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Childhood outcomes of assisted reproductive technology

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ABSTRACT: There is a large population of children conceived via assisted reproductive technology (ART), which continues to increase worldwide, without a clear understanding of associated long-term outcomes. ART children are more likely to be the result of multiple pregnancies, and thus to be born prematurely or low birthweight. There is growing evidence that ART children are phenotypically and biochemically different from naturally conceived children, but the mechanism(s) leading to these changes have not been elucidated. There is a possible increased risk of rare imprinted gene disorders in these children. However, it remains unclear whether more subtle changes in DNA methylation occur commonly, leading to differences in gene expression and phenotype in ART children. Although an increased risk of cancer among ART children has been reported, the role of ART in the development of cancer has not been demonstrated. Further research and ongoing surveillance of ART children is essential to better understand the possible effects of ART on the long-term health of this population.

Key words: assisted reproductive technology / *in vitro* fertilization / intracytoplasmic sperm injection

Introduction

The definition of assisted reproductive technology (ART) varies widely, but the CDC (US Centre for Disease Control and Prevention) defines it as all fertility treatment in which both eggs and sperm are handled (Sunderam *et al.*, 2009). Until relatively recently, IVF made up the majority of ART procedures, but the utilization of ICSI has steadily increased, and it now represents 40–70% of ART procedures (de Mouzon *et al.*, 2010). Further, approximately three-quarters of ART pregnancies are achieved through fresh embryo transfer, and the remainder through frozen embryos that are thawed and then transferred (Sunderam *et al.*, 2009).

From its inception in 1978, there has been a worldwide increase in uptake and demand for ART with recent data suggesting that children conceived by ART comprise anywhere between 1 and 4% of the newborn population in industrialized countries (de Mouzon *et al.*, 2010). There is a continued worldwide increase in the number of ART conceived children due to several possible reasons. These include a shift in reproductive behaviour towards the postponement of childbirth leading to a consequent increase in the proportion of women at risk of infertility; increased availability of treatment and state funding for ART in some countries (Leridon and Slama, 2008; Sunderam *et al.*, 2009). The indications for ART treatment of infertility have also broadened significantly over the past decade, leading to many couples utilizing ART instead of expectant management or other treatment options (Reindollar *et al.*, 2010).

Outcomes of ART-conceived pregnancies are an area of intense scrutiny and research; with vast publications assessing ART treatment success, perinatal outcomes and longer-term outcomes of ART children. The overwhelming majority of studies to date have assessed ART childhood outcomes in comparison with those of the general population. There is emerging evidence that in order to properly determine any actual impact of the ART process itself, ART outcomes may be better compared with those of children born to subfertile parents. Subfertility is generally accepted as the failure to conceive naturally after 12 months or more of unprotected intercourse (Gnoth *et al.*, 2005) and affects between 8 and 20% of couples (Oakley *et al.*, 2008). As the majority of ART parents are subfertile (Zhu *et al.*, 2006; Källén *et al.*, 2010d), it is becoming increasingly evident that underlying maternal or paternal subfertility may be an important factor in obstetric, neonatal and childhood outcomes in the ART population (Williams and Sutcliffe, 2009).

The possible impact of parental subfertility on childhood outcomes is largely accounted for by utilizing naturally conceived children of subfertile parents as a comparison group for ART children. However, this effect is not completely eliminated, as children of subfertile parents were eventually conceived naturally, whereas the ART children were not.

In this review, we examine the evidence to date assessing if outcomes of ART-conceived children are different from those of naturally conceived children, and whether mechanisms such as epigenetics influence these changes.

Multiple births and perinatal outcomes

Multiples births occur more commonly in ART pregnancies. In most developed countries, 30–50% of all twin pregnancies result from ART (Ombelet *et al*., 2005; Pandian *et al*., 2009; Sunderam *et al*., 2009; Gelbaya *et al*., 2010). Multiple pregnancies occur in 25–50% of ART (Martin *et al*., 2009; Sunderam *et al*., 2009; de Mouzon *et al*., 2010) and 5–40% of ovarian stimulation pregnancies (Källén *et al*., 2002; Fauser *et al*., 2005; Wright *et al*., 2008). The wide variation in these figures is due to the differing practices between centres and countries on the number of embryos transferred and ovarian stimulation practices (Maheshwari *et al*., 2011). While double-embryo transfer remains common practice, single embryo transfer (SET) is increasing in popularity; particularly, when involving extended culture to blastocyst to increase the likelihood of successful implantation (Papanikolaou *et al*., 2008). SET in ART significantly reduces the rate of twins and higher order multiples, yet this practice remains sporadic (Bergh, 2005; Ombelet *et al*., 2005; Gerris, 2009). The Belgian project encouraging SET led to a reduction in the rate of twins from 19 to 3% and of higher order multiples to almost 0% (Ombelet *et al*., 2005). A long-term Swedish population study showed that the increasing utilization of SET led to a reduction in twins from 20 to 5%, as well as a corresponding reduction in rates of premature births and low birthweight infants in the ART population (Källén *et al*., 2010d).

