

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

3

4 **Running Title: Obstetric outcomes with frozen versus fresh embryos**

5

6 Abha Maheshwari\*<sup>1</sup>, Shilpi Pandey<sup>2</sup>, Edwin Amalraj Raja<sup>3</sup>, Ashalatha Shetty<sup>1</sup>, Mark  
7 Hamilton<sup>1</sup>, Siladitya Bhattacharya<sup>3</sup>,

8 <sup>1</sup> **Aberdeen Maternity Hospital NHS Grampian , Scotland ,UK**

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

10 <sup>2</sup> CARE Nottingham, UK

11 <sup>3</sup> Institute of Applied Health Sciences, University of Aberdeen, Aberdeen UK

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13	<b>Table of contents</b>
14	
15	• Abstract
16	
17	• Introduction
18	
19	• Methods
20	○ Data sources and searches
21	○ Study selection
22	○ Outcome measures
23	○ Assessment of heterogeneity
24	○ Assessment of reporting
25	○ Statistical analysis
26	
27	• Results
28	○ Results of searches
29	○ Included studies
30	○ Outcomes
31	
32	• Discussion
33	○ Principal findings
34	○ Strengths
35	○ Limitations
36	○ Comparison with other studies
37	○ Explanation of results
38	○ Implications for clinical practice
39	○ Implications for research
40	○ Conclusions
41	
42	
43	

44 **Abstract**

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

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

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

63 **Outcomes:** Twenty-six studies met the inclusion criteria. Singleton babies conceived from  
64 frozen thawed embryos were at lower risk (RR, 95% CI) of preterm delivery (0.90 95% CI:  
65 0.84- 0.97) low birth weight (0.72, 95% CI: 0.67-0.77) and small for gestational age (0.61,  
66 95% CI: 0.56-0.67) compared to those conceived from fresh embryo transfers, but faced an  
67 increased risk (RR, 95% CI) of hypertensive disorders of pregnancy (1.29, 95% CI: 1.07-1.56)

68 large for gestational age (1.54, 95% CI: 1.48-1.61) and high birthweight (1.85, 95% CI: 1.46-  
69 2.33). There was no difference in the risk of congenital anomalies and perinatal mortality  
70 between the two groups. The direction and magnitude of effect for these outcomes have  
71 remained virtually unchanged over time while the degree of precision has improved with  
72 the addition of data from newer studies.

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

85

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

89

90

91 **Introduction**

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

98 The first live birth following the transfer of thawed cryopreserved embryos was reported in  
99 1984. With refinement of technology over the last few decades, the number of frozen  
100 embryo transfers has increased as have pregnancy rates which, according to some authors,  
101 are better than those following the transfer of fresh IVF embryos (Chen et al., 2016).

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

110

111 **Materials and Methods**

112 PRISMA guidelines for systematic reviews were followed

113 (<http://www.plosmedicine.org/article/info:doi%2F10.1371%2Fjournal.pmed.1000097>). The

114 protocol was registered at PROSPERO (CRD42016046131).

115

116 **Data sources and searches**

117 A literature search with no language restrictions was performed (1984-2016) on Medline,  
118 EMBASE, Cochrane Central Register of Clinical Trials, CINAHL and DARE (Appendix 1).  
119 Relevant journals in the specialty (Human Reproduction, Human Reproduction Update, RBM  
120 online and Fertility and Sterility) were searched electronically. Cross references from the  
121 included studies were hand searched. Two review authors (AM, SP) independently  
122 conducted the searches and selected the studies to be included. Differences of opinion were  
123 resolved after team discussion. Contact with authors was attempted wherever additional  
124 information was needed. Data were extracted using pre designed 2x2 tables.

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

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

134

135 **Study selection**

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

139 Exclusion criteria excluded studies if there was no comparator group, obstetric and perinatal  
140 outcomes were not reported or if it was not possible to differentiate the outcomes for  
141 singletons. Case reports and case series were excluded.

142

### 143 **Outcome measures**

144 The following outcome measures were included : small for gestational age (as defined by  
145 the authors of included studies) , very preterm birth (delivery prior to 32 weeks); preterm  
146 birth (delivery prior to 37 weeks); low birth weight (birth weight < 2500gm); very low birth  
147 weight (birth weight< 1500 gm); high birth weight ( > 4000gm); very high birth weight (>  
148 4,500 gm);; large for gestational age (as defined by the authors of the included studies);  
149 antepartum haemorrhage (combination of placenta praevia, placental abruption and other  
150 bleeding) hypertensive disorders of pregnancy (including pregnancy induced hypertension,  
151 pre-eclampsia and eclampsia), congenital anomalies (major & minor), perinatal mortality  
152 (as defined by the authors of the included studies) and admission to neonatal intensive care  
153 unit.

154

### 155 **Assessment of heterogeneity**

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

160

### 161 **Assessment of reporting biases**

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

166

## 167 **Statistical analysis**

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

181

## 182 **Results**

### 183 **Results of the searches**

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



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

193

## 194 **Included studies**

### 195 Methodology of included studies

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

202

### 203 Population in the included studies

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

210 al., 2014; Wennerholm et al., 2014; Opdahl et al., 2015) and beyond 28 weeks (Liu et al.,  
211 2013; Wennerholm et al., 1997; Wikland et al., 2010) and only live deliveries (Piereria et al.,  
212 2016).

213 Three studies (Healy et al., 2010; Wang et al., 2005; Opdahl et al., 2015) provided no  
214 information on the demographic profile of women who had fresh or frozen embryo transfer,  
215 as this comparison was part of a subgroup analysis. The characteristics in the two groups  
216 were similar except in Pinborg et al., 2010 & 2014 (data adjusted at primary analysis);  
217 Pelkonen et al., 2010 & Belva et al., 2008 (mothers in frozen embryo transfer group were  
218 older) and Pelkonen et al., 2014 (higher proportion of nulliparous women in fresh embryo  
219 transfer group). No details on other confounders such as parity, smoking, duration of  
220 infertility and pre-existing medical diseases were available.

221

#### 222 Exposure in the included studies

223 Studies varied in terms of when and how embryos were frozen, and methods used for  
224 endometrial preparation prior to embryo transfer after thawing. Methods of  
225 cryopreservation and the developmental stage at which embryos were frozen (Table I) also  
226 varied within same study especially in registry based datasets. Embryos were frozen either  
227 at day 2/3- cleavage stage (Aflatoonian et al., 2010, 2014; Imudia et al., 2013; Liu et al.,  
228 2013; Pelkonen et al., 2010; Shi et al., 2012; Wada et al., 1994) or day 5/6- blastocyst stage  
229 (Li et al., 2014 Piereria et al., 2016; Roy et al., 2014) or both (Belva et al., 2008; Kato et al.,  
230 2011) using either vitrification (Aflatoonian et al., 2010, 2014; Kato et al., 2011; Piereria et  
231 al., 2016; Shi et al., 2012) or slow freezing (Belva et al., 2008; Imudia et al., 2013; Pelkonen  
232 et al., 2010; Wada et al., 1994) or both techniques (Li et al., 2014; Liu et al., 2013).

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

236

## 237 **Outcomes**

238 Pooled data for outcome measures were as follows.

239

### 240 Small for gestational age

241 Ten studies (n= 53,418 vs. 89,044 pregnancies following frozen vs. fresh cycles) have  
242 reported on the outcome of small for gestational age. This was defined as birth weight less  
243 than 2 standard deviation of mean for that gestation (Ishihara et al., 2014; Pelkonen et al.,  
244 2010; Pinborg et al., 2014; Wennerholm et al., 2013) or less than 10<sup>th</sup> centile (Aflatoonian et  
245 al., 2016; Kato et al., 2012; Li et al., 2014) or birth weight less than 22% of expected mean  
246 birth weight according to gestational age in a reference population (Wikland et al., 2010).

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

252 A statistically significant reduction in small for gestational age babies was first observed in  
253 2010 after first publication (RR, 95% CI- 0.49, 0.33 to 0.75). Although subsequent studies  
254 have increased the precision of the point estimate, no substantive change has occurred in  
255 the direction or magnitude of the treatment effect (Figure 2b).

256

257 Low birth weight (birth weight < 2500 gm)

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

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

268

269 Very low birth weight (birth weight < 1500gm)

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

276 Cumulative meta-analysis shows (Figure 4b) that this evidence has been available since  
277 2012. Although subsequent studies have increased the precision of the point estimate, no  
278 substantive change has occurred in the direction or magnitude of the treatment effect.

279

280 Large for gestational age

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

287 The relative risk (95% CI) of having a large for gestational age baby was higher [1.54 (1.48-  
288 1.61)] in singleton pregnancies subsequent to frozen thawed embryo transfer, as compared  
289 to those conceived following fresh embryo transfer (Figure 5a). There was minimal  
290 heterogeneity amongst the studies ( $I^2 = 11\%$ ). Funnel plot suggest no publication bias  
291 ( $p=0.73$ ).

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

295

#### 296 High birth weight (birth weight > 4000 gm)

297 Three studies reported the outcome of birth weight > 4000gm (n= 48, 026 vs. 113, 241  
298 pregnancies following frozen vs. fresh embryo transfer). There was an increased risk (Figure  
299 6a) of having a baby with birth weight > 4000gm in singleton pregnancies as a result of  
300 frozen embryo transfer when compared to those subsequent to fresh embryo transfer [RR-  
301 1.85; 95% CI (1.46-2.33)].

302 A statistically significant effect was first observed in 2014 after first publication [RR-1.95, ;  
303 95% CI (1.29-2.95)]. Additions of data from subsequent large studies have increased the

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

306

307 Very high birth weight (birth weight > 4500 gm)

308 Four studies have reported the outcome of birth weight > 4500 gm (n= 55,313 vs. 164,542  
309 pregnancies following frozen vs. fresh embryo transfer). There is an increased risk (Figure  
310 7a) of having a baby with birth weight > 4500 gm in singleton pregnancies as a result of  
311 frozen embryo transfer when compared to those subsequent to fresh embryo transfer [RR  
312 1.86; 95% CI (1.58-2.19)].

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

317

318 Preterm delivery (delivery at less than 37 weeks)

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

323 The relative risk of having a delivery at less than 37 weeks was reduced [0.90 (95% CI 0.84,  
324 0.97)] in singleton pregnancies following frozen thawed embryo transfer, when compared to  
325 those after fresh embryo transfers (Figure 8a). There was marked heterogeneity ( $I^2=65\%$ )  
326 amongst the studies. Funnel plot did not reveal any publication bias ( $p=0.73$ ).

