

# Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis

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**BACKGROUND:** Assisted reproduction technology (ART) is used worldwide, at increasing rates, and data show that some adverse outcomes occur more frequently than following spontaneous conception (SC). Possible explanatory factors for the well-known adverse perinatal outcome in ART singletons were evaluated.

**METHODS:** PubMed and Cochrane databases from 1982 to 2012 were searched. Studies using donor or frozen oocytes were excluded, as well as those with no control group or including < 100 children. The main outcome measure was preterm birth (PTB defined as delivery < 37 weeks of gestation), and a random effects model was used for meta-analyses of PTB. Other outcomes were very PTB, low-birthweight (LBW), very LBW, small for gestational age and perinatal mortality.

**RESULTS:** The search returned 1255 articles and 65 of these met the inclusion criteria. The following were identified as predictors for PTB in singletons: SC in couples with time to pregnancy (TTP) > 1 year versus SC singletons in couples with TTP ≤ 1 year [adjusted odds ratio (AOR) 1.35, 95% confidence interval (CI) 1.22, 1.50]; IVF/ICSI versus SC singletons from subfertile couples (TTP > 1 year; AOR 1.55, 95%

CI 1.30, 1.85); conception after ovulation induction and/or intrauterine insemination versus SC singletons where TTP  $\leq$  1 year (AOR 1.45, 95% CI 1.21, 1.74); IVF/ICSI singletons versus their non-ART singleton siblings (AOR 1.27, 95% CI 1.08, 1.49). The risk of PTB in singletons with a 'vanishing co-twin' versus from a single gestation was AOR of 1.73 (95% CI 1.54, 1.94) in the narrative data. ICSI versus IVF (AOR 0.80, 95% CI 0.69–0.93), and frozen embryo transfer versus fresh embryo transfer (AOR 0.85, 95% CI 0.76, 0.94) were associated with a lower risk of PTB.

**CONCLUSIONS:** Subfertility is a major risk factor for adverse perinatal outcome in ART singletons, however, even in the same mother an ART singleton has a poorer outcome than the non-ART sibling; hence, factors related to the hormone stimulation and/or IVF methods *per se* also may play a part. Further research is required into mechanisms of epigenetic modification in human embryos and the effects of cryo-preservation on this, whether milder ovarian stimulation regimens can improve embryo quality and endometrial conditions, and whether longer culture times for embryos has a negative influence on the perinatal outcome.

**Key words:** assisted reproduction technology / perinatal outcome / singleton / subfertility / single embryo transfer

## Introduction

Worldwide, the use of assisted reproduction technology (ART) is expanding and the number of children born after ART has now exceeded 4 million. The estimated number of children born per year in the latest world report from 2003 ranged between 173 000 and 230 000 (Nygren *et al.*, 2011).

The most recent report from the European Society of Human Reproduction and Embryology covering 2006 showed that the proportion of infants born after ART ranged from 0.8 to 4.1% of the total national birth cohorts, with more than 3% being IVF children in several countries (de Mouzon *et al.*, 2010). Different embryo transfer strategies have resulted in very diverging frequencies of multiple deliveries, with rates ranging from 38.3% in Serbia to 5.7% in Sweden. These figures underline that the proportion of ART children is not negligible and will influence the coming generations. Hence, knowledge of the causes of the poorer perinatal outcome in ART singletons is crucial.

Concerns about safety aspects for ART children have been raised since the introduction of IVF and the delivery of the first IVF child, Louise Brown, in 1978. Multiple pregnancies with the high risk of preterm birth (PTB) and the associated higher morbidity have been a major obstacle from the early days of IVF. Moreover, vanishing twin pregnancies, which account for about 10% of IVF singletons conceived using a double embryo transfer (DET) strategy, have also contributed to the increased perinatal risk in children born after IVF (Pinborg *et al.*, 2005, 2007).

The strategy of elective single embryo transfer (eSET) and additional cycles with transfer of frozen/thawed embryos has to a large extent overcome the problems associated with multiple pregnancies and is now used in many countries. In particular, countries with reasonable IVF reimbursement policies have adopted eSET strategies (Martikainen *et al.*, 2001; Gerris *et al.*, 2002; Thurin *et al.*, 2004).

However, eSET is not the only solution to the adverse perinatal outcome in IVF children, as growing evidence has shown that ART singletons also have a higher risk of PTB, low-birthweight (LBW), being small for gestational age (SGA), and an increased rate of congenital malformations and cerebral palsy (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; McGovern *et al.*, 2004; McDonald *et al.*, 2009; Kallen *et al.*, 2010a, b, c; Sazonova *et al.*, 2011a). Even after adjustment for maternal age and parity, significant differences between ART and spontaneously conceived (SC) singletons persist.

The causes of the poorer outcome in ART singletons are probably multifactorial. The major concern is whether the IVF techniques themselves (i.e. embryo culture media and culture time and embryo cryo-preservation) could have negative impacts on the ART offspring. Moreover, the ovarian stimulation resulting in altered endocrine profiles with many corpora lutea in early pregnancy may influence early implantation and placental development. Finally, increasing evidence indicates that the subfertility itself, including the parental characteristics *per se*, seems to be crucial. The aim of this systematic review and meta-analysis was to delineate and discuss possible explanatory factors for the adverse perinatal outcome in ART singletons and to suggest fields for future research.

## Methods

We searched the PubMed and Cochrane databases from January 1982 to April 2012. The main outcome measure was PTB (defined as delivery <37 weeks of gestation), which was used in the meta-analyses for calculating pooled risk estimates.

Other outcome measures were very PTB (defined as <32 weeks of gestation or according to each author), LBW (defined as birthweight <2500 g), very LBW (defined as birthweight <1500 g), SGA (defined as birthweight <10th centile), stillbirth and perinatal mortality (stillbirth or death Day 0–7). The outcomes SGA and stillbirth were defined according to each author. Studies on birth defects were not included in this review. Adjusted odds ratios (AORs) or adjusted relative risks (ARRs) are presented when available, otherwise crude odds ratios/relative risk or absolute values with *P*-values if available.

## Systematic search for evidence

The terms used in the searches are given below.

### Subfertility and ART

Singleton OR singletons OR 'single baby' OR 'single babies' OR 'single infant' OR 'single infants' OR 'single child' OR 'single children' AND birth weight OR birth weight OR premature birth OR perinatal mortality OR stillbirth OR infant, small for gestational age OR 'Pregnancy Outcome'[Mesh] OR children[tw] OR child[tw] OR babies[tw] OR infant[tw] OR infants[tw] OR outcome[tw] AND 'Reproductive Techniques, Assisted'[Mesh] OR ART OR assisted reproductive techn\* OR IVF OR ICSI OR in-vitro fertilisation OR *in vitro* fertilisation OR fertilisation *in vitro* OR intracytoplasmic sperm injection OR ovulation induction OR fertility

[Mesh] OR infertility[Mesh] OR infertility OR infertile OR subfertility OR subfertile OR time to pregnancy[tw].

In addition, two separate searches were performed for culture media and hormonal stimulation.

#### Culture media

Singleton OR singletons OR 'single baby' OR 'single babies' OR 'single infant' OR 'single infants' OR 'single child' OR 'single children' AND birth weight OR birth weight OR premature birth OR perinatal mortality OR stillbirth OR infant, small for gestational age OR 'Pregnancy Outcome'[Mesh] OR children[tw] OR child[tw] OR babies[tw] OR infant[tw] OR infants[tw] OR outcome[tw] AND embryo culture OR culture media OR culture time AND birth weight OR birth weight AND IVF OR ICSI OR in-vitro fertilisation OR *in vitro* fertilisation OR fertilisation *in vitro* OR intracytoplasmic sperm injection OR 'Reproductive Techniques, Assisted'[Mesh] OR ART OR assisted reproductive techn\*.

#### Hormone stimulation

Singleton OR singletons OR 'single baby' OR 'single babies' OR 'single infant' OR 'single infants' OR 'single child' OR 'single children' AND birth weight OR birth weight OR premature birth OR perinatal mortality OR stillbirth OR infant, small for gestational age OR 'Pregnancy Outcome'[Mesh] OR children[tw] OR child[tw] OR babies[tw] OR infant[tw] OR infants[tw] OR outcome[tw] AND hormonal stimulation OR hormone stimulation OR controlled ovarian hyperstimulation OR ovulation induction AND birth weight OR birth weight AND IVF OR ICSI OR in-vitro fertilisation OR *in vitro* fertilisation OR fertilisation *in vitro* OR intracytoplasmic sperm injection OR "Reproductive Techniques, Assisted"[Mesh] OR ART OR assisted reproductive techn\*.

We also searched reference lists of identified articles manually for additional references. Guidelines for meta-analyses and systematic reviews of observational studies were followed (Stroup *et al.*, 2000). Literature searches and abstract screening were performed by three researchers (C.B., A.P. and A.L.) and one librarian. Any disagreement or uncertainty was resolved by discussion to reach a consensus.

### Inclusion and exclusion of studies

Original studies published in the English language reporting perinatal outcomes for singletons after infertility treatment, including ART and ovulation induction (OI) or subfertility without treatment were included. Systematic reviews and meta-analyses were only included as background (Supplementary data, Table S0).

In the case of double publications, the latest study was included. Studies using donor or frozen oocytes were excluded. Studies published only as abstracts were excluded. Studies without a control group or including <100 children in total were excluded.

