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The health of children conceived by ART: "The chicken or the egg?"

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Abstract

Worldwide, more than 7 million children have now been born after ART: these delivery rates are steadily rising and now comprise 2-6% of births in the European countries. To achieve higher pregnancy rates, the transfer of two or more embryos was previously the gold standard in ART. However, recently the practise has moved towards a single embryo transfer policy to avoid multiple births. The positive consequences of the declining multiple birth rates after ART are decreasing perinatal risks and overall improved health for the ART progeny. In this review we summarize the risks for short- and long-term health in ART singletons and discuss if the increased health risks are associated with intrinsic maternal or paternal factors related to subfertility or to the ART treatments *per se.*

Although the risks are modest, singletons born after ART are more likely to have adverse perinatal outcomes compared to spontaneously conceived (SC) singletons dependent on the ART method. Fresh embryo transfer is associated with a higher risk of small for gestational age babies (SGA), low birthweight and preterm birth (PTB), while frozen embryo transfer is associated with large-for-gestational age babies and pre-eclampsia. ICSI may be associated with a higher risk of birth defects and transferral of the poor semen quality to male progeny, while oocyte donation is associated with increased risk of SGA and pre-eclampsia. Concerning long-term health risks, the current evidence is limited but suggests an increased risk of altered blood pressure and cardiovascular function in ART children. The data that are available for malignancies seem reassuring, while results on neurodevelopmental health are more equivocal with a possible association between ART and cerebral palsy. The laboratory techniques used in ART may also play a role, as different embryo culture media give rise to different birthweights and growth patterns in children, while culture to blastocyst stage is associated with PTB. In addition, children born after ART have altered epigenetic profiles, and these alterations may be one of the key areas to explore to improve our understanding of adverse child outcomes after ART.

A major challenge for research into adverse perinatal outcomes is the difficulty in separating the contribution of infertility *per se* from the ART treatment (i.e. “the chicken or the egg”?). Choosing and having access to the appropriate control groups for the ART children in order to eliminate the influence of subfertility *per se* (thereby exploring the pure association between ART and child outcomes) is in itself challenging. However, studies including children of subfertile couples or of couples treated with milder fertility treatments, such as IUI, as controls show that perinatal risks in these cohorts are lower than for ART children but still higher than for SC indicating that both subfertility and ART influence the future outcome. Sibling studies, where a mother gave birth to both an ART and a SC child, support this theory as ART singletons had slightly poorer outcomes.

The conclusion we can reach from the well designed studies aimed at disentangling the influence on child health of parental and ART factors is that both the chicken and the egg matter.

Keywords

ART, IVF, i ICSI, oocyte donation, infertility, frozen embryo transfer, adverse child outcomes, culture media, sibling studies, epigenetics

Introduction

Worldwide, more than 7 million children have now been born after ART (Adamson G. 2017). The health of ART offspring and the risk and safety aspects of specific ART technologies are of importance for the future generations as up to 6% of the European birth cohorts is conceived by ART (Calhaz-Jorge *et al.* 2017). ART includes standard IVF and ICSI, which is the more advanced method originally used for severe male factor infertility. The freezing and thawing of embryos is an additional part of ART, as is oocyte donation (OD). Pregnancies resulting from ART are generally associated with adverse obstetric and perinatal outcomes when compared to spontaneously conceived (SC) pregnancies (Helmerhorst *et al.* 2004; Pinborg *et al.* 2013; Adams *et al.* 2015) mainly related to the higher rate of multiple gestations. However, the decline in multiple birth rates after ART resulting from a move towards single embryo transfer (SET) has considerably reduced perinatal risks for ART children (Henningsen *et al.* 2015). Yet, singletons born after ART have a two- to three-fold increased risk of adverse perinatal outcomes (McDonald *et al.* 2009; Pandey *et al.* 2012).

A major challenge for research into these adverse outcomes is the difficulty in separating the contribution of infertility *per se* from the ART treatment (i.e. “the chicken or the egg”). Furthermore, in order to compare the health of children born after a natural pregnancy with that of children born after ART, an accurate assessment of the evidence is required, including the quality of that evidence. Selecting an appropriate comparison group(s) for children born after ART is vital, and this is a challenge. In most studies, SC children comprise the control group, but it is debatable whether this is the correct group. If parental factors such as infertility *per se* influence health of the offspring, then children conceived naturally by subfertile parents [with a time-to-pregnancy (TTP) of more than 1 year], may constitute a more appropriate control group. Children born after mild ovarian stimulation and IUI could also serve as a better group for comparison. These control groups are, however, difficult to access and most often not included in any registry. To overcome the confounding of parental factors, sibling studies, where a woman gave birth to both a child conceived by ART and a SC child, are valuable. The pure influence of different ART methods can be explored, as several parental factors are kept stable in sibling studies (Romundstad *et al.* 2008; Henningsen *et al.* 2011). The pitfalls in sibling studies are that they may still be confounded by differences in maternal age and birth order of the siblings, unless these factors are being properly adjusted for.

Possible changes in DNA methylation patterns associated with different ART methods may influence development of the placenta and foetus in the early embryonic stages and cause changes in growth patterns. According to the developmental origins of health and disease

(DOHaD) theory, this may result in higher cardiometabolic risk profiles in the ART offspring (Barker 2007).

It is evident that the high rate of multiple births from multiple embryo transfer is the most important cause of poor child outcome in ART (Bergh *et al.* 1999; Pinborg *et al.* 2005). Moreover, SET decreases the short- and long-term risks for the children dramatically, while giving similar live birth rates as double embryo transfer (DET) on a cumulative basis (Thurin *et al.* 2004; Thurin-Kjellberg *et al.* 2009).

In this grand theme review, we will focus on ART singletons, summarizing the short- and long-term risks in singletons conceived by ART, including IVF, ICSI, freezing/thawing and OD (The definitions of all abbreviations used in the review are shown in Table I.) Children conceived by MAR, including IUI, PGD and preimplantation genetic testing for aneuploidy, were all excluded. The influence of different parental lifestyle factors will not be described here. The discussion is focused on studies aiming to disentangle the different modulators of adverse outcomes in ART singletons, including a discussion on causality in terms of “the chicken or the egg” question.

Methods

A literature search was performed on Medline-PubMed, Embase and Cochrane Central Register of Clinical Trials (RCT) to identity eligible English language studies.
The reference lists of relevant articles were reviewed to identify additional qualified studies. No formal quality assessments where made.

Short-term health: perinatal outcomes

IVF/ICSI versus the general population

Several meta-analyses and large cohort studies have compared perinatal outcomes for IVF singletons versus singletons from the general population of naturally conceived children, with adjustment for relevant confounders (at least maternal age and parity) (Helmerhorst *et al.* 2004; Jackson *et al.* 2004; McGovern *et al.* 2004; McDonald *et al.* 2009; Pandey *et al.* 2012; Marino *et al.* 2014; Henningsen *et al.* 2014; Qin *et al.* 2017). Most studies have included both children born after IVF and ICSI as well as fresh embryo transfer and frozen embry transfer (FET). The comparison groups have been children born from SC, but some studies included only SC in a subfertile population as controls, while others excluded the subfertile population. The latest systematic review and meta-analysis from 2017 included more than 180,000 IVF/ICSI singleton pregnancies from 52 cohort studies from all over the world (Qin *et al.* 2017).

The results from these studies are consistent, showing that singletons born after IVF/ICSI have more compromised perinatal outcomes compared to SC (Fig. 2). Significantly higher rates of preterm birth (PTB) have been found for IVF/ICSI singletons with adjusted risks between 1.41 and 2.04 for PTB and 1.68-3.07 for very preterm birth (VPTB). Similarly, higher rates of low birthweight (LBW) and very low birth weight (VLBW) have been found (adjusted risks for LBW 1.6-1.7 and for VLBW 1.8-3.0). Most studies also found an increased risk for being born small for gestational age (SGA) with adjusted risks of around 1.5 and increased risk rates of perinatal mortality with adjusted risks between 1.7 and 2.0. An Australian study found a two-fold increased risk of stillbirth (gestational age [GA] not defined) in pregnancies after ART versus SC (Marino *et al.* 2014). A significantly increased risk for stillbirth in ART singletons was found only for extremely PTB (before 28+0 gestational weeks) in a large Nordic collaborative study (Committee of Nordic ART and Safety, CoNARTaS) including 62,485 ART singletons (adjusted risk 2.0) (Henningsen *et al.* 2014). ART pregnancies have more obstetric complications, such as hypertensive disorders in pregnancy (HDP), placental complications (placenta praevia, abruption and third trimester bleeding), gestational diabetes, interventions (e.g. cesarean section and medical induction of labour) and increased risks of preterm prelabour rupture of the membranes, indicating that there is both a higher risk of indicated PTB and a higher risk of spontaneous PTB in ART pregnancies (Pandey *et al.* 2012; Opdahl *et al.* 2015).

IVF versus ICSI

Since the first child conceived as a result of ICSI was born more than 25 years ago there has been an ongoing debate on the use and safety of the technique (Palermo *et al.* 1992). Initially, ICSI was used to treat severe forms of male factor infertility but today it is also used to treat mild male factor infertility, mixed infertility, unexplained infertility and fertilization failures. Both the latest report from ESHRE and from The International Committee Monitoring Assisted Reproductive Technologies (ICMART) reveal an increasing global use of ICSI, with one-third of fresh ART cycles using standard IVF and two-thirds using ICSI (Dyer *et al.* 2016; Calhaz-Jorge *et al.* 2017). Owing to the invasiveness of the ICSI procedure, the arbitrary selection of the spermatozoon, and genetic and epigenetic parental factors, a concern has been raised over the health of ICSI children.
When comparing ICSI and standard IVF, most large studies have found similar or lower risks of PTB, VPTB, LBW and VLBW and peri/neonatal mortality in singletons born after ICSI.
Pinborg *et al* analysed singletons born after ICSI (fresh or frozen/thawed cycles) versus singletons born after IVF (fresh or frozen/thawed cycles) (Pinborg *et al.* 2013). Ten studies were included in

the analysis with five studies included in a meta-analysis on PTB. The pooled estimate for ICSI versus IVF singletons showed a lower risk of PTB in ICSI singletons (adjusted odds ratio (AOR) 0.80, 95% CI 0.69 to 0.93).
A possible explanation for the better outcome in ICSI singletons may be that in ICSI the majority of the women are reproductively healthy, which could give a more favourable perinatal outcome.

IVF/ICSI fresh versus IVF/ICSI frozen/thawed embryo transfer

The first human pregnancy following the transfer of a frozen/thawed embryo was reported in 1983 (Trounson and Mohr 1983). The number of FETs has now increased, as have pregnancy rates (Calhaz-Jorge *et al.* 2017). Initial systematic reviews and meta-analyses have suggested that perinatal outcomes are better in children conceived following FET as compared with fresh embryo transfers (ET), with reduced risks of PTB and LBW (Wennerholm *et al.* 2009; Maheshwari *et al.* 2012; Pinborg *et al.* 2013; Zhao *et al.* 2016). There was no difference in the risk of stillbirth and perinatal mortality. These reviews did not include outcomes such as macrosomia and large for gestational age (LGA).
Maheshwari *et al* recently published an updated systematic review and cumulative meta-analysis (Maheshwari *et al.* 2018) including 26 studies and almost 300,000 deliveries. It confirmed that singletons conceived from FET were at lower risk of PTB (Relative Risk (RR) 0.90, 95% CI 0.84 to 0.97), LBW (RR 0.72, 95% CI 0.67 to 0.77) and SGA (RR 0.61, 95% CI 0.56 to 0.67) compared to those conceived from fresh ET. However, they also found that singletons born after FET had an increased risk of being born LGA (RR 1.54, 95% CI 1.48 to1.61) and having a birthweight (BW) more than 4000 g (RR 1.85, 95% CI 1.46 to 2.33). There was no difference in the risk of perinatal mortality between children born after FET and fresh ET but the risk of HDP was increased (RR 1.29, 95% CI 1.07 to 1.56) in pregnancies after FET (Maheshwari *et al.* 2018).
Vitrification is an ultrarapid cryopreservation method, which has superseded slow freezing as the dominant method for cryopreservation in recent years. It has been associated with higher post-thaw survival rates and clinical pregnancy rates when compared to slow freezing (AbdelHafez *et al.* 2010; Rienzi *et al.* 2017). However, the high concentrations of cryoprotectants used for vitrification have raised concerns about possible negative health effects for the children. When comparing vitrification and slow freezing of day 3 embryos or blastocysts, a similar or slightly better outcome (higher BW) was found for the vitrified embryo groups (Liu *et al.* 2013; Li *et al.* 2014; Belva *et al.* 2016b).

The implications of the findings of macrosomia and LGA after FET and the consequences for childhood health and risk of obesity are unclear and more follow-up studies are needed. Different explanations for this over-growth have been suggested. It may be a selection process with higher quality embryos surviving the freezing and thawing procedure. Another possible explanation is that the cryopreservation technique may cause epigenetic modifications (heritable alterations that are not caused by changes in DNA sequence but by modifications, such as DNA methylation) at the early embryonic stages and hence in the growth potential of the foetus. A third suggested explanation is that the uterine environment in a FET cycle results in a more natural uterine environment, as the ovarian stimulation used in a fresh IVF cycle is not used in most FET cycles. Recently, the shift to a 'freeze all' strategy in an increasing number of ART treatments in preference to the conventional policy of fresh ET has caused attention. Three large randomised controlled trials (RCTs) have recently been published (Chen *et al.* 2016; Shi *et al.* 2018; Vuong *et al.* 2018). In the Chen *et al* study (n=1508 women), the transfer of vitrified embryos resulted in higher live birth rates than the transfer of fresh embryos in women with infertility associated with polycystic ovary syndrome (PCOS). In the two other RCTs (n=2157 and n=782 women, respectively), which included ovulatory women with no PCOS, the live birth rates were similar in both groups. A higher mean BW was found in the FET group in two of the three RCTs (Chen *et al.* 2016; Vuong *et al.* 2018) and fewer neonates were born SGA in the FET group in one study (Vuong *et al.* 2018).