327 Cumulative meta-analysis (Figure 8) suggests that the evidence favouring frozen embryo  
328 transfer in terms of a reduced risk of preterm delivery was first available in 2005. In 2013  
329 the addition of further data showed that there was no difference in the risk of preterm  
330 delivery between the two groups. However, new results from studies published after 2013  
331 have re-confirmed the reduced risk of preterm delivery. Addition of several studies from  
332 2014-2016 have increased the precision of our estimate without affecting either in the  
333 direction or magnitude of the treatment effect.

334

#### 335 Very preterm birth (delivery at less than 32 weeks)

336 Twelve studies (n=68,927 vs. 184,377 pregnancies following frozen vs. fresh embryo  
337 transfer) reported on deliveries at < 32 weeks. The relative risk (RR, 95% CI) of a delivery at  
338 less than 32 weeks was lower [0.85 (95% CI 0.74-0.97)] in singleton pregnancies following  
339 frozen embryo thawed transfer when compared to those after fresh embryo transfer (Figure  
340 9a). There was moderate heterogeneity ( $I^2 = 38.6\%$ ) amongst the studies. We could not  
341 differentiate between iatrogenic and spontaneous preterm delivery. The funnel plot was  
342 suggestive of a degree of publication bias (p=0.04).

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

346

#### 347 Antepartum haemorrhage (APH)

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

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

355 There was no difference in risk of APH in singleton pregnancies following frozen thawed  
356 embryo transfer when compared to those after fresh embryos (RR 0.82; 95% CI: 0.66-1.03).

357 There was moderate heterogeneity (67.6%) amongst the studies (Figure 10a).

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

363

#### 364 Admission to neonatal intensive care unit (NICU)

365 Five studies reported the outcome of admission to NICU (n= 3,703 vs. 15,862 pregnancies  
366 after frozen vs. fresh embryo transfer). The length and the reasons for NICU admission were  
367 not specified. There was no increase in the risk of admission to NICU (RR 0.99; 95% CI: 0.84-  
368 1.18) in pregnancies following frozen embryos (Figure 11a). There was marked  
369 heterogeneity amongst the studies ( $I^2=54\%$ ).

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

373

#### 374 Congenital anomalies



375 Only 6 studies (n= 25,789 vs. 107,692 pregnancies following frozen vs. fresh embryo  
376 transfer) reported congenital anomalies (one matched cohort study). Both major and minor  
377 anomalies were pooled together. The relative risk of having a congenital anomaly was 1.01  
378 (95% CI 0.87, 1.16) in pregnancies following frozen thawed embryos as compared to fresh  
379 embryos (Figure 12a). There was minimal heterogeneity ( $I^2 = 28\%$ ) amongst the studies.  
380 Cumulative meta-analysis for congenital anomalies showed no clear trend regarding effect  
381 on pregnancies as a result of frozen embryo transfer. This has been consistent over time  
382 despite accrual of fresh data (Figure 12 b).

383

#### 384 Perinatal mortality

385 Twelve studies (n= 25,203 vs. 77,280 pregnancies following frozen vs. fresh embryo transfer)  
386 reported the outcome of perinatal mortality. Still birth and perinatal mortality are  
387 presented together in this report. Some studies reported only neonatal death (Roy et al.,  
388 2014; Shi et al., 2012). Of those who reported perinatal mortality there was a variation in  
389 definition : death of child with a gestational age of more than 20 weeks or up to day 28 of  
390 birth (Aflatoonian et al., 2010, 2016; Pinborg et al., 2010); deaths occurring after the 24th  
391 week of gestation and during the first week of life; after 22 weeks of gestation and first 7  
392 days of life (Kato et al., 2012); stillbirth after 28 weeks of gestation and first 7 days of life (Li  
393 et al., 2014, Liu et al., 2013; Pelkonen et al., 2010; Wennerholm et al., 2013; Wikland et al.,  
394 2010); still birth after 20 weeks, later terminations and all neonatal deaths (Shih et al.,  
395 2008).

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

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

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

403

#### 404 Hypertensive disorders of pregnancy

405 Five studies reported the outcome of hypertensive disorders of pregnancy ( $n = 39,501$  vs.  
406  $59,155$  pregnancies following frozen vs. fresh embryo transfer). The relative risk of  
407 hypertensive disorders of pregnancy were higher in frozen embryo transfer group (Figure  
408 14a) (RR 1.29; 95% CI- 1.07, 1.56). There was moderate heterogeneity ( $I^2 = 66\%$ ).

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

412

### 413 Discussion

414

#### 415 **Principal findings**

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

422

423 **Strengths**

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

430

431 **Limitations**

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

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

451

### 452 **Comparison with other studies**

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

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

470

471 **Explanation of results**

472 Hormonal stimulation of the ovaries in IVF causes a state of hyper-estrogeneism at a time  
473 when fresh embryos are transferred. It has been hypothesized that this leads to abnormal  
474 endometrial angiogenesis leading to reduced implantation as well as abnormal placentation.  
475 This can account for findings of small for gestational age babies, preterm deliveries and low  
476 birthweight babies. Uterine environment in a frozen replacement cycle is a more natural  
477 uterine environment as the effect of ovarian stimulation tends to worn off by the time point  
478 when embryos are replaced (Amor et al., 2009; Healy et al., 2010; Kalra et al., 2011).  
479 However there is as yet no clear explanation for the increased chance of large for  
480 gestational age births. It is possible that higher implantation potential leads to better  
481 placentation and overgrowth of the fetus. Birth order, which is higher in babies, conceived  
482 from frozen thawed embryos, may play a role, but has been challenged by the fact that the  
483 difference has persisted after adjustment for parity in various studies (Maheshwari et al.,  
484 2016; Pinborg et al., 2014). It has also been suggested that the freezing and thawing  
485 procedures may play an independent role for the growth potential of the fetus due to  
486 epigenetic alterations at the early embryonic stages (Pinborg et al., 2014).

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

488

489 **Implications for clinical practice**

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

494 are being electively frozen and reserved for deferred transfer. In fact, in some centres, a  
495 “freeze all” policy followed by thawed frozen embryo transfer has become the norm. It is to  
496 be remembered that both small for gestational age and large for gestational age has  
497 implications for health and diseases later in life. Hence, routine use of freeze all strategy  
498 may have long term implications as well. Moreover all the evidence has been graded as low  
499 quality (Supplementary Table 2) as per GRADE matrix, primarily due to observational data.  
500 We recommend that, on the basis of current evidence, elective freezing of all embryos  
501 should only be performed when there is a definite clinical indication or in the context of a  
502 clinical trial.

503

#### 504 **Implications for research**

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

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

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

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

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

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

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

541

542 **Conclusion**

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

554

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559

560 **Authors' roles**

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

565



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568

569 **Conflict of Interest**

570 AM and SB are co-applicants on the HTA/ NIHR grant, UK (ISCTRN-61225414) for E-Freeze

571 Trial which is a randomized controlled trial comparing elective freezing of embryos with

572 current policy of fresh embryo transfer. Otherwise the authors have no conflict of interest.

573

574

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#	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	
	Fetal Growth Retardation/ or Pregnancy Complications/ or Obstetric Labor		
8	Complications/ or Pregnancy/ or Pregnancy Outcome/ or "obstetric and perinatal complications".mp.	788905	
	Infant, Low Birth Weight/ or Infant, Small for Gestational Age/ or Birth Weight/ or		
9	Gestational Age/ or Infant, Premature/ or " small for gestational age".mp.	144285	
	Diabetes, Gestational/ or Premature Birth/ or "large for gestational age".mp. or		
10	Gestational Age/ or Pregnancy in Diabetics/	93954	
11	Birth Weight/ or Hypertension/ or " high birth weight".mp.	253898	
	Infant, Premature/ or Infant, Small for Gestational Age/ or Infant, Low Birth Weight/ or		
12	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	
	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		
24	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
Fetal Growth Retardation/ or Pregnancy Complications/ or Obstetric Labor	
36 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
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45 Hypertension, Pregnancy-Induced/ or Pre-Eclampsia/ or PIH.mp.	29056
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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	
52 51	1099362
53 32 and 35 and 52	2310
54 Singleton.mp.	14929
55 53 and 54	126

56 from 55 keep 4, 13, 15, 19, 21, 28...

16

**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)	<b>RR 0.61</b> (0.56 to 0.67)	6.1%	<b>3.7%</b> (3.4 to 4.1)	<b>2.4% fewer</b> (2.7 fewer to 2 fewer)	⊕⊕○○ LOW	
Birthweight <2500 gm (low birth weight ) № of participants: 280.19 (20 observational studies)	<b>RR 0.72</b> (0.67 to 0.77)	8.8%	<b>6.3%</b> (5.9 to 6.8)	<b>2.5% fewer</b> (2.9 fewer to 2 fewer)	⊕⊕○○ LOW	
Large for gestational age (LGA) № of participants: 138263 (7 observational studies)	<b>RR 1.54</b> (1.48 to 1.61)	6.1%	<b>9.5%</b> (9.1 to 9.9)	<b>3.3% more</b> (2.9 more to 3.7 more)	⊕⊕○○ LOW	
Preterm deivery (PTL) № of participants: 280622 (20 observational studies)	<b>RR 0.90</b> (0.84 to 0.97)	9.4%	<b>8.4%</b> (7.9 to 9.1)	<b>0.9% fewer</b> (1.5 fewer to 0.3 fewer)	⊕⊕○○ LOW	
Hypertensive disorders of pregnancy (PIH) № of participants: 98656 (5 observational studies)	<b>RR 1.29</b> (1.07 to 1.56)	4.5%	<b>5.9%</b> (4.9 to 7.1)	<b>1.3% more</b> (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

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**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

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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 subgroup 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
<a href="#">Shapiro et al., 2016</a>	<a href="#">Post-hoc analysis of two RCT</a>	<a href="#">Two RCTs from same centre one on hyper responders and one on normal responders</a>	<a href="#">Birth weight outcome; post-hoc analysis</a>	<a href="#">Data obtained through personal communication</a>	NA
Maheshwari et al., 2016	Retrospective analysis	Retrospective analysis of anonymized 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
<a href="#">Opdahl et al., 2015</a>	<a href="#">Unmatched cohort study</a>	<a href="#">Nationwide data from registries of Denmark, Norway and Sweden</a>	<a href="#">Data obtained from health registries</a>	<a href="#">Baseline characteristics for women having fresh or frozen embryo transfer were not compared. As fresh and frozen embryo transfers were one of multiple comparisons</a>	<a href="#">11/12</a>
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
<a href="#">Shapiro et al., 2016</a>	<a href="#">Post hoc analysis of two RCT</a>	<a href="#">Two RCTs from same centre one on hyper responders and one on normal responders</a>	<a href="#">Birth weight outcome; post hoc analysis</a>	<a href="#">Data obtained through personal communication</a>	<a href="#">NA</a>
Shih et al., 2008	Matched cohort with women acting as their own reference	Comparison groups: Frozen versus Fresh IVF/ICSI	Neonatal perinatal statistics unit Australia	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	Retrospective 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 from <del>an</del> singleton and twins <del>can't</del> be <del>seperated</del> <u>separated</u>
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	<del>No difference</del> Higher in Frozen	201 <del>5</del> 4	201 <del>5</del> 4	yes