### Quality appraisal of the evidence

Methodological quality, in terms of risk of bias, of the included studies was assessed by two reviewers (C.B. and U.B.W.) using the tools developed by SBU ([www.sbu.se/sv/Evidensbaserad-varld/Utvardering-av-metoder-i-halso-och-sjukvarden-En-handbok/](http://www.sbu.se/sv/Evidensbaserad-varld/Utvardering-av-metoder-i-halso-och-sjukvarden-En-handbok/)) for original articles, which grade articles as low-, moderate- and high-quality articles. For systematic reviews and meta-analyses the AMSTAR (Shea *et al.*, 2009) tool was used and for quality of evidence, we used the GRADE system (Guyatt *et al.*, 2008). AMSTAR is a tool for checking quality of systematic reviews. It consists of 11 questions concerning research question, literature search, inclusion/exclusion list, quality assessment of studies, formulations of conclusions, test for heterogeneity if pooled results in a meta-analysis, publication bias and conflict of interest report.

The GRADE system evaluates the following variables for all studies combined and per outcome: design, study limitations, consistency, directness, precision, publication bias, magnitude of effect, relative effect and absolute effect. Quality levels are divided into high, moderate, low and very low quality. The quality level is based on our confidence in the effect estimate, which in turn is based on the number of studies, design of studies, consistency of associations between studies, study limitations, directness, precision, publication bias, effect size, and relative and absolute effect.

The quality levels are: very confident = high quality, moderately confident = moderate quality, limited confidence = low quality and very little confidence = very low quality. If conclusions are based on randomized controlled trials (RCTs), GRADE starts at high-quality level (Level 4) but can be downgraded, while if conclusions are based on observational studies GRADE starts at low-quality level (Level 2) but might be upgraded (or downgraded).

The results of the risk of bias and quality assessments for original articles are included in Supplementary data, Tables S0–SXI and the quality assessment is presented in its complete form in Supplementary data, Table SXII.

### Data synthesis

Meta-analyses were performed using Stata for windows version 12.1 with PTB as the outcome measure for Supplementary data, Tables SI–SIX (L.B.R. performed the meta-analyses). Owing to the clinical heterogeneity from different study cohorts, a random effects model was used for meta-analyses of the data. AORs were used to present the effect estimate for all meta-analyses. Heterogeneity was explored using the  $I^2$  statistics and  $\tau^2$ . Where it is not appropriate to conduct meta-analyses, narrative data are presented (Supplementary data, Tables SX and SXI).

## Results

The search returned 1255 articles (1052 from general ART, 64 from culture media/culture time and 139 from hormonal stimulation), of which 65 were included (Supplementary data, Fig. S1). Of these 65 studies, 25 were of high quality, 25 of moderate quality and 15 of low quality. Table 1 shows a summary of all data on the different topics. Supplementary data, Table S0 shows data for IVF/ICSI singletons versus the general population, Supplementary data, Tables SI–SXI present all the different topics, Supplementary data, Table SXII presents the quality and risk of bias assessment of included original articles and Supplementary data, Table SXIII presents the excluded full-text articles with reasons for exclusion.

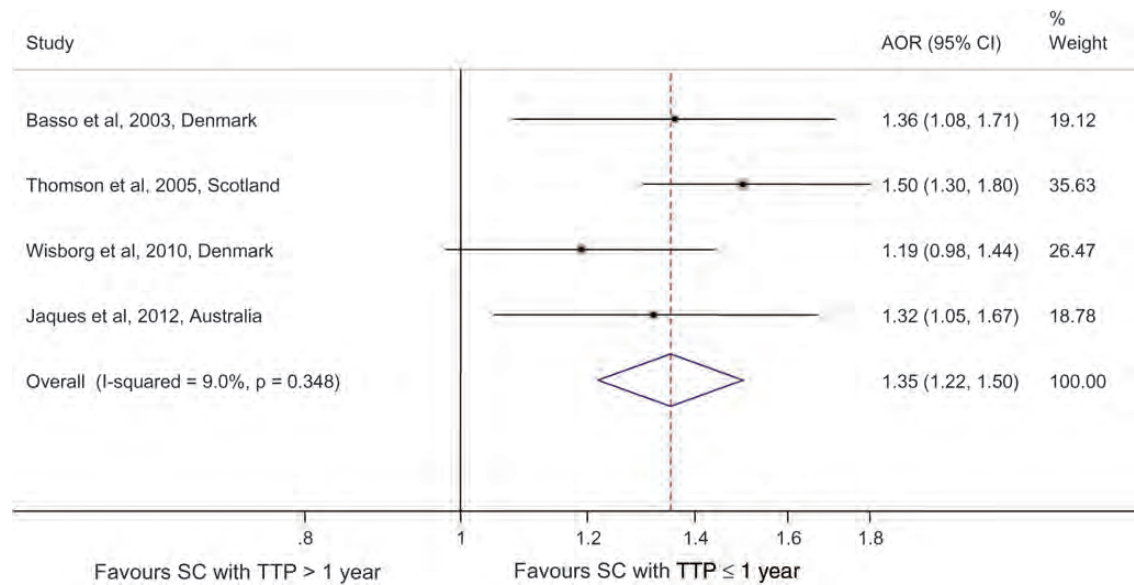
*Singletons born after IVF/ICSI (fresh and frozen/thawed cycles) versus singletons in the general population (Supplementary data, Table S0):* Four meta-analyses and several large cohort studies have compared perinatal outcomes for IVF singletons versus singletons from the general population with adjustment for relevant confounders. Significantly higher rates of PTB have been found for IVF singletons with adjusted risks between 1.41 and 2.04, while the latest study on the youngest IVF singleton cohorts born in 2000–2006 showed improved outcomes with lower AOR (1.15) though still significantly higher than in SC singletons (Sazonova *et al.*, 2011a). The same trend was noticed for very PTB, with a decrease in AOR from 2.27–3.27 in the meta-analyses to 1.19 (0.99–1.43) in the latest Swedish population-based study (Sazonova *et al.*, 2011a). A similar tendency of lower perinatal risks in the later singleton cohorts was seen for LBW, very LBW, SGA and perinatal mortality with SGA and perinatal mortality being no longer significantly increased.

**Table 1** Pooled estimates on the risk of PTB in singletons born in different infertile populations or after different types of fertility treatment, according to Supplementary data, Tables SI–SXI and Figs 1–9.

Table number and figure number	Study population	Control group	Number of original papers included	Number of studies included in meta-analyses	PTB, Range of adjusted risk ratios in individual studies	PTB, Pooled estimate after meta-analysis	Overall quality of evidence (GRADE), quality of individual studies (range)	Final conclusion on evidence
Supplementary data, Table SI and Fig. 1	SC of subfertile women with TTP > 1 year	SC of fertile women with TTP ≤ 1 year	7	4	1.19–1.5	1.35 (1.22, 1.50)	GRADE +++ (Low–high)	There is moderate quality of evidence that SC singletons from subfertile women with TTP > 1 year have increased risk of PTB when compared with SC singletons of fertile women with TTP ≤ 1 year
Supplementary data, Table SII and Fig. 2	IVF/ICSI	SC of subfertile women (TTP > 1 year)	6	2	1.28–1.6	1.55 (1.30, 1.85)	GRADE ++ (Low–high)	There is low quality of evidence that IVF singletons have increased risk of PTB when compared with SC singletons of subfertile women with TTP > 1 year
Supplementary data, Table SIII and Fig. 3	IVF/ICSI sibling	SC sibling	2	2	1.20–1.3	1.27 (1.08, 1.49)	GRADE ++ (High)	There is low quality of evidence that the risk of PTB in a singleton sibling born after ART is higher than in a singleton sibling born after natural conception.
Supplementary data, Table SIV and Fig. 4	OI and/or IUI/IUID or IVF/ICSI	SC of fertile women with TTP ≤ 1 year	12	5	1.16–1.89	1.45 (1.21, 1.74)	GRADE +++ (Low–high)	There is moderate quality of evidence that singletons born after OU/IUI or IVF/ICSI have increased risk of PTB when compared with singletons conceived by fertile couples
Supplementary data, Table SV and Fig. 5	ICSI (fresh and frozen)	IVF (fresh and frozen)	10	5	0.58–0.96	0.80 (0.69, 0.93)	GRADE +++ (Low–high)	There is moderate quality of evidence that singletons born after ICSI when compared with singletons born after IVF have a lower risk of PTB

Supplementary data, Table SVI and Fig. 6	FET	Fresh	10	9	0.69–1.38	0.85 (0.76, 0.94)	GRADE +++ (Low–high)	There is moderate quality of evidence that singletons born after frozen IVF/ICSI when compared with singletons born after fresh IVF/ICSI have a lower risk of PTB
Supplementary data, Table SVII and Fig. 7	FET	SC from general population	4	3	1.05–1.45	1.20 (0.98, 1.46)	GRADE ++ (Low–high)	There is low quality of evidence that singletons born after frozen IVF/ICSI when compared with SC singletons in the general population have no increased risk of PTB
Supplementary data, Table SVIII and Fig. 8	Blastocyst transfer Day 5	Cleavage stage transfer Day 2	4	2	0.93–1.35	1.14 (0.80, 1.64)	GRADE + (conflicting data) (Moderate-high)	There is very low quality of evidence that culture time influences the risk of PTB
Supplementary data, Table SIX and Fig. 9	SET (fresh + frozen)	DET (fresh + frozen)	4	3	0.56–0.99	0.83 (0.64, 1.06)	GRADE ++ (Moderate-high)	There is low quality of evidence that singletons from DET when compared with singletons from SET have no increased risk of PTB
Supplementary data, Table SX	SET (fresh + frozen)	SC from general population	4	No meta-analysis	1.15–2.85	–	GRADE +++ (Low–high)	There is moderate quality of evidence that singletons from SET compared with singletons in the general population have a higher risk of PTB
Supplementary data, Table SXI	IVF/ICSI singletons with a vanishing co-twin	IVF/ICSI singletons from a single gestation	5	No meta-analysis	1.73 (only one study with risk estimate)	–	GRADE +++ (Low–high)	There is moderate quality of evidence that singletons from pregnancies with a vanishing co-twin when compared with singletons from only a single gestation have increased risk of PTB

TTP, time to pregnancy; SET, single embryo transfer; DET, double embryo transfer; FET, frozen embryo transfer; OI, ovulation induction; SC, spontaneous conception; IUI/IUID, intrauterine insemination/donor.