While some reviews suggest that cumulative live birth rates from SET is superior to that of multiple embryo transfer (Pandian *et al*., 2009; Gelbaya *et al*., 2010), a recent meta-analysis concluded that SET yields a lower live birth rate than a double-embryo transfer (27 versus 42%); but that this difference is almost completely overcome by an additional frozen SET cycle in the SET patients (McLernon *et al*., 2010). Increase in the rate of SET has led to greater availability of frozen embryos for future cycles, and it is estimated that over 25% of ART children are now born after cryopreservation of embryos, blastocysts, or oocytes (Källén *et al*., 2010d). Recent evidence suggests that obstetric and perinatal outcomes are comparable irrespective of whether pregnancy is a result of fresh or frozen embryo transfer (Wennerholm *et al*., 2009).

Perinatal outcomes in ART are most significantly influenced by multiple pregnancies, which have more than a 60% risk of low birthweight or premature delivery in the ART population (Sunderam *et al*., 2009). Helmerhorst *et al*. found that ART twins fared better in the peri-natal period in comparison with all naturally conceived twins (monozygotic and dizygotic) (Helmerhorst *et al*., 2004). However, perinatal outcomes of dizygotic are better than those of monozygotic twins, and the proportion of dizygotic twins in the ART population is greater than among naturally conceived twins (Källén *et al*., 2010e). Recent studies examining the outcomes of dizygotic twins in isolation showed that ART twins fare worse than naturally conceived twins in the perinatal period (Hansen *et al*., 2009; Källén *et al*., 2010e).

The contribution of the ART population to the premature and low birthweight population varies between countries, but remains high. Over 41% of all ART infants born in the USA in 2006 were delivered preterm, accounting for 4% of all preterm infants that year, costing more than US$1 billion in neonatal care alone (Martin *et al*., 2009; Sunderam *et al*., 2009). Even in ART singleton pregnancies, there is an almost 2-fold increase in the risk of premature birth, as well as an associated higher rate of small for gestational age (SGA) and low birthweight infants (Helmerhorst *et al*., 2004; Sunderam *et al*., 2009; Källén *et al*., 2010d).

However, the role of ART as a pivotal factor in the increased risk of premature delivery and low birthweight has been called into question; with several studies (Williams *et al*., 1991; Joffe and Li, 1994; Henriksen *et al*., 1997; Draper *et al*., 1999; Basso and Baird, 2003; Ludwig, 2009; Williams and Sutcliffe, 2009) suggesting that background subfertility and time to pregnancy may be more important. For example, a study of singleton ART offspring compared with naturally conceived sibling controls found no differences in rates of low birthweight, premature or SGA births (Romundstad *et al*., 2008). Overall, there is growing evidence that the contribution of parental subfertility and other factors to adverse perinatal outcomes may be equally, if not more important, than the contribution of ART itself.

Neurological and neuro-developmental outcomes

Neurological abnormalities appear to occur more commonly in ART-conceived children. However, there is increasing evidence that factors other than the ART procedure itself, such as perinatal events, may be largely responsible for these findings.

Several studies have found an increased risk of cerebral palsy (CP) or neurological abnormalities among the ART population (Ericson *et al*., 2002; Strömberg *et al*., 2002; Pinborg *et al*., 2004; Källén *et al*., 2005; Lidegaard *et al*., 2005; Hvidtjørn *et al*., 2006, 2010; Klemetti *et al*., 2006; Romundstad *et al*., 2008). However, the authors concluded that these outcomes were generally explained by higher rates of multiple pregnancies, prematurity and low birthweight among ART offspring. The wider use of SET has reduced the number of premature and low birthweight ART infants (Källén *et al*., 2010d). Consequently, it would be expected that a reduction in CP rates would follow, and there is some indication that this is the case (Källén *et al*., 2010a).