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	<a href="#">embryo transfer</a>			
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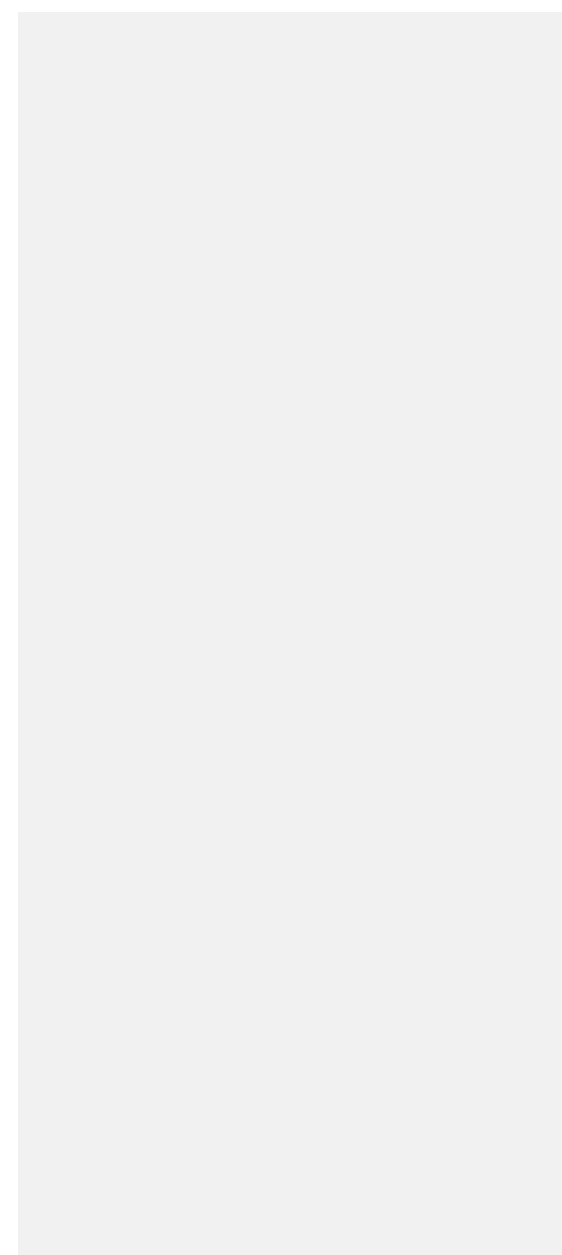


Figure 1: PRISMA flow chart for study selection

Attached as separate file

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

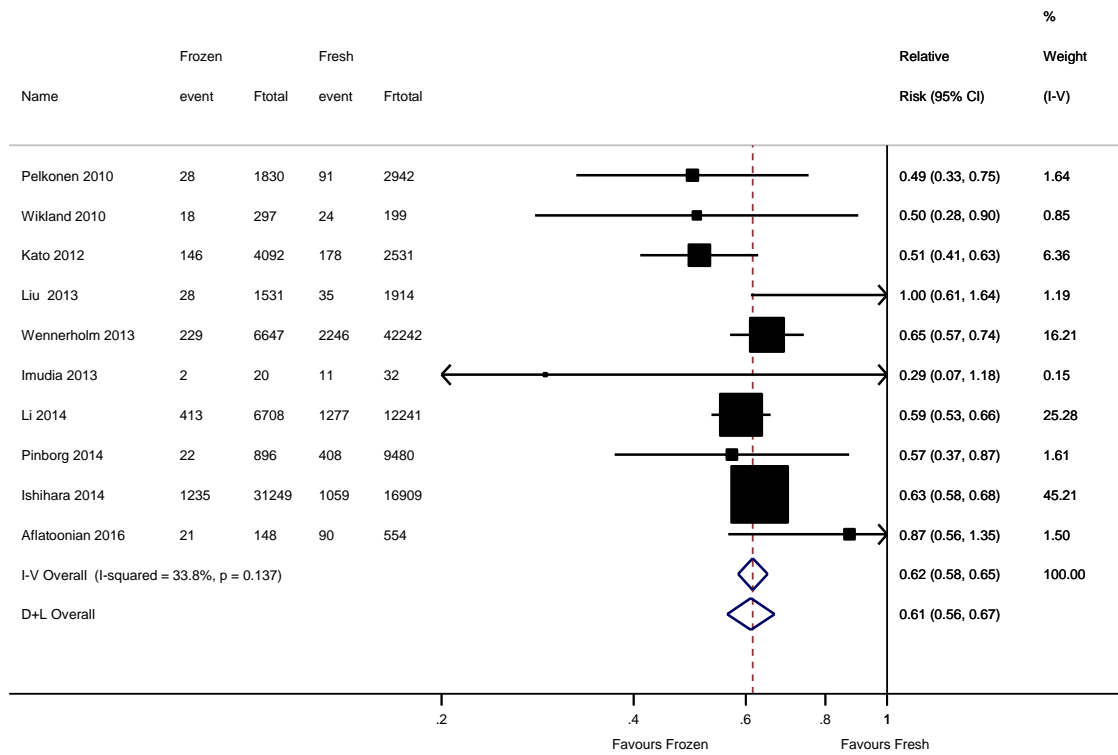


Figure 2b: Small for gestational age- Cumulative meta-analysis

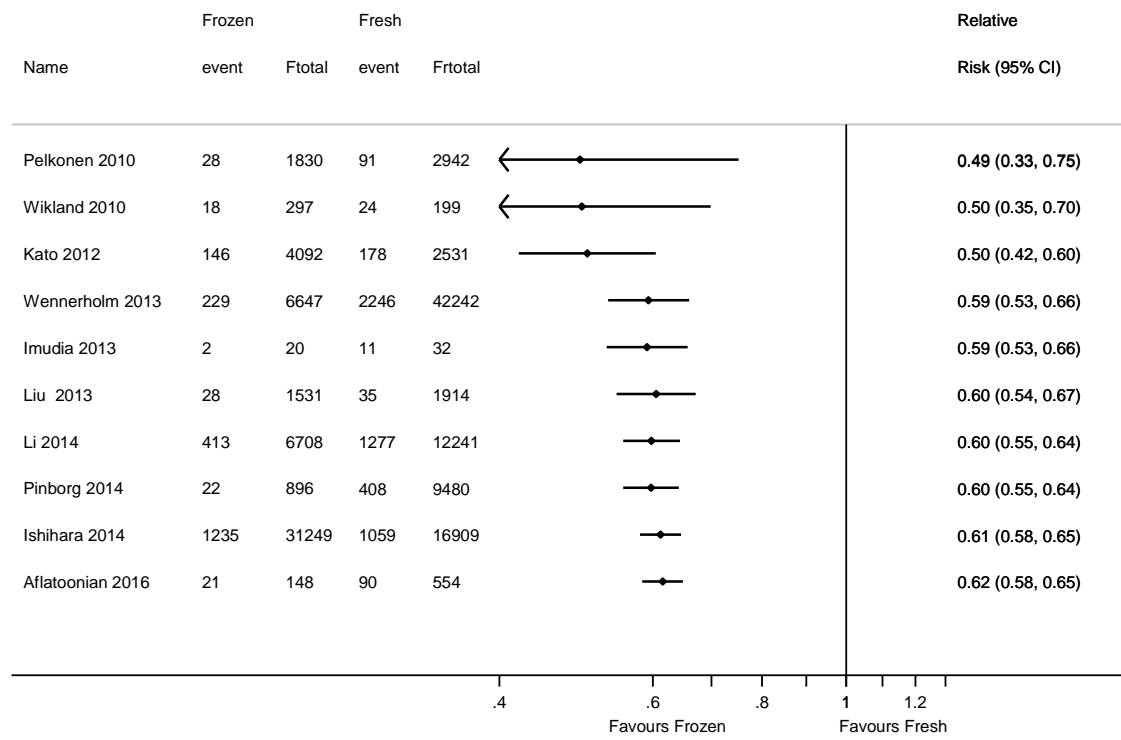






Figure 3b : Low birth weight (Birth-weight less than 2500 gm) : Cumulative meta-analysis