**Figure 1** Pooled estimate on the risk of PTB in SC singletons of subfertile women with TTP > 1 year versus SC singletons of fertile women with TTP ≤ 1 year.  $I^2 = 0.0010$ . SC, spontaneous conception; AOR, adjusted odds ratio; CI, confidence interval.

There is high quality of evidence (GRADE +++) that IVF singletons have worse perinatal outcomes (PTB, very PTB, LBW, very LBW) when compared with singletons in the general population.

## Subfertility

SC singleton born of subfertile women with time to pregnancy (TTP) > 1 year versus SC singletons born of fertile women with TTP ≤ 1 year (Supplementary data, Table SI and Fig. 1): The search returned seven studies, out of which four could be included in the meta-analysis on PTB. The pooled estimate for PTB was AOR 1.35 (95% CI 1.22, 1.50),  $I^2 = 9.0%$ ,  $P = 0.348$ . Apart from two surveys (Wisborg et al., 2010a, b; Cooper et al., 2011), which found no significant differences between the groups, all other studies reported a negative impact of subfertility on perinatal outcome. The risk of LBW varied from AOR 1.18 to 1.44. These results are in line with national data from Sweden, which allowed for adjustment for a variety of confounding factors including duration of infertility. After adjustment for duration of infertility, the perinatal outcomes in IVF singletons were similar to SC singletons (Kallen et al., 2010a).

There is moderate quality of evidence that SC singletons from subfertile women with TTP > 1 year have increased risk of PTB when compared with SC singletons of fertile women with TTP ≤ 1 year (GRADE ++).

Singletons born after IVF/ICSI versus SC singletons of subfertile women with TTP > 1 year (Supplementary data, Table SII and Fig. 2): Six studies compared singletons born after IVF/ICSI versus SC singletons from a subfertile population (TTP > 1 year). Two papers were included in a meta-analysis of PTB revealing a pooled estimate of AOR 1.55 (95% CI 1.30, 1.85),  $I^2 = 0.0%$ ,  $P = 0.379$ . Four studies found significantly higher risk of LBW in IVF/ICSI versus SC singletons from a subfertile population (De Geyter et al., 2006; Kapiteijn et al., 2006; Pelinck et al., 2010; Cooper et al., 2011). No studies found

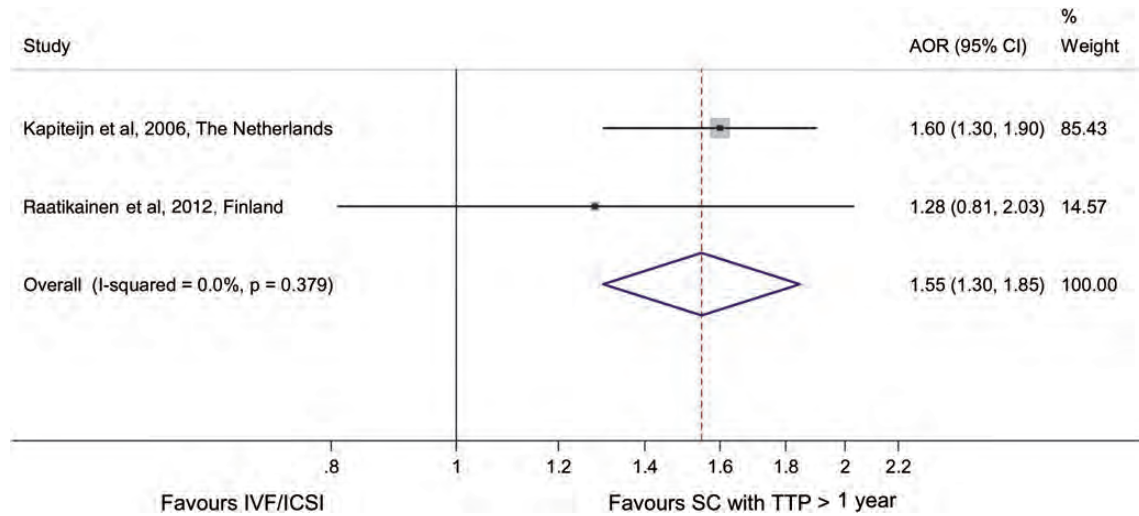
significant differences in the risk of SGA and perinatal mortality between IVF/ICSI and singletons from a subfertile population (Zhu et al., 2007; Raatikainen et al., 2012).

There is low quality of evidence that IVF singletons have increased risk of PTB when compared with SC singletons of subfertile women with TTP > 1 year (GRADE ++).

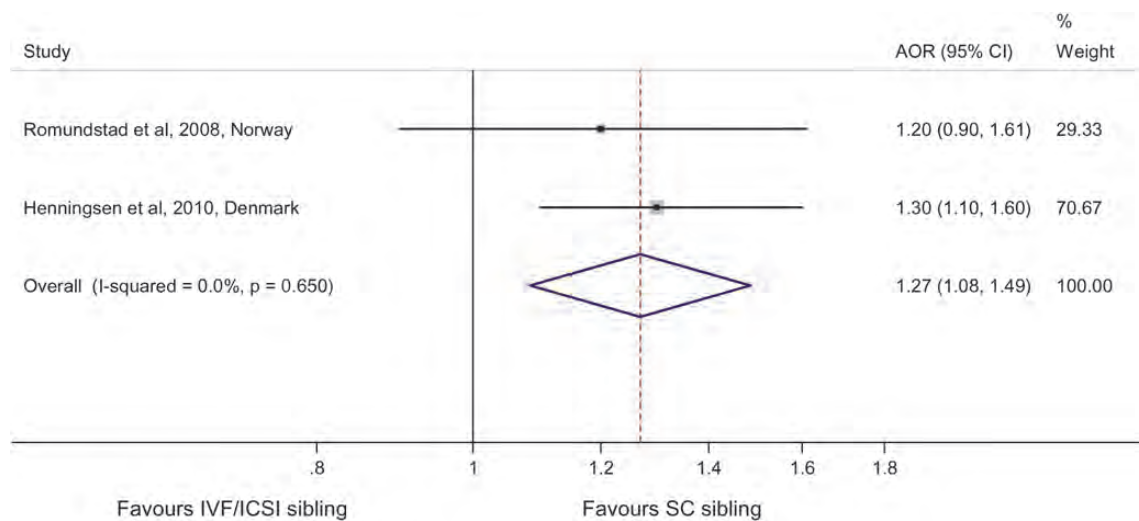
Consecutive singleton sibling pairs, one conceived by ART and the other by spontaneous conception (Supplementary data, Table SIII and Fig. 3): Two studies on perinatal outcome after assisted fertilization used a sib-ship design and both were included in the meta-analysis on PTB with a pooled estimate of AOR 1.27 (95% CI 1.08, 1.49),  $I^2 = 0.0%$ ,  $P = 0.650$  in the ART singleton versus the non-ART singleton sibling. To distinguish the effect related to the reproductive technology from maternal characteristics, Romundstad et al. (2008) analysed data on 2546 ART and non-ART sibling pairs.

In the Norwegian sib-ship comparisons of women who had conceived both spontaneously and after ART (Romundstad et al., 2008), the difference in adjusted mean birthweight was only 9 g (95% CI -18, 36), and for adjusted mean gestational age, the difference was 0.6 days (95% CI -0.5, 1.7) and did not reach statistical significance. The adjusted risk for SGA was fully attenuated to AOR 0.99 (95% CI 0.62, 1.57) in the sib-ship comparison and the AOR for perinatal mortality was 0.36 (95% CI 0.20, 0.67).

In the Danish study, 13 692 pairs of singleton siblings after IVF, ICSI, frozen embryo transfer (FET) and SC were analysed according to the mode of conception (Aaris Henningsen et al., 2011). The mean birthweight was 65 g (95% CI 41, 89) lower in all ART singletons than in their SC siblings. After adjustment for maternal age, parity, year of birth, offspring sex and birth order, the mean birthweight in children born after FET was significantly higher than for siblings born after replacement of fresh embryos, 167 g (95% CI 90–244). In contrast to the Norwegian results, the risk of LBW [AOR 1.4 (95% CI 1.1, 1.7)] was higher in ART siblings after fresh embryo transfer than in



**Figure 2** Pooled estimate on the risk of PTB in singletons born after IVF/ICSI versus SC singletons of subfertile women (TTP > 1 year).  $\tau^2 = 0.0000$ .



**Figure 3** Pooled estimate on the risk of PTB in sibling studies of mothers to consecutive-singleton siblings of an IVF/ICSI child and an SC child.  $\tau^2 = 0.0000$ .

