350 | | | |
| Heterogeneity: $\chi^2$ = 9.51, df = 5 (*P* = .09); $I^2$ = 47% | | | | | | |
| Test for overall effect: Z = 6.53 (*P* < .00001) | | | | | | |
| **Total (95% CI)** | | **8,536** | | **25,800** | **100.0%** | **0.69 [0.62, 0.76]** |
| Total events | 494 | | 2,146 | | | |
| Heterogeneity: $\chi^2$ = 11.16, df = 8 (*P* = .19); $I^2$ = 28% | | | | | | |
| Test for overall effect: Z = 7.53 (*P* < .00001) | | | | | | |
| Test for subgroup differences: $\chi^2$ = 1.42, df = 1 (*P* = .23), $I^2$ = 29.5% | | | | | | |




Low birth weight (birth weight <2,500 g). CI = confidence interval; M-H = Mantel-Haenszel.

*Maheshwari. Frozen versus fresh ET. Fertil Steril 2012.*

## SUPPLEMENTAL FIGURE 6

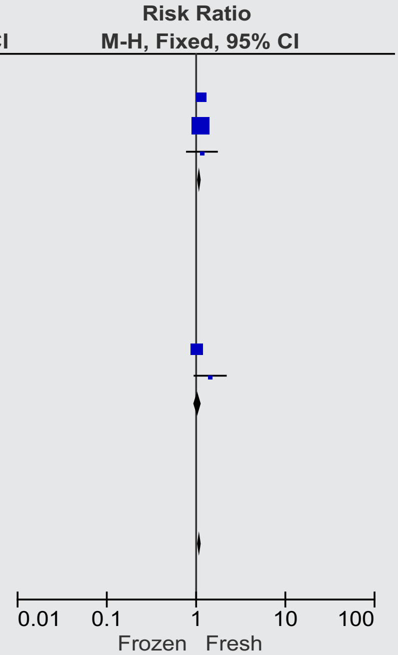



Funnel plot for studies reporting low birth weight.

*Maheshwari. Frozen versus fresh ET. Fertil Steril 2012.*

## SUPPLEMENTAL FIGURE 7

| Study or Subgroup | Frozen Events | Frozen Total | Fresh Events | Fresh Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| **5.1.1 Matched cohort** | | | | | | |
| Pinborg 2010 | 288 | 957 | 2,733 | 10,329 | 21.0% | 1.14 [1.03, 1.26] |
| Shih 2008 | 1,031 | 2,387 | 1,207 | 3,110 | 47.6% | 1.11 [1.04, 1.19] |
| Wennerholm 1997 | 42 | 160 | 36 | 160 | 1.6% | 1.17 [0.79, 1.72] |
| **Subtotal (95% CI)** | | **3,504** | | **13,599** | **70.3%** | **1.12 [1.06, 1.18]** |
| Total events | 1,361 | | 3,976 | | | |
| Heterogeneity: $\chi^2$ = 0.17, df = 2 (*P* = .92); $I^2$ = 0% | | | | | | |
| Test for overall effect: Z = 4.19 (*P* < .0001) | | | | | | |
| **5.1.2 unmatched cohort** | | | | | | |
| Pelkonen 2010 | 519 | 1,830 | 818 | 2,942 | 28.5% | 1.02 [0.93, 1.12] |
| Wikland 2010 | 30 | 101 | 41 | 199 | 1.3% | 1.44 [0.96, 2.16] |
| **Subtotal (95% CI)** | | **1,931** | | **3,141** | **29.7%** | **1.04 [0.95, 1.14]** |
| Total events | 549 | | 859 | | | |
| Heterogeneity: $\chi^2$ = 2.66, df = 1 (*P* = .10); $I^2$ = 62% | | | | | | |
| Test for overall effect: Z = 0.80 (*P* = .42) | | | | | | |
| **Total (95% CI)** | | **5,435** | | **16,740** | **100.0%** | **1.10 [1.05, 1.15]** |
| Total events | 1,910 | | 4835 | | | |
| Heterogeneity: $\chi^2$ = 4.86, df = 4 (*P* = .30); $I^2$ = 18% | | | | | | |
| Test for overall effect: Z = 3.90 (*P* < .0001) | | | | | | |
| Test for subgroup differences: $\chi^2$ = 2.08, df = 1 (*P* = .15), $I^2$ = 51.9% | | | | | | |




Cesarean section. CI = confidence interval; M-H = Mantel-Haenszel.