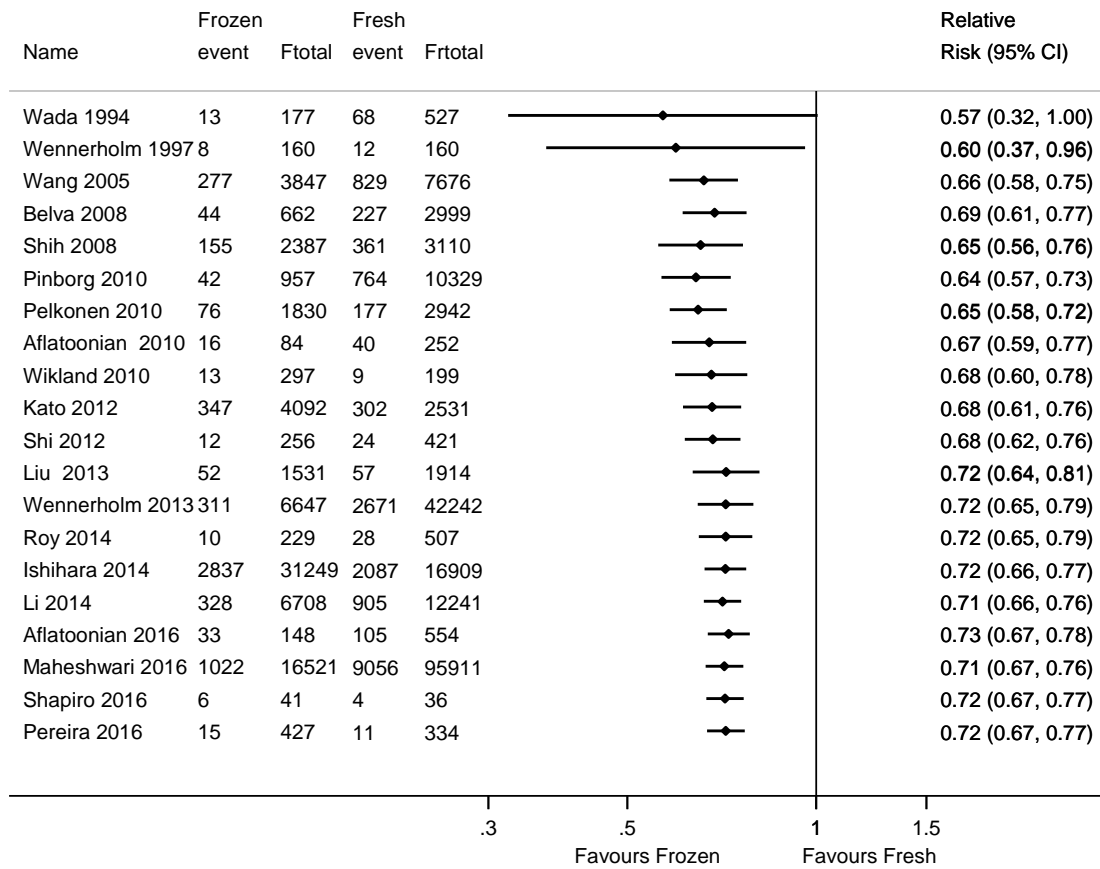






Figure 5a : Large for gestational age- Meta-analysis

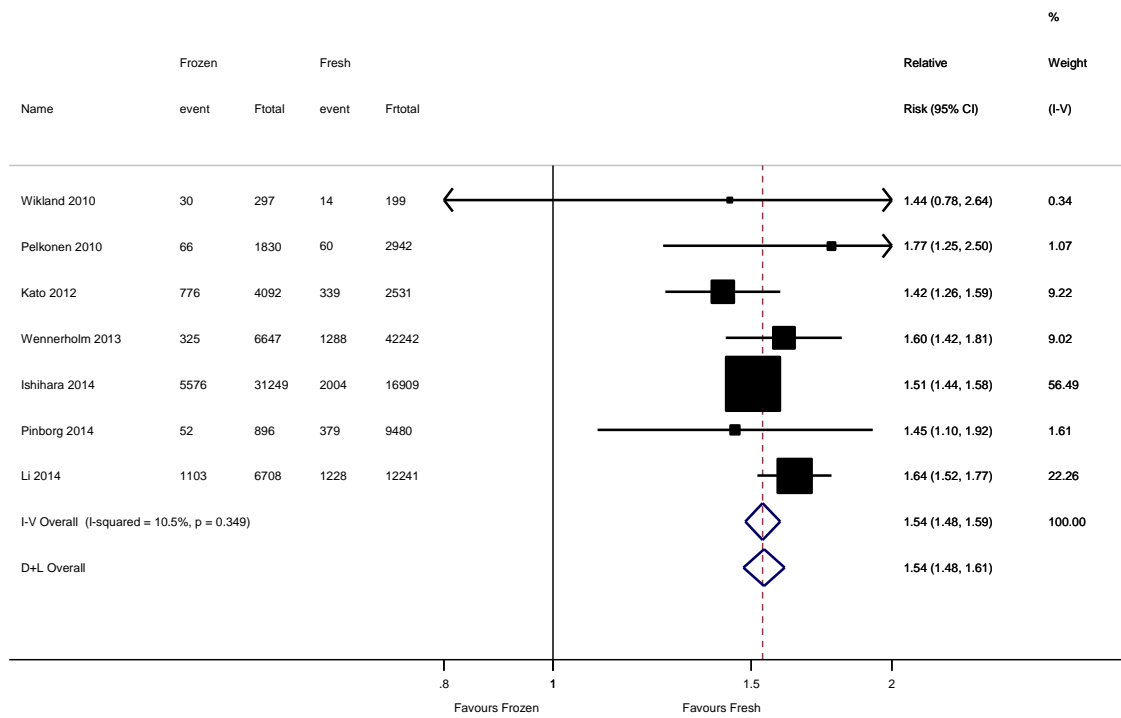


Figure 5b : Large for gestational age- Meta-analysis

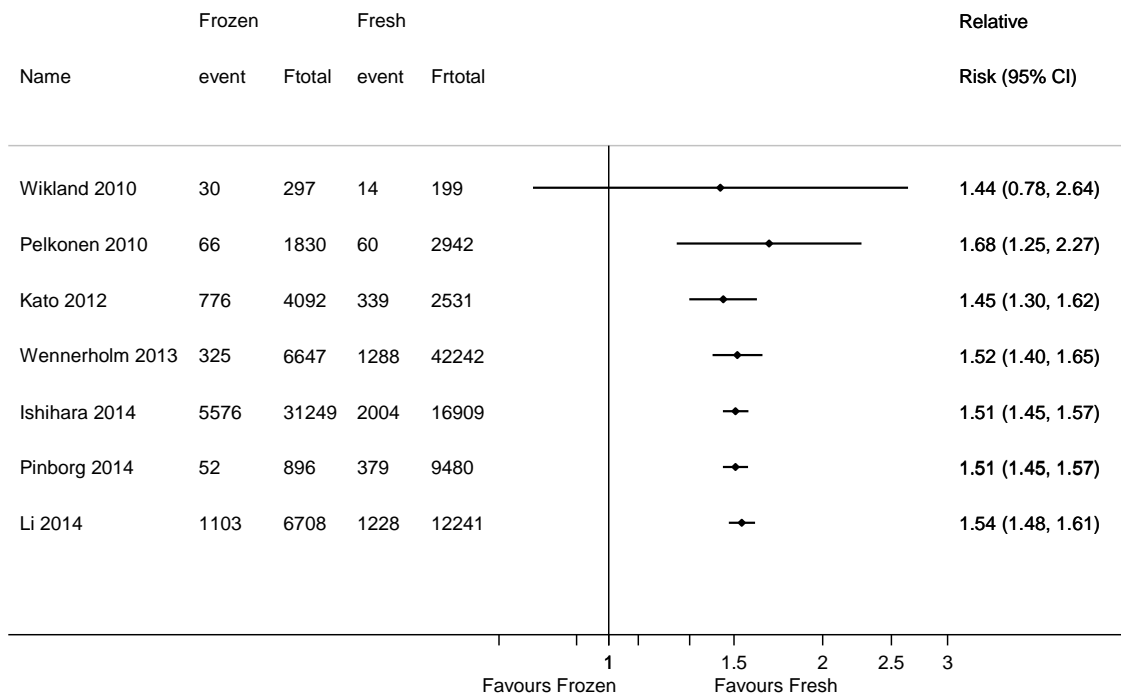


Figure 6a : High birth weight (birth weight > 4000 gm): meta-analysis

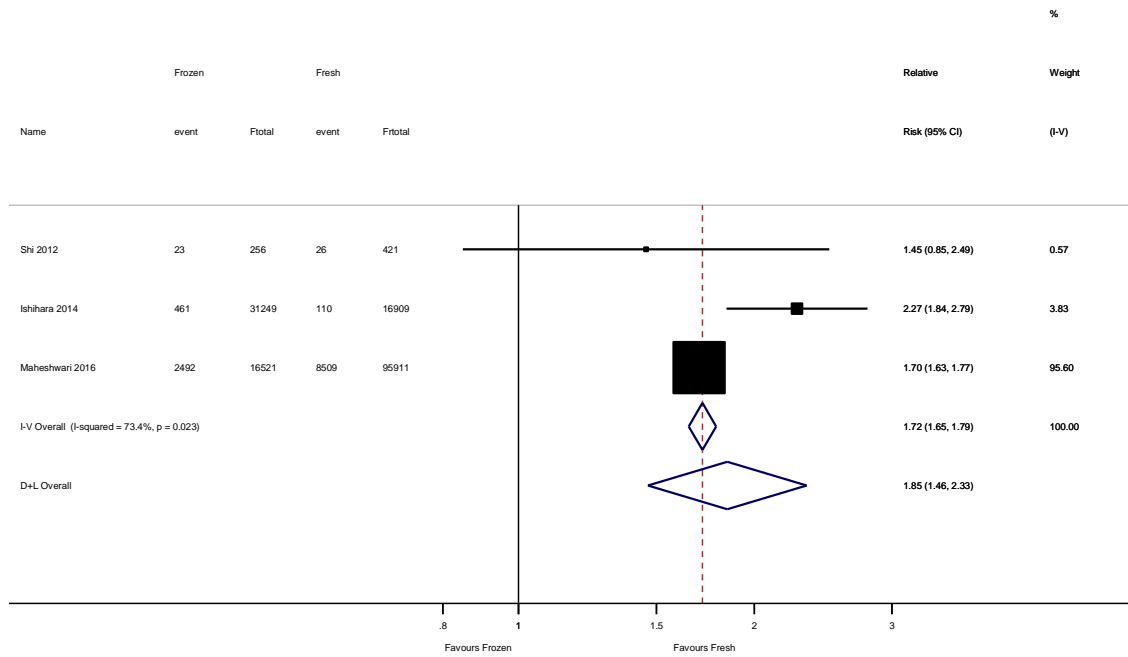


Figure 6b : High birth weight (birth weight > 4000 gm): Cumulative meta-analysis

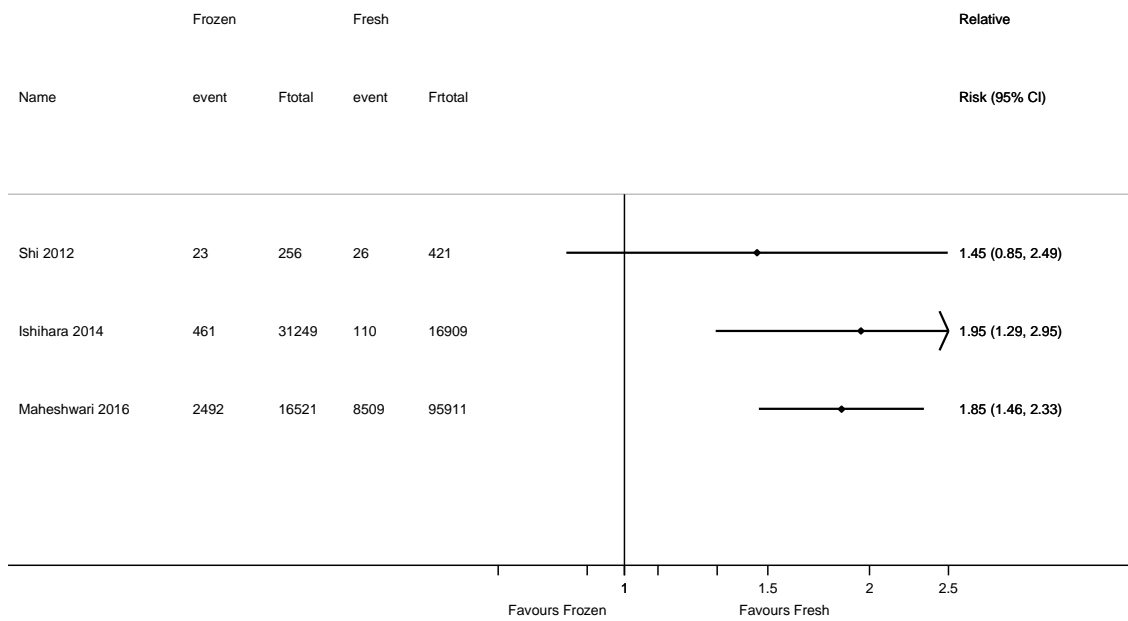