Trends over time in perinatal outcomes

Recently, a study of the perinatal outcome of 62,379 ART singletons born between 1988 and 2007 in four Nordic countries from the CoNARTaS (**AUTHOR:** can this name be defined?- it is defined in the above text in line 241) group was published (Henningsen *et al.* 2015). The ART singletons were compared with a control group of 362,215 SC singletons. The rates of several adverse perinatal outcomes were stratified into the following time periods: 1988–1992, 1993–1997, 1998–2002 and 2003–2007. For singletons conceived after ART but not for singletons born after SC, a substantial decline in the risk of PTB and VPTB was observed. The proportion of ART singletons born with LBW and VLBW also decreased, as well as the stillbirth and infant death rates. There are several possible explanations for the positive development such as a change in the number of women seeking ART with a shorter duration of infertility, more ICSI/male factor and fewer embryos being transferred during recent years.

Summary

Singletons born after ART have worse perinatal outcomes compared with singletons born after SC. However, these differences are less pronounced for children born after ICSI and FET and show a clear trend of improved outcomes during recent years.

Birth defects and chromosomal anomalies

Classification of birth defects

When reporting birth defects, different classification methods have been used. Major birth defects could be defined as causing functional impairment or requiring surgical correction. This is the classification used by Bonduelle and coworkers in their follow-up studies of ART infants (Bonduelle *et al.* 2005). However, the definition is problematic because it is difficult to replicate. Another classification, which is frequently used, is to include any condition listed in the international classification of diseases (ICD) chapter entitled “Congenital anomalies”. The ICD code alone does not differentiate between major and minor defects (http://apps.who.int/classifications/icd10/browse/2016/en#/XVII) and studies may suffer from problems associated with underreporting and/or variable reporting of minor defects. Classifications of birth defects can also be based on pathology or etiology rather than organ system (Wellesley *et al.* 2005). Furthermore, other systems used in dedicated birth defects registers could include reference to the classification system used by European Surveillance of Congenital Anomalies (EUROCAT) for example (http://www.eurocat-network.eu). These limitations show that the same classification and methodology should be used for subject groups that are compared in a specific study. In addition, as there are considerable differences in the reporting of minor birth defects, the inclusion of major birth defects only is preferable. Many studies include birth defects recorded in live-born children only. However, as many of the most severe birth defects may result in elective pregnancy termination, the total prevalence of birth defects, including those observed in terminated pregnancies, should be reported for all pregnancies, not just those following ART. Furthermore, if ART pregnancies are monitored more closely,such that birth defects are more likely to be detected prenatally, this may introduce bias in terms of differential case ascertainment between ART and non-ART births. The direction of this bias may also depend on whether couples using ART are any more (or less) likely to undergo pregnancy termination of an affected fetus. This important issue has not received much attention in the literature. A single study on congenital heart defects in

France showed no difference in either prenatal diagnosis or the termination of pregnancies between ART and non-ART births (Tararbit *et al.* 2015).

Birth defects

A higher rate of birth defects has been reported in children conceived through ART compared with children conceived naturally (Zhu *et al.* 2006; Pandey *et al.* 2012; Wen *et al.* 2012; Hansen *et al.* 2013; Qin *et al.* 2015). From the meta-analysis by Hansen and coworkers, a 32% increased risk of birth defects in ART-children compared with SC infants (RR 1.32, 95% CI 1.24 to 1.42) was reported, and a slightly increased risk of 36% when singleton births were examined separately (RR 1.36, 95% CI 1.30 to 1.43). When the studies were restricted to major birth defects, the increased risk was 42% (Hansen *et al.* 2013). An increased risk of defects was confirmed by the meta-analysis of Qin and co-workers in 2015, which compared the risk for congenital birth defects in singleton ART-children with SC singletons (RR 1.37, 95% CI 1.29 to 1.45) (Qin *et al.* 2015). According to the cohort study by Davies, including close to 303,000 SC births and 6163 ART births, the increased risk of birth defects associated with standard IVF was no longer significant after adjustment for parental factors such as maternal age, parity, year of birth, maternal smoking during pregnancy and parental occupation (Davies *et al.* 2012). The risk of birth defects associated with ICSI remained increased after multivariable adjustment, although the possibility of residual confounding could not be excluded. Fresh IVF cycles were associated with a lower risk than fresh ICSI cycles. This is contrary to the findings of both Lie and Wen in their meta-analyses where ICSI did not increase the risk for birth defects compared with standard IVF (Lie *et al.* 2005; Wen *et al.* 2012).

A recent Nordic study including more than 90,000 ART children observed an increased risk although with a lower AOR for major birth defects, comparing singleton ART children with children born after SC (AOR 1.14, 95% CI 1.08 to 1.20). No difference was reported between ART twins and SC twins. During the time period 1988-2007, the RR of a major birth defect between ART-children and SC-children remained unchanged with the most common birth defect being cardiovascular defects (Henningsen *et al.* 2018).

A higher prevalence of genital malformations, including hypospadia, was found in singletons born to infertile couples after fertility treatment than in SC singletons (Zhu *et al.* 2006). Evidence suggests that men with testicular failure have an increased prevalence of chromosomal abnormalities (Bernardini *et al.* 2000; Dul *et al.* 2012) as well as cryptorchidism and testicular cancer (Olesen *et al.* 2017). The frequency of malformations in the genitourinary system, in particular hypospadias, has been reported to be about five times higher with ICSI using testicular

sperm (Fedder *et al.* 2007) compared with the risk of hypospadias in the general population (Toppari *et al.* 2001).

Two systematic reviews (Holte *et al.* 2007; Woldringh *et al.* 2010) did not report any difference in the rate of birth defects in children conceived by ICSI using non-ejaculated sperm compared with ICSI using ejaculated sperm. However, most previous studies have compared pregnancy and neonatal outcome based on whether the spermatozoa originated from the testicle, the epididymis or from the ejaculate (Woldringh *et al.* 2011; Belva *et al.* 2011). The possible significance that the specific diagnosis of the azoospermic male partner has (e.g. obstructive azoospermia (OA), non-obstructive azoospermia (NOA) or aspermia) on birth defect rate, however, has hardly been investigated. There was no difference in the major birth defect rate in children conceived when the fathers were diagnosed with NOA compared with OA or aspermia but the numbers were too small to be conclusive (Belva *et al.* 2011; Oldereid *et al.* 2014).

The rate of birth defects in children born after FET with slow freezing procedures seems comparable to that of other ART children (Wennerholm *et al.* 2009; Pelkonen *et al.* 2014; Maheshwari *et al.* 2016). In the study by Belva and co-workers from 2008, a two-fold increase in rate of major malformations was reported in the children born after ICSI compared with IVF, and when compared with fresh transfer and ICSI (Belva *et al.* 2008). However, a more recent Belgian study reported that the prevalence of birth defects in singletons and twins born after embryo vitrification were similar to or slightly lower than after fresh ET (Belva *et al.* 2016b).

Chromosomal anomalies

A significantly higher rate of *de novo*, non-inherited chromosomal abnormalities in children born after ICSI was observed compared with the rate in the general population (1.6% versus 0.5%) (Bonduelle *et al.* 2002). When the father suffered from a reduced sperm concentration, the incidence of *de novo* abnormalities in the children was higher compared to children born to fathers with a normal sperm concentration (2.1% versus 0.24%, respectively) (Bonduelle *et al.* 2002). In another study, an increased risk for chromosomal anomalies seemed to be similar among different infertility groups, but was related to reduced sperm concentration and/or motility (Jozwiak *et al.* 2004). However, the incidence of the *de novo* chromosomal anomalies was comparable in children conceived from non-ejaculated sperm versus ejaculated sperm, and also when comparing OA with NOA in a study of ICSI and 530 children conceived with testicular sperm, 194 with epididymal sperm and 2516 with ejaculated sperm (Belva *et al.* 2011).

Possible mechanisms

Whether this increase in birth defects with ART is attributable to patient characteristics related to infertility or to the IVF/ICSI technique is uncertain. Subfertility, with or without assisted conception, is significantly associated with a higher incidence of birth defects (Zhu *et al.* 2006; Davies *et al.* 2012). Fertility treatment options in general (including standard IVF and ICSI, as well as IUI, hormone treatment, surgery and 'alternatives' i.e. treatments not usually pooled under the heading ART) were found to increase the risk of being diagnosed with a birth defect and the overall prevalence of birth defects increased with increasing time to pregnancy (Zhu *et al.* 2006).
Severely impaired sperm count may rarely be explained by genetic causes such as microdeletions of the Y-chromosome, various chromosomal translocations, mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene or Klinefelter's syndrome (Krausz 2011). However, there is still limited knowledge about the possible mutations and genes involved in male infertility (Bracke *et al.* 2018). ICSI is the method of choice for couples with severe male-factor infertility, giving these men the ability to become genetic fathers. With ART, these genetic abnormalities may be inherited from the infertile father as seen in fathers carrying a deletion in the Y chromosome (Kleiman *et al.* 1999).
As with the effect observed for other adverse perinatal outcomes in ART infants, both infertility and ART treatment factors seem to contribute to the excess risk of birth defects after ART conceptions. It is difficult to obtain information about all individual aspects of ART treatment and their association with a certain birth defect, while holding all the other variables constant in sufficiently large studies (Hansen *et al.* 2013).

Summary

Subfertility as well as infertility treatment with or without ART is associated with a modestly increased risk of birth defects when compared with SC. On an individual level this increase in risk is small.

Long-term health

Neurodevelopmental health

Psychomotor and language development, behavior and social functioning

Three systematic reviews found comparable psychomotor development, overall social functioning, language development and behavior between children born after ART and SC controls (Middelburg *et al.* 2008; Bay *et al.* 2013b; Hart and Norman 2013b).

In a Danish National Birth Cohort study (n= 45, 557), SC singletons born of couples with an increased TTP showed a modest delay in psychomotor development compared with singletons born of fertile couples. Furthermore, infertility treatment was associated with a slight delay in some developmental milestones (Zhu *et al.* 2009).

Cognitive development

In the recent systematic review focusing on cognitive development following ART (Rumbold *et al.* 2017) it was shown that most studies on the subject had methodological limitations, making it difficult to draw reliable conclusions. However, there was some high-quality evidence to suggest that certain ART treatments influence cognitive development (Stromberg *et al.* 2002; Knoester *et al.* 2008; Sandin *et al.* 2013).

In a study including 5680 children born after IVF (3228 singletons), a four-fold increase (95% CI 1.9 to 8.3) in risk of developmental delay and an increased risk of needing habilitation services [odds ratio (OR) 1.7, 95% CI 1.3 to 2.2] compared to SC children was reported (Stromberg *et al.* 2002). However, when only singeltons were included, the increased risk of developmental delay was no longer significant (OR 2.0, 95% CI 0.7 to 5.4) although the OR was 2.0 and the risk of needing habilitation services in singletons was not significant (OR 1.4, 95% CI 1.0 to 2.1). In a Dutch study, ICSI singletons were shown to have significantly lower scores in tests of intelligence (on average 5–7 IQ points lower) compared with SC singletons. However, the number of children included was limited (ICSI n=83, IVF n=83) (Knoester *et al.* 2008).

In a large prospective population-based registry study (n= 30,959 ART children), *Sandin et al.* found a small but significantly increased risk of mental retardation in ART children when compared to SC children (RR 1.18, 95% CI 1.01 to 1.36). When restricting the analysis to singletons the statistical significance disappeared. In a supplementary subanalysis in singletons, *Sandin et al.* found an association between mental retardation and ICSI-FET (RR 2.36, 95% CI 1.04 to 5.36), but not between mental retardation and ICSI-fresh ET (RR 1.60, 95% CI 1.00 to 2.57) (Sandin *et al.* 2013).

Pinborg *et al.* reported no difference in risk of neurological deficits or special needs between IVF/ICSI children (singletons, n= 634) and SC children (Pinborg *et al.* 2003). Wagenaar *et al.* found no difference in cognitive function in 139 IVF conceived singletons aged 8-18 years when compared with SC singletons (Wagenaar *et al.* 2009) and Bay *et al.* reported a comparable risk of mental retardation in IVF conceived (n=139) and SC singletons (Bay *et al.* 2013a).

A Danish study on academic performance, including 4766 adolescents born after ART, reported a higher crude mean test score in ART children (singletons and twins) compared to SC controls. However, for ART singletons the adjusted mean overall test score was slightly lower. No difference

was found in the adjusted analysis for twins (Spangmose *et al.* 2017) Similar results were found in a large population-based Swedish study of 8,323 ART singletons (Norrman *et al.* 2018). The effect of FET versus fresh ET on the school performance of children was reported in a recent Danish study from the same group and no differences were observed (Spangmose *et al.* 2018).

