Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer?

Running Title: Obstetric outcomes with frozen versus fresh embryos

Abha Maheshwari*[1], Shilpi Pandey[2], Edwin Amalraj Raja[3], Ashalatha Shetty[1], Mark Hamilton[1], Siladitya Bhattacharya[3],

[1] Aberdeen Maternity Hopsital NHS Grampian , Scotland ,UK

*corresponding author abha.maheshwari@abdn.ac.uk

[2] CARE Nottingham, UK

[3] Institute of Applied Health Sciences, University of Aberdeen, Aberdeen UK

Table of contents

Abstract

Background: Initial observational studies and a systematic review published five years ago have suggested that obstetric and perinatal outcomes are better in offspring conceived following frozen rather than fresh embryo transfers, with reduced risks of preterm birth, small for gestational age, low birth weight and preeclampsia. More recent primary studies are beginning to challenge some of these findings. We therefore conducted an updated systematic review and cumulative meta-analysis to examine if these results have remained consistent over time.

Objective and Rationale: The aim of this study was to perform a systematic review and cumulative meta-analysis (trend with time) of obstetric and perinatal complications in singleton pregnancies following the transfer of frozen thawed and fresh embryos generated through in-vitro fertilisation.

Search Methods: Data Sources from Medline, EMBASE, Cochrane Central Register of Clinical Trials DARE and CINAHL (1984-2016) were searched using appropriate key words. Observational and randomized studies comparing obstetric and perinatal outcomes in singleton pregnancies conceived through IVF using either fresh or frozen thawed embryos. Two independent reviewers extracted data in 2x2 tables and assessed the methodological quality of the relevant studies using CASP scoring. Both aggregated as well as cumulative meta-analysis was done using STATA.

Outcomes: Twenty-six studies met the inclusion criteria. Singleton babies conceived from frozen thawed embryos were at lower risk (RR, 95% CI) of preterm delivery (0.90 95% CI: 0.84- 0.97) low birth weight (0.72, 95% CI: 0.67-0.77) and small for gestational age (0.61, 95% CI: 0.56-0.67) compared to those conceived from fresh embryo transfers, but faced an increased risk (RR, 95% CI) of hypertensive disorders of pregnancy (1.29, 95% CI: 1.07-1.56)

large for gestational age (1.54, 95% CI: 1.48-1.61) and high birthweight (1.85, 95% CI: 1.46-2.33). There was no difference in the risk of congenital anomalies and perinatal mortality between the two groups. The direction and magnitude of effect for these outcomes have remained virtually unchanged over time while the degree of precision has improved with the addition of data from newer studies.

Wider Implications: The results of this cumulative systematic review confirm that the decreased risks of small for gestational age, low birth weight and preterm delivery and increased risks of large for gestational age and high birth weight associated with pregnancies conceived from frozen embryos have been consistent in terms of direction and magnitude of effect over several years, with increasing precision around the point estimates. Replication in a number of different populations has provided external validity for the results, for outcomes of birthweight and preterm delivery. Meanwhile, caution should be exercised about embarking on a policy of electively freezing all embryos in IVF as there are increased risks for large for gestational age babies and hypertensive disorders of pregnancy, Therefore elective freezing should ideally be undertaken in specific cases such as ovarian hyperstimulation syndrome, fertility preservation or in the context of randomised trials.

Key Words: IVF, ICSI, obstetric outcomes, perinatal outcomes, frozen replacement cycles, preterm delivery, fresh embryo transfer, cryopreservation, large for gestational age, small for gestational age

Introduction

In-vitro fertilisation involves hormonal stimulation of ovaries followed by surgical retrieval of oocytes and their insemination in the laboratory. Conventionally, embryos created by this process are transferred within the uterus after 2-5 days in culture, while any remaining embryos are frozen for subsequent use. Cryopreserved embryos are usually thawed and replaced in a natural or hormonally manipulated cycle in women in whom a fresh embryo transfer fails to result in a pregnancy or in those who return for a second baby.

The first live birth following the transfer of thawed cryopreserved embryos was reported in 1984. With refinement of technology over the last few decades, the number of frozen embryo transfers has increased as have pregnancy rates which, according to some authors, are better than those following the transfer of fresh IVF embryos (Chen et al., 2016).

Initial observational studies and a systematic review based on these which was published five years ago, have suggested that obstetric and perinatal outcomes are better in those conceived following frozen rather than fresh embryo transfers (Maheshwari et al., 2012), with reduced risks of preterm birth, small for gestational age babies, low birth weight babies and preeclampsia. Subsequent primary studies (Chen et al., 2016; Maheshwari et al., 2016) are beginning to challenge some of these initial findings. We therefore conducted a new systematic review incorporating all the published studies and including a cumulative meta-analysis to examine whether the results have remained consistent over time.

Materials and Methods

PRISMA guidelines for systematic reviews were followed (http://www.plosmedicine.org/article/info:doi%2F10.1371%2Fjournal.pmed.1000097). The protocol was registered at PROSPERO (CRD42016046131).

Data sources and searches

A literature search with no language restrictions was performed (1984-2016) on Medline, EMBASE, Cochrane Central Register of Clinical Trials, CINAHL and DARE (Appendix 1). 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 (AM, SP) 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 pre designed 2x2 tables.

Quality assessment of included studies was performed independently by two authors (SP and AM). Any disagreement regarding type and quality of the study was resolved after discussion.

Checklists from the critical appraisal skills programme (CASP) (http://www.phru.nhs.uk/pages/phd/resources.htm) were used to assess and assign a quality score. CASP is critical appraisal tool consist of 12 questions to appraise a cohort study, systematically in three board domains Are the results of the study valid? (Section A) What are the results? (Section B); Will the results help locally? (Section C). A score is then allocated out of 12.(supplementary table)

Study selection

Inclusion criteria included ll published observational studies and randomized trials comparing obstetric and perinatal outcomes in singleton pregnancies following transfer of fresh and frozen embryos.

Exclusion criteria excluded studies 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. Case reports and case series were excluded.

Outcome measures

The following outcome measures were included : small for gestational age (as defined by the authors of included studies) , very preterm birth (delivery prior to 32 weeks); preterm birth (delivery prior to 37 weeks); low birth weight (birth weight < 2500gm); very low birth weight (birth weight< 1500 gm); high birth weight (> 4000gm); very high birth weight (> 4,500 gm);; large for gestational age (as defined by the authors of the included studies); antepartum haemorrhage (combination of placenta praevia, placental abruption and other bleeding) hypertensive disorders of pregnancy (including pregnancy induced hypertension, pre-eclampsia and eclampsia), congenital anomalies (major & minor), perinatal mortality (as defined by the authors of the included studies) and admission to neonatal intensive care unit.

Assessment of heterogeneity

We assessed whether there was sufficient similarity between the eligible studies in their design and clinical characteristics to ensure that pooling was valid. I^2 statistic was used to assess the impact of the heterogeneity on the meta-analysis. I^2 > 50% was labelled as marked heterogeneity (Higgins et al., 2011).

Assessment of reporting biases

Funnel plots were constructed to test the small study effect where a statistically significant difference was obtained in outcome measure, if at least 5 studies reported that outcome. Egger's regression test (Egger et al., 1997) was used to investigate whether the difference was due to publication or reporting bias.

Statistical analysis

For each outcome, data were extracted in 2x2 tables. When there was an outcome with no events in one of the groups a correction factor of 0.5 was added to all cells in a 2 by 2 table in the calculation of risk ratio (Sweeting et al., 2004). The summary measures for each study were Risk Ratio / Relative Risk (RR) with 95% Confidence intervals (CI). The 'fresh embryo transfer' group was considered as reference group. The pooled estimates were obtained using both standard and cumulative meta-analysis. Although we analysed the data using both the fixed effect models and random effect models, results in text are only reported from random effect models due to underlying heterogeneity in the studies. Cumulative meta-analyses (Lau et al., 1992) were carried to track the accumulation of evidence on the obstetrics and perinatal outcomes in singleton pregnancies subsequent to frozen embryo over the period of time. The statistical analyses were carried out using Stata MP version 14. GRADE PRO software was used to generate the summary of finding table as well as quality of evidence.

Results

Results of the searches

The literature search yielded 126 citations. Of these, 106 were excluded after reading the title and the abstract. Full text versions of 20 articles were obtained, of which 16 were

included, while another 10 publications were identified from hand searches of cross references and checking for advance access publications as well as articles in press. Hence, a total of 26 studies were included (Figure 1). Studies from the same research group/ region were carefully examined for any overlapping data. Authors were contacted if the information was unclear. Studies with overlapping data were excluded. Table I summarises details of all included studies; while excluded studies along with reasons for exclusion are listed in Table II.

Included studies

Methodology of included studies

Of the 26 included studies, one was a post hoc analysis of two RCTs (Shapiro et al., 2016), while the rest were cohort studies. Most (n=21) were unmatched cohort studies. A high proportion of studies (n=16) scored high (≥10) on the CASP scoring system. Data were obtained from databases and data linkage of routinely collected data and case notes except in 3 studies where clinical information was reported only by questionnaires filled by patients (Kato et al., 2011; Liu et al., 2013; Shi et al., 2012).

Population in the included studies

Although all studies were based on outcomes of pregnancies conceived through IVF/ICSI using fresh or frozen embryos, they varied in terms of the duration of pregnancy at which women were included: all clinical pregnancies (Belva et al., 2008, Imudia et al., 2013); all births beyond 20 weeks (Aflatoonian et al., 2010; 2016 Hayley et al., 2010 Li et al., 2014; Rallis et al., 2013; Shih et al., 2008; Wada et al., 1994; Wang et al., 2005); beyond 21 weeks (Ishihara et al., 2014); beyond 22 weeks (Kato et al., 2011; Pelkonen et al., 2010 Pelkonen et

al., 2014; Wennerholm et al., 2014; Opdahl et al., 2015) and beyond 28 weeks (Liu et al., 2013; Wennerholm et al., 1997; Wikland et al., 2010) and only live deliveries (Piereria et al., 2016).

Three studies (Healy et al., 2010; Wang et al., 2005; Opdahl et al., 2015) provided no information on the demographic profile of women who had fresh or frozen embryo transfer, as this comparison was part of a subgroup analysis. The characteristics in the two groups were similar except in Pinborg et al., 2010 & 2014 (data adjusted at primary analysis); Pelkonen et al., 2010 & Belva et al., 2008 (mothers in frozen embryo transfer group were older) and Pelkonen et al., 2014 (higher proportion of nulliparous women in fresh embryo transfer 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

Studies varied in terms of when and how embryos were frozen, and methods used for endometrial preparation prior to embryo transfer after thawing. Methods of cryopreservation and the developmental stage at which embryos were frozen (Table I) also varied within same study especially in registry based datasets. Embryos were frozen either at day 2/3- cleavage stage (Aflatoonian et al., 2010, 2014; Imudia et al., 2013; Liu et al., 2013; Pelkonen et al., 2010; Shi et al., 2012; Wada et al., 1994) or day 5/6- blastocyst stage (Li et al., 2014 Piereria et al., 2016; Roy et al., 2014) or both (Belva et al., 2008; Kato et al., 2011) using either vitrification (Aflatoonian et al., 2010, 2014; Kato et al., 2011; Piereria et al., 2016; Shi et al., 2012) or slow freezing (Belva et al., 2008; Imudia et al., 2013; Pelkonen et al., 2010; Wada et al., 1994) or both techniques (Li et al., 2014; Liu et al., 2013).

Frozen thawed embryos were transferred in women following additional hormones to prepare the endometrium (Aflatoonian et al., 2010, 2014; Imudia et al., 2013) or in natural unstimulated cycles (Belva et al., 2008; Rallis et al., 2013) (Table I).

Outcomes

Pooled data for outcome measures were as follows.

Small for gestational age

Ten studies (n= 53,418 vs. 89,044 pregnancies following frozen vs. fresh cycles) have reported on the outcome of small for gestational age. This was defined as birth weight less than 2 standard deviation of mean for that gestation (Ishihara et al., 2014; Pelkonen et al., 2010; Pinborg et al., 2014; Wennerholm et al., 2013) or less than 10^{th} centile (Aflatoonian et al., 2016; Kato et al., 2012; Li et al., 2014) or birth weight less than 22% of expected mean birth weight according to gestational age in a reference population (Wikland et al., 2010).

The risk of having a small for gestational age baby was significantly less in singleton pregnancies subsequent to frozen thawed embryo transfer as compared to those after fresh embryo transfer [RR (95% CI) - 0.61 (0.56-0.67) (Figure 2a)]. There was minimal heterogeneity amongst the studies (I^2 =33.8%). The funnel plot does not suggest any publication bias (p=0.77).

A statistically significant reduction in small for gestational age babies was first observed in 2010 after first publication (RR, 95% CI- 0.49, 0.33 to 0.75). Although subsequent studies have increased the precision of the point estimate, no substantive change has occurred in the direction or magnitude of the treatment effect (Figure 2b).

Low birth weight (birth weight < 2500 gm)

Meta-analysis of the data based on 20 studies (n= 78,250 vs. 201,794 pregnancies following frozen vs. fresh cycles) shows that the risk of having a baby with birth weight < 2500gm is significantly less (Figure 3a) in singleton pregnancies following frozen thawed embryos, when compared to those following fresh embryos [RR (95% CI)- 0.72 (0.67-0.77)]. There was moderate heterogeneity (I^2 =55%) amongst the studies. Funnel plot did not reveal any publication bias (P=0.15).

The evidence that frozen embryo transfer reduces the risk of low birth weight babies has been available since 1997 (Figure 3b). Although subsequent studies have increased the precision of the point estimate, no substantive change has occurred in the direction or magnitude of the treatment effect.

Very low birth weight (birth weight < 1500gm)

Thirteen studies (n=71, 218 vs. 189, 008 pregnancies following frozen vs. fresh embryo transfer) reported proportion of deliveries with birth weight less than 1500gm. The relative risk (95% CI) of having a baby with birth weight < 1500gm was less [0.76 (0.69-0.82)], following singleton pregnancies subsequent to frozen thawed embryo transfer as compared to those following fresh embryo transfer (Figure 4a). There was no heterogeneity (I^2 = 0%) amongst the studies. Funnel plot does not suggest publication bias (p=0.16).

Cumulative meta-analysis shows (Figure 4b) that this evidence has been available since 2012. Although subsequent studies have increased the precision of the point estimate, no substantive change has occurred in the direction or magnitude of the treatment effect.

Large for gestational age

Seven studies (n= 51,719 vs. 86,544 pregnancies following frozen vs. fresh cycles) have reported on outcome of large for gestational age. This was defined as birth weight greater than 2 standard deviations of the mean for that gestation (Ishihara et al., 2014; Pelkonen et al., 2010; Pinborg et al., 2014; Wennerholm et al., 2013) or more than 90th centile (Kato et al., 2012; Li et al., 2014) or birth weight more than 22% of expected mean birth weight according to gestational age in a reference population (Wikland et al., 2010).

The relative risk (95% CI) of having a large for gestational age baby was higher [1.54 (1.48-1.61)] in singleton pregnancies subsequent to frozen thawed embryo transfer, as compared to those conceived following fresh embryo transfer (Figure 5a). There was minimal heterogeneity amongst the studies (I^2 = 11%). Funnel plot suggest no publication bias (p=0.73).

Cumulative meta-analysis suggests that this evidence has been available since 2012 with further precision of point estimate provided by additional data without changing the direction and magnitude of the effect (Figure 5b).

<u>High birth weight (birth weight > 4000 gm)</u>

Three studies reported the outcome of birth weight > 4000gm (n= 48, 026 vs. 113, 241 pregnancies following frozen vs. fresh embryo transfer). There was an increased risk (Figure 6a) of having a baby with birth weight > 4000gm in singleton pregnancies as a result of frozen embryo transfer when compared to those subsequent to fresh embryo transfer [RR-1.85; 95% CI (1.46-2.33)].

A statistically significant effect was first observed in 2014 after first publication [RR-1.95, ; 95% CI (1.29-2.95)]. Additions of data from subsequent large studies have increased the

precision of the point estimate, no change has occurred in the direction or magnitude of the treatment effect (Figure 6 b).