Figure 7a : Very High birth weight (birth weight > 4500 gm): meta-analysis

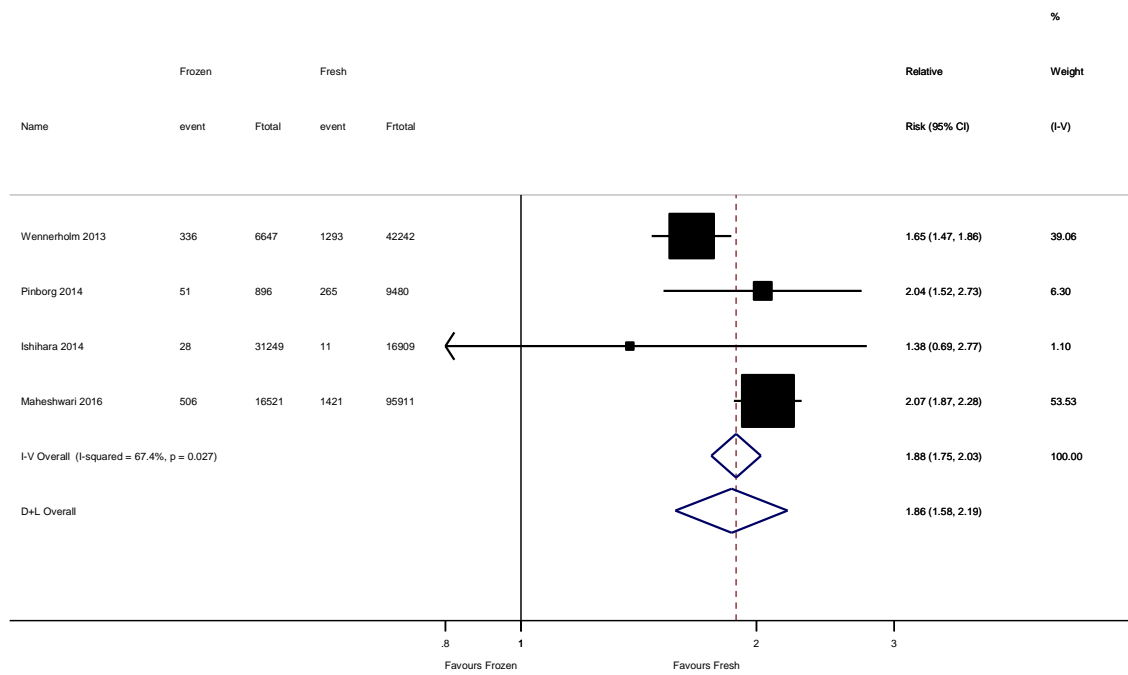


Figure 7b : Very High birth weight (birth weight > 4500 gm): Cumulative meta-analysis

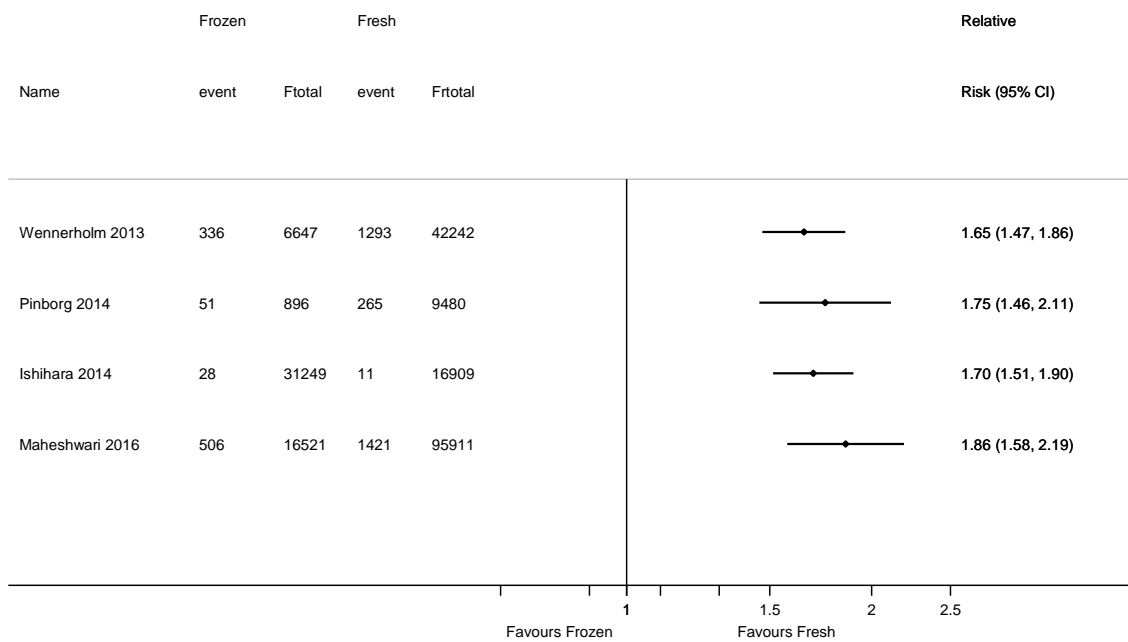


Figure 8a: Preterm delivery (Delivery at less than 37 weeks): Meta-analysis

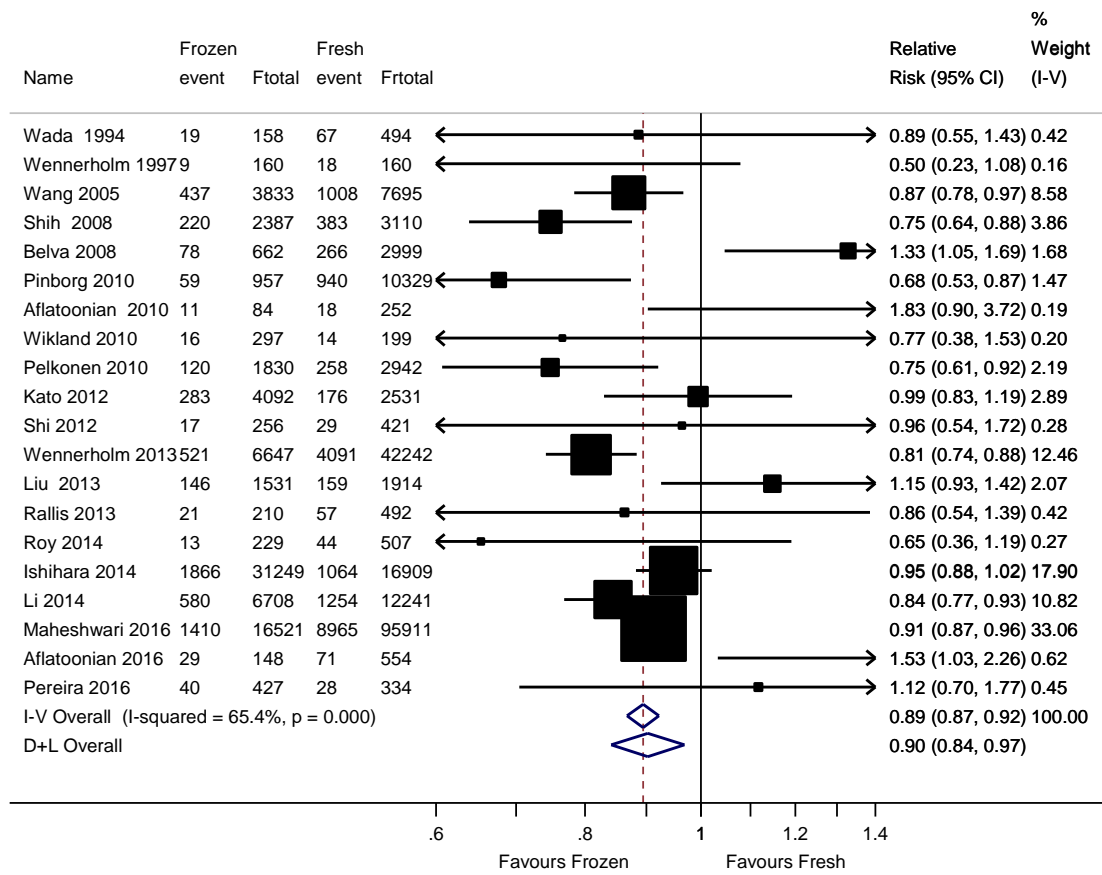




Figure 8b: Preterm delivery (Delivery at less than 37 weeks): Cumulative Meta-analysis