Attention deficit hyperactivity disorder and autism spectrum disease

Attention deficit hyperactivity disorder (ADHD) was found to be weakly associated with IVF in a Swedish study including 28,158 multiples and singletons born after IVF (OR 1.18, 95% CI 1.03 to 1.36). However, after adjusting for length of involuntary childlessness the statistical significance was lost. Furthermore, no association was found when the analyses included only singletons (Kallen *et al.* 2011). A Danish study including 124,269 children born to women with fertility problems reported a significantly higher risk of ADHD [hazard ratio (HR) 1.36, 95% CI 1.29 to 1.45] in these children compared with children born to women without fertility problems, although no adjustments were made for multiple gestations (Svahn *et al.* 2015).

When adjusted for multiplicity, analyses reported by Hvidtjørn *et al.* (n= 33,139) and later Sandin *et al.* (n=30,959) found no increased risk of autism spectrum disease (ASD) for ART treatment overall (Hvidtjorn *et al.* 2011; Sandin *et al.* 2013). Fountain *et al.* (Fountain et al. 2015) identified an elevated incidence of diagnosed ASD amongst 48,865 Californian multiples and singletons conceived by ART. However, the adjusted risk was not elevated among singletons alone. From the same Californian cohort, Kissin *et al.* reported a higher risk of ASD in ICSI singletons compared to standard IVF with fresh ET (adjusted hazard risk ratio (HRR) 1.65, 95% CI 1.08 to 2.52) (ART singletons n= 19,790 (Kissin *et al.* 2015).

In a recent meta-analysis Liu *et al.* concluded that ART was associated with a greater risk of ASD when comparing the complete ART group with SC children (RR 1.35, 95% CI 1.09 to 1.68, $P = 0.007$). However, in the subgroup analysis for singletons only there was no increased risk of ASD (RR 0.94, 95% CI 0.77 to 1.14) (Liu *et al.* 2017).

Cerebral palsy

An increased risk of cerebral palsy (CP) was found for singletons compared with naturally conceived controls in a Swedish study including 5680 children born after IVF (OR 2.8, 95% CI 1.3 to 5.8) (Stromberg *et al.* 2002). In another Swedish study including 16,280 children born after IVF (multiples and singletons), a higher risk for CP was found in the crude analysis but disappeared after adjustment for year af birth, maternal age, parity, smoking and years of unwanted childlessness (AOR 1.14, 95% CI 0.72 to 1.81) (Kallen *et al.* 2005). An increased risk for CP found among 33,139 IVF children (HR 2.34, 95% CI 1.81 to 3.01) in a Danish study disappeared when

adjusted for multiplicity and GA (Hvidtjorn *et al.* 2010). Another Danish study found an increased risk of CP in singletons after fresh ET, but not FET, compared to SC (AOR 2.44, 95% CI 1.15 to 5.22, *P* =0.02) (Pinborg *et al.* 2010).
In a recent Australian register study including 2914 children born after ART, the prevalence of CP was more than doubled in ART singletons at <32 weeks GA compared to SC singletons at <32 weeks GA (AOR 2.7, 95% CI 1.0 to 6.9) (Goldsmith *et al.* 2018).

Summary
For most neurodevelopmental health variables conflicting results exist concerning a possible association with ART. Many of the identified risk associations disappeared after adjustment for multiple births or were only observed in subgroups.

Cardiovascular function and metabolism

Higher cardiovascular and metabolic risk profiles in ART offspring is of importance for individuals as well as for society, as this may influence the health of future generations. As ART is still a relatively new technology the follow-up time is limited to childhood and early adulthood, with limited numbers of children in the older age cohorts, and the studies are based on few cases with mostly small and highly selected control groups.
Cardiovascular and metabolic profiles of offspring conceived by ART have been summarized in a systematic review (Hart and Norman 2013a) and also recently reported in a systematic review and meta-analysis (Guo *et al.* 2017). The (Hart and Norman 2013a) systematic review concludes that the limited data on ART offspring suggest a potential increase in blood pressure (BP), elevated fasting glucose and increase in total body fat composition (Hart and Norman 2013a). The recent meta-analysis included 19 studies with a total of 2,112 IVF/ICSI and 4,096 SC offspring and concluded that systolic BP (SBP) and diastolic BP (DBP) were higher in ART than in SC offspring (weighted mean differences (WMD): 1.88; 95% CI 0.27 to 3.49 for SBP and 1.51; 95%CI 0.33 to 2.70 for DBP) (Guo *et al.* 2017). However, the higher WMD in SBP and DBP was only observed in the cohorts born in 1990-1999 and not for those born in 2000-2009 and was not linked to bias introduced by younger participants or a larger ICSI proportion in the younger cohorts. ART stimulation protocols and techniques have improved over the two timeperiods; milder ovarian stimulation, single embryo transfer and the laboratory tecniques have developed. These are all changes that may cause less epigenetic modification at the gamete and embryonal stages. Meta-analyses showed comparable BMI, low-density lipoprotein, cholesterol and fasting insulin levels for ART and SC children (Guo *et al.* 2017).

Cardiovascular function was reported in five studies including 50-128 ART children and 50-100 SC controls in the age range 1 month to 12 years (Guo *et al.* 2017). Owing to the heterogeneity in the methods used to assess cardiovascular function, no meta-analysis was performed on this outcome. A consistently suboptimal cardiac diastolic function was reported in all studies especially under stressful conditions such as high altitude(Scherrer *et al.* 2012; Valenzuela-Alcaraz *et al.* 2013; Zhou *et al.* 2014; Liu *et al.* 2015; von Arx *et al.* 2015). Two studies showed higher aortic and carotid intima-media thickness among ART offspring (Scherrer *et al*. 2012; Valenzuela-Alcaraz *et al. 2013)*

Recently, a Swiss follow-up of the Scherrer *et al.* 2012 study, including adolescents aged 16-17 years found that the average BP and BP variability were markedly higher in ART-conceived adolescents than in SC controls(Meister *et al.* 2018). Eight of 52 ART participants, but only one of the 43 controls had arterial hypertension (BP > 130/80) (Meister *et al.* 2018).

Summary

Limited data suggest a potential increase in BP and deteriorated metabolic profiles in ART children as well as suboptimal cardiovascular function.

Growth

Ceelen *et al.* assessed the growth from birth to 8-18 years in 233 ART versus SC children and observed a rapid catch-up growth from birth, which gave rise to concern as rapid growth is associated with an increased risk of disease later in life, in particular cardiovascular disease (Barker 2007; Ceelen *et al.* 2009).

Although children born after ART are more likely to be born preterm, most studies have shown similar growth patterns in ART and SC children (Hart and Norman 2013b; Chen and Heilbronn 2017). A recent US prospective follow-up study compared 969 singletons conceived after fertility treatment (ART and ovulation induction +/- IUI) with 2,471 SC children and found comparable growth and development up to 3 years of age (Yeung *et al.* 2016) and similar observations have been seen in European cohorts (Olivennes *et al.* 1997; Bonduelle *et al.* 2005; Basatemur *et al.* 2010).

Summary

Although we recognize the paucity of studies, the overall results on growth in children born after ART are reassuring.

Respiratory and allergic disorders

Both PTB and LBW are known as risk factors for asthma (Jaakkola *et al.* 2006). A Swedish registry study of 2,628,728 children born in 1982-2007 and including 31,918 conceived by ART revealed an increased risk for asthma in children born after ART (AOR 1.28, 95% CI 1.23 to 1.34), increasing the absolute risk from 4.4% to 5.6% (Kallen *et al.* 2013). The risk was higher for preterm than term singletons. The effect of IVF on the risk of asthma was low and statistically non-significant for children with a low Apgar score, respiratory diagnoses, mechanical ventilation, continuous positive airway pressure or neonatal sepsis. Adjustment for the duration of infertility eliminated the effect, and removal of infants whose mothers had used anti-asthmatic drugs in early pregnancy reduced the risk.

A UK-wide prospective study, the Millennium Cohort Study, of 18,818 singletons recruited at 9 months of age was based on a follow-up survey at 5 years of age (response rates of 79 and 70%, respectively). Children born to subfertile parents were found to be more likely to experience asthma (AOR 1.39, 95%CI 1.07 to 1.80), wheezing (AOR 1.27, 95 %CI 1.00 to 1.63) and to be taking anti-asthmatic drugs at 5 years of age (AOR 1.90, 95% CI 1.32 to 2.74) compared with SC children (Carson *et al.* 2013).The association was mainly related to children born after ART (AOR 2.65, 95% CI 1.48 to 4.76; AOR 1.97, 95% CI 1.10 to 3.53 and AOR 4.67, 95% CI 2.20 to 9.94 for asthma, wheezing and taking anti-asthmatics, respectively). However, only 104 children conceived by ART were included in the study. Although diminished, the association was also present in 7-year-old children. The data were adjusted for a wide range of possible confounding and mediating factors and data were weighted for non-response to minimize any selection bias.

Summary

The main risk factor for the association between asthma and ART is parental subfertility, but neonatal morbidity and maternal asthma may act as mediators. Studies are limited in this area.

Malignancies

Only a few large cohort studies with sufficient power concerning risk assessment for malignancies currently exist. In a population-based British cohort study from 2013, including 106,013 ART children, no increase in the overall risk of cancer was found when compared with the expected risk standardized incidence ratio (SIR) for singleton births (1.04, 95% CI 0.80 to 1.33) (Williams *et al.* 2013). A retrospective population-based cohort study combining data from four Nordic countries (CoNARTaS) including 91,796 children (singletons and multiples) born after ART also concluded

that there was no increase in overall cancer rates when compared with children born after SC; adjusted HR 1.08 (95% CI 0.91 to 1.27) (Sundh *et al.* 2014). No adjustments were made for plurality. In a study from Israel including a cohort of 9,042 children conceived after ART and 211,763 SC children born from 1997 to 2004, no elevated risk for overall types of cancer in singletons (RR 1.32, 95% CI 0.80 to 2.17) was found (Lerner-Geva *et al.* 2017). In a Norwegian study including 25,782 children conceived after ART, where 51 cases of cancers were observed, the HR of cancer in ART versus the control population was 1.21 (95% CI 0.90 to 1.63) (Reigstad *et al.* 2016). Maternal use of fertility drugs and risk for cancer was examined in a cohort of 123,322 children in a Danish study from 2015 (Hargreave *et al.* 2015). No increased risk for cancer was found in childhood (HR 0.94, 95% CI 0.60 to 1.46) or in young adulthood (HR 0.89,95% CI 0.36 to 2.24)) after maternal use of fertility drugs. No adjustments were made for plurality.
In line with the results found in children born after non-donor ART (described above), a recent study identified no overall increased risk of cancer in a cohort of 12,137 children born after donor ART (donor oocytes, sperm or embryos); standardized incidence ratio for singletons 0.91 (95% CI 0.37 to 1.87) (Williams *et al.* 2018).

Summary

Large cohort studies consistently show no increased risk of overall cancer in children conceived by ART. However, studies on specific cancers are very limited by sample size.

Reproductive health

The first study exploring semen quality in the oldest ICSI offspring cohort was reported recently. The authors found that among 54 young adult men conceived by ICSI because of severe male infertility, the median sperm concentration, total sperm count, and total motile sperm count were significantly lower than in SC men, even after adjusting for relevant confounders. The young ICSI men were almost three times more likely to have sperm concentrations <15 million/ml and four times more likely to have total sperm count <39 million. However, no clear correlation between semen parameters of the young ICSI men and their fathers was found (Belva *et al.* 2016a). In another study, the same group showed that reproductive hormones in these young adult ICSI men (n = 54) were comparable to SC controls (Belva *et al.* 2017a). In a cohort of young adult women conceived by ICSI because of male factor infertility (n = 71), the antral follicle count, and anti-Müllerian hormone (AMH), FSH, LH, and dehydroepiandrosterone (DHEAS) levels in serum (were similar in SC offspring (Belva *et al.* 2017b).

Summary

The limited data published on reproductive health in ART offspring indicate some deterioration in sperm counts in male offspring conceived by ICSI, while in female offspring no adverse effects have been identified.

Controlled ovarian stimulation

Few studies report perinatal outcome and controlled ovarian stimulation (COS) regimens. In a large trial (MERIT trial) comparing recombinant FSH and urinary hMG (Andersen *et al.* 2006), there were no apparent differences between highly purified hMG and recombinant FSH with respect to neonatal health among live-born children. Follow up studies from RCTs (Bonduelle *et al.* 2012) and a Cochrane review (Pouwer *et al.* 2015) compared long-acting FSH versus daily FSH. In all, 806 children were assessed in the follow-up analysis
(Bonduelle *et al.* 2012) and they found that the PTB rate was 8.3% and 9.3% in the long-acting FSH and conventional daily FSH group, respectively, while LBW rates were 6.6% and 6.8%, respectively. Major birth defects were in the expected range and did not differ between the groups (Bonduelle *et al.* 2012).
Few studies, with contradictory results, have investigated whether the number of oocytes retrieved after COS might be associated with negative perinatal outcomes. A large cohort study from the UK including more than 65,000 singleton births after IVF found an association between excessive ovarian response (>20 oocytes retrieved for IVF) to COS and an increased risk of PTB and LBW (Sunkara *et al.* 2015). However, another study investigating factors affecting obstetric outcome after IVF found no association between VLBW or SGA and the number of oocytes retrieved, although fewer deliveries were included (Sazonova *et al.* 2011). COS for IVF that results in a high serum estradiol (E_2) peak on the day of administration of hCG, as opposed to a more moderate rise in E_2, has been found to be a risk factor for LBW (Pereira *et al.* 2015). Finally, a large registry study from Sweden including 27,000 singletons from fresh cycles found no indication of an adverse perinatal outcome associated with the number of oocytes retrieved (Magnusson *et al.* 2018).