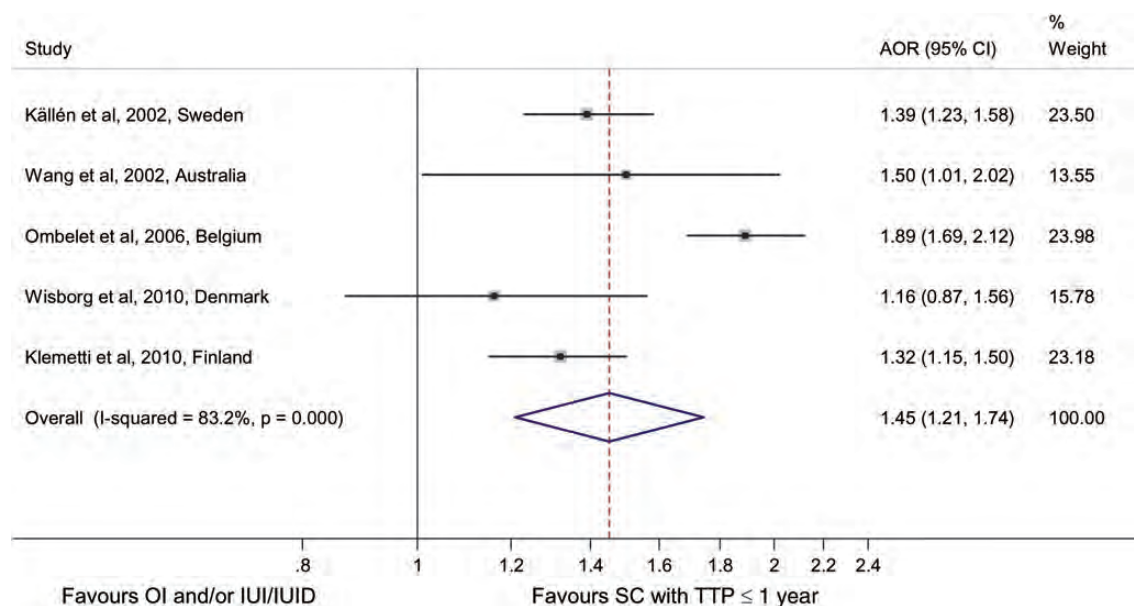
the SC siblings after adjustment for maternal age and birth order (Aaris Henningsen *et al.*, 2011).

In a retrospective Australian study including 8179 singleton births, the outcome in two successive singleton births was compared in a subgroup of 1219 women. The effects of fresh embryo transfer and FET on differences in birthweight of second versus first singleton birth was examined. They found a parity effect on birthweight with a higher mean birthweight of the second child independent of whether the embryo was fresh or frozen. However, this effect was more prominent if the first child was born after fresh embryo transfer and the second child after FET, when compared with the reverse situation (Shih *et al.*, 2008).

There is low quality of evidence that the risk of PTB in a singleton sibling born after ART is higher than in a singleton sibling born after natural conception (GRADE ++).

### Controlled ovarian stimulation

Singletons born after OI and/or intrauterine insemination (IUI/IUID) or IVF/ICSI versus SC singletons born of fertile women with TTP  $\leq$  1 year (Supplementary data, Table SIV and Fig. 4): Twelve studies were identified and five were suitable for meta-analysis. Raatikainen *et al.* (2012) were not included in the meta-analysis as they only compared IVF/ICSI versus fertile women and had no comparisons with singletons born after OI and/or IUI. The pooled estimate for PTB in OI/IUI versus SC



**Figure 4** Pooled estimate on the risk of PTB in singletons born after OI and/or intrauterine insemination/donor (IUI/IUID) versus SC singletons of fertile women with TTP  $\leq$  1 year.  $I^2 = 83.2\%$ ,  $P = 0.000$ .

singletons of fertile couples was AOR 1.45 (95% CI 1.21, 1.74),  $I^2 = 83.2\%$ ,  $P = 0.000$ . One study also allowed for adjustment of length of involuntary childlessness, which resulted in a diminished AOR from 1.39 (95% CI 1.23, 1.58) to 1.24 (95% CI 1.07, 1.45) for PTB (Kallen et al., 2002).

Apart from a Danish survey (Wisborg et al., 2010a), all studies comparing singletons born after OI and/or IUI versus singletons born to fertile couples reported a higher adjusted risk of LBW in OI/IUI singletons. In the Danish survey, the authors found similar AOR for PTB, very PTB and stillbirth in OI/IUI versus SC singletons from fertile couples, but higher risks in singletons conceived after IVF/ICSI versus SC singletons of fertile couples (Wisborg et al., 2010a, b). Another study included a case-matched control cohort of IVF/ICSI children and showed a higher risk of PTB in IVF/ICSI compared with OI/IUI children (Wang et al., 2002).

There is moderate quality of evidence that singletons born after OI/IUI or IVF/ICSI have increased risk of PTB when compared with singletons conceived by fertile couples (GRADE +++).

*Singletons born after mild stimulation regimens [controlled ovarian stimulation (COS) only or modified natural cycle (MNC)-IVF] versus SC singletons of subfertile women (TTP > 1 year) (Supplementary data, Table SII, no meta-analysis):* Only two small studies compared perinatal outcome in singletons born after mild stimulation protocols versus singletons born after SC in a subfertile population (Kapiteijn et al., 2006; Pelinck et al., 2010). Kapiteijn et al. (2006) compared 84 singletons born after COS only versus 6343 SC singletons born to women with TTP > 1 year and found no significant differences between the two groups. 'MNC-IVF' is the term used for an IVF cycle with minimal FSH stimulation in the late follicular phase simultaneous with a GnRH antagonist. Pelinck et al. (2010) compared 158 MNC-IVF with 132 SC singletons from subfertile couples and found similar mean gestational age, but a lower mean birthweight in the MNC-IVF group.

There is very low quality of evidence that singletons born after mild stimulation regimens when compared with SC singletons of subfertile women (TTP > 1 year) do not have a worse perinatal outcome (GRADE +).

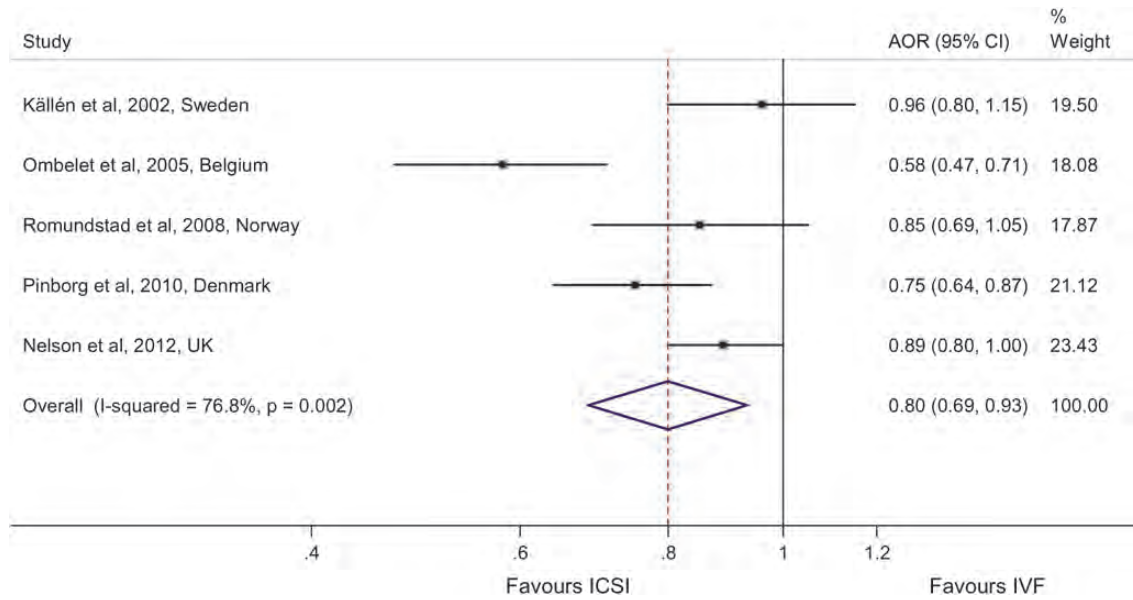
*Perinatal outcome in IVF/ICSI singletons according to the dose of gonadotrophins or the number of oocytes retrieved:* Few studies have investigated a possible association between the dose of gonadotrophins used for COS and the perinatal outcome. In a recent US study including both singleton and multiple pregnancies, ovarian hyperstimulation syndrome (OHSS) (often with a higher number of oocytes retrieved compared with non-OHSS pregnancies) was associated with poorer perinatal outcome assessed by PTB and/or LBW, even after adjusting for relevant confounders (Chung et al., 2006). However, when analyses were restricted to singletons only, the effect of OHSS was not statistically significant for poor perinatal outcome. In another more recent larger study from Society for Assisted Reproductive Technologies, OHSS compared with no OHSS was associated with increased AOR for LBW (Luke et al., 2010). In a large national cohort study from Germany, the authors failed to demonstrate an association between the total dose of gonadotrophins and birthweight (Griesinger et al., 2008). A recent Swedish national cohort study found no association between the number of oocytes retrieved and obstetric outcome (Sazonova et al., 2011b).

There is low quality of evidence that the dose of gonadotrophins or the number of oocytes retrieved does not affect perinatal outcome (GRADE ++).