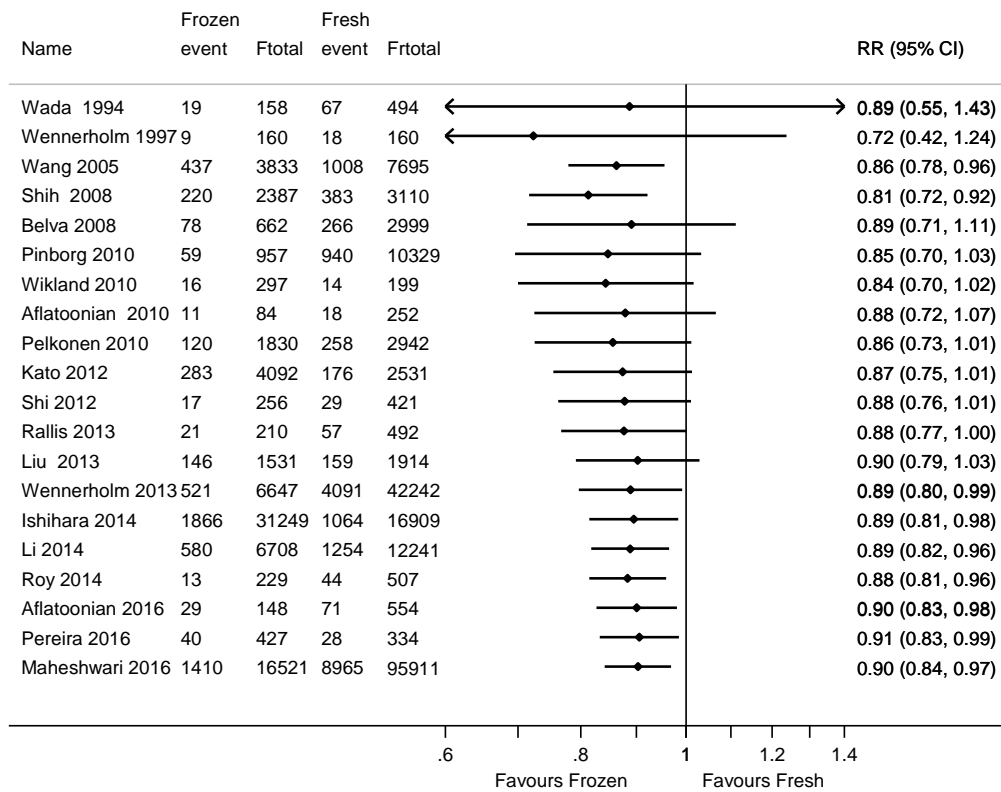


Figure 9a: Very Preterm delivery (Delivery at less than 32 weeks): Meta-analysis

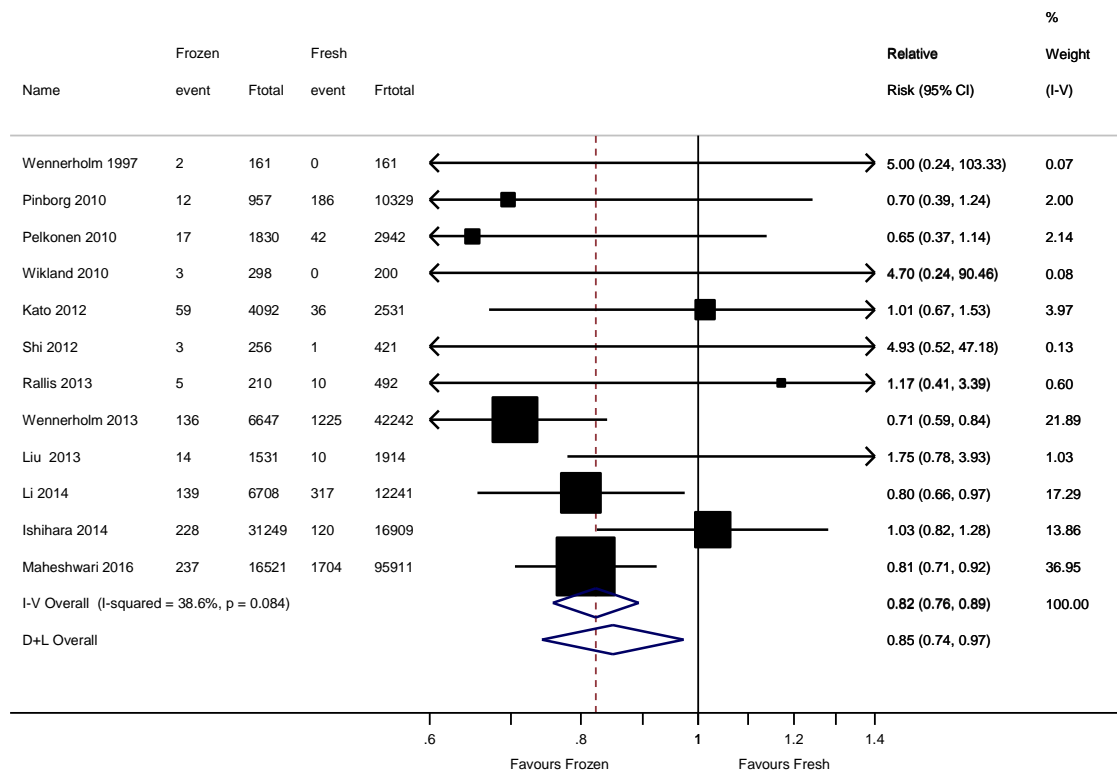


Figure 9b: Very Preterm delivery (Delivery at less than 32 weeks): Cumulative Meta-analysis