Very high birth weight (birth weight > 4500 gm)

Four studies have reported the outcome of birth weight > 4500 gm (n= 55,313 vs. 164,542 pregnancies following frozen vs. fresh embryo transfer). There is an increased risk (Figure 7a) of having a baby with birth weight > 4500 gm in singleton pregnancies as a result of frozen embryo transfer when compared to those subsequent to fresh embryo transfer [RR 1.86; 95% CI (1.58-2.19)].

There was significant heterogeneity (1^2= 67%). Cumulative meta-analysis (Figure 7b) suggests that significantly increased risk of very high birth weight babies was first reported in 2013 with no change in direction, estimate and precision by adding further data over the years.

Preterm delivery (delivery at less than 37 weeks)

Twenty studies (n=78,386 vs. 202,236 pregnancies following frozen vs. fresh cycles) reported the proportion of deliveries occurring at less than 37 weeks of gestation. Definition of preterm labour/delivery was delivery prior to 37 weeks in all studies. There are no data on how many of them were spontaneous or induced preterm labour.

The relative risk of having a delivery at less than 37 weeks was reduced [0.90 (95% CI 0.84, 0.97)] in singleton pregnancies following frozen thawed embryo transfer, when compared to those after fresh embryo transfers (Figure 8a). There was marked heterogeneity (I^2 =65%) amongst the studies. Funnel plot did not reveal any publication bias (p=0.73).

Cumulative meta-analysis (Figure 8) suggests that the evidence favouring frozen embryo transfer in terms of a reduced risk of preterm delivery was first available in 2005. In 2013 the addition of further data showed that there was no difference in the risk of preterm delivery between the two groups. However, new results from studies published after 2013 have re-confirmed the reduced risk of preterm delivery. Addition of several studies from 2014-2016 have increased the precision of our estimate without affecting either in the direction or magnitude of the treatment effect.

Very preterm birth (delivery at less than 32 weeks)

Twelve studies (n=68,927 vs. 184,377 pregnancies following frozen vs. fresh embryo transfer) reported on deliveries at < 32 weeks. The relative risk (RR, 95% CI) of a delivery at less than 32 weeks was lower [0.85 (95% CI 0.74-0.97)] in singleton pregnancies following frozen embryo thawed transfer when compared to those after fresh embryo transfer (Figure 9a). There was moderate heterogeneity (I^2 =38.6%) amongst the studies. We could not differentiate between iatrogenic and spontaneous preterm delivery. The funnel plot was suggestive of a degree of publication bias (p=0.04).

Cumulative meta-analysis suggests that the evidence in support of a reduced risk of very preterm delivery in singleton pregnancies after thawed frozen embryo transfer has only become available since 2016 (Figure 9b).

Antepartum haemorrhage (APH)

Five studies were included in the meta-analysis (n=36,911 vs. 26,244 pregnancies after frozen vs. fresh embryo transfer). Hayley et al. (2010) reported a comparison between fresh embryo transfer (stimulated) versus frozen embryo transfer (natural cycles only). They

reported antepartum haemorrhage, postpartum haemorrhage and placenta praevia as well as accreta separately. Shi et al. (2012) reported all antepartum haemorrhage together, Ishihara et al. (2014), Liu et al. (2013), Pelkonen et al. (2010) reported placenta praevia, abruption and accreta separately.

There was no difference in risk of APH in singleton pregnancies following frozen thawed embryo transfer when compared to those after fresh embryos (RR 0.82; 95% CI: 0.66-1.03). There was moderate heterogeneity (67.6%) amongst the studies (Figure 10a).

Cumulative meta-analysis (Figure 10b) suggest that data available by 2010-2013 suggested that the risk of antepartum haemorrhage was lower in singleton pregnancies in women who underwent frozen embryo transfer; however, by 2014 this outcome had changed to no difference following the accrual of fresh data. No studies after 2014 have reported this outcome.

Admission to neonatal intensive care unit (NICU)

Five studies reported the outcome of admission to NICU (n= 3,703 vs. 15,862 pregnancies after frozen vs. fresh embryo transfer). The length and the reasons for NICU admission were not specified. There was no increase in the risk of admission to NICU (RR 0.99; 95% CI: 0.84-1.18) in pregnancies following frozen embryos (Figure 11a). There was marked heterogeneity amongst the studies (I^2 =54%).

Cumulative meta-analysis for admission to neonatal unit showed no clear trend regarding effect on singleton pregnancies as a result of frozen embryo transfer. This has not changed over the years with accrual of fresh data over time (Figure 11b).

Congenital anomalies

Only 6 studies (n= 25,789 vs. 107,692 pregnancies following frozen vs. fresh embryo transfer) reported congenital anomalies (one matched cohort study). Both major and minor anomalies were pooled together. The relative risk of having a congenital anomaly was 1.01 (95% CI 0.87, 1.16) in pregnancies following frozen thawed embryos as compared to fresh embryos (Figure 12a). There was minimal heterogeneity ($I^2 = 28\%$) amongst the studies. Cumulative meta-analysis for congenital anomalies showed no clear trend regarding effect on pregnancies as a result of frozen embryo transfer. This has been consistent over time despite accrual of fresh data (Figure 12 b).

Perinatal mortality

Twelve studies (n= 25,203 vs. 77,280 pregnancies following frozen vs. fresh embryo transfer) reported the outcome of perinatal mortality. Still birth and perinatal mortality are presented together in this report. Some studies reported only neonatal death (Roy et al., 2014; Shi et al., 2012). Of those who reported perinatal mortality there was a variation in definition : death of child with a gestational age of more than 20 weeks or up to day 28 of birth (Aflatoonian et al., 2010, 2016; Pinborg et al., 2010); deaths occurring after the 24th week of gestation and during the first week of life; after 22 weeks of gestation and first 7 days of life (Kato et al., 2012); stillbirth after 28 weeks of gestation and first 7 days of life (Li et al., 2014, Liu et al., 2013; Pelkonen et al., 2010; Wennerholm et al., 2013; Wikland et al., 2010); still birth after 20 weeks, later terminations and all neonatal deaths (Shih et al., 2008).

There was no difference in perinatal mortality (RR 0.92; 95% CI- 0.78, 1.08) in singleton pregnancies after frozen thawed embryo transfers, when compared to those after fresh

embryos (Figure 13a). There was no heterogeneity amongst the studies (I^2 =0.8%). There was no publication bias (p= 0.41).

Cumulative meta-analysis for perinatal mortality showed no clear trend regarding effect on pregnancies as a result of frozen embryo transfer despite addition of fresh data over time (Figure 13b).

Hypertensive disorders of pregnancy

Five studies reported the outcome of hypertensive disorders of pregnancy (n= 39,501 vs. 59,155 pregnancies following frozen vs. fresh embryo transfer). The relative risk of hypertensive disorders of pregnancy were higher in frozen embryo transfer group (Figure 14a) (RR 1.29; 95% CI- 1.07, 1.56). There was moderate heterogeneity (I^2=66%).

Cumulative meta-analysis suggests that the evidence in support of an increased risk of hypertensive disorders in singleton pregnancies after thawed frozen embryo transfer has only become available since 2015 (Figure 14b).

Discussion

Principal findings

Singleton pregnancies following frozen embryo transfer face a reduced risk of preterm birth, small for gestational age and low birth weight babies but a higher risk of large for gestational age babies as well as hypertensive disorders of pregnancies. Although more recent studies have increased the precision of the point estimate, no substantive change has occurred in the direction or magnitude of the treatment effect for these outcomes over time.

Strengths

This is a definitive, updated date systematic review on a key topic in assisted reproduction, at a time when frozen embryo transfer rates are rising sharply. In addition to conventional meta-analysis, we are also able to present a cumulative meta-analysis to assess temporal trends which might be influenced by improvements in freezing and thawing techniques over the years. The consistency in direction and magnitude of the treatment effect for the key outcomes confirms the validity of the published data.

Limitations

As there are no randomized controlled trials (except Shapiro et al., 2016, where birth weight was done as a post hoc analysis- data obtained by personal communication, that could be included) who reported perinatal outcomes in singleton pregnancies, this review is limited to data from observational studies. Hence the evidence is being graded as low despite large numbers (supplementary Table 2) There are variations in the studies whose data have been complied together not only in design but population, interventions (method of freezing and regimens in replacement cycles) ascertainment of outcomes (Table 1) . , which. We were unable to adjust for confounders such as age, smoking, parity, duration of infertility and pre-existing medical illness.. Without individual patient data, we are unable to determine if the risks are different for embryos frozen by slow freezing and vitrification and whether embryos were frozen at cleavage or blastocyst stage of development or protocols used for endometrial preparation. . Although our cumulative meta-analysis is stratified by year of publication, the paper in 2016 contains data from 1997 (Maheshwari et al., 2016), hence the true effect of changes in freezing techniques over time cannot be fully captured.

We have combined both major and minor fetal abnormalities together, as separate data for these were not available for most of the studies that did report of this.. It is also acknowledged that authors might use different classification systems for fetal abnormalities, and that some studies may have included terminations for these abnormalities while others might not have. This data is again not available in the studies included.

Comparison with other studies

The findings of low and high birth weight are consistent with the published literature and our previous systematic review (Maheshwari et al., 2012). The incidence of preterm delivery was reported to be lower in frozen embryo transfer in this as well as the previous review. However, a recent randomized trial (Chen et al., 2016) did not find any difference in preterm birth rates, and neither did an analysis of a large national U.K. dataset (Maheshwari et al., 2016). Addition of results from this large dataset (Maheshwari et al., 2016) did not change the direction and magnitude of the effect for key outcomes in the cumulative meta-analysis. This provides a degree of confidence in the reliability of the existing data for the outcomes of birth weight and preterm delivery. Increased risk of hypertensive disorders of pregnancy in pregnancies following frozen embryo transfer in this report is similar to the findings in large randomized controlled trial (Chen et al., 2016).

Outcomes of antepartum haemorrhage, congenital anomalies, perinatal mortality, and admission to neonatal units are similar in pregnancies conceived from fresh and frozen embryos. As these outcomes have not been reported by all studies, the overall numbers are much lower. There is a possibility that addition of further data may change the current estimate of risk, especially for rarer outcomes such as perinatal mortality and congenital anomalies, where the number of observations is low.

Explanation of results

Hormonal stimulation of the ovaries in IVF causes a state of hyper-estrogeneism at a time when fresh embryos are transferred. It has been hypothesized that this leads to abnormal endometrial angiogenesis leading to reduced implantation as well as abnormal placentation. This can account for findings of small for gestational age babies, preterm deliveries and low birthweight babies. Uterine environment in a frozen replacement cycle is a more natural uterine environment as the effect of ovarian stimulation tends to worn off by the time point when embryos are replaced (Amor et al., 2009; Healy et al., 2010; Kalra et al., 2011). However there is as yet no clear explanation for the increased chance of large for gestational age births. It is possible that higher implantation potential leads to better placentation and overgrowth of the fetus. Birth order, which is higher in babies, conceived from frozen thawed embryos, may play a role, but has been challenged by the fact that the difference has persisted after adjustment for parity in various studies (Maheshwari et al., 2016; Pinborg et al., 2014). It has also been suggested that the freezing and thawing procedures may play an independent role for the growth potential of the fetus due to epigenetic alterations at the early embryonic stages (Pinborg et al., 2014).

There is no obvious biological explanation for increase in hypertensive disorders.

Implications for clinical practice

Data from this review provides reassurance for embryo cryopreservation programmes in IVF, while, at the same time, suggesting a need for caution due to higher risk of large for gestational age babies as well as increased risk of hypertension in pregnancy. This is especially relevant as the threshold for freezing is falling and increasing numbers of embryos

are being electively frozen and reserved for deferred transfer. In fact, in some centres, a "freeze all" policy followed by thawed frozen embryo transfer has become the norm. It is to be remembered that both small for gestational age and large for gestational age has implications for health and diseases later in life. Hence, routine use of freeze all strategy may have long term implications as well. Moreover all the evidence has been graded as low quality (Supplementary Table 2) as per GRADE matrix, primarily due to observational data.

We recommend that, on the basis of current evidence, elective freezing of all embryos should only be performed when there is a definite clinical indication or in the context of a clinical trial.

Implications for research

There have been a number of observational studies published over years to evaluate obstetric and perinatal outcomes in singleton pregnancies following thawed frozen embryo transfer. As is clear from the summary table (Table III) the data for birth weight and preterm delivery has reached saturation to the extent that even large datasets are not able to shift the magnitude and direction of the effect. Replication of data from different databases, geographical areas and populations, proved the validity of the findings. Therefore, we do not feel that more data from observational studies are needed for the outcomes of preterm delivery and birth weight. Due to observational data the quality of evidence has been graded as low, despite large numbers (Supplementary table). This will not alter by adding more observational data.

For other outcomes, especially rarer outcomes (neonatal death, congenital anomalies), it is important that an IPD MA (individual patient data meta-analysis) is done from registries across the world. This will help for e.g. in the analysis of major and minor congenital

anomalies separately. It will also help in doing subgroup analysis for a specific group of patients, which is not possible in current report.

Although IPD-MA of registry data will be ideal this will not be without considerable investment and collaboration. There will be difficulties of data transfer due to local governances as well the format (all data are in different format and collect different variables).

As the threshold for freezing has fallen, some clinics are choosing to opt for “freeze all” programmes for an increasing number of IVF treatments in preference to the conventional policy of elective fresh embryo transfer. While the data generally provides reassurance for the safety of thawed frozen embryo transfers, there are some lingering concerns related to the risk of large for gestational age babies. This has created a state of equipoise which makes this an ideal time to conduct randomized controlled trials to comparing an elective “freeze all” policy with usual care, in terms of clinical and cost effectiveness.

Across the world, a number of trials with live birth as the primary outcome are either ongoing or have recently been completed (ACTRN 12616000643471;NCT01841528; NCT02746562; NCT02570386; NTR3187; ISCTRN- 61225414; NCT00963625; NCT00963079; NCT02471573; NCT01954758). Follow up of offspring from these trials provides an opportunity to minimise bias in any future comparison of pregnancy outcomes such as preterm delivery, low and high birth weight, while an individual patient data meta-analysis approach permits outcomes in clinically relevant subgroups (e.g. older versus younger women) to be compared.

Further mechanistic studies are needed to identify the biological reason of increase in hypertensive disorders in pregnancies subsequent to frozen embryo transfer.

Conclusion

This systematic review confirms that singleton babies conceived by frozen embryo transfers are at lower risk of preterm delivery, small for gestational age and low birth weight. The direction and magnitude of effect for these outcomes have remained virtually unchanged over time while the degree of precision has improved with the addition of data from newer studies. Our results also show that frozen embryo transfer is associated with an increased risk of hypertensive disorders of pregnancy and large for gestational age in singleton babies. Although replication of the research by several groups has added to the external validity of the results, the data from our cumulative meta-analyses suggest that further analyses of observational data from published studies are unlikely to change them. Given the current challenges around research funding, resources should be concentrated on following up pregnancy outcomes of relevant randomized trials and IPD MA of registry data.

Acknowledgements

The authors would like to thank authors of primary studies who responded to queries and provided additional data required to complete this review (Professors Anja Pinborg, Forest Gardner and Bruce Shapiro).

Authors' roles

AM and SB conceived the manuscript. AM and SP did searches, quality assessment and data collation. EAR did all the statistical analysis. MH, SB and AS provided intellectual input from the protocol stage right through all versions of the manuscript. All authors contributed to the final version of the manuscript.

Funding

No external funding was sought in preparing this manuscript

Conflict of Interest

AM and SB are co-applicants on the HTA/ NIHR grant, UK (ISCTRN-61225414) for E-Freeze Trial which is a randomized controlled trial comparing elective freezing of embryos with current policy of fresh embryo transfer. Otherwise the authors have no conflict of interest.

References

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.

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 ; 27:357-363.

Aflatoonian A, Karimzadeh Maybodi MA, Aflatoonian N, Tabibnejad N, Amir-Arjmand MH, Soleimani M, Aflatoonian B, Aflatoonian A. Perinatal outcome in fresh versus frozen embryo transfer in ART cycles. *Int J Reprod Biomed* 2016 ;14:167-172.

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 ; 24:1330-1338.

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: 2619-2624.

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 ; 23: 2227-2238.

Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y et al., Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome *N Engl J Med* 2016;375:523-533.

Egger, M.,G. Smith Davey,. Schneider M, and Minder C. Bias in meta-analysis detected by a simple, graphical test *BMJ* 1997; 315: 629-634.

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* 1989 ;160 :209-213.

613 Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, et al. Prevalence and
614 risk factors for obstetric haemorrhage in 6730 singleton births after assisted
615 reproductive technology in Victoria Australia. *Hum Reprod* 2010 ; 25 :265-274.
616
617 Henningsen A-A, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN.
618 Perinatal outcome of singleton siblings born after assisted reproductive technology and
619 spontaneous conception: Danish national sibling-cohort study. *Fertil Steril* 2011 ; 95
620 :959-963.
621
622 Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-
623 analyses. *BMJ*. 2003; 327 :557–560.
624
625 Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective
626 cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients
627 at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric
628 outcomes: a preliminary study. *Fertil Steril*. 2013 ;99: 168-173.
629
630 Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-
631 thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of
632 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril*. 2014
633 ;101:128-133.
634

635 Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden:
636 infant outcome after different IVF fertilization methods. *Fertil Steril* 2005 **a** ;84 :611-
637 617.
638
639 Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk
640 for congenital malformations after different IVF methods. *Birth Defects Research* 2005 **b**
641 ; 73:162-169.
642
643 Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal
644 morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertil Steril*
645 2011 ;95: 548-553.
646
647 Kato O, Kawasaki N, Bodri D, Kuroda T, Kawachiya S, Kato K, Takehara Y. Neonatal
648 outcome and birth defects in 6623 singletons born following minimal ovarian
649 stimulation and vitrified versus fresh single embryo transfer. *Eur J Obstet Gynecol*
650 *Reprod Biol*. 2012 ;161:46-50
651
652 Ku PY, Lee RK, Lin SY, Lin MH, Hwu YM. Comparison of the clinical outcomes between
653 fresh blastocyst and vitrified-thawed blastocyst transfer. *J Assist Reprod Genet*. 2012
654 ;29:1353-1356.
655
656 Lau J E M Antman, J Jimenez-silva et al. Cumulative meta-analysis of Therapeutic trails
657 for Myocardial Infectoin. *N Engl J Med* 1992; 327: 248-254.
658

659 Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following
660 cryopreservation of blastocysts by vitrification or slow freezing: a population-based
661 cohort study. *Hum Reprod*. 2014 ; 29 :2794-2801.
662
663 Liu SY, Teng B, Fu J, Li X, Zheng Y, Sun XX. Obstetric and neonatal outcomes after transfer
664 of vitrified early cleavage embryos. *Hum Reprod* 2013; 28: 2093–2100.
665
666 Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and Perinatal
667 outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus
668 fresh embryos generated through in-vitro fertilisation treatment. A systematic review
669 and meta-analysis. *Fertil Steril* 2012; 98 : 366-377.
670
671 Maheshwari A, Raja EA, Bhattacharya S Obstetric and perinatal outcomes after either
672 fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies
673 recorded in the Human Fertilisation and Embryology Authority anonymized dataset.
674 *Fertil Steril*. 2016;106: 1703-1708.
675
676 Opdahl S, Henningsen AA, Tiitinen A, et al. Risk of hypertensive disorders in pregnancies
677 following assisted reproductive technology: a cohort study from the CoNARTaS group.
678 *Hum Reprod* 2015; 30: 1724-31.
679
680 Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari A-, Hyden-Granskog C,
681 et al. Perinatal outcome of children born after frozen and fresh embryo transfer: The
682 Finnish cohort study 1995-2006. *Hum Reprod* 2010 ;25: 914-923.

683

684 Pelkonen S, Hartikainen AL, Ritvanen A, Koivunen R, Martikainen H, Gissler M, Tiitinen A.
685 Major congenital anomalies in children born after frozen embryo transfer: a cohort
686 study 1995-2006.*Hum Reprod* 2014 ;29:1552-1557.

687

688 Pereira N, Petrini AC, Lekovich JP, Schattman GL, Rosenwaks Z. Comparison of perinatal
689 outcomes following fresh and frozen-thawed blastocyst transfer. *Int J Gynaecol Obstet*.
690 2016 ; 135: 96-100

691

692 Pinborg A, Loft A, Aaris Henningsen A-, Rasmussen S, Andersen AN. Infant outcome of
693 957 singletons born after frozen embryo replacement: The Danish National Cohort Study
694 1995-2006. *Fertil Steril* 2010 ;94:1320-1327.

695

696 Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby
697 syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal
698 factors or the cryotechnique? *Hum Reprod*. 2014 ; 29:618-627.

699

700 Rallis A, Tremellen K. Controlled ovarian hyper-stimulation during IVF treatment does
701 not increase the risk of preterm delivery compared to the transfer of frozen-thawed
702 embryos in a natural cycle. *Aust N Z J Obstet Gynaecol.* 2013 ;53:165-169.

703

704 Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of
705 impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: A

706 prospective randomized trial comparing fresh and frozen-thawed embryo transfer in
707 normal responders. *Fertil Steril* 2011 ;96 :344-348.
708
709 Shapiro BS, Daneshmand ST, Bedient CE, Garner FC. Comparison of birth weights in
710 patients randomly assigned to fresh or frozen-thawed embryo transfer. *Fertil Steril*. 2016
711 ; 106:317-21.
712
713 Sweeting M J, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of
714 continuity corrections in meta-analysis of spare data. *Stat Med* 2004; 23: 1351-1375.
715
716 Shi Wenhao, Xia Xue, Silin Zhang, Wanqiu Zhao, Shan Liu, Hanying Zhou, Min Wang, and
717 Juanzi Shi, Perinatal and neonatal outcomes of 494 babies delivered from 972 vitrified
718 embryo transfers. *Fertil Steril* 2012;97: 1338–42.
719
720 Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting
721 low birthweight after assisted reproduction technology: Difference between transfer of
722 fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum*
723 *Reprod* 2008 ;23:1644-1653.
724
725 Roy TK , Bradley CK, Bowman MC, McArthur SJ. Single-embryo transfer of vitrified-
726 warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes
727 compared with fresh transfers . *Fertil Steril* 2014;101: 1294–1301.
728

729 Takeshima K, Jwa SC, Saito H, Nakaza A, Kuwahara A, Ishihara O, Irahara M, Hirahara F,
730 Yoshimura Y, Sakumoto T. Impact of single embryo transfer policy on perinatal outcomes
731 in fresh and frozen cycles-analysis of the Japanese Assisted Reproduction Technology
732 registry between 2007 and 2012. *Fertil Steril.* 2016 ;105:337-346.
733
734 Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR. Birth characteristics and
735 perinatal outcome of babies conceived from cryopreserved embryos. *Hum Reprod* 1994;
736 9:543-546.
737
738 Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth
739 weight after assisted reproductive technology-related pregnancy in Australia between
740 1996 and 2000. *Fertil Steril*. 2005 ;83:1650-1658.
741
742 Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth
743 weight after assisted reproductive technology-related pregnancy in Australia between
744 1996 and 2000. Fertil Steril 2005 ;83:1650-1658.
745 Wennerholm U-, Hamberger L, Nilsson L, Wennergren M, Wikland M, Bergh C. Obstetric
746 and perinatal outcome of children conceived from cryopreserved embryos. *Hum Reprod*
747 1997 ;12:1819-1825.
748
749 Wennerholm WB. Cryopreservation of embryos and oocytes: obstetric outcome and
750 health in children. *Hum Reprod* 2000 ;15(Suppl 5):18-25.
751

752 Wennerholm UB, SoderstromAnttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, et al.
753 Children born after cryopreservation of embryos or oocytes: a systematic review of
754 outcome data. *Hum Reprod* 2009 ;24 :2158-2172.
755 Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R,
756 Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after
757 frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Hum*
758 *Reprod*. 2013 ; 28:2545-53
759
760 Wikland M, Hardarson T, Hillensjo T, Westin C, Westlander G, Wood M, et al. Obstetric
761 outcomes after transfer of vitrified blastocysts. *Hum Reprod* 2010 ;25:1699-1707

#	Searches	Results	Annotations
1	Embryo Transfer/ or Fertilization in Vitro/ or IVF.mp.	37303	
2	" in-vitro fertilisation".mp.	1599	
3	ICSI.mp. or Sperm Injections, Intracytoplasmic/	7723	
4	1 or 2 or 3	40160	
5	Cryopreservation/ or "fresh embryo transfer".mp.	20105	
6	" frozen embryo transfer".mp.	305	
7	5 or 6	20232	
8	Fetal Growth Retardation/ or Pregnancy Complications/ or Obstetric Labor Complications/ or Pregnancy/ or Pregnancy Outcome/ or "obstetric and perinatal complications".mp.	788905	
9	Infant, Low Birth Weight/ or Infant, Small for Gestational Age/ or Birth Weight/ or Gestational Age/ or Infant, Premature/ or " small for gestational age".mp.	144285	
10	Diabetes, Gestational/ or Premature Birth/ or "large for gestational age".mp. or Gestational Age/ or Pregnancy in Diabetics/	93954	
11	Birth Weight/ or Hypertension/ or " high birth weight".mp.	253898	
12	Infant, Premature/ or Infant, Small for Gestational Age/ or Infant, Low Birth Weight/ or SGA.mp.	65525	
13	Fetal Macrosomia/ or Diabetes, Gestational/ or LGA.mp.	9895	
14	Obstetric Labor, Premature/ or " preterm delivery".mp.	17502	
15	" very preterm delivery".mp.	100	
16	" very low birth weight".mp. or Infant, Very Low Birth Weight/	9807	
17	Hypertension, Pregnancy-Induced/ or Pre-Eclampsia/ or PIH.mp.	29056	
18	Cesarean Section/	38260	
19	" perinatal mortality".mp. or Fetal Death/ or Perinatal Mortality/	30491	
20	Congenital Abnormalities/ or " still birth".mp.	33057	
21	"antepartum haemorrhage".mp. or Uterine Hemorrhage/	9129	
22	Placenta Previa/	2532	
23	Abruptio Placentae/	2077	
24	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23	1099362	
25	4 and 7 and 24	2310	
26	Singleton.mp.	14929	
27	25 and 26	126	

28	from 27 keep 4, 13, 15, 19, 21, 28...	16
29	Embryo Transfer/ or Fertilization in Vitro/ or IVF.mp.	37303
30	" in-vitro fertilisation".mp.	1599
31	ICSI.mp. or Sperm Injections, Intracytoplasmic/	7723
32	29 or 30 or 31	40160
33	Cryopreservation/ or "fresh embryo transfer".mp.	20105
34	" frozen embryo transfer".mp.	305
35	33 or 34	20232
36	Fetal Growth Retardation/ or Pregnancy Complications/ or Obstetric Labor Complications/ or Pregnancy/ or Pregnancy Outcome/ or "obstetric and perinatal complications".mp.	788905
37	Infant, Low Birth Weight/ or Infant, Small for Gestational Age/ or Birth Weight/ or Gestational Age/ or Infant, Premature/ or " small for gestational age".mp.	144285
38	Diabetes, Gestational/ or Premature Birth/ or "large for gestational age".mp. or Gestational Age/ or Pregnancy in Diabetics/	93954
39	Birth Weight/ or Hypertension/ or " high birth weight".mp.	253898
40	Infant, Premature/ or Infant, Small for Gestational Age/ or Infant, Low Birth Weight/ or SGA.mp.	65525
41	Fetal Macrosomia/ or Diabetes, Gestational/ or LGA.mp.	9895
42	Obstetric Labor, Premature/ or " preterm delivery".mp.	17502
43	" very preterm delivery".mp.	100
44	" very low birth weight".mp. or Infant, Very Low Birth Weight/	9807
45	Hypertension, Pregnancy-Induced/ or Pre-Eclampsia/ or PIH.mp.	29056
46	Cesarean Section/	38260
47	" perinatal mortality".mp. or Fetal Death/ or Perinatal Mortality/	30491
48	Congenital Abnormalities/ or " still birth".mp.	33057
49	"antepartum haemorrhage".mp. or Uterine Hemorrhage/	9129
50	Placenta Previa/	2532
51	Abruptio Placentae/	2077
52	36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51	1099362
53	32 and 35 and 52	2310
54	Singleton.mp.	14929
55	53 and 54	126

Summary of findings:

Frozen versus fresh embryo transfer

Patient or population: IVF
Setting:
Intervention: Frozen embryo transfer
Comparison: fresh embryo transfer

Outcome № of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Quality	What happens
		Without Frozen embryo transfer	**With Frozen embryo transfer**	**Difference**		
small for gestational age (SGA) № of participants: 142462 (10 observational studies)	**RR 0.61** (0.56 to 0.67)	6.1%	**3.7%** (3.4 to 4.1)	**2.4% fewer** (2.7 fewer to 2 fewer)	⨁⨁◯◯ LOW	
Birthweight <2500 gm (low birth weight) № of participants: 280.19 (20 observational studies)	**RR 0.72** (0.67 to 0.77)	8.8%	**6.3%** (5.9 to 6.8)	**2.5% fewer** (2.9 fewer to 2 fewer)	⨁⨁◯◯ LOW	
Large for gestational age (LGA) № of participants: 138263 (7 observational studies)	**RR 1.54** (1.48 to 1.61)	6.1%	**9.5%** (9.1 to 9.9)	**3.3% more** (2.9 more to 3.7 more)	⨁⨁◯◯ LOW	
Preterm deivery (PTL) № of participants: 280622 (20 observational studies)	**RR 0.90** (0.84 to 0.97)	9.4%	**8.4%** (7.9 to 9.1)	**0.9% fewer** (1.5 fewer to 0.3 fewer)	⨁⨁◯◯ LOW	
Hypertensive disorders of pregnancy (PIH) № of participants: 98656 (5 observational studies)	**RR 1.29** (1.07 to 1.56)	4.5%	**5.9%** (4.9 to 7.1)	**1.3% more** (0.3 more to 2.5 more)	⨁⨁◯◯ LOW	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Table 1: Tables of included studies

Study ID	Design of study	Population	Method of data collection	Risk of Bias	Scoring
Aflatoonian et al., 2010	Un matched Cohort study	500 pregnancies obtained after the transfer of fresh ET and 200 pregnancies after FET from March 2006 to March 2008.	Questionnaires filled by gynaecologists, paediatrics and women regarding perinatal and obstetric outcomes	The characteristics of two groups were similar All pregnancies were included	9/12
Aflatoonian et al., 2016	Un matched Cohort study	300 women using FET and 1150 women undergoing fresh embryo transfer over a 4-years period between December 2010 and December 2014 No overlap with previous study	Data were collected from the hospital records. In addition, a telephone questionnaire consists of data on maternal and neonatal factors was administered by a trained nurse based on patients and their husbands' information.	The characteristics of two groups were similar All pregnancies were included	9/12
Belva et al., 2008	Un matched Cohort Study	Exposed cohort: all cryo pregnancies irrespective of cryo procedure used were consecutively included. Unexposed cohort: fresh IVF/ICSI cycles	Data on pregnancies, deliveries and neonatal history was obtained by gynaecologists, paediatricians and double checked with parents, when child was 2 months old.	Mode of delivery and Duration of infertility was significantly different in the two groups.	10/12
Healy et al., 2010.	Retrospective Unmatched Cohort	fresh vs. Frozen comparison was a sub group analysis Jan 1991-Dec 2004	Data was collected using record linkage of national databases	Includes first singleton birth only, delivered after 20 weeks No data on demographic profile of the women in FET vs. fresh group	10/12
Imudia et al., 2013	Retrospective cohort study	Twenty women who underwent elective cryopreservation of all embryos with subsequent cryothaw ET and 32 similar women with elevated peak E2 during controlled ovarian hyperstimulation for IVF who underwent a fresh ET.	Data was collected from Medical records	Study adjusted for confounders (body mass index, antral follicle count, peak serum E2 level) Excluded peak serum E2> 4500pgm/ml	9/12
Ishihara et al., 2014	Cohort	Registered from 2008 through 2010 undergoing single embryo transfer cycles. Only singleton ongoing pregnancies >21 weeks of gestation were included.	Japanese nationwide registry of assisted reproductive technology (ART) with mandatory reporting for all ART clinics in Japan.	Japanese registry is cycle based with complete anonymity, they didn't know when oocytes were retrieved and fertilized for consecutive FET cycles. Detailed background of the patients who underwent ART, e.g., gravidity, parity, previous uterine surgery was not available.	11/12