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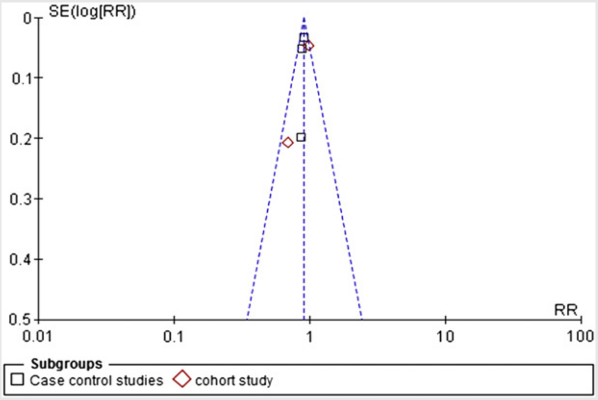
## SUPPLEMENTAL FIGURE 8

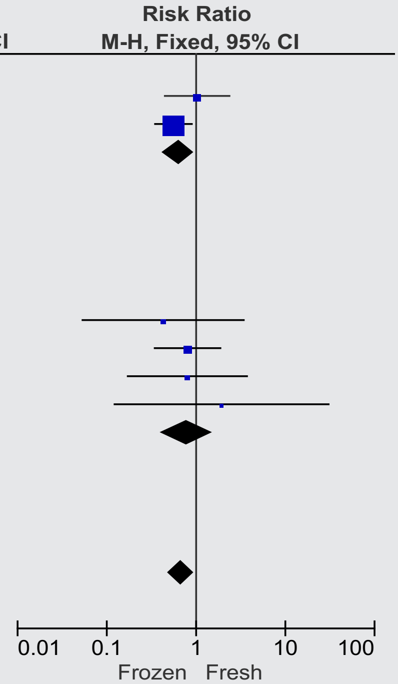



Funnel plot for studies reporting on cesarean sections.

*Maheshwari. Frozen versus fresh ET. Fertil Steril 2012.*

## SUPPLEMENTAL FIGURE 9

| Study or Subgroup | Frozen Events | Frozen Total | Fresh Events | Fresh Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| **5.7.1 matched cohort** | | | | | | |
| Pinborg 2010 | 6 | 957 | 63 | 10,329 | 13.4% | 1.03 [0.45, 2.37] |
| Shih 2008 | 24 | 2,387 | 56 | 3,110 | 61.1% | 0.56 [0.35, 0.90] |
| **Subtotal (95% CI)** | | **3,344** | | **13,439** | **74.5%** | **0.64 [0.43, 0.97]** |
| Total events | 30 | | 119 | | | |
| Heterogeneity: $\chi^2 = 1.55$, df = 1 ($P$ = .21); $I^2 = 36\%$ | | | | | | |
| Test for overall effect: Z = 2.11 ($P$ = .03) | | | | | | |
| **5.7.2 unmatched cohort** | | | | | | |
| Aflatoonian2010 | 1 | 84 | 7 | 252 | 4.4% | 0.43 [0.05, 3.43] |
| Pelkonen 2010 | 8 | 1,830 | 16 | 2,942 | 15.4% | 0.80 [0.34, 1.87] |
| Wada et al., 1994 | 2 | 185 | 8 | 592 | 4.8% | 0.80 [0.17, 3.73] |
| Wikland 2010 | 1 | 103 | 1 | 199 | 0.9% | 1.93 [0.12, 30.57] |
| **Subtotal (95% CI)** | | **2,202** | | **3,985** | **25.5%** | **0.78 [0.40, 1.52]** |
| Total events | 12 | | 32 | | | |
| Heterogeneity: $\chi^2 = 0.74$, df = 3 ($P$ = .86); $I^2 = 0\%$ | | | | | | |
| Test for overall effect: Z = 0.74 ($P$ = .46) | | | | | | |
| **Total (95% CI)** | | **5,546** | | **17,424** | **100.0%** | **0.68 [0.48, 0.96]** |
| Total events | 42 | | 151 | | | |
| Heterogeneity: $\chi^2 = 2.54$, df = 5 ($P$ = .77); $I^2 = 0\%$ | | | | | | |
| Test for overall effect: Z = 2.19 ($P$ = .03) | | | | | | |
| Test for subgroup differences: $\chi^2 = 0.22$, df = 1 ($P$ = .64), $I^2 = 0\%$ | | | | | | |




Perinatal mortality. CI = confidence interval; M-H = Mantel-Haenszel.

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