Summary

The data on the impact of COS, although limited with respect to different COS regimes and perinatal outcomes in ART children, are reassuring.

Laboratory factors

Culture media

At the beginning of the IVF era the individual IVF laboratories produced their own culture media whereas nowadays a number of different commercially available culture media are used, and although a description of the media components is made available by the manufacturers, the exact composition is often not revealed (Sunde *et al.* 2016). Further, the type of culture media used in a specific ART cycle is only rarely recorded in ART registers, which has also hampered research into the safety of this aspect of ART.

In 2010, Dumoulin *et al.* published the first data suggesting that culture media (Vitrolife GI.3 versus Cook K-SIMC) influenced the BW of singletons conceived by ART with fresh transfer of cleavage stage embryos (BW±SEM: Vitrolife GI.3 3453±53g versus CooK-SIMC 3208±61g; *P*=0.003). Subsequent prospective studies from the Dutch group with random allocation to two culture media groups showed a similar BW of the infants and altered growth patterns of the fetus from the second trimester of pregnancy and at 2 years of age between the different culture media (Dumoulin *et al.* 2010; Nelissen *et al.* 2012; Kleijkers *et al.* 2014). A recent Dutch study observed that two culture media were associated with differences in body weight, BMI, truncal adiposity, waist circumference and waist/hip ratio in 9-year-old children conceived after ART, while no significant differences were observed regarding cardiovascular development (Zandstra *et al.* 2018). Two other studies showed culture medium-dependent differences in mRNA expression levels of genes involved in apoptosis, protein degradation, metabolism and cell-cycle regulation (Kleijkers *et al.* 2015a) and that the age of the embryo culture media was inversely associated with the BW of singletons born after ART (Kleijkers *et al.* 2015b). In 2016, Kleijkers et al. published a multicenter, double-blind RCT of 836 couples comparing human tubal fluid (HTF) with Vitrolife G5 embryo culture media and confirmed their finding of an association between culture media and BW (HTF group versus G5 group: mean difference of 158g, 95% CI 42 to 275g), and for singletons alone the Z-score was significantly elevated after adjustment for GA and gender (Kleijkers *et al.* 2016).

On the other hand, seven retrospective cohort studies and one RCT found no difference between two different culture media in terms of BW of the infants after transfer of cleavage stage embryos. However, the retrospective studies all have methodological limitations and the RCT included only 49 children in each arm.

Summary

Few prospective, controlled studies indicate that some embryo culture media may affect the BW of children conceived by ART.

Culture time

Compared to cleavage stage culture (day 2-4), blastocyst culture (day 5-6) implies an extended *in-vitro* embryo culture time that goes beyond embryonic genomic activation, potentially having adverse effects on the embryo and offspring. Furthermore, the culture media used for the extended culture period differ from those used for growth to cleavage stage (Sunde *et al.* 2016). A possible advantage of an extended culture period is a self-selection of the most viable embryo, which potentially may result in more healthy infants born after transfer of blastocysts rather than cleavage stage embryos.

Three systematic reviews and meta-analyses on perinatal outcomes after transfer of blastocyst versus cleavage stage embryo have been published recently (Martins *et al.* 2016; Wang *et al.* 2017; Alviggi *et al.* 2018). Alviggi *et al.* and Wang *et al.* included 14 and 12 studies, respectively, and the analyses were performed for singletons with stratified analyses on fresh cycles, frozen cycles and both fresh and frozen cycles. Martin *et al.* included 12 studies with singletons after both fresh and frozen cycles. All three meta-analyses included more than 100,000 singletons and found a significantly higher rate of PTB (<37 weeks of gestation) (RR 1.12-1.16) and VPTB (<32 weeks of gestation) (RR 1.14-1.16) after blastocyst compared to cleavage stage transfer. There was no difference with respect to LBW or VLBW. However, the risk of SGA was significantly lower for infants born after blastocyst transfer (RR 0.83-0.84) (Martins *et al.* 2016; Wang *et al.* 2017; Alviggi *et al.* 2018). Regarding LGA, the meta-analysis, which pooled fresh and frozen cycles (Martins *et al.* 2016; Wang *et al.* 2017), found an increased risk after blastocyst transfer (RR 1.12 (95%CI 1.03 to1.21), while the the two meta-analysis including only fresh cycles found no significant association [RR 1.22 (95%CI 1.00 to1.48) and RR 1.14 (95%CI 0.97 to 1.35)] (Wang *et al.* 2017; Alviggi *et al.* 2018) . Two of the three reviews included birth defects and found no increased risk of birth defects after extended culture time (Martins *et al.* 2016; Alviggi *et al.* 2018).

The causes of the increased risk of PTB after blastocyst transfer are not clear, but the possibility of genetic and epigenetic changes in the trophectoderm cells resulting in abnormal placentation and implantation has been considered and has raised concerns. Still, one must keep in mind that the results above are based on retrospective observational studies with high heterogeneity and that relevant confounder adjustment has not been performed.

In an extensive review, Wale and Gardner (Wale and Gardner 2016) have pointed out that during *in-vitro* culturing the embryo is constantly exposed to stressors, which are not present *in-vivo*. In

the IVF laboratory, chemical and physical factors (e.g. incubator gases, temperature, pH, oxygen concentration, culture oils, the act of pipetting itself) not related to the culture medium and culture time *per se* may also have potentially adverse effect on embryos. Hence it is crucial to investigate the impact of these factors in order to optimize the *in-vitro* environment.
The risk of monozygotic (MZ) twinning after blastocyst transfer compared to cleavage stage embryo transfer was studied by Hviid *et al.* in a systematic review including 38 studies with a rate of MZ twins from zero to 13.3%. Their meta-analysis showed an elevated risk after blastocyst transfer compared with cleavage stage ET (OR 2.18, 95% CI 1.93 to 2.48). The authors pointed out that the underlying mechanism for the increased risk of MZ twinning is not fully understood and besides extended culture time, culture media and maternal age, the authors hypothesized that the age of the oocytes may also be a potential risk factor for MZ twinning after ART (Hviid *et al.* 2018).

Summary
Studies suggest that an extended culture time for embryos is associated with an increased risk of some adverse perinatal outcomes, particularly PTB. Further a higher rate of MZ twinning has been observed after culture to blastocyst stage.

Number of embryos transferred

In ART singleton pregancies with a vanishing twin the risk of PTB is increased and furthermore, there is a higher risk of CP that increases the later in pregnancy the spontaneous reduction occurs (Pinborg *et al.* 2005). Thus, the consequences of multiple ET are not only observed in twin pregnancies but also in singleton deliveries, where the loss of a twin occurs in one in ten pregnancies in case of a DET policy (Pinborg *et al.* 2005). Most ART twins originate from DET and the considerable increased short- and long-term risks in ART twins should encourage a SET policy as the bench marking standard, as one fresh ET + one frozen ET results in similar live birth rates tot DET (Thurin *et al.* 2004; Thurin-Kjellberg *et al.* 2009). With the improved freezing programs, SET should be the gold standard as DET mainly adds a risk to both singleton and twin offspring and does not improve cumulative live birth rates.
A large UK cohort study showed that the vanishing twin phenomenon is associated with an increased risk of PTB and LBW in the surviving singletons conceived after ART (Kamath *et al.* 2018). The same study showed that when a single gestational sac was seen at ultrasonography no increased risk of PTB and LBW was observed, even following the transfer of multiple embryos.

Oocyte donation and gestational surrogacy

Oocyte donation

The number of OD treatments has increased over time. Nowadays in Europe, more than 40,000 transfers are performed using donated oocytes and 63 % of the recipients are 40 years or older (Calhaz-Jorge *et al.* 2017). In the USA, more than 20,000 donor oocyte ETs are performed yearly with the percentage of cycles increasing sharply after age 40 years (CDC 2015).

It is well known that there is an increased risk of obstetric and perinatal complications after OD treatment. The rate of HDP is 16-40 %, which is two to three times higher than in standard IVF/ICSI pregnancies (van der Hoorn *et al.* 2010; Malchau *et al.* 2013; Nejdet *et al.* 2016; Savasi *et al.* 2016; Masoudian *et al.* 2016; Storgaard *et al.* 2017). Several studies have also shown that the risk of PTB and LBW is increased two- to three-fold more often after OD compared to standard IVF conception (Adams *et al.* 2015; Nejdet *et al.* 2016; Storgaard *et al.* 2017). Only a few studies have reported rates on birth defects after OD, with no differences found compared to autologous IVF (Soderstrom-Anttila *et al.* 1998; Malchau *et al.* 2013).

The high occurrence of obstetric complications after use of donated oocytes has been associated to the couples´s infertility, primiparity and to advanced maternal age. This is probably not the only explanation because young oocyte recipients have similar rates of HDP to women of more advanced age (Keegan *et al.* 2007). One hypothesis is that in these HDP pregnancies the genetically foreign fetus induces immunological reactions and placental pathology, such as villitis, chronic deciduitis, and ischemic changes, in the oocyte recipient (Gundogan *et al.* 2010; van der Hoorn *et al.* 2010; Savasi *et al.* 2016). Lashley *et al.* suggested that HLA matching between donor and recipient might prevent the immunologic mismatch between mother and fetus thereby decreasing the risk of HDP and other obstetric and perinatal complications (Lashley *et al.* 2015).

Gestational surrogacy

The transfer of an embryo originating from a genetically foreign oocyte also occurs in gestational surrogacy (GS). However, the rate of HDP has generally been reported to be much lower (between 4.3 and 10 %) in singleton GS pregnancies compared to that in OD pregnancies (Parkinson *et al.* 1999; Dar *et al.* 2015; Soderstrom-Anttila *et al.* 2016). In a large dataset comparing outcomes after GS and autologous IVF, there was no increased risk of PTB, LBW or birth defects after GS compared to autologous IVF (Sunkara *et al.* 2017). Based on the limited data on GS outcome available, there has been speculation that a healthy carrier with a normal reproductive background might somehow compensate for perinatal risks associated with a foreign embryo and IVF. The

surrogates might have a more hospitable uterine environment than infertile oocyte recipients (Gibbons *et al.* 2011).

In a recent study pregnancy complications and perinatal outcomes were compared in live born singletons conceived with the use of embryos from intended parents (n =103) versus SC (n = 249) carried in the same woman (Woo *et al.* 2017). Children born after GS had a lower mean gestational duration, higher rates of PTB (10.7% versus 3.1%, respectively), and higher rates of LBW (7.8% versus 2.4%, respectively). The authors concluded that the results showed that ART may potentially affect embryo quality and that even a proven healthy uterine environment cannot compensate for this negative impact (Woo *et al.* 2017). However, many factors might have affected the study outcome, for example an artificial replacement cycle versus natural cycle, number of embryos transferred, and the time interval between the SC and surrogate pregnancies (Spandorfer 2017).

Long-term follow up

Surprisingly, there are only a few long-term follow-up studies on the health of OD children. In a Finnish follow-up study of 164 children born after OD and aged 1-14 years, the incidence of severe neurological disability was 1.8%. Nine children (5.5%) had a diagnosis of an endocrine or autoimmune disease, including premature adrenarche, diabetes, growth delay, hypothyreosis, parathyroid disorder, juvenile rheumatitis and coeliac disease (Soderstrom-Anttila *et al.* 2010).

Summary

Perinatal outcomes are poorer for singletons conceived after OD compared to both singletons conceived after autologous IVF and SC. The extent to which the poorer perinatal outcomes after OD relates to immunological factors associated with a genetically foreign oocyte or to other confounding factors, such as advanced maternal age, maternal co-morbidity, uterine environment and ART laboratory procedures, is unknown. Pregnancies achieved through OD are high-risk pregnancies and SET is highly recommended as multiple pregnancies further add to the perinatal risks.

Oocyte vitrification

One rapidly expanding field in ART is the use of frozen oocytes. Reports from OD programmes using young donors show similar clinical pregnancy rates after transfer of embryos created from fresh or vitrified oocytes (Cobo *et al.* 2008; Cobo *et al.* 2010). As mentioned earlier, concern has arisen regarding the oocyte vitrification procedure and its possible health risks for the offspring

(Cobo *et al.* 2014). The main worries are associated with use of the high concentrations of cryoprotectants that are required to avoid ice crystal formation in the oocytes.

In 2009, Noyes *et al.* reported data on 936 infants born after use of either slow-freezing or vitrification of autologous or donor oocytes. Data were gathered from 23 case reports, 35 case series and from seven fertility centers around the world. As the multiple birth status was not well reported, the PTB and LBW rate could not be properly assessed. In all, 1.3% (12/936) had a birth defect, the most common of which was a ventricular septal defect. The birth defect rate was 1.1% after slow freeze and 1.5% after the use of vitrified embryos, similar to that reported in SC children (Noyes *et al.* 2009).

The largest set of data to date included approximately 1000 children born after vitrification of donor oocytes (88%) or own oocytes (12%) (Cobo *et al.* 2014). Of the infants born, 43% originated from multiple pregnancies. No differences in GA, BW, Apgar scores, birth defects, and perinatal mortality were found when comparing the use of vitrified or fresh oocytes (Cobo *et al.* 2014).

Summary

Perinatal outcomes after oocyte vitrification are reassuring, although the evidence is scarce. There is no apparent increase in birth defects after oocyte vitrification.