Kato et al., 2011	Retrospective Cohort	Single-centre retrospective cohort study of 6623 consecutive delivered singletons following 29,944 single-embryo transfers. January 2006 and December 2008	Two-part questionnaire filled by patient at the 20th pregnancy week and after delivery.	There was no difference in baseline characteristics in both group.	11/12
Li et al., 2014	Retrospective Cohort study	Reterospective population based cohort study from Jan 2009- Dec 2011 of autologous fresh and frozen cycles in Australia and New Zealand	ART treatment information and perinatal outcomes were obtained from the Australian and New Zealand Assisted Reproduction Database (ANZARD).		9/12
Liu et al., 2013	Retrospective single centre analysis	retrospective, single-centre study of children born after Day 3 embryo transfer from fresh, slow frozen or vitrified embryos during the period January 2006 to May 2011	Data obtained via patient filled questionnaire at 12 weeks	Baseline characteristics for women having fresh or frozen embryo transfer were not compared. Comparisons were made between vitrified versus fresh and vitrified versus slow freezing	8/12
~~Shapiro et al., 2016~~	~~Post hoc analysis of two RCT~~	~~Two RCTs from same centre one on hyper responders and one on normal responders~~	~~Birth weight outcome; post hoc analysis~~	~~Data obtained through personal communication~~	~~NA~~
Maheshwari et al., 2016	Retrospective analysis	Retrospective analysis of annonymized HFEA data	Data taken from HFEA database (which gets reported to HFEA by clinics as part of regulatory requirement)	Age in database was in age bands rather than continuous Many confounders were not reported in database- smoking and BMI	11/12
Opdahl et al., 2015	Unmatched cohort study	Nationwide data from registries of Denmark, Norway and Sweden	Data obtained from health registries	Baseline characteristics for women having fresh or frozen embryo transfer were not compared. As fresh and frozen embryo transfers were one of multiple comparisons	11/12
Pelkonen et al., 2010	Unmatched Cohort study (1995-2006)	Exposed cohort: FET resulting in singleton pregnancy 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	Mothers in FET group were slightly older. Proportion of women having first pregnancy were 35% in FET group as compared to 52% in fresh embryo transfer group. The data on variables of pregnancy complications are incomplete in Finnish Medical Birth Register before 2004	11.5/12
Pelkonen et al., 2014	Register based cohort study	Exposed cohort: FET resulting in singleton pregnancy Controls= Fresh IVF/ICSI treatment	Linkage of fertility, birth and congenital anomalies registries	There was a higher proportion of nulliparous women in fresh ET group	11/12

Piereria et al., 2016	Retrospective review	Consecutive live deliveries from all patients who began IVF cycles at the single centre between January 1, 2010 and September 30, 2013.	Data collected by retrospective review of patients charts	Patients were of similar age, BMI, infertility diagnosis, endometrial thickness and there was no difference in the grading of blastocysts.	11/12
Pinborg et al., 2010	Matched Cohort study	Exposed cohort: Singletons born after FET (Jan 1995- Dec 2006) Unexposed cohort = singletons born after fresh IVF/ICSI within the same time frame	Danish IVF and Danish Birth Register	Age and parity showed statistically significant difference in the groups But the data adjusted for age, parity child gender and year of birth	11/12
Pinborg et al., 2014	The national register–based controlled cohort study	two populations of FET singletons The first population consisted of all FET singletons (compared with singletons born after Fresh embryo transfer (Fresh) from 1997 to 2006. The second population (B: Sibling FET cohort) included all sibling pairs, where one singleton was born after FET and the consecutive sibling born after Fresh embryo transfer or vice versa from 1994 to 2008.	Registry data	Age and parity showed statistically significant difference in the groups But the data adjusted for age, parity child gender and year of birth overlapping data with 2010. outcomes not available in 2010 are taken from this (LGA,SGA, Macrosomic babies and PP); This was checked by personal communication with corresponding authors.	11/12
Rallis et al., 2013	Retrospective review	Single centre private IVF centre in Adelaide Australia from 2008-2009 Only singleton pregnancies beyond 20 weeks, after single embryo transfer were included	Clinic based data, case records , database	Basic demographic data other than age group was not available confounding factors for preterm birth such as previous pregnancy outcomes were not available.	10/12
Roy et al., 2014	Retrospective cohort	Single centre Assisted Reproduction clinic between March 2010 and November 2011	Private IVF Clinic database	Data for the fresh group were restricted to the patients with three or fewer stimulation cycles who had single blastocyst transferred and one blastocyst cryopreserved.	10/12
Shapiro et al., 2016	Post hoc analysis of two RCT	Two RCTs from same centre one on hyper responders and one on normal responders	Birth weight outcome; post hoc analysis	Data obtained through personal communication	NA
Shih et al., 2008	Matched cohort with women acting as their own reference	Comparison groups: Frozen versus Fresh IVF/ICSI	Neonatal perinatal statistics unit Austraila	All pregnancies after 20 weeks were recorded. Fresh IVF/ICSI conception could be first/ second one	10/12
Shi et al.,	Retrospective	Single centre Assisted Reproduction	The outcome data were obtained from a	All baseline parameters were similar	8/12

2012	data	clinic	postal questionnaire of parents after delivery.	between both groups Obstetric outcomes were preterm delivery and pregnancy complications and neonatal outcomes evaluated were birth weight,	
Wada et al., 1994	Unmatched cohort	232 consecutive deliveries following embryo cryopreservation between 1985-1991. Fresh IVF data – 763 consecutive deliveries	Data was collected from medical records		7/12
Wang et al., 2005	Unmatched cohort study	Infants conceived through ART Procedures and born in Australia during 1996-2000	The study used data from two national collections. Assisted conception data collection & Australian national perinatal data collection	Fresh and frozen pregnancies were subgroup analysis. Hence not matched for the confounders.	9/12
Wennerholm et al., 1997	Matched cohort	Unexposed cohort: IVF conception with fresh embryos between 1990- 1995 with frozen embryos. Exposed Cohort: Births between 1990-1995 with frozen embryos.	Data was collected after medical records review	Controls were matched for age and parity	10.5/11
Wennerholm et al., 2013	Reterospective Matched cohort study	Retrospective Nordic population-based cohort study of all singletons conceived after FET in Denmark, Norway and Sweden until December 2007 were included Exposed cohort:Singletons born after FET (n 6647) Un exposed cohort singletons born after fresh IVF and ICSI (n 42 242)	Data on perinatal outcomes were obtained by linkage to the national Medical Birth Registries.	Adjustments were made for maternal age, parity, year of birth, offspring sex and country of origin. Data on embryo freezing methods were not available. They were not able to control for confounding factors, such as BMI, smoking and reason for, or length of, infertility	11/12
Wikland et al., 2010	Unmatched Cohort Study	Unexposed cohort : fresh blastocyst transfer Exposed Cohort : Pregnancies after transfer of vitrified, blastocyst	Data for obstetric and perinatal complications was collected from maternity records	Fresh versus frozen blastocysts only Although no matching was done but the characteristics were similar in two groups .	11/12

Table 2: Table of excluded studies

Study	Reason for exclusion
Aytoz et al., 1999	Data from Singleton and twins could not be separated
Aflatoonian et al., 2010	No data on obstetric and perinatal outcomes
Frydman et al., 1989	There is no control group
Henningsen et al 2011	Overlapping data with Pinborg 2010
Kallen et al., 2005a	2x2 table cannot be made
Kallen et al., 2005b	Data for singleton cannot be separated
Kalra et al., 2011	Data on singletons cannot be separated
Ku et al., 2012	No obstetric and perinatal outcomes reported
Wang et al., 2005	Overlapping data with Shih 2008
Shapiro et al., 2011	No data on obstetric and perinatal outcomes
Chen et al., 2016	Data fro~~n~~m singleton and twins ~~cant~~can't be ~~seperated~~separated
Takesima et al., 2016	Data for singletons cannot be separated to generate 2x2 table
Wennerholm et al., 2000	Overlapping data from Wennerholm et al., 1997
Wennerholm et al., 2009	Systematic review

Table III: Summary of findings from cumulative meta-analysis

Risk of Outcome	Evidence	Evidence available by year	No further change in precision, magnitude or direction	More observational data needed
Small for gestational age	Lower in Frozen embryo transfer	2010	2014	no
Low birth weight	Lower in Frozen embryo transfer	1997	2014	no
Very low birth weight	Lower in Frozen embryo transfer	2013	2016	no
Large for gestational age	Higher in Frozen embryo transfer	2010	2014	no
High birth weight	Higher in Frozen embryo transfer	2014	2016	no
Very high birth weight	Higher in Frozen embryo transfer	2013	2014	no
Preterm delivery	Lower in Frozen embryo transfer	2005	2014	no
Very preterm delivery	Lower in Frozen embryo transfer	2016	2016	no
Antepartum haemorrhage	No difference	2010	2014	yes
Admission to NICU	No difference	2012	2013	yes
Congenital anomalies	No difference	2014	2016	yes
Perinatal mortality	No difference	2014	2014	yes
Hypertensive disorders of pregnancy	~~No difference~~ Higher in Frozen	2015~~4~~	2015~~4~~	yes

Formatted Table

		embryo transfer			

Figure 1: PRISMA flow chart for study selection

Attached as separate file

Figure 2a : Small for gestational age- meta-analysis

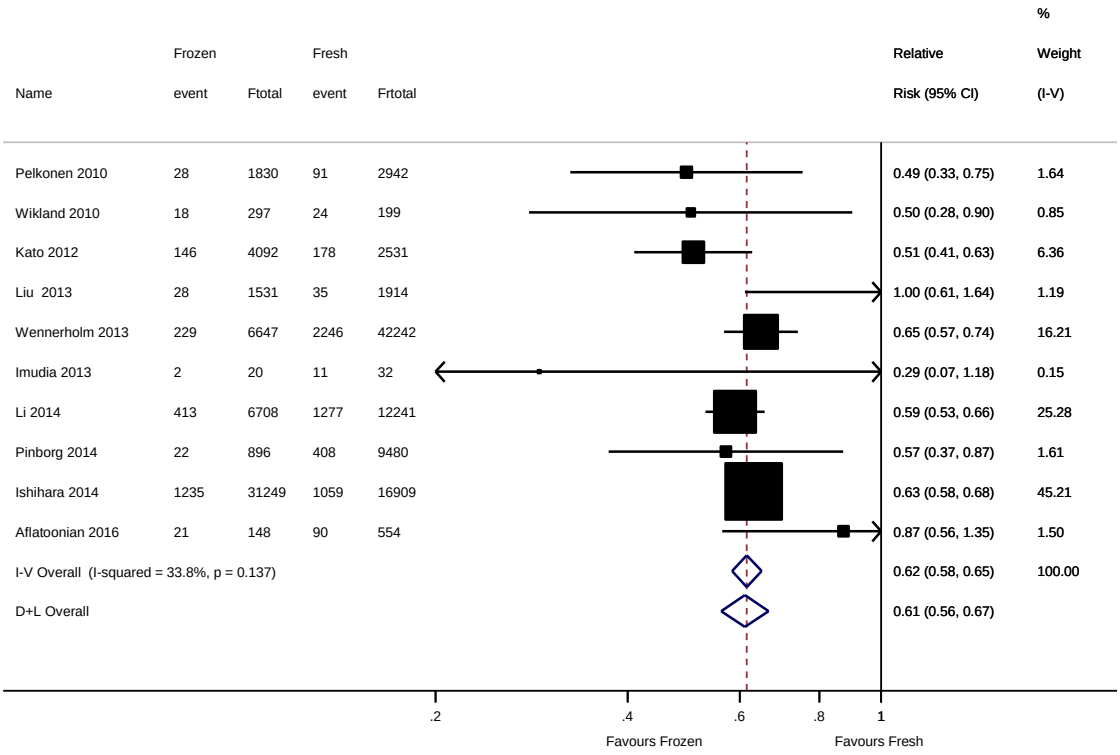


Figure 2b: Small for gestational age- Cumulative meta-analysis

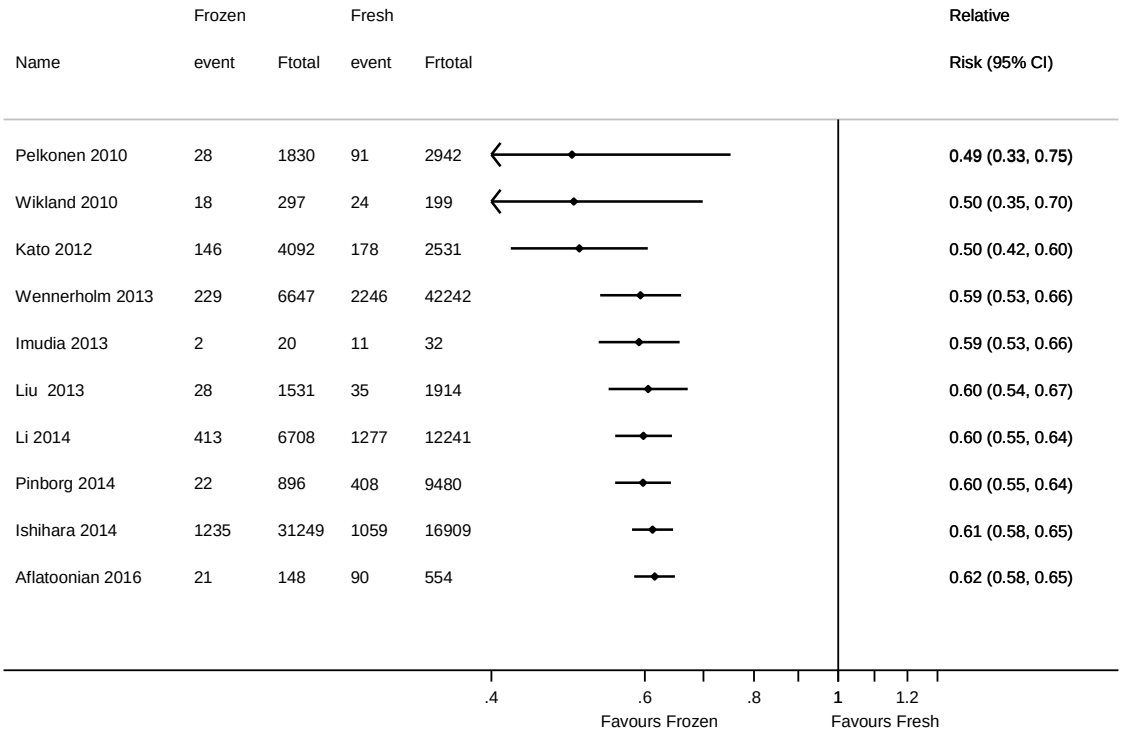


Figure 3a : Low birth weight (Birth-weight less than 2500 gm) : meta-analysis

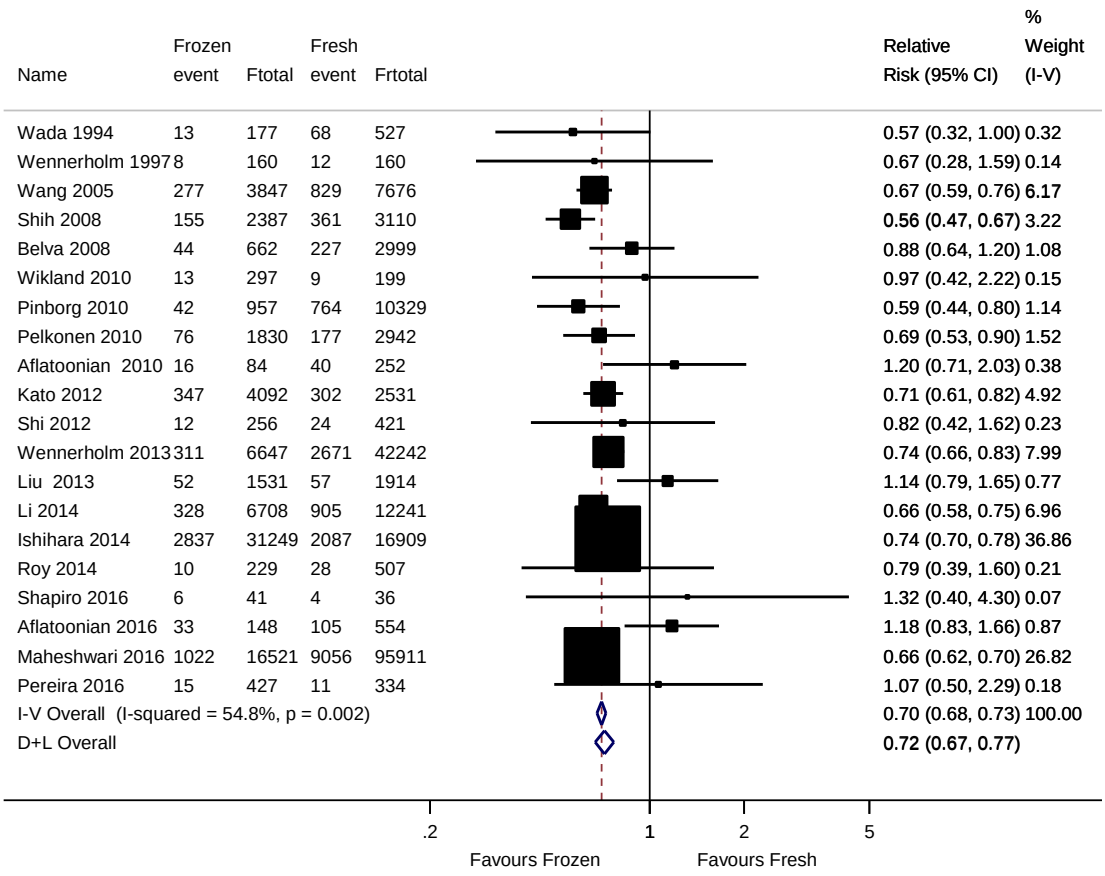


Figure 3b : Low birth weight (Birth-weight less than 2500 gm) : Cumulative meta-analysis