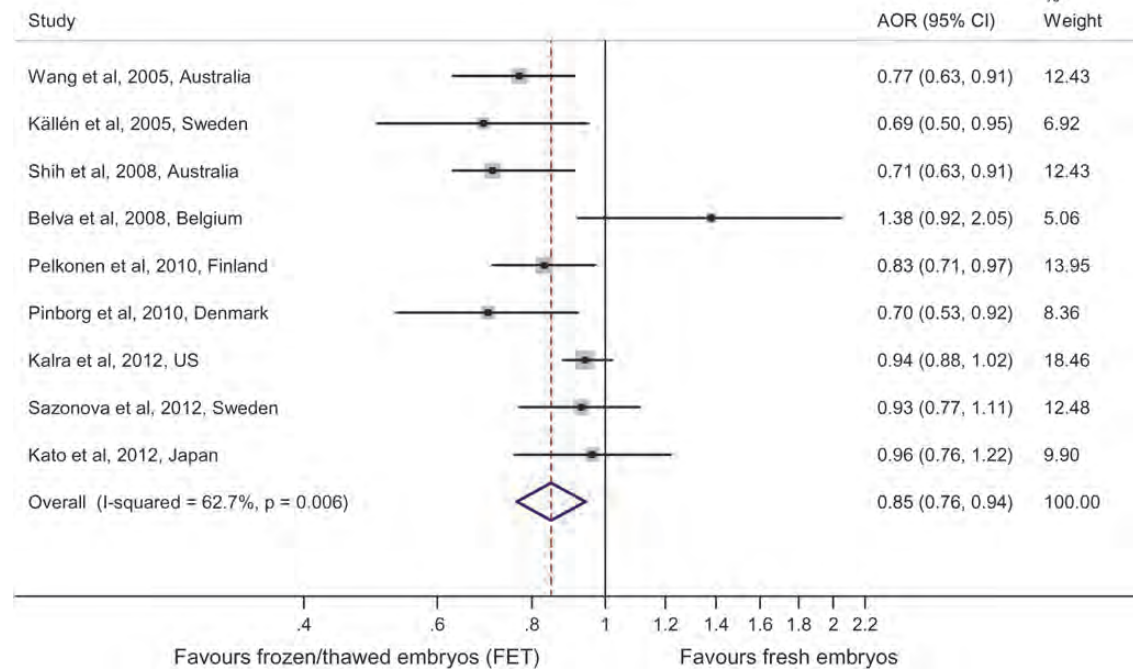
## IVF Laboratory procedures

*Singletons born after ICSI in (fresh or frozen/thawed cycles) versus singletons born after IVF (fresh or frozen/thawed cycles) (Supplementary data, Table SV and Fig. 5):* Ten studies were included with five papers suitable





**Figure 5** Pooled estimate on the risk of PTB singletons born after ICSI (fresh and frozen/thawed) cycles versus singletons born after IVF (fresh and frozen/thawed) cycles.  $\tau^2 = 0.0232$ .

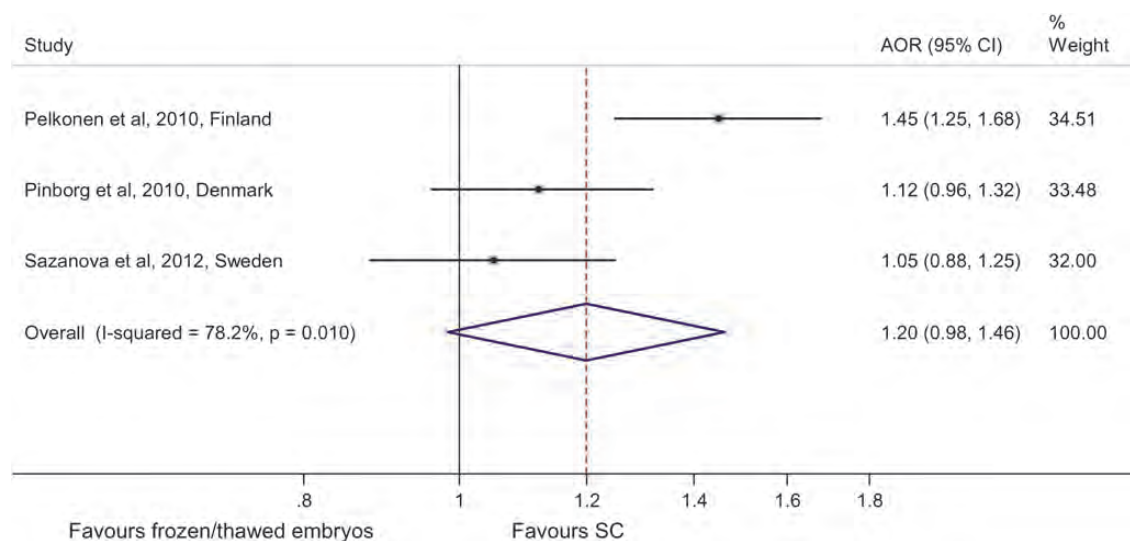


**Figure 6** Pooled estimate on the risk of PTB in singletons born after IVF/ICSI in frozen/thawed cycles versus singletons born after IVF/ICSI in fresh cycles.  $\tau^2 = 0.0138$ .

for the meta-analysis on PTB. The pooled estimate for ICSI versus IVF singletons showed a lower risk of PTB in ICSI singletons [AOR 0.80 (95% CI 0.69, 0.93),  $I^2 = 76.8\%$ ,  $P = 0.002$ ]. The majority of studies showed a lower risk of LBW in ICSI, while only in one was this difference statistically significant (Ombelet et al., 2005).

There is moderate quality of evidence that singletons born after ICSI when compared with singletons born after IVF have a lower risk of PTB (GRADE +++).

Singletons born after IVF/ICSI in frozen/thawed cycles (FET) versus singletons born after IVF/ICSI in fresh cycles and versus singletons in the



**Figure 7** Pooled estimate on the risk of PTB in singletons born after IVF/ICSI in frozen/thawed cycles versus SC singletons in the general population.  $\tau^2 = 0.0240$ .

general population (Supplementary data, Table SVI and Fig. 6 and Supplementary data, Table SVII and Fig. 7): Four studies compared PTB in FET versus singletons from the general population and three were suitable for meta-analysis. The pooled estimate was AOR 1.20 (0.98, 1.46),  $I^2 = 78.2\%$ ,  $P = 0.010$ . Except for the Finnish study all other studies showed similar risk of LBW in FET and SC singletons, however the Finnish study found a lower risk of SGA in FET (Pelkonen et al., 2010).

Ten studies compared PTB in FET versus singletons from fresh embryo transfer and nine were included in the meta-analyses with an AOR 0.85 (0.76, 0.94),  $I^2 = 62.7\%$ ,  $P = 0.006$ . Similarly, all studies apart from Belva et al. (2008) found significantly lower risk of LBW in FET versus fresh embryo transfer.

Most studies are performed on cleavage-stage embryos and after slow freezing, and very few have been published on the perinatal outcome after vitrification, both for cleavage-stage embryos and blastocysts. One recent study compared child outcome after using vitrified blastocysts, fresh blastocysts and slow freezing of early cleavage-stage embryos (Wikland et al., 2010), and found no significant differences in the rates of PTB, LBW or mortality between the groups. A higher rate of SGA was noted in singletons born after fresh blastocysts when compared with children born after vitrified blastocysts.

There is low quality of evidence that singletons born after frozen IVF/ICSI when compared with SC singletons in the general population have no increased risk of PTB (GRADE ++).

There is moderate quality of evidence that singletons born after frozen IVF/ICSI when compared with singletons born after fresh IVF/ICSI have a lower risk of PTB (GRADE +++).

## Embryo culture

IVF/ICSI singletons born after blastocyst transfer versus Cleavage-stage transfer (Day 5 versus Day 2 embryo culture; Supplementary data, Table SVIII and Fig. 8): The search returned four studies out of which two were appropriate for the meta-analysis on PTB, and the pooled

estimate was AOR 1.14 (95% CI 0.80, 1.64),  $I^2 = 68.2\%$ ,  $P = 0.076$  for PTB in singletons from Day 5 versus Day 2 embryo culture.

In the first Swedish study, where an unknown proportion of singletons came from FET cycles, the rate of PTB was higher among singletons after blastocyst transfer than after cleavage-stage transfer (Kallen et al., 2010d). In the first study from Australia with no adjustments, a significantly lower crude rate of LBW and PTB were noted for children born after blastocyst transfer when compared with cleavage-stage transfer, despite the fact that the multiple birth rates were significantly higher in the blastocyst group (5.0 versus 3.4%,  $P < 0.01$ ; Wang et al., 2009). In the two recent studies similar perinatal outcomes were found for Day 5 versus Day 2 culture (Sazonova et al., 2011b; Fernando et al., 2012).