Epigenetics

Genome-wide epigenetic reprogramming occurs during gametogenesis and early embryogenesis (Inbar-Feigenberg *et al.* 2013). Epigenetic marks are erased in the primordial germ cells (the precursor cells of sperm and oocytes). Subsequently, epigenetic reprogramming occurs before and after fertilization, including parent-of-origin controlled activity of specific genes (genomic imprinting). Many imprinted genes are found in clusters on chromosomes and some genes are expressed from the paternally inherited allele and others from the maternally inherited allele (Ferguson-Smith 2011). Differentially methylated regions in imprinted genes are protected from the global wave of demethylation that occurs in the early embryo during preimplantation development (Feng *et al.* 2010). Epimutation refers to an aberrant DNA methylation or histone modification pattern (Horsthemke 2010) (Fig. 4). A majority of imprinting defects are primary epimutations, which occur in the absence of DNA mutation (Horsthemke 2010).

Genetic factors in infertile couples and the variables involved in ART (hormones, culture media, prolonged embryo culture, cryopreservation) may have adverse effects on these epigenetic processes, which are then transmitted to the offspring (Horsthemke and Ludwig 2005; Ceelen *et al.* 2008; Jiang *et al.* 2017). After ART, changes in DNA methylation and/or transcription of some transposable elements and imprinted genes were found in placenta samples while transcription modifications for some transposable elements were also discovered in cord blood (Choux *et al.* 2018). Song and coworkers compared placental DNA methylation levels at 37 CpG sites in 16 previously identified candidate genes in SC children and ART children conceived either from autologous oocytes or from donor oocytes obtained from young, healthy, fertile donors. Significant differences in placental DNA methylation levels were demonstrated between the donor oocyte ART and fertile control groups. The authors concluded that the observed methylation differences seem to be associated with some aspect of the ART procedures, not simply the underlying infertility (Song *et al.* 2015). A recent Finnish study on human placentas looked at DNA methylation changes together with phenotypic findings and suggested that an intronic rs10732516 polymorphism, which is in the sixth binding site for CCCTC-binding factor in the *H19* imprinting control region,,associates with the effects of ART in a parent-of-origin manner. The authors conclude that this polymorphism should be considered when the effects of environmental factors on embryonic development are studied (Marjonen *et al.* 2018).

Indirect supporting data on epigenetic programming comes from epidemiological and animal studies. Periconceptional exposure to famine during the Dutch Hunger Winter in 1944-45 was associated, six decades later, with less DNA methylation of the imprinted insulin growth factor 2 gene (*IGF2)* in individuals conceived at that time compared to their unexposed siblings (Heijmans *et al.* 2008). Epigenetic change in the IGF2 receptor (*IGFR2)* gene was associated with fetal overgrowth after sheep embryo culture, although the mechanism was not analogous to Beckwith-Wiedemann syndrome (BWS) in humans (Young *et al.* 2001). In addition, superovulation has been shown to perturb genomic imprinting of both maternally and paternally expressed genes in a mouse model (Market-Velker *et al.* 2010).

Imprinting disorders

Imprinting disorders currently comprise a group of 12 rare congenital diseases that are defined by phenotype and/or an association with molecular disturbances at specific imprinted loci (Soellner *et al.* 2017). A majority of these disorders are characterized by aberrant pre- and/or postnatal growth, hypo- or hyperglycemia, abnormal feeding behavior in early childhood and later, behavioral difficulties, mental retardation, and precocious puberty.

A systematic review and meta-analysis by Lazaraviciute and coworkers has demonstrated an excess of imprinting disorders in children born after IVF and ICSI (Lazaraviciute *et al.* 2014). The combined OR (95%CI) of any imprinting disorder in children born after ART was 3.67 (1.37 to 9.74) compared to SC children. Their results regarding the methylation within individual imprinted genes were inconclusive owing to the small number of studies and their heterogeneity. In a registry study, Lidegaard and coworkers compared the frequency of imprinting diseases in 6052 children born after IVF with the incidence in 442,349 SC children born in 1995-2001 in Denmark (Lidegaard *et al.* 2005) and no increased risk of imprinting disorders after IVF was detected.

BWS is associated with abnormalities in imprinted genes at 11p15.5 (Soellner *et al.* 2017). Using eight epidemiological studies, Vermeiden and Bernardus found a positive association between IVF/ICSI and BWS with a RR of 5.2, 95%CI 1.6 to 7.4 (Vermeiden and Bernardus 2013). They calculated that with the population prevalence of 1:13,700, among every 2700 IVF/ICSI births one BWS child would be born. In BWS primary epimutations at the imprinting control center 2 (IC2) have been associated with ART (Dias and Maher 2013). Tenorio and coworkers carried out a molecular analysis and obtained complete data regarding conception for 156 patients with clinical features of BWS from The Spanish Overgrowth Syndrome Registry (17 born after ART, 139 after SC). They demonstrated that the chance of having an epimutation at 11p15 was 7.18 times higher in patients with BWS born after ART than in those born after SC (Tenorio *et al.* 2016).

Silver-Russell syndrome (SRS) is a clinical mirror of BWS, also associated with imprinted genes at 11p15.5 (Soellner *et al.* 2017). Up to 50% of children with SRS have hypomethylation of the paternal IC1 resulting in silencing of both alleles of *IGF2*. Vermeiden and Bernardus suggested a positive association of SRS with IVF or ISCI, but data were too sparse to draw a conclusion (Vermeiden and Bernardus 2013). Several sporadic reports have been published linking SRS with ART (Cocchi *et al.* 2013).

Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are caused by a deficiency in imprinted gene expression from the paternal or maternal chromosome 15q11-q13, respectively, and the patients display different phenotypes (Camprubi *et al.* 2007). A subgroup (1-4%) of PWS and AS patients has an imprinting defect, the majority of which is associated with epimutation (Buiting *et al.* 2003). Vermeiden and Bernardus found no associations between the incidence of AS and PWS and IVF or ICSI treatments (Vermeiden and Bernardus 2013). Johnson and coworkers detected one AS patient with an imprinting error in a series of prenatal samples from 949 IVF pregnancies (Johnson *et al.* 2018).

Summary

Imprinting disorders have been associated with ART but discriminating the influence of the variable ART procedures *per se* and the effect of the reproductive disease of the parents remains a challenge.

The chicken or the egg?

The choice of a proper comparison group for children born after ART is a challenge. Most studies have used groups of children born of healthy, fertile women in the general population as controls thus making it difficult to separate the contribution of infertility *per se* from the ART treatment (i.e. the chicken or the egg). However, a variety of different approaches have been carried out to explain the gap between the outcomes of singletons conceived by ART and SC.

Subfertility and sibling studies

In a systematic review and meta-analysis, singletons born after ART were compared with SC singletons of subfertile women with pregnancy TTP >1 year with the inclusion of six studies (Pinborg *et al.* 2013). Two of the papers were included in a meta-analysis of PTB, showing a pooled AOR of 1.55 (95% CI 1.30 to 1.85).

A large cohort study compared ART singletons (n = 10,149) and a subfertile population (n = 8,054) used as reference with a fertile population (n = 441,420). Risks for adverse infant outcomes were increased for the ART group with adjusted risks ranging from 1.21 to 1.26 for LBW and PTB, respectively, and 1.40 to 1.44 for VLBW and VPTB, respectively (Luke *et al.* 2017). Risks were lower for the fertile population than for the subfertile population for all outcomes.

Another approach has been to study potential differences in outcome related to causes of infertility. In a review, Luke analysed the effect of infertility diagnoses (male factor, endometriosis, ovulation disorders, tubal factors, inflammation). Women with an infertility-related diagnosis, who conceived with or without ART had higher risks for adverse perinatal outcomes compared to women without an infertility diagnosis. This gives support for the primary role of the underlying infertility disorder rather than the ART treatment in causing the increased risk of adverse perinatal outcome (Luke 2017). The chance of conceiving naturally with regard to the cause of infertility is poorly explored. Thus, the absolute indications for fertility treatment are still not well defined. With the great number

of underlying reasons for subfertility and the gap in knowledge about impact of these on adverse outcomes, caution must be taken if grouping all types of subfertility into one category.
When adjusting for "years of infertility" as a simple confounder, there is a risk of effect modification, therefore studies that do use this adjustment should instead perform a stratified analyses comparing risk in ART versus non-ART couples and using subgroups of women with 1-2 years of subfertility, 2-3 years and 4-5+ years. In addition, subgroups of treated women have been studied by comparing "low technology treatment" (IUI) with ART and SC (Wang *et al.* 2002). A 50% increased risk of PTB in the IUI group and a two-fold higher risk in the ART group were found (Wang et al. 2002). In a meta-analysis, PTB in singletons born after ovulation induction and/or IUI were compared with SC singletons, where TTP was ≤ 1 year. The results showed a higher risk of PTB in the low technology treatment group (AOR 1.45, 95% CI 1.21 to 1.74), indicating that infertility *per se* is an important factor in ART outcome (Pinborg *et al.* 2013).

Sibling studies

Comparisons of siblings that are discordant for mode of conception have been used in observational studies. In the sibship design, where a mother gives birth to both one singleton conceived by ART and one singleton conceived by SC, the mothers are their own controls and adjustment can be made for changes in maternal age and parity between the two deliveries and birth order. In this design, factors such as biology (genetics), lifestyle, and socio-economic situation are relatively constant during a woman's reproductive life. Thus, the major strength of this design is the steadiness of maternal and paternal factors.
A meta-analysis of PTB in ART versus SC siblings including two Nordic studies showed an increased risk for PTB in the ART sibling (AOR 1.27, 95% CI 1.08 to 1.49), indicating a risk attributable to the ART treatment *per se* (Pinborg *et al.* 2013). A sibship study from the USA also showed that subfertile women, with or without ART, were at increased risk for adverse perinatal outcomes when compared with fertile women (Luke *et al.* 2016). Women whose fertility status declined from their first to their second singleton pregnancy had increased risks for adverse outcomes, and the highest risks were observed in those women with the steepest decline in their fertility status (Luke *et al.* 2016). Thus, subfertility plays a significant role in the adverse outcome in ART although it is not the only contributor. Recently, potential limitations to the sibling comparisons have been debated (Frisell *et al.* 2012). First, the fact that siblings may not share the same environment (i.e. that a mother may change life style behaviour between pregnancies) cannot always be measured and can therefore bias the results. This risk of bias is one of the limitations that are being discussed and it has been claimed that it is the nonsystematic aspects of the unshared environment that are the most difficult to measure and therefore tend to be uncontrolled.

On the other hand, others claim that a sibling design is appropriate as evidence suggests that the unshared environmental factors contribute only little to the interindividual variation in the outcome of interest (Keyes *et al.* 2013). In a recent UK study looking at cognitive development following ART, the authors highlighted the methodological considerations regarding the choice of comparison group and confounding and mediating factors (Carson *et al.* 2010). Cognitive development test scores in ART children at the age of 3 years was increased, but was explained by the women treated with ART having higher socioeconomic status and longer education than women that did not have ART treatment. The authors emphasized that the comparison groups should be carefully selected with regard to the study question one aims to address, and further variables should be considered as a potential confounder or mediating factor as this can help to identify the underlying mechanism of an observed association. For future studies, discussion of potential biases that accompany evolving study designs will continue to improve the quality of the research and the use of the sib-ship design will give further insight to the "chicken or the egg" debate.

Summary

Singletons born of subfertile women are preferable as the control group for ART singletons, as infertility *per se* is a contributing factor to the adverse outcomes in the ART offspring. Future research is needed on the comparability of subfertile couples that eventually conceive naturally and couples conceiving by the use of ART. To identify a potential effect of the reproductive technology *per se*, the sib-ship design may contribute, given that the important limitations of the design are being considered.

Future research

We still have a considerable gap in knowledge concerning the long-term health of children conceived by ART and we need to explore the influence of different ART methods, including the freezing of embryos and other laboratory procedures, on the long-term health outcomes. In order to cover this field, both epidemiological and clinical research is needed; international and national databases and registry studies that combine ART cycle data with health care information (i.e. hospital diagnosis codes, drug prescription registries and specific registries on childhood diabetes and malignancies) with the possibility of linking the parents to the child can elucidate some of the questions in the coming years.

However, large clinical studies with thorough medical investigations of the children including cardiometabolic (e.g. BP, right and left cardiovascular function), endocrine and reproductive function (e.g. deterioration in sperm count in ICSI offspring) and epigenetic mapping are necessary to fully investigate the long-term health consequencs and underlying mechanisms responsible for the possible negative influence of ART. Other areas that need further research are neurodevelopmental health (increased risk of CP, ASD and mental retardation in ICSI offspring), male infertility diagnosis (e.g. azoospermia) and risk of birth defects in ICSI, the consequences of the chosen stimulation regimen, and the effect of different culture media and culture time. The exact composition of the commercial media ought to be available for future research. Furthermore, during *in-vitro* culture other chemical and physical factors, besides media and time, could have an impact on the offspring and should be investigated. Moreover, differences between ART and non-ART parents in life style factors, such as smoking and BMI, may also bias the health outcome risks in ART children and should be included as a confounding factors in future research.
In general, this research will help us to understand the influence of early changes in the embryo and foetal environment on later development. A research programme such as this requires resources and these should be prioritized and facilitated in the reproductive, medical and political spheres, both at a national and international level.

Conclusion

In general, the short-term health of singletons conceived by ART shows modestly increased risks of adverse outcomes, including LBW, PTB and birth defects. Studies on long-term health are still too sparse to allow robust conclusions to be reached. Limited data indicate that cardiovascular and metabolic risk profiles may be altered after ART.