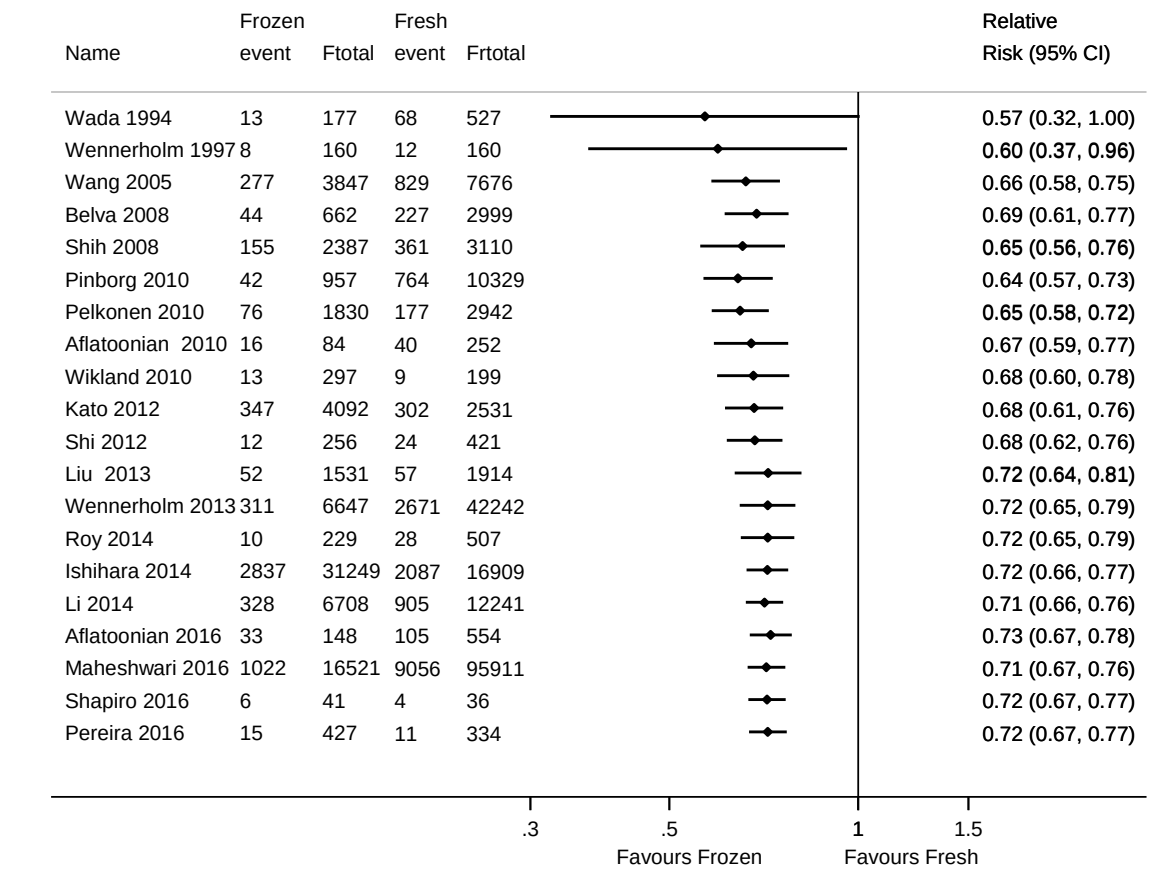


Figure 4a: Very low birth weight (Birth-weight less than 1500gms): Meta-analysis

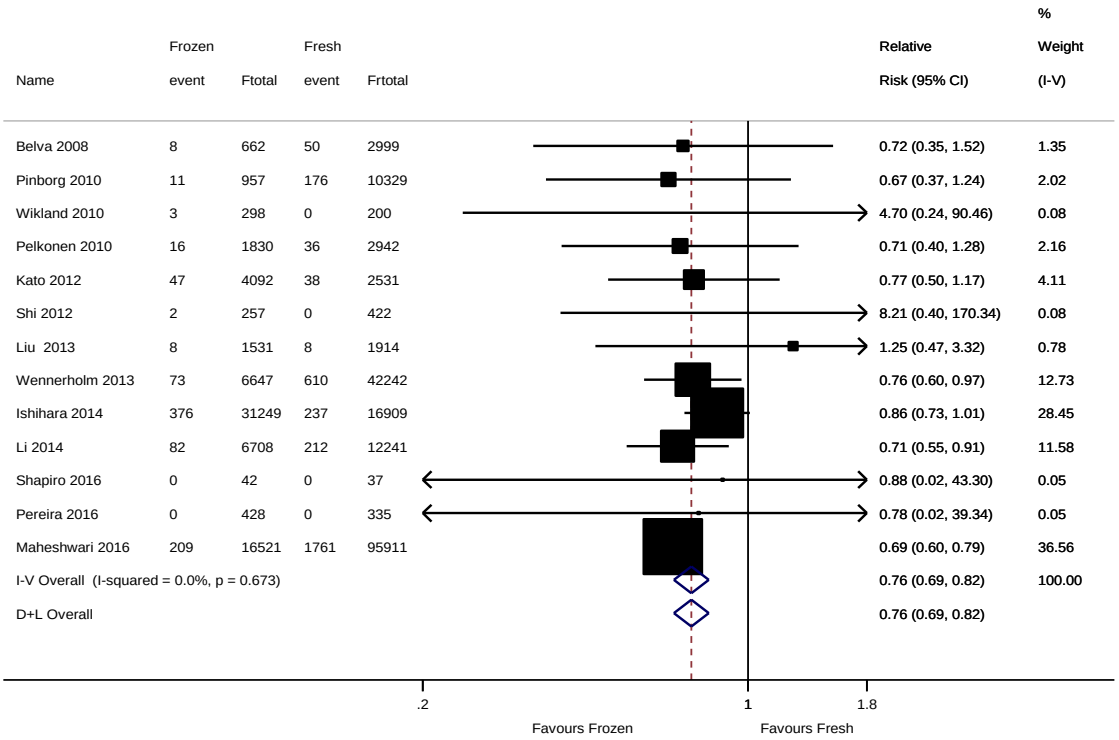


Figure 4b: Very low birth weight (Birth-weight less than 1500gms): Cumulative Meta-analysis