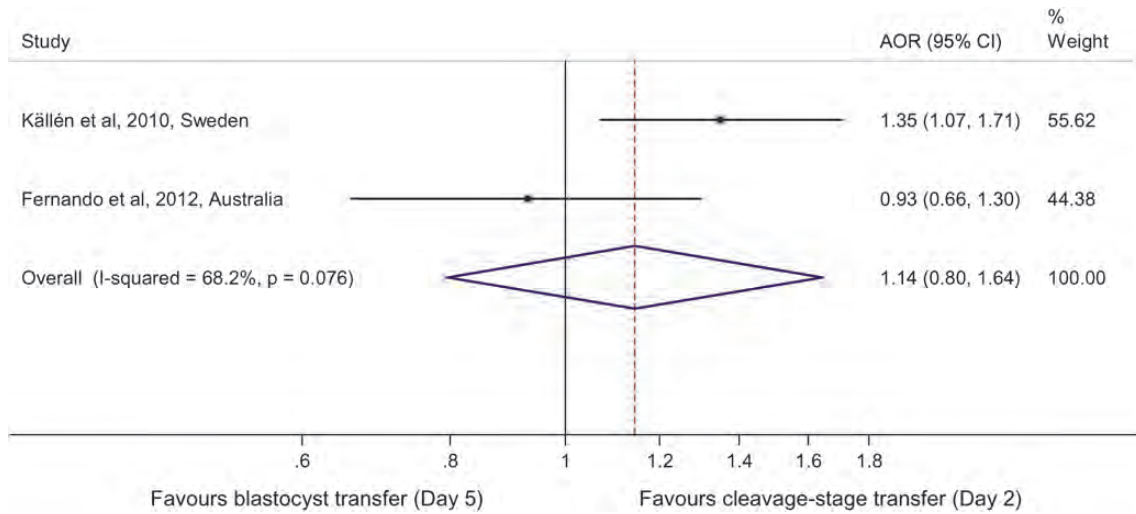
The possible effects of two different culture media were investigated in a Dutch study (Dumoulin et al., 2010). The mean birthweight was around 250 g higher after one medium versus the other, which was statistically significant even with adjustment for gestational age and sex.

There is very low quality of evidence that culture time influences the risk of PTB (conflicting data; GRADE +).

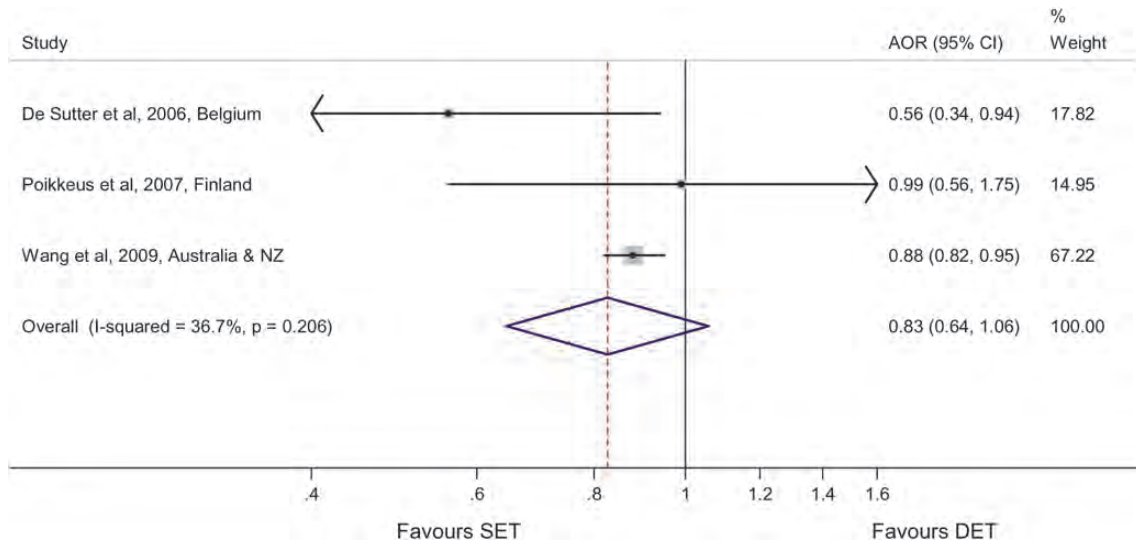
## Number of embryos transferred

Singletons born after SET and eSET (fresh and frozen/thawed cycles) versus singletons born after DET (fresh and frozen/thawed cycles; Supplementary data, Table SIX and Fig. 9): Four studies were identified with three included in the meta-analysis on PTB for SET/eSET versus DET singletons, with a pooled estimate of AOR 0.83 (95% CI 0.64, 1.06),  $I^2 = 36.7\%$ ,  $P = 0.206$ .

In two studies a better outcome was found for SET singletons (De Sutter et al., 2006; Wang et al., 2009), while the small Finnish study found no differences between the two groups (Poikkeus et al., 2007). Comparable absolute values for SET and DET were found in the Swedish study for all perinatal outcomes (Sazonova et al., 2011a).



**Figure 8** Pooled estimate on the risk of PTB in IVF/ICSI singletons born after blastocyst transfer versus IVF/ICSI singletons born after cleavage-stage transfer (Day 5 versus Day 2 culture).  $\tau^2 = 0.0473$ .



**Figure 9** Pooled estimate on the risk of PTB in singletons born after SET in fresh and frozen/thawed (FET) cycles versus singletons born after DET in fresh and FET cycles.  $\tau^2 = 0.0224$ .

There is low quality of evidence that singletons from DET when compared with singletons from SET have no increased risk of PTB (GRADE ++).

*Singletons born after SET or eSET in (fresh and frozen/thawed) cycles versus singletons in the general population (Supplementary data, Table SX, no meta-analysis):* The search revealed four studies, of which only two performed adjusted analyses. Owing to high heterogeneity between the studies no meta-analysis was performed. In the Finnish study, significantly higher rates of both PTB and LBW were detected in the SET group versus the general population (Poikkeus et al., 2007). In the largest study, crude OR for eSET versus the general population

was significantly increased for all obstetric outcomes except perinatal mortality but only remained for PTB after confounder adjustment (Sazonova et al., 2011a).

There is moderate quality of evidence that singletons from SET compared with singletons in the general population have a higher risk of PTB (GRADE +++).

*Singletons with a vanishing co-twin versus singletons from a single gestation (Supplementary data, Table SXI, no meta-analysis):* All published studies showed worse perinatal outcome in singletons after a 'vanishing twin' or a vanishing gestation when compared with singletons from pregnancies with only one gestation initially. The largest

study showed AOR for PTB at 1.73(95% CI 1.54, 1.94) and risks of other perinatal outcomes ranging from AOR 1.73 to 2.88 in singletons from vanishing twin pregnancies versus singletons with only one gestation (Luke et al., 2009). Significantly higher rates of SGA singletons in vanishing twin pregnancies were observed in two studies (Pinborg et al., 2007; Shebl et al., 2008).

There is moderate quality of evidence that singletons from pregnancies with a vanishing co-twin when compared with singletons from only a single gestation have increased risk of PTB (GRADE +++).

## Discussion

According to the extensive literature there are clear increased risks of adverse gestational (placenta previa, etc.) and perinatal outcomes in ART versus SC singletons. Basal risks associated with pregnancy in a population vary greatly with time and place and the professions need to be pro-active to determine ART risks as early as possible and to seek strategies to prevent them (Kalra and Barnhart, 2011). Risks can be categorized into intrauterine growth disturbances, perinatal adverse events and birth defects. They manifest themselves in an increase in morbidity and mortality, either in pregnancy, perinatally or later in life, according to the theory of fetal origins of adult disease (Barker, 2007; Calkins and Devaskar, 2011). Non-iatrogenic risks derive from patient characteristics and include life-style factors that may be preventable by the patients themselves, possibly after proper professional information and advice. Additive, iatrogenic ART risks may come from ART *per se*, for example, from its various applications: ovarian stimulation, ovum retrieval strategies, laboratory technologies and embryo transfer strategies. It has now been clearly demonstrated that drastic reductions in iatrogenic, multiple pregnancies, by adopting SET as the norm results in a clinically significant decrease of risk for mother and child after ART, including pre-eclampsia for the mother and cerebral palsy for the children (Finnstrom et al., 2011).

Still ART singletons have a higher risk of adverse perinatal outcome than SC singletons even after adjustment for maternal age and parity. The causes of this adverse outcome are, however, poorly understood.

One intriguing finding is that recent Swedish population-based studies show that perinatal outcome in ART singletons improves over time, with fewer risks in the more recent populations of ART children when compared with the earlier ART populations (Kallen et al., 2010a; Sazonova et al., 2011a). This trend over time can be attributable to many factors including a shift towards less severe reproductive disease in the couples, milder ovarian stimulation, SET, and improvements in the laboratory techniques and better culture media over time may also have an impact.

The present systematic review included studies with a focus on factors influencing perinatal risks in ART singletons. Results in terms of risk parameters were delineated into four major subheadings: subfertility *per se*, COS, laboratory procedures and number of embryos transferred. These four subheadings are discussed in detail below.

### Subfertility

TTP and 'years of involuntary childlessness' can be interpreted as pseudo-markers for the severity of reproductive abnormalities in a couple. With a moderate quality of evidence, the meta-analysis for

PTB demonstrated that AOR was significantly higher in SC singletons from subfertile couples versus singletons from fertile couples.

Furthermore, the Swedish national data have revealed that the higher perinatal risks observed in IVF singletons decline after adjusting for 'years of involuntary childlessness' (Kallen et al., 2010a). This negative effect of subfertility on perinatal outcome in ART has also been shown for other outcomes, such as congenital malformations (Basso and Olsen, 2005; Zhu et al., 2006, 2009). The only study not showing significantly poorer perinatal outcome in SC singletons from subfertile versus fertile couples was a Danish survey but owing to the questionnaire design selection bias between the subfertile and fertile population may be present (Wisborg et al., 2010a).