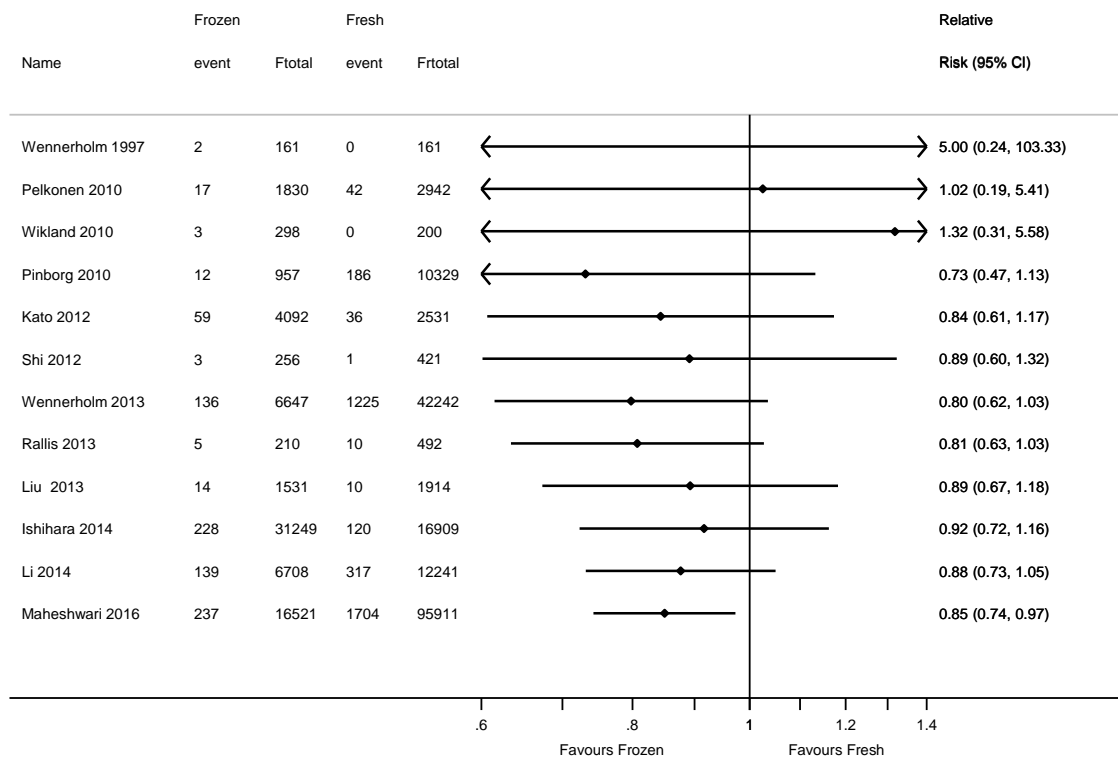


Figure 10 a: Antepartum Haemorrhage : Meta-analysis

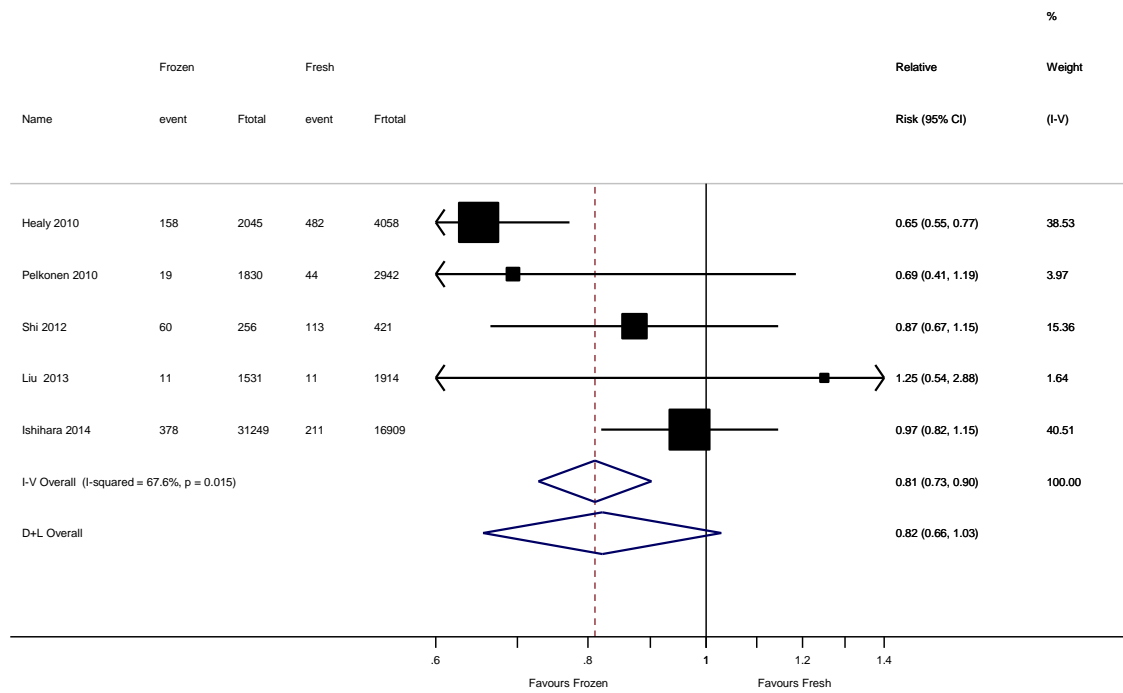


Figure 10 b: Antepartum Haemorrhage : Cumulative Meta-analysis

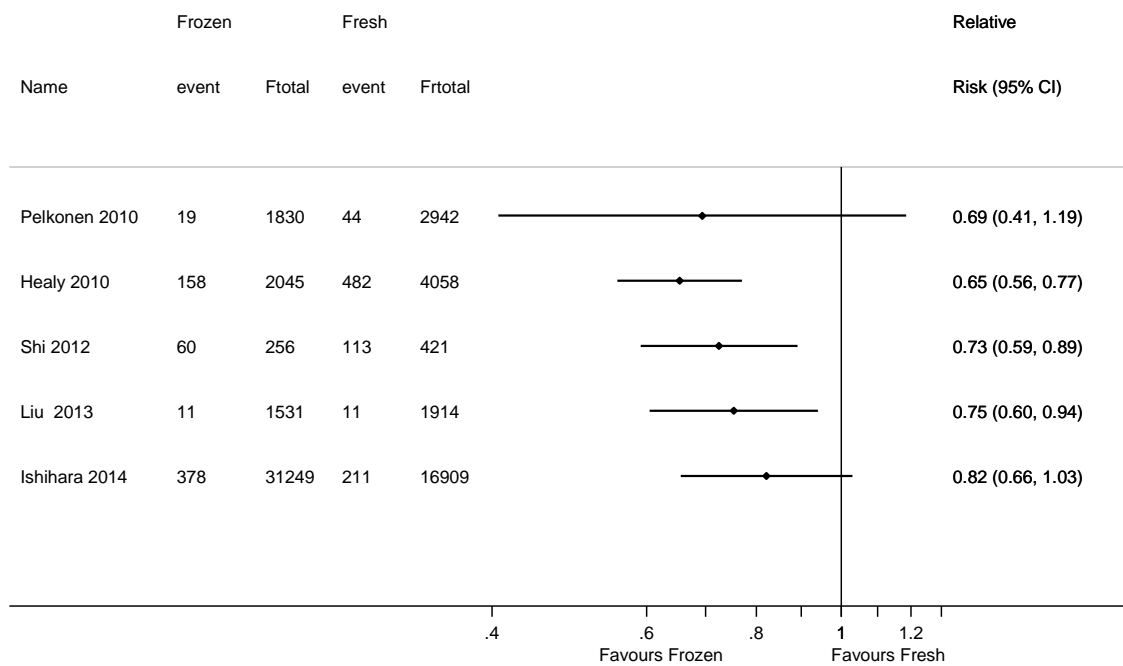


Figure 11a: Admission to Neonatal Intensive care Unit (NICU): Meta-Analysis

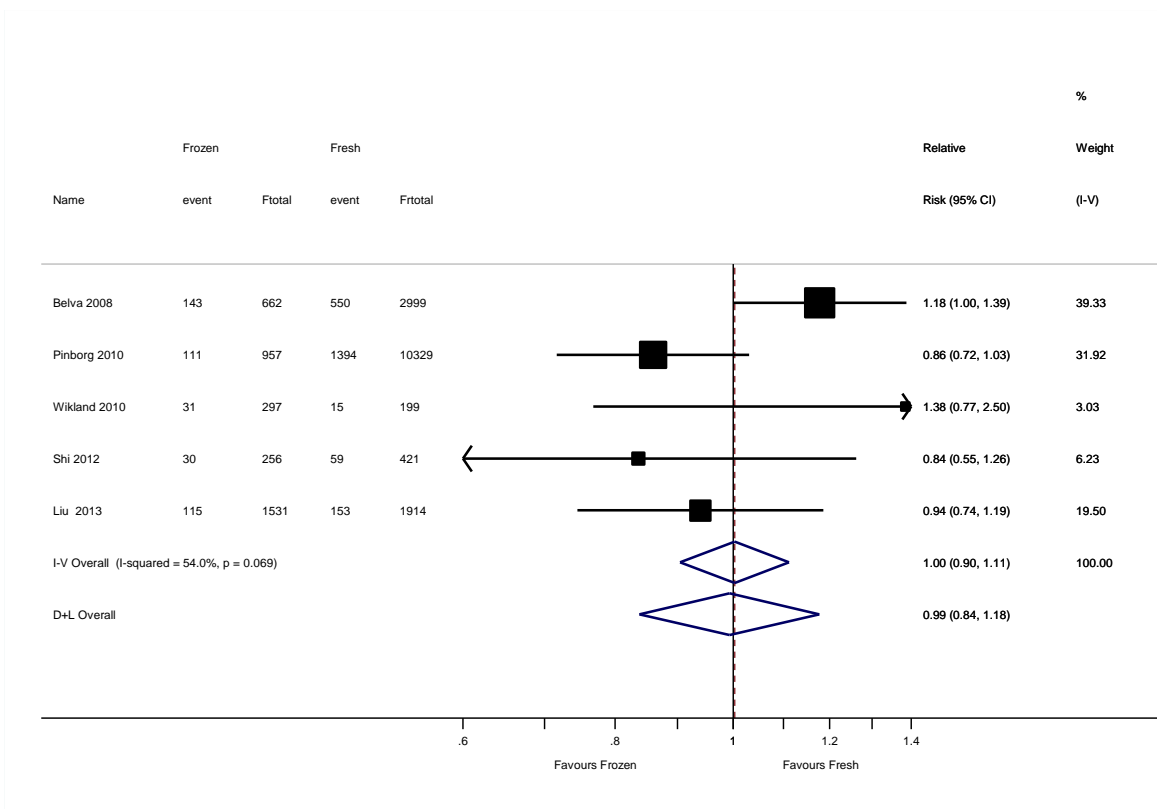


Figure 11b: Admission to Neonatal Intensive care Unit (NICU): Cumulative Meta-Analysis