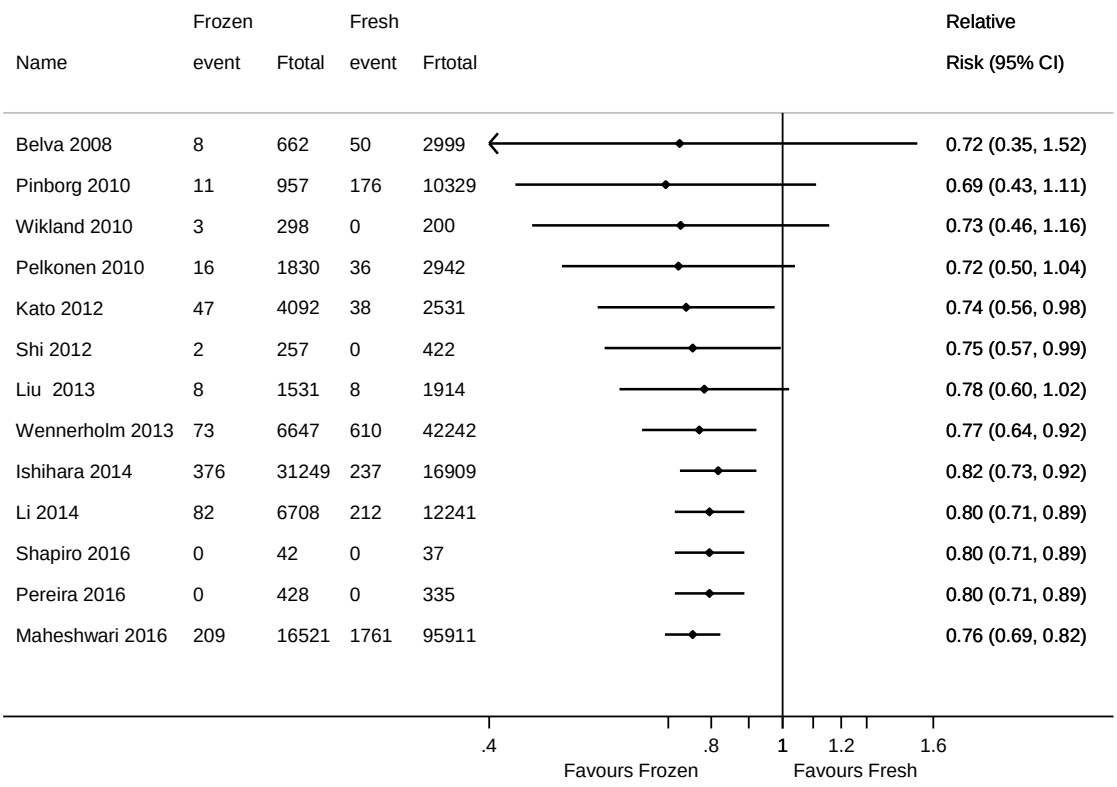


Figure 5a : Large for gestational age- Meta-analysis

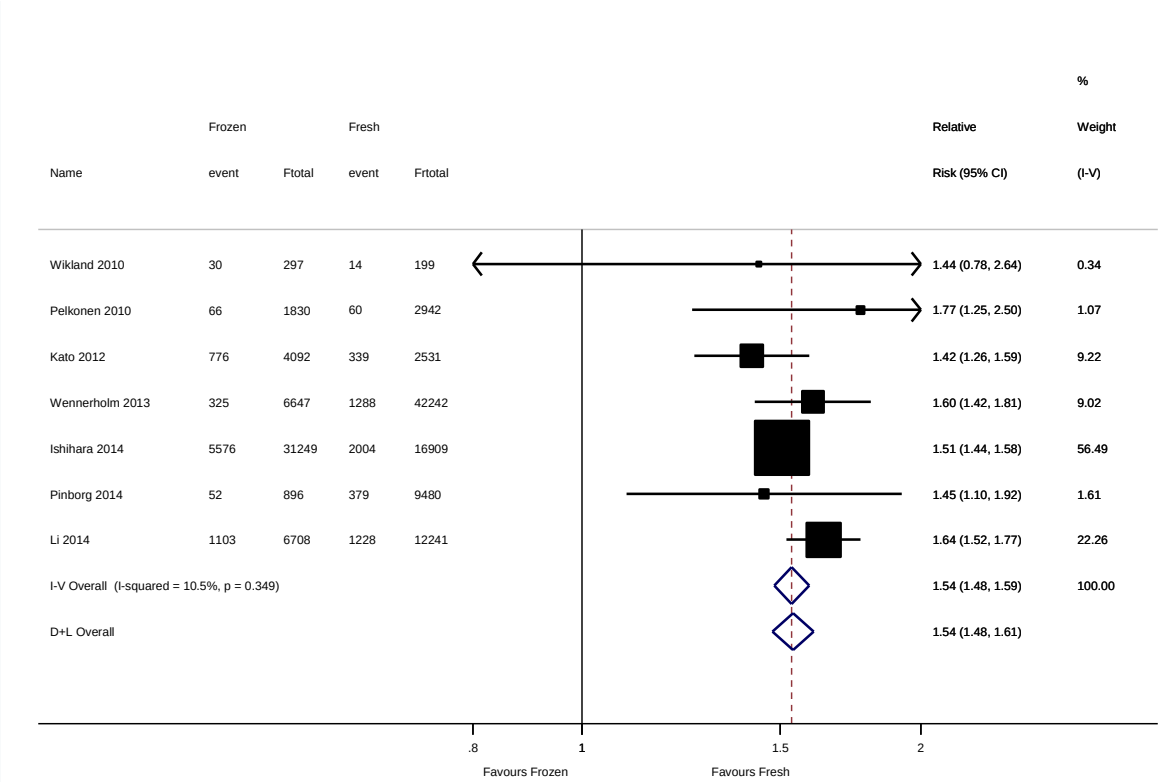


Figure 5b : Large for gestational age- Meta-analysis

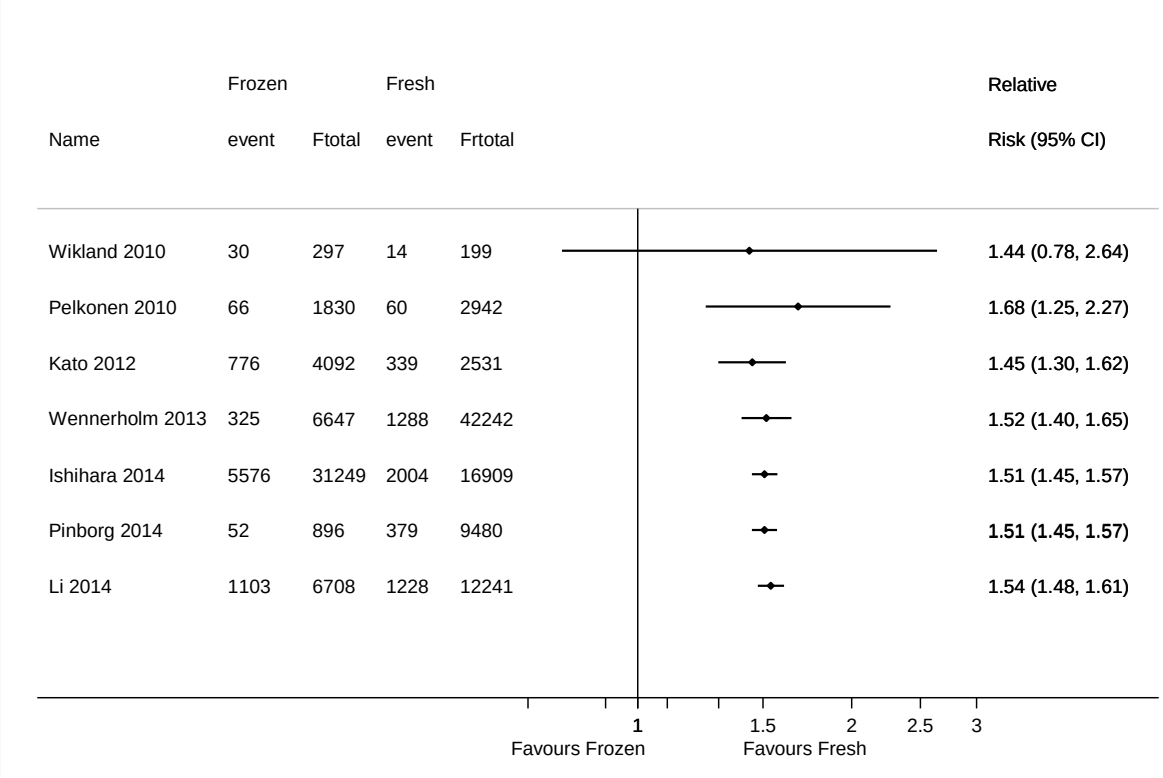


Figure 6a : High birth weight (birth weight > 4000 gm): meta-analysis

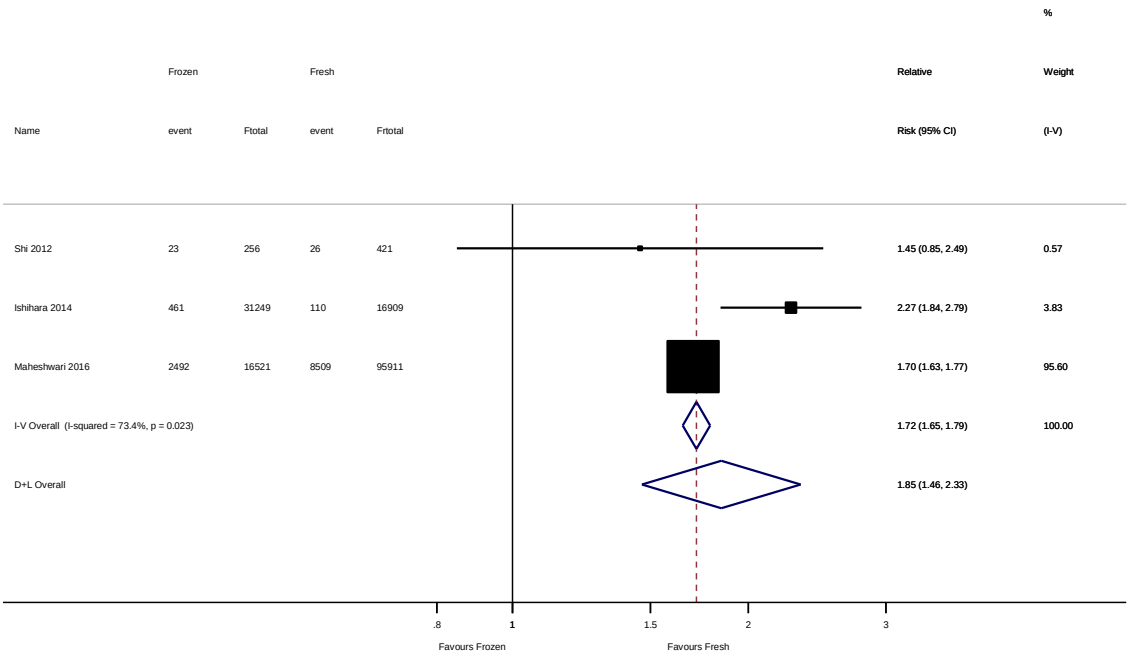


Figure 6b : High birth weight (birth weight > 4000 gm): Cumulative meta-analysis

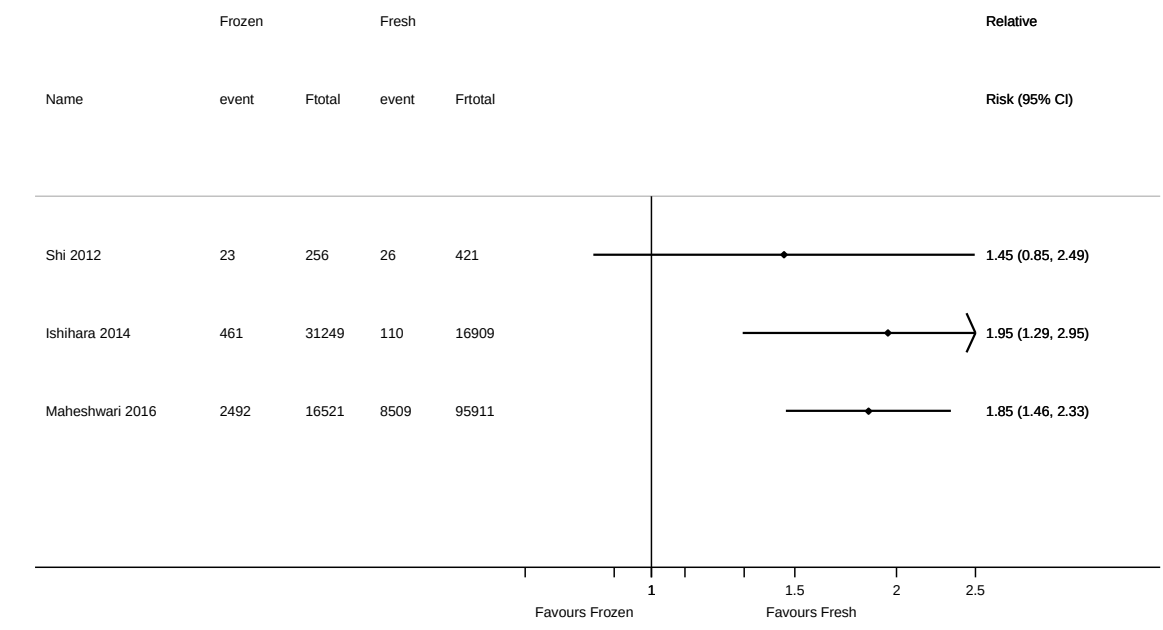


Figure 7a : Very High birth weight (birth weight > 4500 gm): meta-analysis

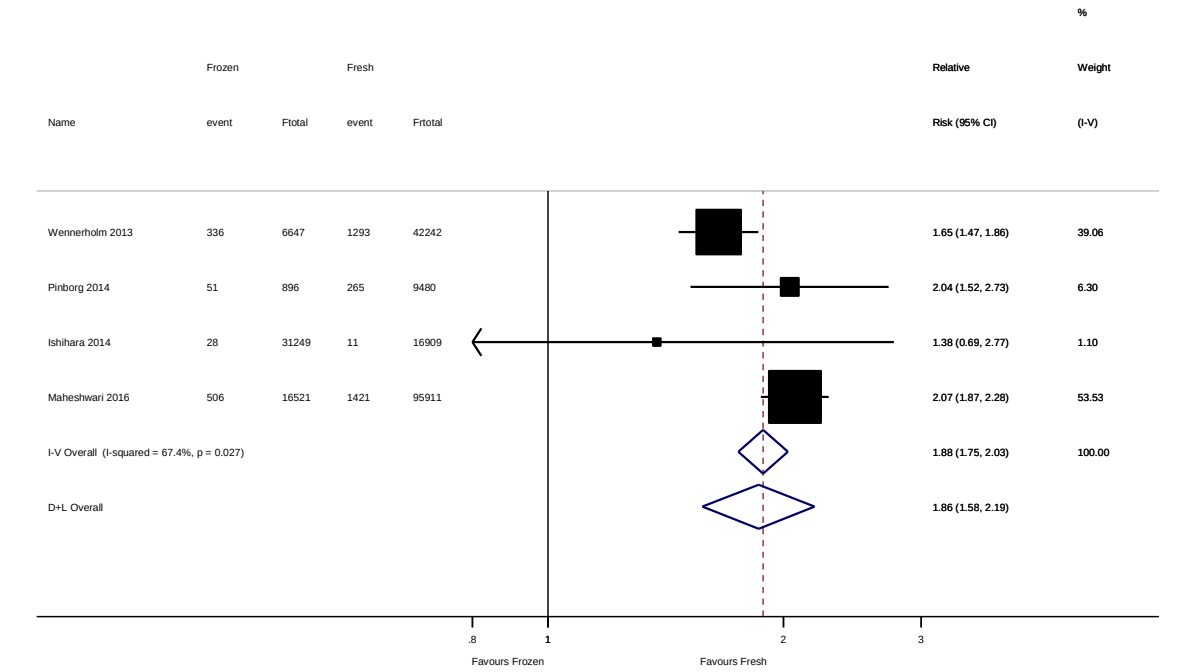


Figure 7b : Very High birth weight (birth weight > 4500 gm): Cumulative meta-analysis

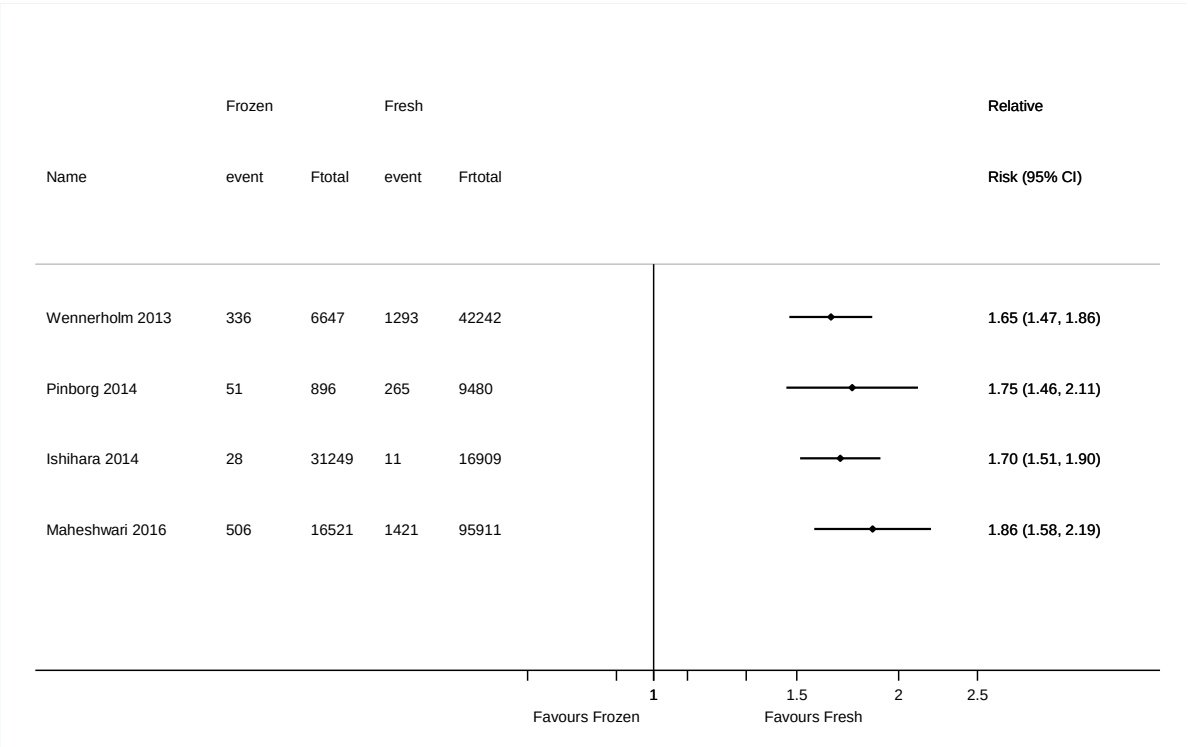


Figure 8a: Preterm delivery (Delivery at less than 37 weeks): Meta-analysis

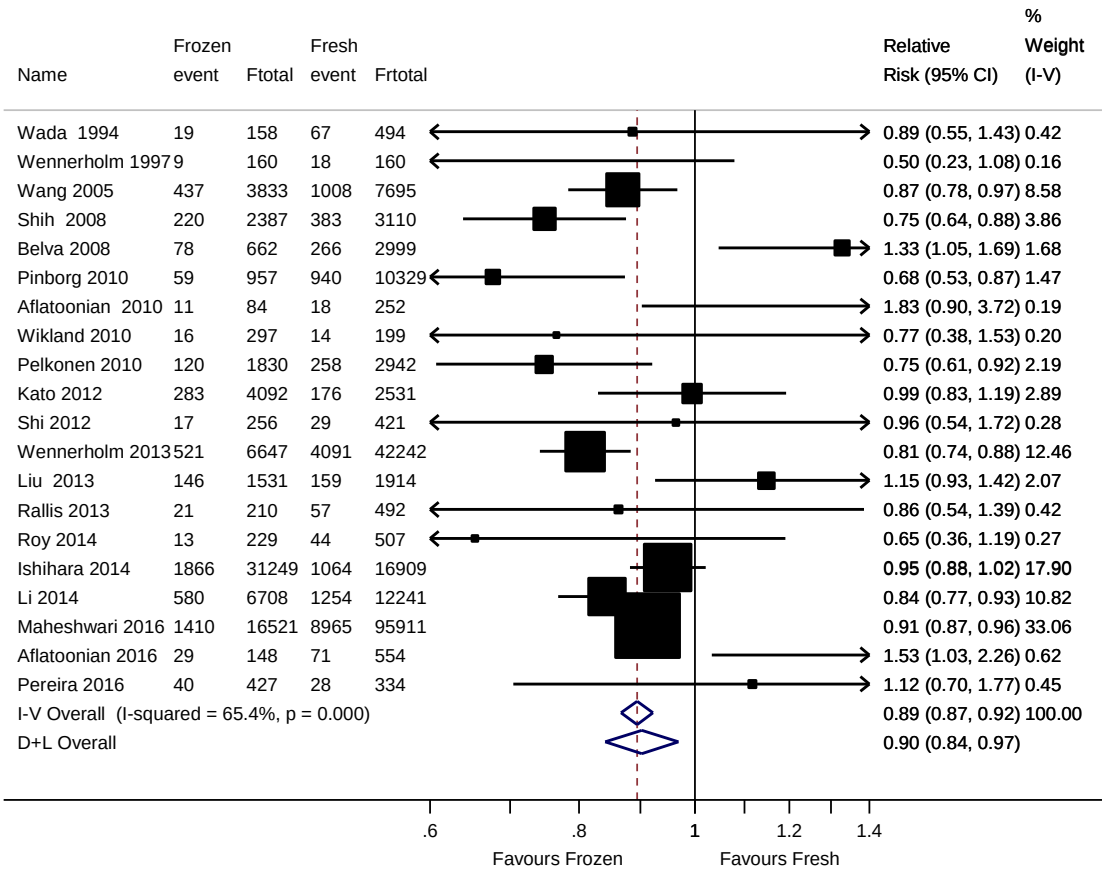


Figure 8b: Preterm delivery (Delivery at less than 37 weeks): Cumulative Meta-analysis