The surveillance of ART children by ART registries and national health care registries are of utmost importance for monitoring the long-term health of children conceived by ART and to monitor the health of children born after the introduction of new ART techniques, such as preimplantation genetic testing, vitrification and blastocyst transfer.

The causes of the poorer outcomes seem to be a combination of parental subfertility and the ART techniques *per se*. However, the multiple pregnancies after ART are still by far the most important contributor to adverse health outcomes in ART offspring and we should not lose focus on recommending SET. In spite of this knowledge, twin and higher-order multiple pregnancy rates are still high in many counries because the routine transfer of several cleaved embryos or blastocysts continues.

Authors' roles

S.B., V.S.A., U.B.W., H.L, A.L., N.B.O., L.B.R., C.B. and A.P. contributed to the design of the study, screened articles, selected articles and wrote the manuscript. All authors approved the final version for submission.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

References

AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, and Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. *Reprod Biomed Online* 2010: **20**; 209-222.

Adams DH, Clark RA, Davies MJ, and de Lacey S. A meta-analysis of neonatal health outcomes from oocyte donation. *J Dev Orig Health Dis* 2015; 1-16.

Adamson G. Z-HF, Dyer S., Chambers G., Ishihara O., Mansour R., Banker M., de Mouzon J. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) World Report on ART, 2014 and The International Glossary on Infertility and Fertility Care, 2017. ICMART World Report 2014 2017.

Alviggi C, Conforti A, Carbone IF, Borrelli R, de Placido G, and Guerriero S. Influence of cryopreservation on perinatal outcome after blastocyst- vs cleavage-stage embryo transfer: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018: **51**; 54-63.

Andersen AN, Devroey P, and Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. *Hum Reprod* 2006: **21**; 3217-3227.

Barker DJ. The origins of the developmental origins theory. *J Intern Med* 2007: **261**; 412-417.

Basatemur E, Shevlin M, and Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12years of age. *Reprod Biomed Online* 2010: **20**; 144-149.

Bay B, Mortensen EL, Hvidtjorn D, and Kesmodel US. Fertility treatment and risk of childhood and adolescent mental disorders: register based cohort study. *BMJ* 2013a: **347**; f3978.

Bay B, Mortensen EL, and Kesmodel US. Assisted reproduction and child neurodevelopmental outcomes: a systematic review. *Fertil Steril* 2013b: **100**; 844-853.

Belva F, Bonduelle M, Roelants M, Michielsen D, Van Steirteghem A, Verheyen G, and Tournaye H. Semen quality of young adult ICSI offspring: the first results. *Hum Reprod* 2016a: **31**; 2811-2820.

Belva F, Bonduelle M, Roelants M, Verheyen G, and Van Landuyt L. Neonatal health including congenital malformation risk of 1072 children born after vitrified embryo transfer. *Hum Reprod* 2016b: **31**; 1610-1620.

Belva F, De Schrijver F, Tournaye H, Liebaers I, Devroey P, Haentjens P, and Bonduelle M. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. *Hum Reprod* 2011: **26**; 1752-1758.

Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, Liebaers I, Haentjens P, and Bonduelle M. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. *Hum Reprod* 2008: **23**; 2227-2238.

Belva F, Roelants M, De Schepper J, Van Steirteghem A, Tournaye H, and Bonduelle M. Reproductive hormones of ICSI-conceived young adult men: the first results. *Hum Reprod* 2017a: **32**; 439-446.

Belva F, Roelants M, Vloeberghs V, Schiettecatte J, Evenepoel J, Bonduelle M, and de Vos M. Serum reproductive hormone levels and ultrasound findings in female offspring after intracytoplasmic sperm injection: first results. *Fertil Steril* 2017b: **107**; 934-939.

Bergh T, Ericson A, Hillensjo T, Nygren KG, and Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. *Lancet* 1999: **354**; 1579-1585.

Bernardini L, Gianaroli L, Fortini D, Conte N, Magli C, Cavani S, Gaggero G, Tindiglia C, Ragni N, and Venturini PL. Frequency of hyper-, hypohaploidy and diploidy in ejaculate, epididymal and testicular germ cells of infertile patients. *Hum Reprod* 2000: **15**; 2165-2172.
Bonduelle M, Mannaerts B, Leader A, Bergh C, Passier D, and Devroey P. Prospective follow-up of 838 fetuses conceived after ovarian stimulation with corifollitropin alfa: comparative and overall neonatal outcome. *Hum Reprod* 2012: **27**; 2177-2185.
Bonduelle M, Van Assche E, Joris H, Keymolen K, Devroey P, Van Steirteghem A, and Liebaers I. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002: **17**; 2600-2614.
Bonduelle M, Wennerholm UB, Loft A, Tarlatzis BC, Peters C, Henriet S, Mau C, Victorin-Cederquist A, Van Steirteghem A, Balaska A*, et al.* A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum Reprod* 2005: **20**; 413-419.
Bracke A, Peeters K, Punjabi U, Hoogewijs D, and Dewilde S. A search for molecular mechanisms underlying male idiopathic infertility. *Reprod Biomed Online* 2018: **36**; 327-339.
Buiting K, Gross S, Lich C, Gillessen-Kaesbach G, el-Maarri O, and Horsthemke B. Epimutations in Prader-Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *Am J Hum Genet* 2003: **72**; 571-577.
Calhaz-Jorge C, De Geyter C, Kupka MS, de Mouzon J, Erb K, Mocanu E, Motrenko T, Scaravelli G, Wyns C, and Goossens V. Assisted reproductive technology in Europe, 2013: results generated from European registers by ESHRE. *Hum Reprod* 2017: **32**; 1957-1973.
Camprubi C, Coll MD, Villatoro S, Gabau E, Kamli A, Martinez MJ, Poyatos D, and Guitart M. Imprinting center analysis in Prader-Willi and Angelman syndrome patients with typical and atypical phenotypes. *Eur J Med Genet* 2007: **50**; 11-20.
Carson C, Kurinczuk JJ, Sacker A, Kelly Y, Klemetti R, Redshaw M, and Quigley MA. Cognitive development following ART: effect of choice of comparison group, confounding and mediating factors. *Hum Reprod* 2010: **25**; 244-252.
Carson C, Sacker A, Kelly Y, Redshaw M, Kurinczuk JJ, and Quigley MA. Asthma in children born after infertility treatment: findings from the UK Millennium Cohort Study. *Hum Reprod* 2013: **28**; 471-479.
CDC. Assisted Reproductive Technology National Summary Report. 2015. https://www.cdc.gov/art/reports/2015/national-summary.html.
Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JP, Spreeuwenberg M, van Leeuwen FE, and Delemarre-van de Waal HA. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to subfertile parents. *Hum Reprod* 2009: **24**; 2788-2795.
Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, and Delemarre-van de Waal HA. Growth and development of children born after in vitro fertilization. *Fertil Steril* 2008: **90**; 1662-1673.
Chen M and Heilbronn LK. The health outcomes of human offspring conceived by assisted reproductive technologies (ART). *J Dev Orig Health Dis* 2017: **8**; 388-402.
Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, Yang J, Liu J, Wei D, Weng N*, et al.* Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *N Engl J Med* 2016: **375**; 523-533.
Choux C, Binquet C, Carmignac V, Bruno C, Chapusot C, Barberet J, Lamotte M, Sagot P, Bourc'his D, and Fauque P. The epigenetic control of transposable elements and imprinted

genes in newborns is affected by the mode of conception: ART versus spontaneous conception without underlying infertility. *Hum Reprod* 2018: **33**; 331-340.
Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, and Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril* 2008: **89**; 1657-1664.
Cobo A, Meseguer M, Remohi J, and Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod* 2010: **25**; 2239-2246.
Cobo A, Serra V, Garrido N, Olmo I, Pellicer A, and Remohi J. Obstetric and perinatal outcome of babies born from vitrified oocytes. *Fertil Steril* 2014: **102**; 1006-1015 e1004.
Cocchi G, Marsico C, Cosentino A, Spadoni C, Rocca A, De Crescenzo A, and Riccio A. Silver-Russell syndrome due to paternal H19/IGF2 hypomethylation in a twin girl born after in vitro fertilization. *Am J Med Genet A* 2013: **161A**; 2652-2655.
Dar S, Lazer T, Swanson S, Silverman J, Wasser C, Moskovtsev SI, Sojecki A, and Librach CL. Assisted reproduction involving gestational surrogacy: an analysis of the medical, psychosocial and legal issues: experience from a large surrogacy program. *Hum Reprod* 2015: **30**; 345-352.
Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, and Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012: **366**; 1803-1813.
Dias RP and Maher ER. Genes, assisted reproductive technology and trans-illumination. *Epigenomics* 2013: **5**; 331-340.
Dul EC, Groen H, van Ravenswaaij-Arts CM, Dijkhuizen T, van Echten-Arends J, and Land JA. The prevalence of chromosomal abnormalities in subgroups of infertile men. *Hum Reprod* 2012: **27**; 36-43.
Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP*, et al.* Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010: **25**; 605-612.
Dyer S, Chambers GM, de Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, and Adamson GD. International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. *Hum Reprod* 2016: **31**; 1588-1609.
Fedder J, Gabrielsen A, Humaidan P, Erb K, Ernst E, and Loft A. Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm. *Hum Reprod* 2007: **22**; 1080-1085.
Feng S, Jacobsen SE, and Reik W. Epigenetic reprogramming in plant and animal development. *Science* 2010: **330**; 622-627.
Ferguson-Smith AC. Genomic imprinting: the emergence of an epigenetic paradigm. *Nat Rev Genet* 2011: **12**; 565-575.
Fountain C, Zhang Y, Kissin DM, Schieve LA, Jamieson DJ, Rice C, and Bearman P. Association between assisted reproductive technology conception and autism in California, 1997-2007. *Am J Public Health* 2015: **105**; 963-971.
Frisell T, Oberg S, Kuja-Halkola R, and Sjolander A. Sibling comparison designs: bias from non-shared confounders and measurement error. *Epidemiology* 2012: **23**; 713-720.
Gibbons WE, Cedars M, Ness RB, and Society for Assisted Reproductive Technologies Writing G. Toward understanding obstetrical outcome in advanced assisted reproduction: varying sperm, oocyte, and uterine source and diagnosis. *Fertil Steril* 2011: **95**; 1645-1649 e1641.

Goldsmith S, McIntyre S, Badawi N, and Hansen M. Cerebral palsy after assisted reproductive technology: a cohort study. *Dev Med Child Neurol* 2018: **60**; 73-80.
Gundogan F, Bianchi DW, Scherjon SA, and Roberts DJ. Placental pathology in egg donor pregnancies. *Fertil Steril* 2010: **93**; 397-404.
Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, and Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. *Fertil Steril* 2017: **107**; 622-631 e625.
Hansen M, Kurinczuk JJ, Milne E, de Klerk N, and Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. *Hum Reprod Update* 2013: **19**; 330-353.
Hargreave M, Jensen A, Nielsen TS, Colov EP, Andersen KK, Pinborg A, and Kjaer SK. Maternal use of fertility drugs and risk of cancer in children--a nationwide population-based cohort study in Denmark. *Int J Cancer* 2015: **136**; 1931-1939.
Hart R and Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment: Part I--General health outcomes. *Hum Reprod Update* 2013a: **19**; 232-243.
Hart R and Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment. Part II--Mental health and development outcomes. *Hum Reprod Update* 2013b: **19**; 244-250.
Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, and Lumey LH. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A* 2008: **105**; 17046-17049.
Helmerhorst FM, Perquin DA, Donker D, and Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004: **328**; 261.
Henningsen AA, Bergh C, Skjaerven R, Tiitinen A, Wennerholm UB, Romundstad LB, Gissler M, Opdahl S, Nyboe Andersen A, Lidegaard O*, et al.* Trends over time in congenital malformations in live-born children conceived after assisted reproductive technology. *Acta Obstet Gynecol Scand* 2018.
Henningsen AA, Gissler M, Skjaerven R, Bergh C, Tiitinen A, Romundstad LB, Wennerholm UB, Lidegaard O, Nyboe Andersen A, Forman JL*, et al.* Trends in perinatal health after assisted reproduction: a Nordic study from the CoNARTaS group. *Hum Reprod* 2015: **30**; 710-716.
Henningsen AA, Wennerholm UB, Gissler M, Romundstad LB, Nygren KG, Tiitinen A, Skjaerven R, Nyboe Andersen A, Lidegaard O, Forman JL*, et al.* Risk of stillbirth and infant deaths after assisted reproductive technology: a Nordic study from the CoNARTaS group. *Hum Reprod* 2014: **29**; 1090-1096.
Henningsen AK, Romundstad LB, Gissler M, Nygren KG, Lidegaard O, Skjaerven R, Tiitinen A, Andersen AN, Wennerholm UB, and Pinborg A. Infant and maternal health monitoring using a combined Nordic database on ART and safety. *Acta Obstet Gynecol Scand* 2011: **90**; 683-691.
Holte TO, Hofmann B, Lie RT, Norderhaug IN, Romundstad P, Saeterdal I, Orstavik KH, and Tanbo T. Male Infertility: Intracytoplasmic Sperm Injection (ICSI) Using Surgically Retrieved Sperm from the Testis or the Epididymis. 2007. Oslo, Norway.
Horsthemke B. Mechanisms of imprint dysregulation. *Am J Med Genet C Semin Med Genet* 2010: **154C**; 321-328.
Horsthemke B and Ludwig M. Assisted reproduction: the epigenetic perspective. *Hum Reprod Update* 2005: **11**; 473-482.
http://apps.who.int/classifications/icd10/browse/2016/en - /XVII. ICD-10.
http://www.eurocat-network.eu. EUROCAT.