Recent studies have included children of subfertile parents as a comparison group to ART children, finding similar neurological outcomes (Middelburg *et al*., 2009; Middelburg *et al*., 2010). However, a recent large population study suggested that ART may be a more important factor than parental subfertility in CP risk (Zhu *et al*., 2010). Further studies are needed to clarify the possible contribution of background subfertility to neurological outcomes in ART children.

The vast majority of studies assessing neuro-cognitive outcomes (i.e. locomotor, cognitive, speech and language and behaviour) in ART children showed no differences between ART-conceived and naturally conceived singletons; when adjusted for recognized confounding factors such as low birthweight and prematurity (Koivurova *et al*., 2003; Place and Englert, 2003; Ponjaert-Kristoffersen *et al*., 2005; Leunens *et al*., 2008; Hvidtjørn *et al*., 2009). Similarly, there is no evidence of an increased risk of autism among ART children (Maimburg and Væth, 2007; Hvidtjørn *et al*., 2009, 2011). Current

evidence suggests that the ART process itself does not lead to an increased risk of adverse neuro-cognitive outcomes or autism.

A weak association between ART and drug-treated attention deficit hyperactivity disorder has been reported, but the association is no longer significant with adjustment for subfertility (Källén *et al*., 2011). Further, there are no concerns about psycho-social issues or family relationships in the ART population, with a suggestion that ART children may fare a little better than naturally conceived children in this area (Barnes *et al*., 2004; Basatemur and Sutcliffe, 2008; Colpin and Bossaert, 2008; Wagenaar *et al*., 2008; Wagenaar *et al*., 2011).

Growth

The large offspring syndrome is a well-recognized adverse consequence of cultured embryos in cattle and sheep (Young *et al*., 1998). This is proposed to be due to altered expression of the insulin-like growth factor (IGF)-II receptor, which is imprinted in animals but not humans (Young *et al*., 2001). These observations have triggered interest in the growth patterns of ART-conceived children.

There are several studies that have examined the growth and weight patterns of ART-conceived children, and these have revealed conflicting results. Most of these studies included children that were born prematurely, SGA or as a result of multiple pregnancies. This is not surprising considering the higher rate of multiple and premature births associated with ART (Sunderam *et al*., 2009; de Mouzon *et al*., 2010). At one extreme, earlier studies reported lower weight and length percentiles in ART-conceived children at 2 and 3 years of age (Brandes *et al*., 1992; Koivurova *et al*., 2003). However, a large uncontrolled study of 400 IVF-conceived children aged 6–13 years found no difference in height or weight compared with population-based growth data (Olivennes *et al*., 1997). In addition, inclusion of ICSI in the IVF process did not influence children's height or weight compared with controls (Belva *et al*., 2007). Further, a large European multi-centre study found no difference in height or weight among IVF, ICSI and naturally conceived children at 5 years of age (Bonduelle *et al*., 2005). Kai *et al*. assessed two cohorts of ART children (longitudinally from 0–36 months and a cross-sectional cohort at 5 years of age) finding that ICSI children were shorter than IVF and naturally conceived controls at 3 years, but no different at 5 years (Kai *et al*., 2006). They also found a lower serum IGF-1 level in ICSI boys and IVF-conceived girls at 3 months, but this difference did not persist into childhood (Kai *et al*., 2006). In contrast, others found that ART children were taller than naturally conceived controls or the general population (Saunders *et al*., 1996; Pruksananonda, 2001; Miles *et al*., 2007; Makhoul *et al*., 2009).

Few studies have provided longitudinal assessment of growth in childhood. Celeen *et al*. analysed height and weight data (from 3 months to 4 years) of IVF-conceived children compared with naturally conceived controls of subfertile parents (Ceelen *et al*., 2009). They found that ART infants had a lower weight, height and BMI standard deviation score (SDS) at 3 months, and a lower weight SDS at 6 months of age (Ceelen *et al*., 2009). ART children then showed greater growth velocity than controls in late infancy, but with no difference in height at 3 years of age (Ceelen *et al*., 2009). However, this study did acknowledge the potential effect of parental subfertility on growth outcome. We have identified only one other study that utilized this important comparison group (Makhoul *et al*., 2009).

In a recent study of growth in ART children, Basatemur *et al*. found no difference in height or weight in IVF or ICSI subjects between 5 and 12 years (Basatemur *et al*., 2010). However, the study population did include children who were low birthweight and born as early as 32 weeks gestation.