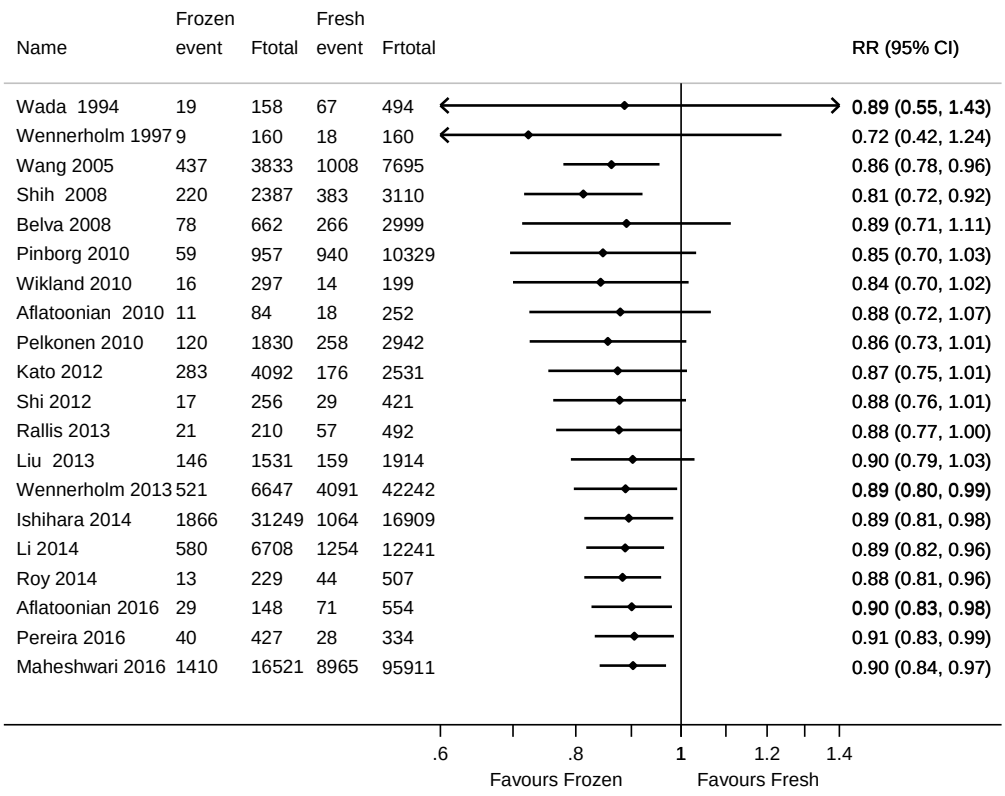


Figure 9a: Very Preterm delivery (Delivery at less than 32 weeks): Meta-analysis

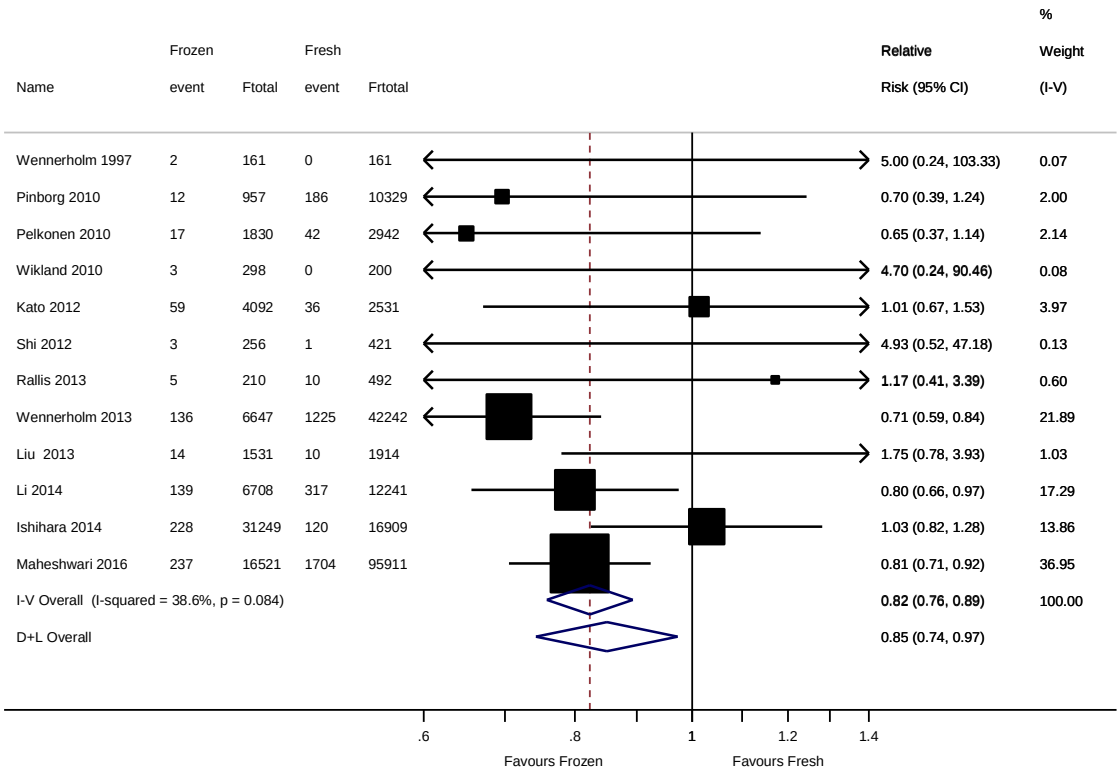


Figure 9b: Very Preterm delivery (Delivery at less than 32 weeks): Cumulative Meta-analysis

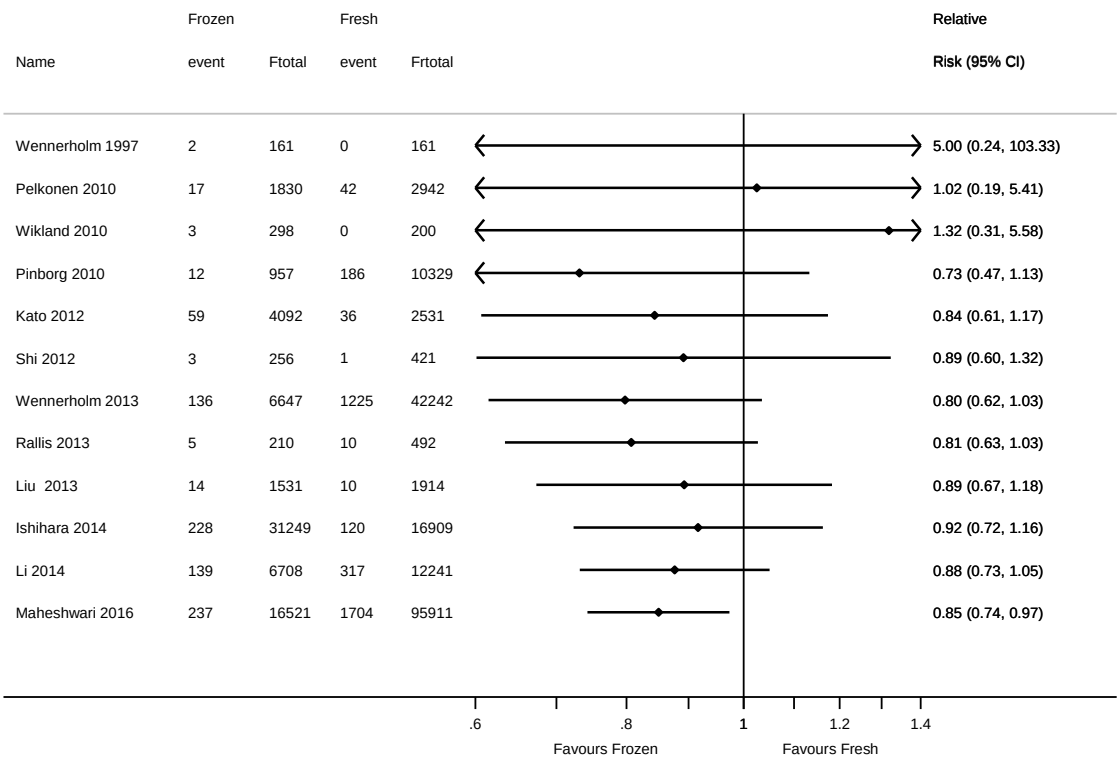


Figure 10 a: Antepartum Haemorrhage : Meta-analysis

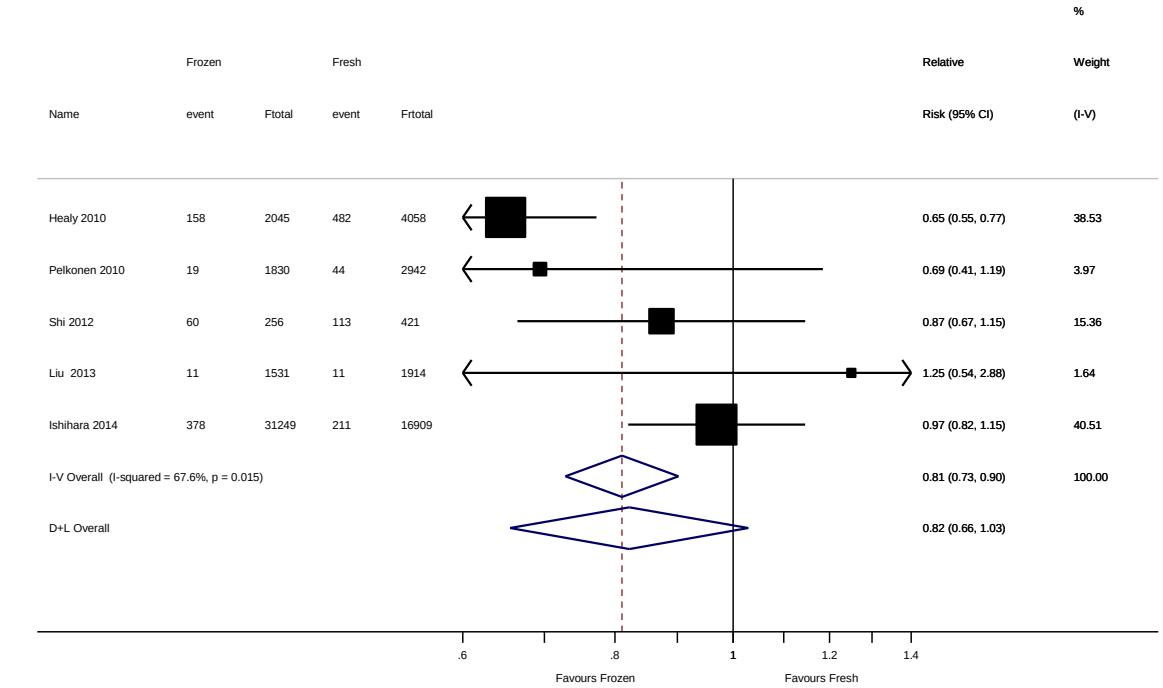


Figure 10 b: Antepartum Haemorrhage : Cumulative Meta-analysis

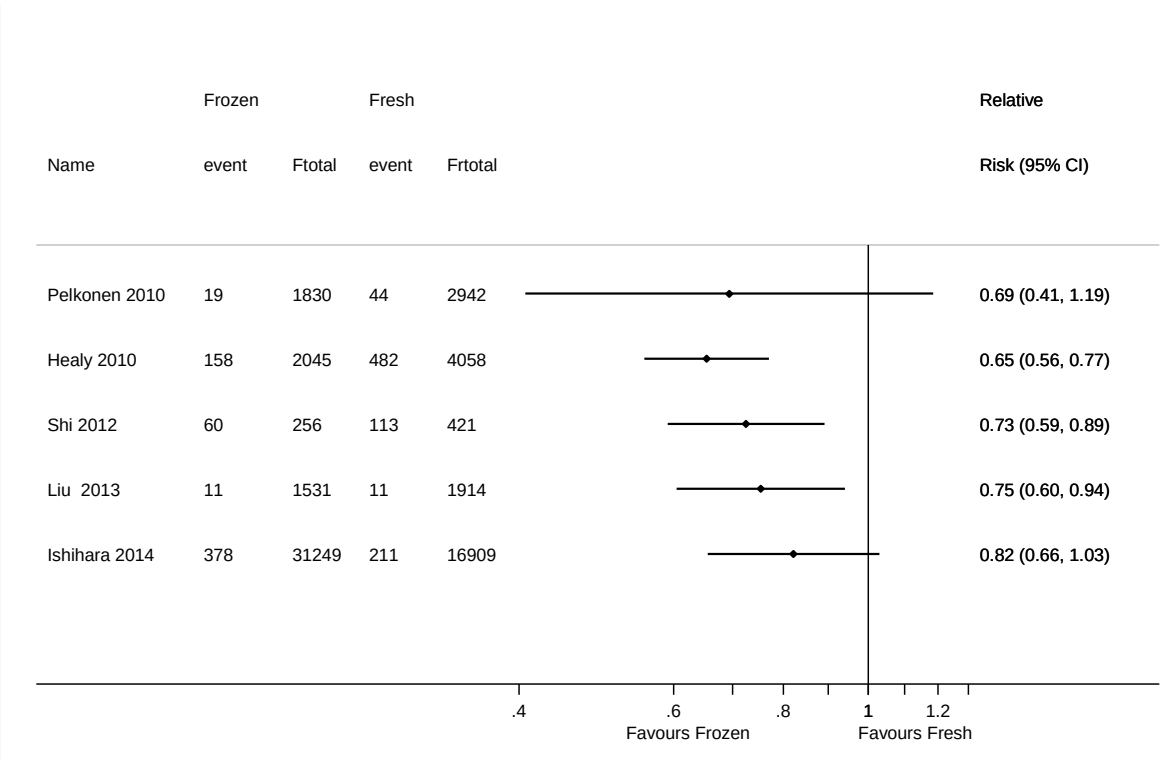


Figure 11a: Admission to Neonatal Intensive care Unit (NICU): Meta-Analysis

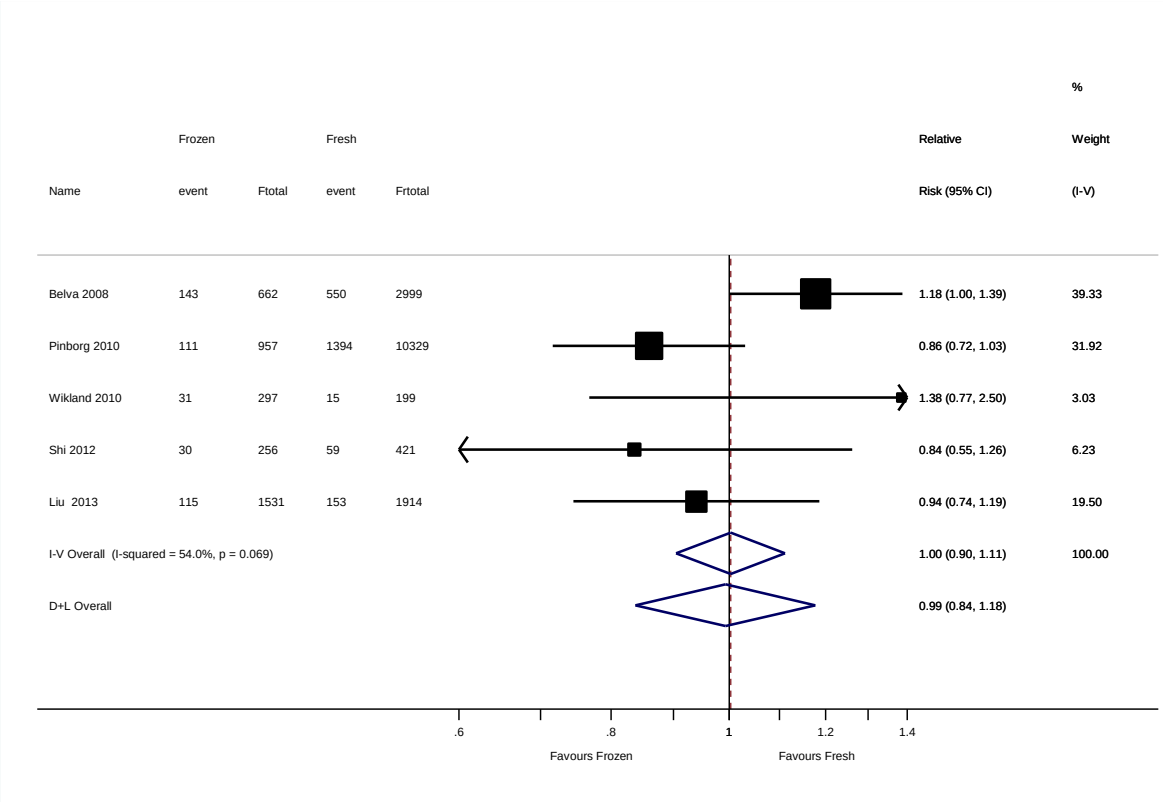


Figure 11b: Admission to Neonatal Intensive care Unit (NICU): Cumulative Meta-Analysis