Studies on perinatal outcomes of ART singletons have been criticized owing to problems of comparability between subfertile mothers and groups of healthy, fertile mothers in the general population. Therefore, it has not been possible to disentangle whether the increased risk of adverse perinatal outcome after ART is attributed to the reproductive technology *per se* or to factors related to the inherent infertility. Two studies tried to overcome this by using a sib-ship design with comparisons of perinatal outcomes in ART versus non-ART singleton siblings, hence keeping the maternal and paternal factors in a 'steady-state'. The meta-analysis on ART versus the non-ART siblings demonstrated significantly higher risk of PTB in the ART sibling (Fig. 3). The Norwegian study concluded that the increased perinatal risk observed after ART compared with the general population was related to the factors that led to infertility rather than to parameters of the reproductive technology, as the differences in perinatal outcome between ART and non-ART were absent in the sib-ship adjusted analyses (Romundstad et al., 2008). In contrast, the Danish sib-ship analyses demonstrated declining but persisting significantly increased perinatal risk in ART versus non-ART singleton siblings, indicating that also factors related to the treatment *per se* adversely affect ART singleton outcome (Aaris Henningsen et al., 2011). Further, the meta-analysis on PTB in IVF singletons versus SC singletons of subfertile couples (TTP > 1 year) showed significantly higher risk of PTB in the IVF population (Fig. 2) also indicating a risk attributable to the IVF treatment *per se*. In line with this, two studies found that singletons from ICSI couples with a non-affected female partner carried a lower perinatal risk compared with IVF (Ombelet et al., 2005; Wang et al., 2005).

Obviously, further studies with sib-ship designs or studies with singletons from subfertile couples as comparison groups would add significantly to our knowledge in this field.

According to the current literature, subfertility *per se* has a documented negative influence on perinatal outcomes. As this is a non-iatrogenic effect it cannot be prevented directly. However, a changing patient mix towards a more reproductively healthy subfertile population over time as well as an improved lifestyle in couples seeking fertility treatment may, as previously mentioned, diminish perinatal risks in the offspring (Nygren et al., 2007). In summary, subfertility *per se* plays a significant role in the poorer perinatal outcome in ART singletons, however most studies indicate that it is not the only contributor.

### Controlled ovarian stimulation

COS results in marked endocrine changes owing to multiple follicular maturation and development of multiple corpus lutea. These

modifications in the endocrine profile may have a downstream negative effect on implantation and early pregnancy (Pelinck *et al.*, 2010). In high-dose COS, non-optimal oxygenation of the rapidly growing follicles has been suggested to disturb normal oocyte development (Plachot, 2001), and multiple oocyte maturation seems to lead to an increased proportion of chromosomal anomalies (Baart *et al.*, 2007). Furthermore, the indication and duration of infertility is often different for OI/IUI versus IVF and almost never described in the papers although extremely important.

With a moderate level of evidence the meta-analysis demonstrated that singletons from OI and/or IUI are at a higher risk of PTB than singletons from a fertile population ( $TTP \leq 1$  year; Fig. 4). This may, however, be interpreted as being caused by the subfertility of the mothers to OI/IUI singletons. On the other hand, studies have shown that COS/IUI singletons have better outcome than IVF singletons, which can again be attributed to a negative impact of the IVF laboratory procedures or more aggressive COS regimens (Wang *et al.*, 2002; De Sutter *et al.*, 2005). The appropriate populations for determining the specific effects of COS on perinatal outcome are comparisons between COS/IUI singletons and SC singletons from a subfertile population. This has, to our knowledge, only been performed in two very small series with diverging results (Kapiteijn *et al.*, 2006; Pelinck *et al.*, 2010). Kapiteijn *et al.* (2006) reported that the only negative effect of COS was a borderline significant higher risk of very LBW, while Pelinck *et al.* (2010) showed significantly lower mean birthweight in MNC-IVF versus SC singletons of subfertile couples. A Swedish population-based study adjusted for years of involuntary childlessness and still found a significantly increased risk of PTB and LBW in OI singletons versus SC singletons from the general population, indicating that COS *per se* has an independent effect on the perinatal outcome (Kallen *et al.*, 2002). The Danish survey failed to show any significant difference between OI/IUI and SC singletons of subfertile couples, which may be related to selection bias as mentioned previously (Wisborg *et al.*, 2010a, b).

In contrast to IVF with fresh embryo transfer, FET is usually performed in minimally stimulated or natural cycles. Several studies showed that FET singletons have significantly lower adjusted risks of LBW and SGA than singletons born after IVF/ICSI with fresh embryo transfer (Supplementary data, Table SVI). This lower risk after FET may be attributable to a luteal phase which mirrors the natural cycle, with favourable effects on the endometrium and early implantation.

A recent Swedish national cohort study found, in correspondence with earlier reports (Chung *et al.*, 2006; Griesinger *et al.*, 2008), no association between the number of oocytes retrieved and obstetric outcome (Sazonova *et al.*, 2011b). To conclude, there is low quality of evidence that the total dose of gonadotrophins or the number of oocytes retrieved do not affect perinatal outcome.

## Laboratory procedures

There is some evidence that factors other than parental characteristics play a role for the adverse perinatal outcome in ART singletons. Recently, focus has been on the effects of the laboratory procedures *per se*. Aspects of laboratory procedures include culture media composition and the duration of embryo culture, specific laboratory procedures, such as ICSI, as well as freezing/thawing techniques

including slow freezing and vitrification. As discussed above, it is very difficult to distinguish the effects of the COS from the effects of the IVF laboratory procedures *per se*.

### ICSI versus IVF

With moderate level of evidence the meta-analysis showed a lower risk of PTB in ICSI versus IVF singletons. Three studies found significantly lower risk of PTB or LBW in ICSI versus IVF, while the rest showed similar outcomes and none showed adverse outcomes with regard to ICSI (Supplementary data, Table SV). Wang *et al.* (2005) showed higher perinatal risks in children from couples with female factor infertility (mainly treated with IVF) compared with children from couples with male factor infertility (mainly treated with ICSI), indicating a beneficial role of a non-affected mother. It is in the nature of ICSI that the majority of the female partners are reproductively healthy, which may affect the outcome favourably. Concerning assisted hatching/zona drilling, no studies were identified on these procedures and perinatal outcomes.

### Frozen/thawed versus fresh embryos

The increased use of FET has intensified the awareness of the safety aspects of this procedure (de Mouzon *et al.*, 2010). According to the meta-analyses, FET singletons have a similar risk of PTB as singletons in the general population however a lower risk compared with IVF/ICSI singletons from fresh embryo transfer with low and moderate levels of evidence, respectively. The literature on the outcome after newer cryopreservation techniques, such as vitrification of embryos, is too limited to draw any firm conclusions regarding the outcome.

Ten years ago, a French study demonstrated that the rate of SGA singletons originating from frozen–thawed embryos was half that of singletons coming from fresh embryo transfers (Olivennes *et al.*, 2002). Correspondingly, an Australian group reported lower mean z-scores in singletons born from fresh compared with those born from cryopreserved embryos (Shih *et al.*, 2008). The z-score or standard deviation is a measure of the dispersion of birthweight data. The lower the z-score, the more children with LBW at a given gestational age. Three recent cohort studies demonstrated a lower risk of being SGA in singletons from FET versus fresh embryo transfer (Pelkonen *et al.*, 2010; Pinborg *et al.*, 2011; Kato *et al.*, 2012), while a recent Swedish study showed a not significantly lower risk of SGA in FET singletons (Sazonova *et al.*, 2012). Altogether, the lower rates of PTB and SGA were hitherto considered reassuring and explained by the mild or even absent ovarian stimulation in FET cycles and/or the positive selection of embryos and patients.

However, three reports also revealed a significantly higher adjusted risk of being large for gestational age (LGA) in FET singletons when compared with singletons from fresh embryo transfer (AOR 1.6, 1.7; Pelkonen *et al.*, 2010; Pinborg *et al.*, 2011; Sazonova *et al.*, 2012), while the increased risk of LGA in the Japanese study disappeared after adjustment for maternal age, BMI, parity, type of stimulation protocol, ICSI versus conventional IVF fertilization, blastocyst versus cleavage-stage embryo culture and infant sex (Kato *et al.*, 2012). In two of the populations the risk of being LGA in FET singletons even exceeded that of the SC singletons, with AOR 1.5 (Pinborg *et al.*, 2011; Sazonova *et al.*, 2012). In the Swedish study, they aimed to distinguish between FET singletons after either SET or DET and found

similar increased risk of LGA (greater than +2 SD) also in FET singletons from SET versus the general population (Sazonova et al., 2012). For LGA greater than +3 SD they found no increased risk of LGA compared with either the general population or to singletons from fresh embryo transfer. The Danish sibling cohort study with one singleton sibling born after fresh transfer and the other after FET showed that the higher mean birthweight in FET singletons remained even in the same mother after adjustment for maternal age and birth order (Aaris Henningsen et al., 2011). This indicates that factors apart from maternal characteristics also influence the higher birthweight in FET singletons. Thus, the cryopreservation techniques may induce changes in the developmental processes in the early embryo stages and hence in the intrauterine growth potential. Macrosomic babies are at increased risk of adverse perinatal outcomes, such as stillbirth, birth asphyxia, shoulder dystocia, hypoglycaemia, respiratory distress and perinatal mortality (Henriksen, 2008). Macrosomia has also been reported as a risk factor for childhood cancer (Kallen et al., 2010e).