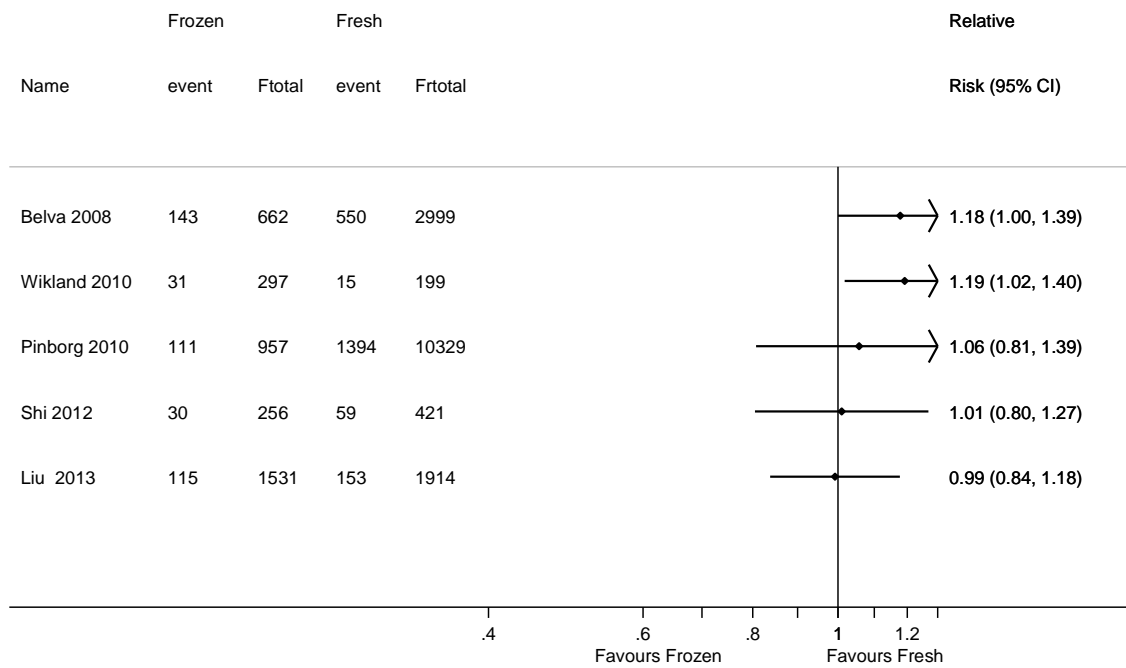


Figure 12 a: Congenital anomalies : Meta-analysis

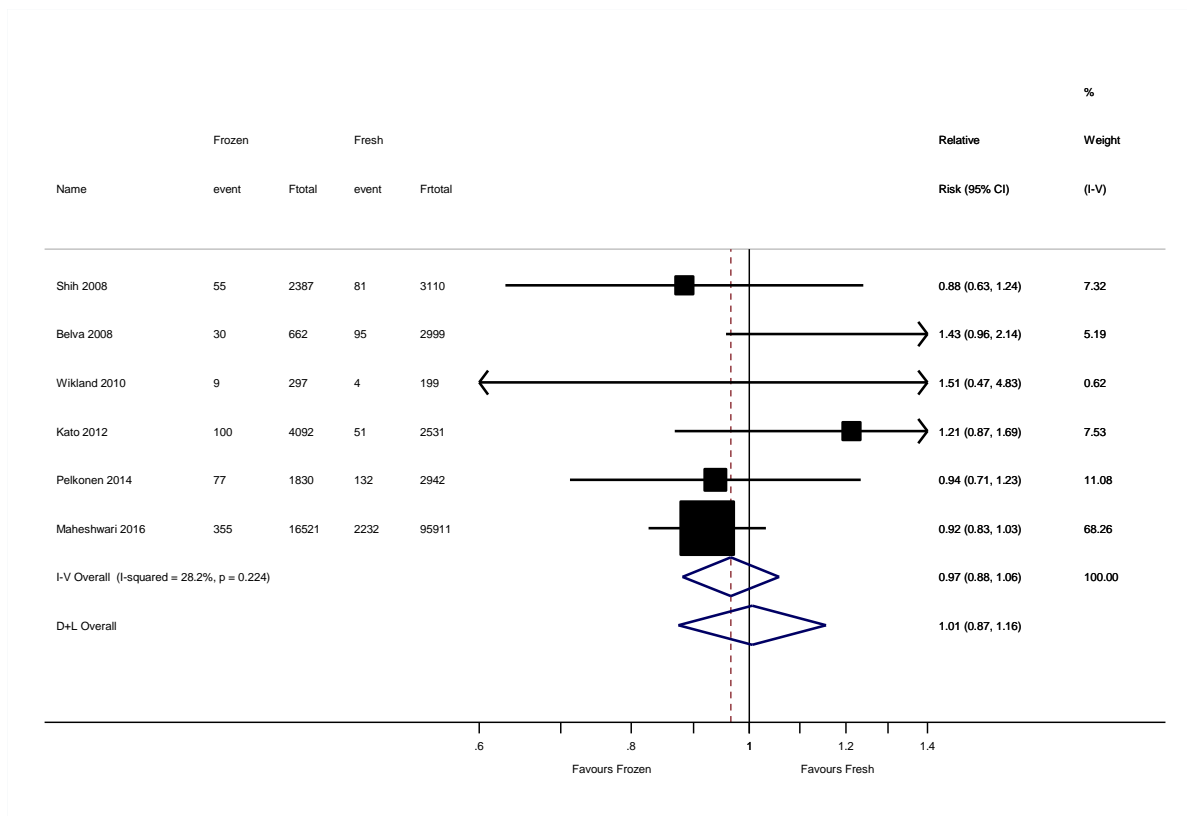


Figure 12 b: Congenital anomalies : Cumulative Meta-analysis

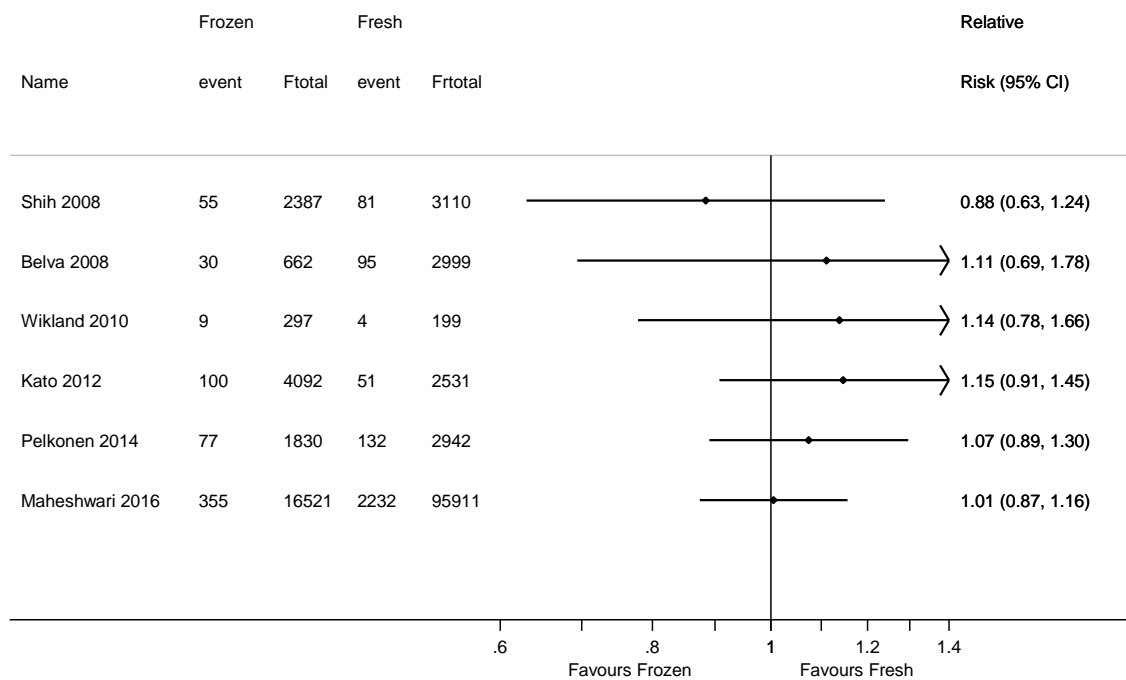


Figure 13a: Perinatal Mortality: Meta-analysis

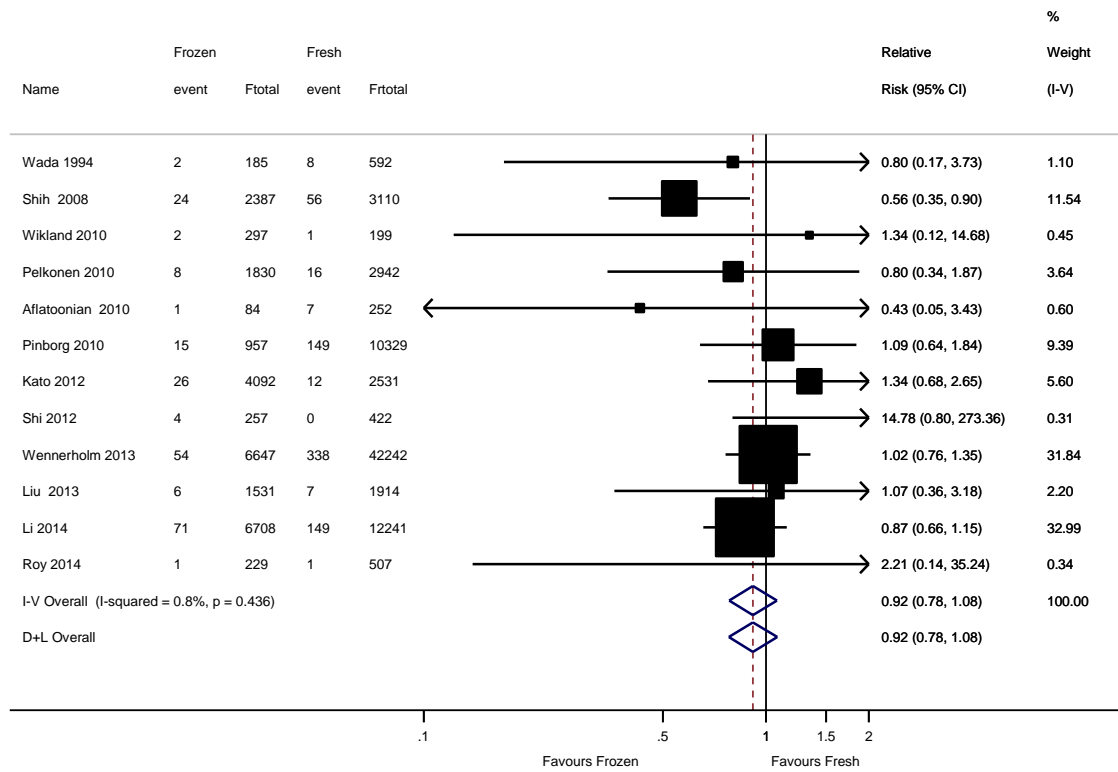


Figure 13b: Perinatal Mortality: Cumulative Meta-analysis

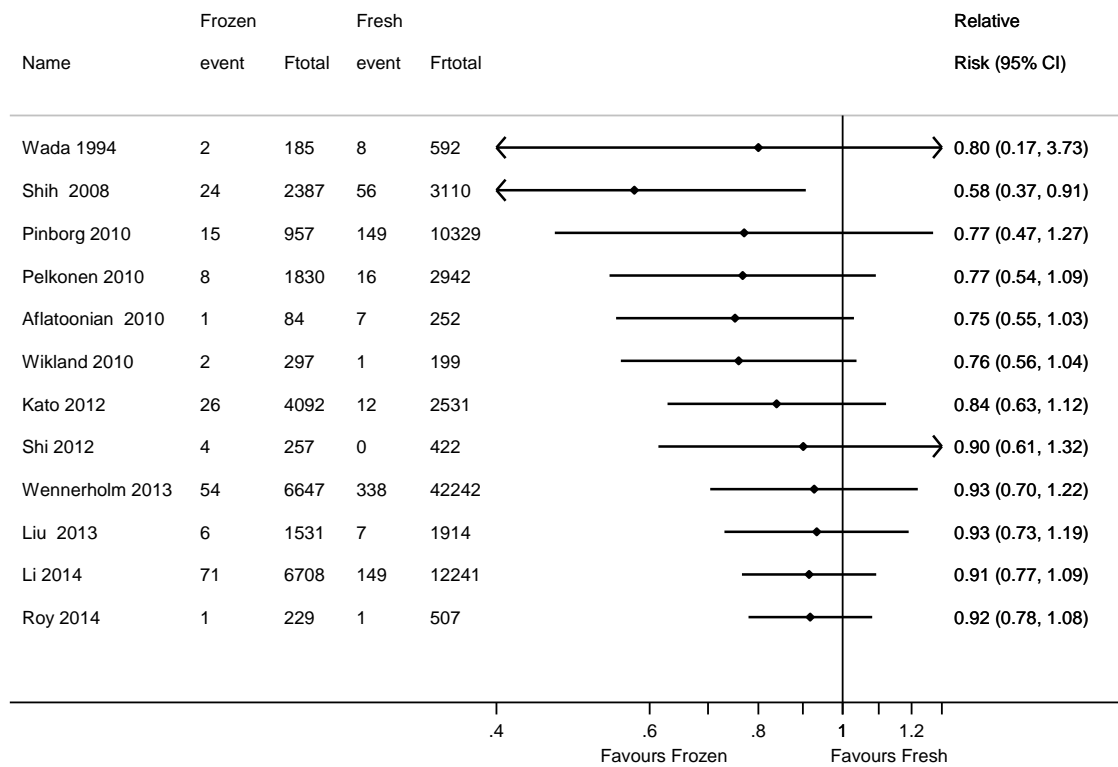




Figure 14 a: Hypertensive disorders of pregnancy: Meta-Analysis

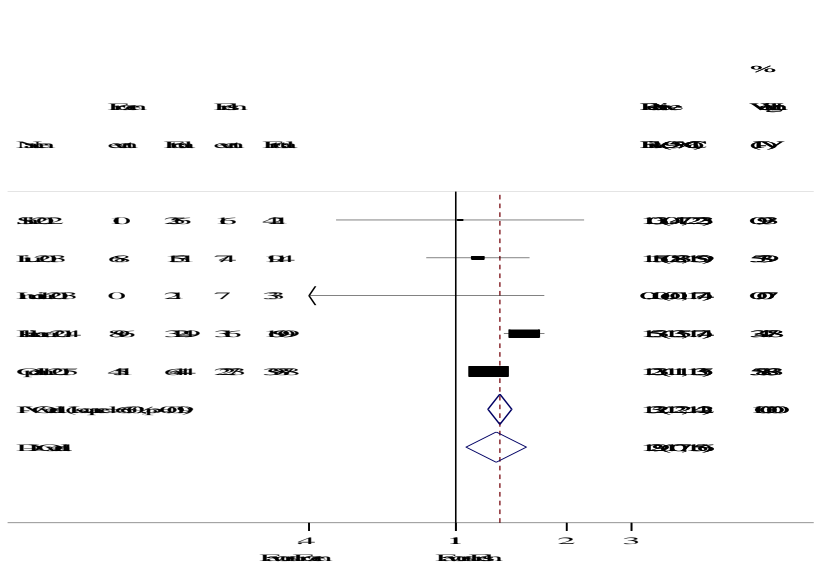
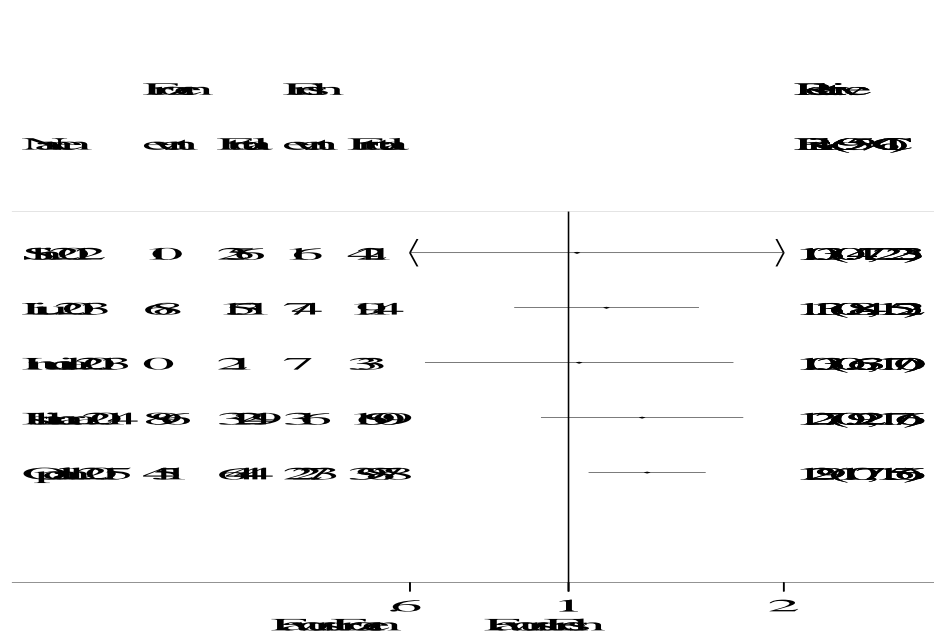


Figure 14 b: Hypertensive disorders of pregnancy: Cumulative Meta-Analysis





## PRISMA 2009 Flow Diagram