Hvidtjorn D, Grove J, Schendel D, Schieve LA, Svaerke C, Ernst E, and Thorsen P. Risk of autism spectrum disorders in children born after assisted conception: a population-based follow-up study. *J Epidemiol Community Health* 2011: **65**; 497-502.

Hvidtjorn D, Grove J, Schendel D, Svaerke C, Schieve LA, Uldall P, Ernst E, Jacobsson B, and Thorsen P. Multiplicity and early gestational age contribute to an increased risk of cerebral palsy from assisted conception: a population-based cohort study. *Hum Reprod* 2010: **25**; 2115-2123.

Hviid KVR, Malchau SS, Pinborg A, and Nielsen HS. Determinants of monozygotic twinning in ART: a systematic review and a meta-analysis. *Hum Reprod Update* 2018: **24**; 468-483.

Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M, and Weksberg R. Basic concepts of epigenetics. *Fertil Steril* 2013: **99**; 607-615.

Jaakkola JJ, Ahmed P, Ieromnimon A, Goepfert P, Laiou E, Quansah R, and Jaakkola MS. Preterm delivery and asthma: a systematic review and meta-analysis. *J Allergy Clin Immunol* 2006: **118**; 823-830.

Jackson RA, Gibson KA, Wu YW, and Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004: **103**; 551-563.

Jiang Z, Wang Y, Lin J, Xu J, Ding G, and Huang H. Genetic and epigenetic risks of assisted reproduction. *Best Pract Res Clin Obstet Gynaecol* 2017: **44**; 90-104.

Johnson JP, Schoof J, Beischel L, Schwancke C, Goldberg J, Black L, Ross L, and Bhatt S. Detection of a case of Angelman syndrome caused by an imprinting error in 949 pregnancies analyzed for AS following IVF. *J Assist Reprod Genet* 2018.

Jozwiak EA, Ulug U, Mesut A, Erden HF, and Bahceci M. Prenatal karyotypes of fetuses conceived by intracytoplasmic sperm injection. *Fertil Steril* 2004: **82**; 628-633.

Kallen AJ, Finnstrom OO, Lindam AP, Nilsson EM, Nygren KG, and Otterblad Olausson PM. Is there an increased risk for drug treated attention deficit/hyperactivity disorder in children born after in vitro fertilization? *Eur J Paediatr Neurol* 2011: **15**; 247-253.

Kallen B, Finnstrom O, Nygren KG, and Olausson PO. In vitro fertilization in Sweden: child morbidity including cancer risk. *Fertil Steril* 2005: **84**; 605-610.

Kallen B, Finnstrom O, Nygren KG, and Otterblad Olausson P. Asthma in Swedish children conceived by in vitro fertilisation. *Arch Dis Child* 2013: **98**; 92-96.

Kamath MS, Antonisamy B, Selliah HY, and Sunkara SK. Perinatal outcomes of singleton live births with and without vanishing twin following transfer of multiple embryos: analysis of 113 784 singleton live births. *Hum Reprod* 2018: **33**; 2018-2022.

Keegan DA, Krey LC, Chang HC, and Noyes N. Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes. *Fertil Steril* 2007: **87**; 776-781.

Keyes KM, Smith GD, and Susser E. On sibling designs. *Epidemiology* 2013: **24**; 473-474.

Kissin DM, Zhang Y, Boulet SL, Fountain C, Bearman P, Schieve L, Yeargin-Allsopp M, and Jamieson DJ. Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. *Hum Reprod* 2015: **30**; 454-465.

Kleijkers SH, Eijssen LM, Coonen E, Derhaag JG, Mantikou E, Jonker MJ, Mastenbroek S, Repping S, Evers JL, Dumoulin JC*, et al.* Differences in gene expression profiles between human preimplantation embryos cultured in two different IVF culture media. *Hum Reprod* 2015a: **30**; 2303-2311.

Kleijkers SH, Mantikou E, Slappendel E, Consten D, van Echten-Arends J, Wetzels AM, van Wely M, Smits LJ, van Montfoort AP, Repping S*, et al.* Influence of embryo culture medium (G5 and HTF) on pregnancy and perinatal outcome after IVF: a multicenter RCT. *Hum Reprod* 2016: **31**; 2219-2230.

Kleijkers SH, van Montfoort AP, Smits LJ, Coonen E, Derhaag JG, Evers JL, and Dumoulin JC. Age of G-1 PLUS v5 embryo culture medium is inversely associated with birthweight of the newborn. *Hum Reprod* 2015b: **30**; 1352-1357.

Kleijkers SH, van Montfoort AP, Smits LJ, Viechtbauer W, Roseboom TJ, Nelissen EC, Coonen E, Derhaag JG, Bastings L, Schreurs IE*, et al.* IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod* 2014: **29**; 661-669.

Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Paz G, Lessing JB, Yaron Y, and Yavetz H. Three-generation evaluation of Y-chromosome microdeletion. *J Androl* 1999: **20**; 394-398.

Knoester M, Helmerhorst FM, Vandenbroucke JP, van der Westerlaken LA, Walther FJ, Veen S, and Leiden Artificial Reproductive Techniques Follow-up P. Cognitive development of singletons born after intracytoplasmic sperm injection compared with in vitro fertilization and natural conception. *Fertil Steril* 2008: **90**; 289-296.

Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Best Pract Res Clin Endocrinol Metab* 2011: **25**; 271-285.

Lashley LE, Haasnoot GW, Spruyt-Gerritse M, and Claas FH. Selective advantage of HLA matching in successful uncomplicated oocyte donation pregnancies. *J Reprod Immunol* 2015: **112**; 29-33.

Lazaraviciute G, Kauser M, Bhattacharya S, Haggarty P, and Bhattacharya S. A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously. *Hum Reprod Update* 2014: **20**; 840-852.

Lerner-Geva L, Boyko V, Ehrlich S, Mashiach S, Hourvitz A, Haas J, Margalioth E, Levran D, Calderon I, Orvieto R*, et al.* Possible risk for cancer among children born following assisted reproductive technology in Israel. *Pediatr Blood Cancer* 2017: **64**.

Li Z, Wang YA, Ledger W, Edgar DH, and Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. *Hum Reprod* 2014: **29**; 2794-2801.

Lidegaard O, Pinborg A, and Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod* 2005: **20**; 950-954.

Lie RT, Lyngstadaas A, Orstavik KH, Bakketeig LS, Jacobsen G, and Tanbo T. Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis. *Int J Epidemiol* 2005: **34**; 696-701.

Liu H, Zhang Y, Gu HT, Feng QL, Liu JY, Zhou J, and Yan F. Association between assisted reproductive technology and cardiac alteration at age 5 years. *JAMA Pediatr* 2015: **169**; 603-605.

Liu L, Gao J, He X, Cai Y, Wang L, and Fan X. Association between assisted reproductive technology and the risk of autism spectrum disorders in the offspring: a meta-analysis. *Sci Rep* 2017: **7**; 46207.

Liu SY, Teng B, Fu J, Li X, Zheng Y, and Sun XX. Obstetric and neonatal outcomes after transfer of vitrified early cleavage embryos. *Hum Reprod* 2013: **28**; 2093-2100.

Luke B. Pregnancy and birth outcomes in couples with infertility with and without assisted reproductive technology: with an emphasis on US population-based studies. *Am J Obstet Gynecol* 2017: **217**; 270-281.

Luke B, Gopal D, Cabral H, Diop H, and Stern JE. Perinatal outcomes of singleton siblings: the effects of changing maternal fertility status. *J Assist Reprod Genet* 2016: **33**; 1203-1213.

Luke B, Gopal D, Cabral H, Stern JE, and Diop H. Pregnancy, birth, and infant outcomes by maternal fertility status: the Massachusetts Outcomes Study of Assisted Reproductive Technology. *Am J Obstet Gynecol* 2017: **217**; 327 e321-327 e314.

Magnusson A, Wennerholm UB, Kallen K, Petzold M, Thurin-Kjellberg A, and Bergh C. The association between the number of oocytes retrieved for IVF, perinatal outcome and obstetric complications. *Hum Reprod* 2018: **33**; 1939-1947.

Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, and Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2018: **24**; 35-58.

Maheshwari A, Pandey S, Shetty A, Hamilton M, and Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012: **98**; 368-377 e361-369.

Maheshwari A, Raja EA, and Bhattacharya S. Obstetric and perinatal outcomes after either fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies recorded in the Human Fertilisation and Embryology Authority anonymized dataset. *Fertil Steril* 2016: **106**; 1703-1708.

Malchau SS, Loft A, Larsen EC, Aaris Henningsen AK, Rasmussen S, Andersen AN, and Pinborg A. Perinatal outcomes in 375 children born after oocyte donation: a Danish national cohort study. *Fertil Steril* 2013: **99**; 1637-1643.

Marino JL, Moore VM, Willson KJ, Rumbold A, Whitrow MJ, Giles LC, and Davies MJ. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. *PLoS One* 2014: **9**; e80398.

Marjonen H, Auvinen P, Kahila H, Tsuiko O, Koks S, Tiirats A, Viltrop T, Tuuri T, Soderstrom-Anttila V, Suikkari AM*, et al.* rs10732516 polymorphism at the IGF2/H19 locus associates with genotype-specific effects on placental DNA methylation and birth weight of newborns conceived by assisted reproductive technology. *Clin Epigenetics* 2018: **10**; 80.

Market-Velker BA, Zhang L, Magri LS, Bonvissuto AC, and Mann MR. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Hum Mol Genet* 2010: **19**; 36-51.

Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia CR, and Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. *Hum Reprod* 2016: **31**; 2561-2569.

Masoudian P, Nasr A, de Nanassy J, Fung-Kee-Fung K, Bainbridge SA, and El Demellawy D. Oocyte donation pregnancies and the risk of preeclampsia or gestational hypertension: a systematic review and metaanalysis. *Am J Obstet Gynecol* 2016: **214**; 328-339.

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A, and Knowledge Synthesis G. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009: **146**; 138-148.

McGovern PG, Llorens AJ, Skurnick JH, Weiss G, and Goldsmith LT. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. *Fertil Steril* 2004: **82**; 1514-1520.

Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, Scherrer U, and Rexhaj E. Association of Assisted Reproductive Technologies With Arterial Hypertension During Adolescence. *J Am Coll Cardiol* 2018: **72**; 1267-1274.

Middelburg KJ, Heineman MJ, Bos AF, and Hadders-Algra M. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI-a systematic review. *Hum Reprod Update* 2008: **14**; 219-231.

Nejdet S, Bergh C, Kallen K, Wennerholm UB, and Thurin-Kjellberg A. High risks of maternal and perinatal complications in singletons born after oocyte donation. *Acta Obstet Gynecol Scand* 2016: **95**; 879-886.

Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, Land JA, Evers JL, and Dumoulin JC. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012: **27**; 1966-1976.

Norrman E, Petzold M, Bergh C, and Wennerholm UB. School performance in singletons born after assisted reproductive technology. *Hum Reprod* 2018: **33**; 1948-1959.

Noyes N, Porcu E, and Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. *Reprod Biomed Online* 2009: **18**; 769-776.

Oldereid NB, Hanevik HI, Bakkevig I, Romundstad LB, Magnus O, Hazekamp J, Hentemann M, Eikeland SN, Skrede S, Reitan IR*, et al.* Pregnancy outcome according to male diagnosis after ICSI with non-ejaculated sperm compared with ejaculated sperm controls. *Reprod Biomed Online* 2014: **29**; 417-423.

Olesen IA, Andersson AM, Aksglaede L, Skakkebaek NE, Rajpert-de Meyts E, Joergensen N, and Juul A. Clinical, genetic, biochemical, and testicular biopsy findings among 1,213 men evaluated for infertility. *Fertil Steril* 2017: **107**; 74-82 e77.

Olivennes F, Kerbrat V, Rufat P, Blanchet V, Fanchin R, and Frydman R. Follow-up of a cohort of 422 children aged 6 to 13 years conceived by in vitro fertilization. *Fertil Steril* 1997: **67**; 284-289.

Opdahl S, Henningsen AA, Tiitinen A, Bergh C, Pinborg A, Romundstad PR, Wennerholm UB, Gissler M, Skjaerven R, and Romundstad LB. Risk of hypertensive disorders in pregnancies following assisted reproductive technology: a cohort study from the CoNARTaS group. *Hum Reprod* 2015: **30**; 1724-1731.

Palermo G, Joris H, Devroey P, and Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992: **340**; 17-18.

Pandey S, Shetty A, Hamilton M, Bhattacharya S, and Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012: **18**; 485-503.

Parkinson J, Tran C, Tan T, Nelson J, Batzofin J, and Serafini P. Perinatal outcome after in-vitro fertilization-surrogacy. *Hum Reprod* 1999: **14**; 671-676.

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

Pereira N, Reichman DE, Goldschlag DE, Lekovich JP, and Rosenwaks Z. Impact of elevated peak serum estradiol levels during controlled ovarian hyperstimulation on the birth weight of term singletons from fresh IVF-ET cycles. *J Assist Reprod Genet* 2015: **32**; 527-532.

Pinborg A, Lidegaard O, la Cour Freiesleben N, and Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod* 2005: **20**; 2821-2829.