In animals, the association between IVF and 'large offspring syndrome', in particular related to cattle and sheep, is well known (Young et al., 1998; Grace and Sinclair, 2009). However, the 'large offspring syndrome' in animals is closely related to more serious conditions including organ and placental abnormalities with polyhydramnios (Young et al., 1998). There is no reason to assume that the higher risk of LGA in FET singletons mirrors the 'large offspring syndrome' observed in animals, as no increased risk of malformations has been observed after transfer of frozen/thawed embryos in humans. In a review from 2009 it is claimed that subtle epigenetic modifications to non-imprinted loci in gametes and the preimplantation embryo may have health-related consequences that do not manifest until adulthood (Grace and Sinclair, 2009). Accumulating evidence from animal studies indicates that such effects do exist, at least in animals.

A second explanation for the higher rate of LGA in FET singletons could be the possible asynchrony between the endometrium and embryo, which may alter the subsequent trajectory of fetal growth and development (Grace and Sinclair, 2009). A third explanation may be that IVF culture in humans as well as in animals is prone to *in utero* overgrowth but the ovarian stimulation and the altered steroid hormone profiles in fresh embryo transfer cycles inhibit this overgrowth in humans. The mechanisms behind epigenetic modification in human embryos and the relation to cryopreservation remain to be explored.

#### Culture time and culture media composition

There is very low quality of evidence that embryo culture time influences perinatal outcome (conflicting data). Only two studies with contradictory results were appropriate for meta-analysis. One study showed a higher risk of PTB after blastocyst culture (Kallen et al., 2010d), while the other showed a similar risk of PTB in the two groups (Fernando et al., 2012; Fig. 8). Wang et al. (2009) showed a lower crude percentage of PTB after blastocyst versus cleavage-stage culture.

The proportion of FET cycles in each group was not reported in the Swedish report and it may bias the results towards a higher PTB risk in the blastocyst group, if the proportion of FET cycles was higher in the cleavage-stage group (Kallen et al., 2010d). In another recent Swedish study, no effects were found from blastocyst culture on the adjusted

risk of very LBW and SGA in singletons from fresh embryo transfers only (Sazonova et al., 2011b).

Only one RCT of sufficient sample size has examined the effects of the culture media composition and found a significant difference in mean birthweight between two culture media (Dumoulin et al., 2010). Many changes in gene expression and epigenetic modifications take place in the preimplantation embryo and may therefore affect outcome (Natale et al., 2001; Lonergan et al., 2003). In animal models, preimplantation embryo culture has been shown to affect methylation and expression of imprinted genes (Sinclair et al., 2000). In mice embryos, it has been shown that *in vitro* culture of preimplantation embryos in the presence of serum can affect the regulation of imprinted genes leading to diminished growth and disturbances of the development (Khosla et al., 2001). Developmental and behavioural alterations have also been identified in adult mice derived from *in vitro* produced embryos, lending support to the possible long-term effects (Ecker et al., 2004; Fernandez-Gonzalez et al., 2007). A recent review concluded that studies in both animals and humans have made it increasingly clear that proper epigenetic regulation of both imprinted and non-imprinted genes is important to placental development. Disturbance, which can be caused by various environmental factors including *in vitro* culture, can lead to abnormal placental development and function with possible consequences for maternal morbidity, fetal development and disease susceptibility in later life (Nelissen et al., 2011).

From the current literature primarily based on animal models, ART technologies, particularly *in vitro* culture of embryos, can affect gene expression and epigenetic modification of the embryonic genome. However, it is not known whether these changes have long-term effects and if the results from other species are applicable to human embryos. These findings, however, do implicate a strong need for future research in the field of embryo culture and epigenetic alterations in the human placenta and fetus.

### Number of embryos transferred

Even in countries with SET as the norm, 20–50% of transfers are still DET. There is moderate quality of evidence that the vanishing twin phenomenon remains an issue, leading to growth disturbances and to non-optimal perinatal outcomes including a higher risk of perinatal mortality among ART singletons (Supplementary data, Table SXI). The vanishing twin phenomenon can be prevented by using SET. Vanishing twin pregnancies involve about 10% of pregnancies with a DET-only strategy and a much higher proportion in a 'three-or-more-embryo-transfer' situation (Pinborg et al., 2005). The meta-analysis exploring PTB in eSET/SET versus DET singletons showed no significant difference in PTB (Fig. 9). Both studies comparing singletons from SET versus singletons from the general population found a higher risk for PTB in the SET singletons (Supplementary data, Table SX).

SET is highly recommended to reduce rates of multiple pregnancy and vanishing twins, hence minimizing perinatal risks for ART singletons. However, it seems that SET cannot completely eradicate the higher perinatal risks in ART singletons, as a certain risk increase may remain even in SET singletons.

#### Oocyte donation and surrogacy

OD and surrogacy pregnancies were not included in this systematic literature search. Briefly, since the 1990s, it has been known that there is

a high risk of obstetric complications in OD pregnancies. In most studies, donor oocyte recipients have an increased incidence of preterm or LBW (Soderstrom-Anttila, 2001; Sheffer-Mimouni *et al.*, 2002; Nelson and Lawlor, 2011). As in standard IVF, a high number of multiple pregnancies and vanishing twins contribute to this adverse perinatal outcome (Rodriguez-Gonzalez *et al.*, 2002). However, the risk of delivering a preterm or LBW baby is also increased in singleton OD pregnancies and it seems to be higher than in conventional IVF (Soderstrom-Anttila *et al.*, 1998; Gibbons *et al.*, 2011). Comparisons of perinatal outcome in singleton pregnancies from IVF, OD and gestational carrier cycles show the lowest birth-weight among oocyte recipients and the highest among gestational carriers, suggesting that the uterine environment is more important to this outcome than oocyte quality (Gibbons *et al.*, 2011). It also indicates that, compared with standard IVF, the effect of maternal health conditions in OD might be more prominent in relation to the adverse perinatal outcome. Uterine vascular dysfunction associated with premature ovarian insufficiency might affect pregnancy negatively.

#### Other possible iatrogenic causes

It is well known that the frequency of placenta previa in ART singleton pregnancies is higher than after SC, which may be related to the embryo transfer procedure (Jackson *et al.*, 2004; Kallen *et al.*, 2005b; Romundstad *et al.*, 2006; Healy *et al.*, 2010; Sazonova *et al.*, 2011b). Further, more precautions might be taken in ART pregnancies, which may lead to higher rates of 'iatrogenic' Caesarean sections and medical inductions of labour causing a lower mean gestational age in ART singletons. Again it is very difficult to disentangle iatrogenic causes from other causes of adverse outcomes. One might restrict the comparison to spontaneous vaginal singleton births after ART versus spontaneous vaginal singleton births after natural conception. In a sub-analysis this restriction was performed in the Norwegian study (Romundstad *et al.*, 2008) and the results clearly indicate that the more rigorous obstetric interventions in ART pregnancies also contribute to an increased risk of a shorter mean gestational length in ART pregnancies. However, the obstetric management of singleton ART pregnancies seems to be approaching the conventional management of SC singletons (Romundstad *et al.*, 2009).

## Concluding remarks

In summary, one important finding from this review is that a substantial proportion of the increased risks in ART singletons can be attributed to parental characteristics, including subfertility *per se*, but although they play an important role, parental factors are not the only risk parameter for ART singletons. FET singletons have a lower risk of PTB than singletons from fresh embryo transfer, indicating that one or more of the ovarian stimulation, embryo selection or freezing/thawing procedures affect outcome. Additionally culture medium may also exert an influence on outcome. However, distinguishing the influence of each of these parameters is still a difficult task. Pregnancies with an ultrasound-verified vanishing twin are significantly related to poorer perinatal outcome in the surviving co-twin, indicating that the vanishing gestation in singletons conceived after DET is one of the explanations for the poorer outcome in ART singletons. ICSI singletons seem to have slightly better perinatal outcome than singletons from conventional IVF. Contradictory results are reported for ART

singletons regarding the influence of the number of culture days and the dosage of the gonadotrophin stimulation.

We suggest the following prevention strategies for ART singletons:

(i) Preconceptional counselling to optimize the general health status of the pre-pregnant women, i.e. appropriate medication for other diseases and lifestyle advice to stop smoking and to optimize BMI, (ii) eSET to prevent the burden of multiple pregnancies and to reduce the prevalence of vanishing twins, (iii) milder ovarian stimulation strategies to prevent OHSS, (iv) continuous surveillance of laboratory procedures and (v) minimizing iatrogenic causes of PTB by improving antenatal care strategies with fewer medically induced births and Caesarean sections on vague indications.

More research is needed to investigate whether milder COS regimens can improve embryo quality and endometrial conditions. Laboratory strategies should be revised according to investigations of culture media composition. Whether a longer culture time for embryos has a negative influence on perinatal outcome still needs to be elucidated. One of the future challenges for the profession is to develop appropriate test systems for culture media and additives (e.g. growth factors) in order to prove their safety and quality with regard to embryo development and epigenetic modifications before their application in human ART.

## Supplementary data

Supplementary data are available at <http://humupd.oxfordjournals.org/>.

## Authors' roles

A.P., U.B.W., L.B.R., A.L. and C.B. conceptualized the review. A.P., U.B.W., A.L. and C.B. searched databases, selected articles and performed data-extraction and statistical analysis. L.B.R. provided statistical support and data meta-analysis. A.P., U.B., L.B.R., A.L. and C.B. took the lead in writing the review. K.A., V.S.A., K.G.N and J.H. did critical appraisal, data interpretation and revising of the report. All authors approved the final version for submission.

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## Conflict of interest statement

None declared.

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