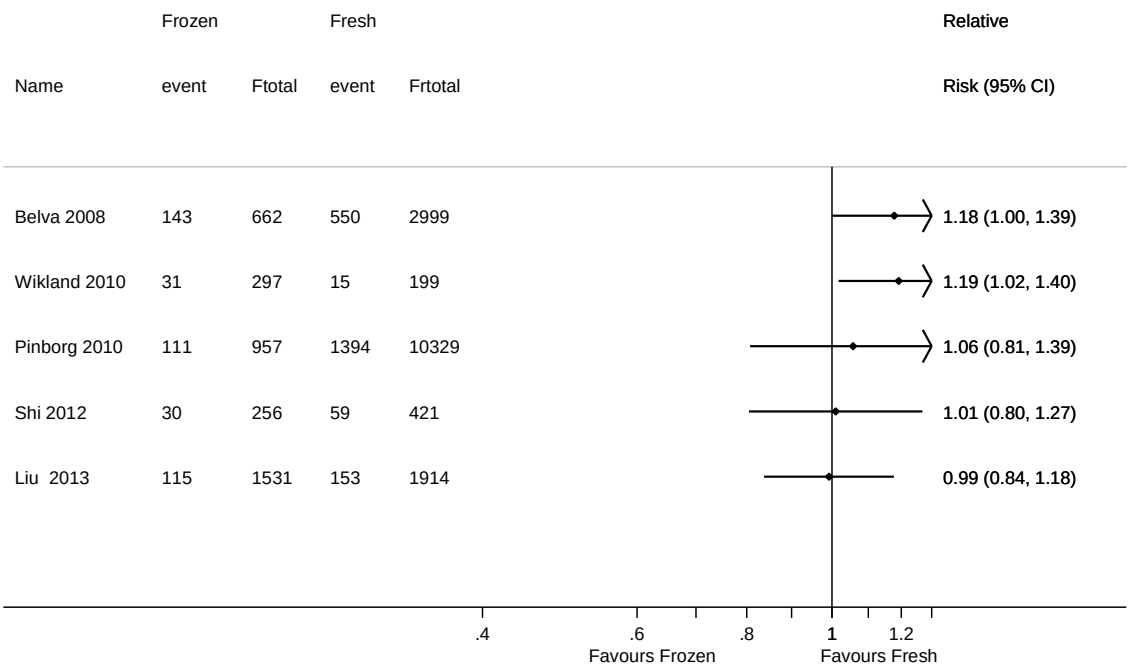


Figure 12 a: Congenital anomalies : Meta-analysis

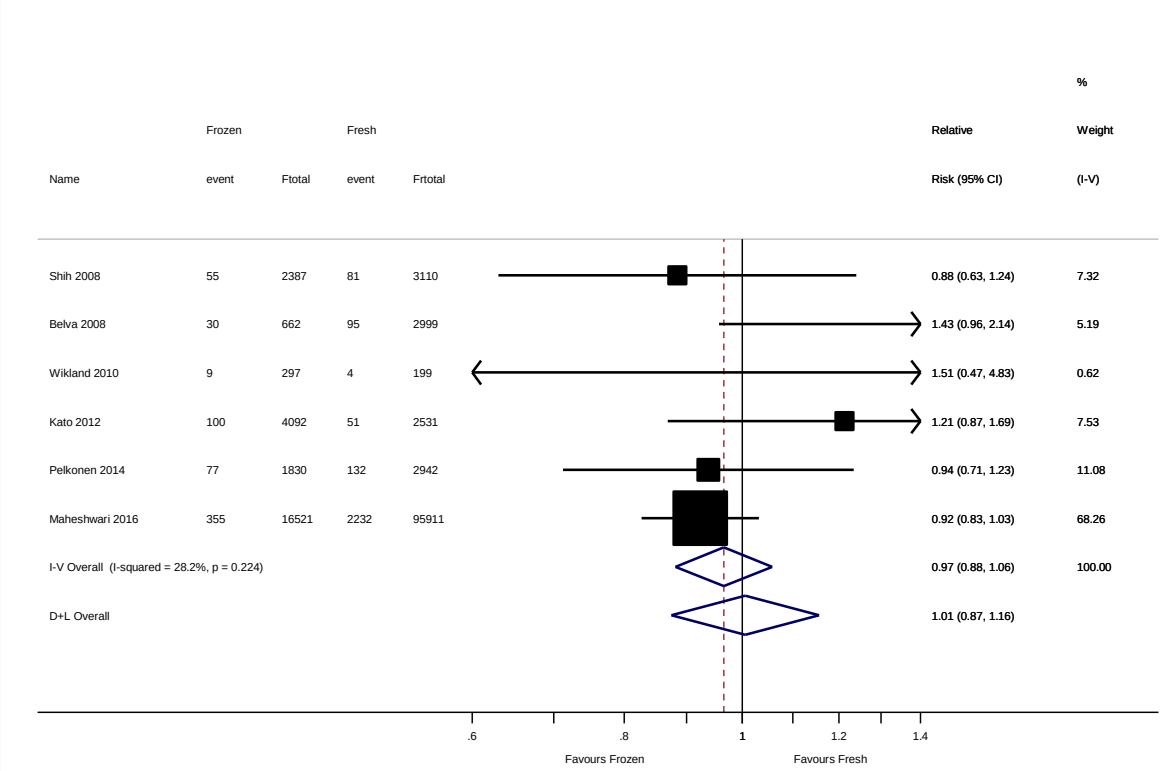


Figure 12 b: Congenital anomalies : Cumulative Meta-analysis

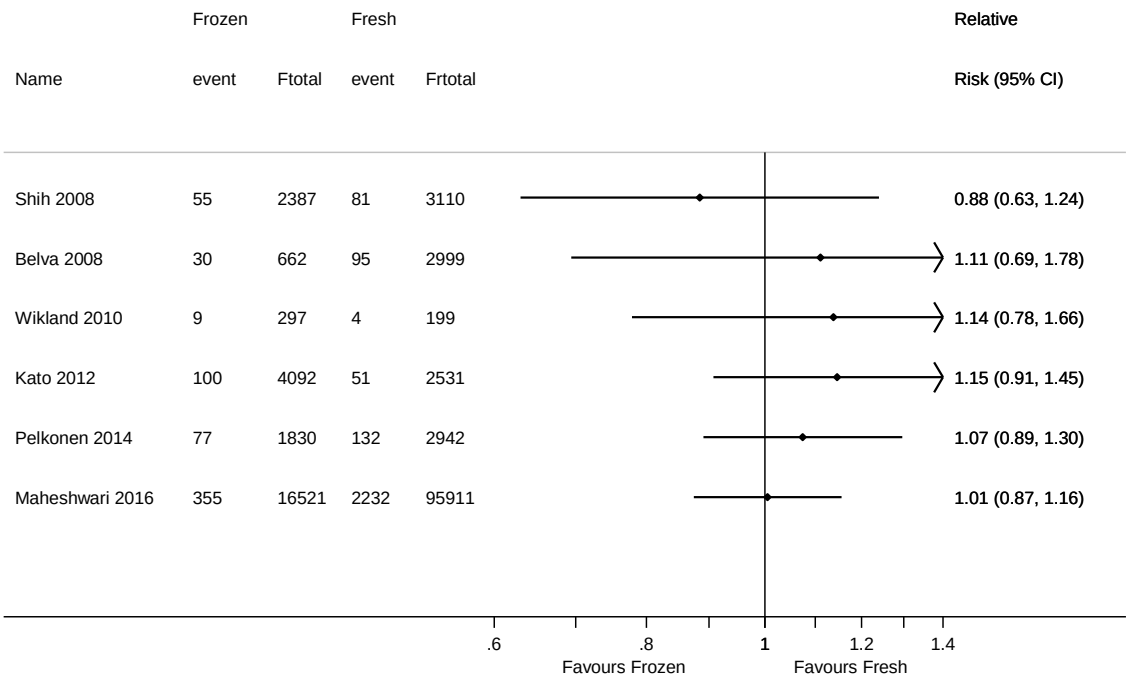


Figure 13a: Perinatal Mortality: Meta-analysis

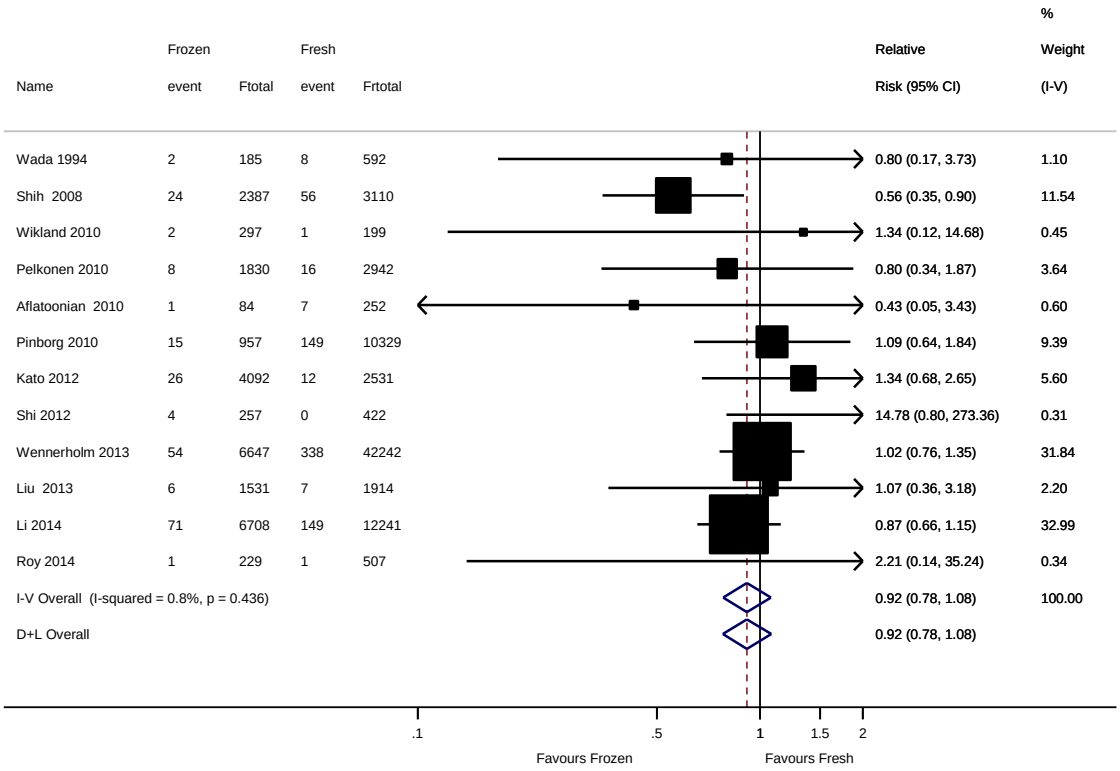


Figure 13b: Perinatal Mortality: Cumulative Meta-analysis

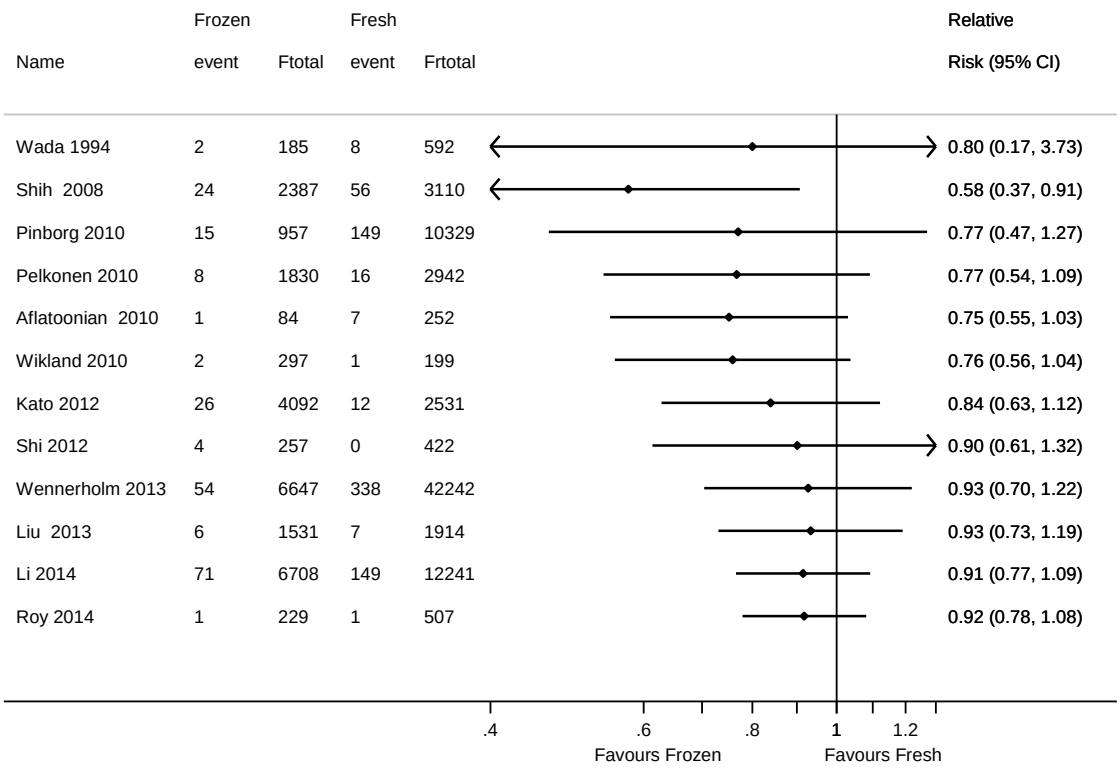


Figure 14 a: Hypertensive disorders of pregnancy: Meta-Analysis

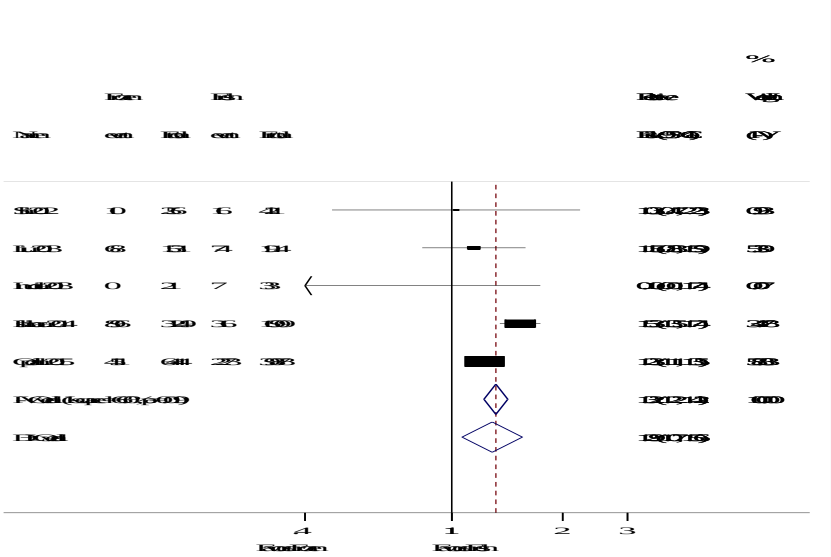
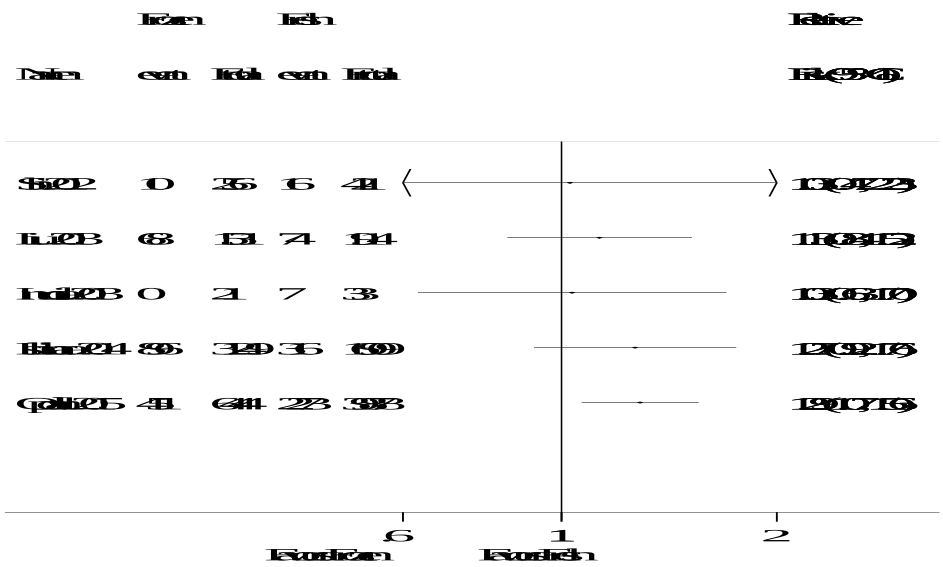


Figure 14 b: Hypertensive disorders of pregnancy: Cumulative Meta-Analysis



PRISMA 2009 Flow Diagram