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

Pinborg A, Loft A, Schmidt L, and Andersen AN. Morbidity in a Danish national cohort of 472 IVF/ICSI twins, 1132 non-IVF/ICSI twins and 634 IVF/ICSI singletons: health-related and social implications for the children and their families. *Hum Reprod* 2003: **18**; 1234-1243.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Soderstrom-Anttila V, Nygren KG, Hazekamp J, and Bergh C. Why do singletons conceived after assisted reproduction

technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013: **19**; 87-104.
Pouwer AW, Farquhar C, and Kremer JA. Long-acting FSH versus daily FSH for women undergoing assisted reproduction. *Cochrane Database Syst Rev* 2015; CD009577.
Qin J, Wang H, Sheng X, Liang D, Tan H, and Xia J. Pregnancy-related complications and adverse pregnancy outcomes in multiple pregnancies resulting from assisted reproductive technology: a meta-analysis of cohort studies. *Fertil Steril* 2015: **103**; 1492-1508 e1491-1497.
Qin JB, Sheng XQ, Wu D, Gao SY, You YP, Yang TB, and Wang H. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2017: **295**; 285-301.
Reigstad MM, Larsen IK, Myklebust TA, Robsahm TE, Oldereid NB, Brinton LA, and Storeng R. Risk of Cancer in Children Conceived by Assisted Reproductive Technology. *Pediatrics* 2016: **137**; e20152061.
Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S, and Racowsky C. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update* 2017: **23**; 139-155.
Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Gunnell D, and Vatten LJ. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet* 2008: **372**; 737-743.
Rumbold AR, Moore VM, Whitrow MJ, Oswald TK, Moran LJ, Fernandez RC, Barnhart KT, and Davies MJ. The impact of specific fertility treatments on cognitive development in childhood and adolescence: a systematic review. *Hum Reprod* 2017: **32**; 1489-1507.
Sandin S, Nygren KG, Iliadou A, Hultman CM, and Reichenberg A. Autism and mental retardation among offspring born after in vitro fertilization. *JAMA* 2013: **310**; 75-84.
Savasi VM, Mandia L, Laoreti A, and Cetin I. Maternal and fetal outcomes in oocyte donation pregnancies. *Hum Reprod Update* 2016: **22**; 620-633.
Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, and Bergh C. Factors affecting obstetric outcome of singletons born after IVF. *Hum Reprod* 2011: **26**; 2878-2886.
Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, de Marchi SF, Nicod P, Germond M, Allemann Y*, et al.* Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. *Circulation* 2012: **125**; 1890-1896.
Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, Zhu Y, Deng X, Qi X, Li H*, et al.* Transfer of Fresh versus Frozen Embryos in Ovulatory Women. *N Engl J Med* 2018: **378**; 126-136.
Soderstrom-Anttila V, Salevaara M, and Suikkari AM. Increasing openness in oocyte donation families regarding disclosure over 15 years. *Hum Reprod* 2010: **25**; 2535-2542.
Soderstrom-Anttila V, Tiitinen A, Foudila T, and Hovatta O. Obstetric and perinatal outcome after oocyte donation: comparison with in-vitro fertilization pregnancies. *Hum Reprod* 1998: **13**; 483-490.
Soderstrom-Anttila V, Wennerholm UB, Loft A, Pinborg A, Aittomaki K, Romundstad LB, and Bergh C. Surrogacy: outcomes for surrogate mothers, children and the resulting families-a systematic review. *Hum Reprod Update* 2016: **22**; 260-276.
Soellner L, Begemann M, Mackay DJ, Gronskov K, Tumer Z, Maher ER, Temple IK, Monk D, Riccio A, Linglart A*, et al.* Recent Advances in Imprinting Disorders. *Clin Genet* 2017: **91**; 3-13.

Song S, Ghosh J, Mainigi M, Turan N, Weinerman R, Truongcao M, Coutifaris C, and Sapienza C. DNA methylation differences between in vitro- and in vivo-conceived children are associated with ART procedures rather than infertility. *Clin Epigenetics* 2015: **7**; 41.

Spandorfer SD. Unlocking the cause of increased adverse singleton pregnancy outcomes: the role of the assisted reproductive technology derived embryo. *Fertil Steril* 2017: **108**; 953-954.

Spangmose AL, Malchau SS, Henningsen AA, Forman JL, Rasmussen S, Loft A, Schmidt L, and Pinborg A. Academic performance in adolescents aged 15-16 years born after frozen embryo transfer compared with fresh embryo transfer: a nationwide registry-based cohort study. *BJOG* 2018.

Spangmose AL, Malchau SS, Schmidt L, Vassard D, Rasmussen S, Loft A, Forman J, and Pinborg A. Academic performance in adolescents born after ART-a nationwide registry-based cohort study. *Hum Reprod* 2017: **32**; 447-456.

Storgaard M, Loft A, Bergh C, Wennerholm UB, Soderstrom-Anttila V, Romundstad LB, Aittomaki K, Oldereid N, Forman J, and Pinborg A. Obstetric and neonatal complications in pregnancies conceived after oocyte donation: a systematic review and meta-analysis. *BJOG* 2017: **124**; 561-572.

Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, and Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 2002: **359**; 461-465.

Sunde A, Brison D, Dumoulin J, Harper J, Lundin K, Magli MC, Van den Abbeel E, and Veiga A. Time to take human embryo culture seriously. *Hum Reprod* 2016: **31**; 2174-2182.

Sundh KJ, Henningsen AK, Kallen K, Bergh C, Romundstad LB, Gissler M, Pinborg A, Skjaerven R, Tiitinen A, Vassard D*, et al.* Cancer in children and young adults born after assisted reproductive technology: a Nordic cohort study from the Committee of Nordic ART and Safety (CoNARTaS). *Hum Reprod* 2014: **29**; 2050-2057.

Sunkara SK, Antonisamy B, Selliah HY, and Kamath MS. Perinatal outcomes after gestational surrogacy versus autologous IVF: analysis of national data. *Reprod Biomed Online* 2017: **35**; 708-714.

Sunkara SK, La Marca A, Seed PT, and Khalaf Y. Increased risk of preterm birth and low birthweight with very high number of oocytes following IVF: an analysis of 65 868 singleton live birth outcomes. *Hum Reprod* 2015: **30**; 1473-1480.

Svahn MF, Hargreave M, Nielsen TS, Plessen KJ, Jensen SM, Kjaer SK, and Jensen A. Mental disorders in childhood and young adulthood among children born to women with fertility problems. *Hum Reprod* 2015: **30**; 2129-2137.

Tararbit K, Lelong N, Jouannic JM, Goffinet F, Khoshnood B, and Group ES. Is the probability of prenatal diagnosis or termination of pregnancy different for fetuses with congenital anomalies conceived following assisted reproductive techniques? A population-based evaluation of fetuses with congenital heart defects. *BJOG* 2015: **122**; 924-931.

Tenorio J, Romanelli V, Martin-Trujillo A, Fernandez GM, Segovia M, Perandones C, Perez Jurado LA, Esteller M, Fraga M, Arias P*, et al.* Clinical and molecular analyses of Beckwith-Wiedemann syndrome: Comparison between spontaneous conception and assisted reproduction techniques. *Am J Med Genet A* 2016: **170**; 2740-2749.

Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, and Bergh C. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med* 2004: **351**; 2392-2402.

Thurin-Kjellberg A, Olivius C, and Bergh C. Cumulative live-birth rates in a trial of single-embryo or double-embryo transfer. *N Engl J Med* 2009: **361**; 1812-1813.

Toppari J, Kaleva M, and Virtanen HE. Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Hum Reprod Update* 2001: **7**; 282-286.

Trounson A and Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature* 1983: **305**; 707-709.

Valenzuela-Alcaraz B, Crispi F, Bijnens B, Cruz-Lemini M, Creus M, Sitges M, Bartrons J, Civico S, Balasch J, and Gratacos E. Assisted reproductive technologies are associated with cardiovascular remodeling in utero that persists postnatally. *Circulation* 2013: **128**; 1442-1450.

van der Hoorn ML, Lashley EE, Bianchi DW, Claas FH, Schonkeren CM, and Scherjon SA. Clinical and immunologic aspects of egg donation pregnancies: a systematic review. *Hum Reprod Update* 2010: **16**; 704-712.

Vermeiden JP and Bernardus RE. Are imprinting disorders more prevalent after human in vitro fertilization or intracytoplasmic sperm injection? *Fertil Steril* 2013: **99**; 642-651.

von Arx R, Allemann Y, Sartori C, Rexhaj E, Cerny D, de Marchi SF, Soria R, Germond M, Scherrer U, and Rimoldi SF. Right ventricular dysfunction in children and adolescents conceived by assisted reproductive technologies. *J Appl Physiol (1985)* 2015: **118**; 1200-1206.

Vuong LN, Dang VQ, Ho TM, Huynh BG, Ha DT, Pham TD, Nguyen LK, Norman RJ, and Mol BW. IVF Transfer of Fresh or Frozen Embryos in Women without Polycystic Ovaries. *N Engl J Med* 2018: **378**; 137-147.

Wagenaar K, van Weissenbruch MM, Knol DL, Cohen-Kettenis PT, Delemarre-van de Waal HA, and Huisman J. Information processing, attention and visual-motor function of adolescents born after in vitro fertilization compared with spontaneous conception. *Hum Reprod* 2009: **24**; 913-921.

Wale PL and Gardner DK. The effects of chemical and physical factors on mammalian embryo culture and their importance for the practice of assisted human reproduction. *Hum Reprod Update* 2016: **22**; 2-22.

Wang JX, Norman RJ, and Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. *Hum Reprod* 2002: **17**; 945-949.

Wang X, Du M, Guan Y, Wang B, Zhang J, and Liu Z. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. *Reprod Biol Endocrinol* 2017: **15**; 36.

Wellesley D, Boyd P, Dolk H, and Pattenden S. An aetiological classification of birth defects for epidemiological research. *J Med Genet* 2005: **42**; 54-57.

Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, Liu J, and Hu Z. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. *Fertil Steril* 2012: **97**; 1331-1337 e1331-1334.

Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aittomaki K, Hazekamp J, Nygren KG, Selbing A, and Loft A. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009: **24**; 2158-2172.

Williams CL, Bunch KJ, Murphy MFG, Stiller CA, Botting BJ, Wallace WH, Davies MC, and Sutcliffe AG. Cancer risk in children born after donor ART. *Hum Reprod* 2018: **33**; 140-146.

Williams CL, Bunch KJ, Stiller CA, Murphy MF, Botting BJ, Wallace WH, Davies M, and Sutcliffe AG. Cancer risk among children born after assisted conception. *N Engl J Med* 2013: **369**; 1819-1827.

Woldringh GH, Besselink DE, Tillema AH, Hendriks JC, and Kremer JA. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review. *Hum Reprod Update* 2010: **16**; 12-19.

Woldringh GH, Horvers M, Janssen AJ, Reuser JJ, de Groot SA, Steiner K, D'Hauwers KW, Wetzels AM, and Kremer JA. Follow-up of children born after ICSI with epididymal spermatozoa. *Hum Reprod* 2011: **26**; 1759-1767.

Woo I, Hindoyan R, Landay M, Ho J, Ingles SA, McGinnis LK, Paulson RJ, and Chung K. Perinatal outcomes after natural conception versus in vitro fertilization (IVF) in gestational surrogates: a model to evaluate IVF treatment versus maternal effects. *Fertil Steril* 2017: **108**; 993-998.

Yeung EH, Sundaram R, Bell EM, Druschel C, Kus C, Xie Y, and Buck Louis GM. Infertility treatment and children's longitudinal growth between birth and 3 years of age. *Hum Reprod* 2016: **31**; 1621-1628.

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, and Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001: **27**; 153-154.

Zandstra H, Brentjens L, Spauwen B, Touwslager RNH, Bons JAP, Mulder AL, Smits LJM, van der Hoeven M, van Golde RJT, Evers JLH*, et al.* Association of culture medium with growth, weight and cardiovascular development of IVF children at the age of 9 years. *Hum Reprod* 2018: **33**; 1645-1656.

Zhao J, Xu B, Zhang Q, and Li YP. Which one has a better obstetric and perinatal outcome in singleton pregnancy, IVF/ICSI or FET?: a systematic review and meta-analysis. *Reprod Biol Endocrinol* 2016: **14**; 51.

Zhou J, Liu H, Gu HT, Cui YG, Zhao NN, Chen J, Gao L, Zhang Y, and Liu JY. Association of cardiac development with assisted reproductive technology in childhood: a prospective single-blind pilot study. *Cell Physiol Biochem* 2014: **34**; 988-1000.

Zhu JL, Basso O, Obel C, Bille C, and Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. *BMJ* 2006: **333**; 679.

Zhu JL, Basso O, Obel C, Hvidtjorn D, and Olsen J. Infertility, infertility treatment and psychomotor development: the Danish National Birth Cohort. *Paediatr Perinat Epidemiol* 2009: **23**; 98-106.

FIGURE LEGENDS

Figure 1 Factors with a possiple adverse effect on the offspring born after ART.

Figure 2 Perinatal outcomes in singletons born after ART versus singletons born without ART.

Outcomes are depicted as bars showing the adjusted risks (x-axes) with 95% CI (error bars).
PTB: preterm birth, LBW: low birthweight, SGA: small for gestational age, PNM: perinatal mortality

Figure 3 Time trends in preterm birth rates.

A. Time trends in preterm birth rates among children (singletons and twins) born after ART in four Nordic countries.

B. Time trends in preterm birth rates according to plurality in children born after ART and naturally in four Nordic countries.

Figure 4 Basic epigenetic mechanisms involved in the regulation of gene expression and development.

Figure 6 Factors affecting the short- and long-term health in offspring born after ART.