As previously stated, most studies included children born prematurely, SGA or from multiple pregnancies, factors that are known to be associated with impaired childhood growth (Leger *et al*., 1997; Fewtrell *et al*., 2000; Peralta-Carcelen *et al*., 2000). Thus, ART populations including these subjects would likely be shorter than naturally conceived populations that have far fewer low birthweight or prematurely born children. Therefore, it is surprising that these studies largely show no difference in height between IVF- and naturally conceived offspring.

There have been only a handful of studies that have corrected for prematurity and birthweight to determine any actual effects of the ART process itself on childhood growth (Miles *et al*., 2007; Makhoul *et al*., 2009; Green *et al*., 2010). In a matched control study, Miles *et al*. analysed the growth and metabolic parameters of 69 ART-conceived children from fresh embryo transfers (Miles *et al*., 2007). Compared with controls, ART-conceived girls were taller, had higher IGF binding protein 3 levels and displayed a trend towards higher IGF-1 levels. ART children also had higher high-density lipoprotein (HDL) and lower triglyceride levels than controls. The findings were particularly relevant as it was the first study to correct for parental heights and exclude children born premature or with low birthweight. The same group also assessed ART-conceived children from frozen embryo transfers, again showing that ART-conceived girls were taller than matched controls (Green *et al*., 2010).

Recently, Makhoul *et al*. published findings on the follow-up of children with very low birthweight aged 6 to 10 years conceived by ART or fertility medications (Makhoul *et al*., 2009). This group accounted for the genetic height and the low birthweight status in their analyses, and found that the ART and fertility medication-conceived groups were significantly taller than controls (Makhoul *et al*., 2009). Currently, there is no clear explanation for the taller stature or the gender bias observed.

It is clear that many studies on the growth of children conceived by ART had confounding factors and methodological weaknesses. Larger studies including appropriately matched groups are needed to confirm these latter findings. Further, given that ICSI and/or frozen embryo transfer are common major additions to ART, the effects of these processes on offspring outcome also need to be clarified.

Metabolism and gonadal function

There are limited and conflicting data on the metabolic and hormonal profiles of ART children, as summarized by a recent review (Kanaka-Gantenbein *et al*., 2010). Examples of observed differences in ART children compared with controls include higher fasting glucose (Ceelen *et al*., 2008b), higher HDL and lower or higher triglycerides (Miles *et al*., 2007; Sakka *et al*., 2010). Such differences were minor and remained well within the normal range.

Marginal increases in systolic and diastolic blood pressure have also been observed in ART children (Ceelen *et al*., 2008b). Although not

clinically significant in childhood, subtle blood pressure increases may be amplified in adulthood (Law *et al*., 1993).

There is evidence of an increased rate of early adrenarche and polycystic ovarian syndrome in women who were born prematurely or SGA (Pandolfi *et al*., 2008; Melo *et al*., 2010). However, only one study has assessed puberty in ART children, finding no difference in pubertal staging in comparison with children of subfertile couples (Ceelen *et al*., 2008a). Interestingly, ART-conceived girls had a significantly higher dehydroepiandrosterone sulfate and luteinizing hormone levels than naturally conceived controls. Although the cause for this was unclear, there were no apparent clinical effects (Ceelen *et al*., 2008a). The group was not evaluated further for disorders such as polycystic ovarian syndrome.

There are potential concerns for the future fertility of ART offspring, but these are yet to be clarified as the majority of this population is still relatively young. Current concern relates mostly to the future fertility of ICSI-conceived boys. Limited evidence suggest that ICSI boys have an increased rate of genital abnormalities (Ludwig *et al*., 2009), lower serum testosterone levels at 3 months (Mau Kai *et al*., 2007), but have salivary testosterone levels in puberty comparable with controls (Belva *et al*., 2011). A proportion of adult males requiring ICSI have very low sperm counts, which is associated with a greater risk of carrying chromosomal abnormalities (Aittomäki *et al*., 2004; Marchina *et al*., 2007).There is limited evidence that paternal sex chromosomal disorders, including micro-deletions, are rarely transmitted to male ART offspring (Feng *et al*., 2008; Mau Kai *et al*., 2008). Their potential effects on the gonadal function of ART boys is yet to be determined. While current evidence is inconclusive; the future fertility of ART offspring, particularly ICSI-conceived males, warrants further research.

Congenital abnormalities

There is a higher incidence of congenital abnormalities following ART (Rimm *et al*., 2004; Hansen *et al*., 2005; Lie *et al*., 2005), with considerable speculation as to the causes. Meta-analyses indicate a 30% increased risk of major malformations in children conceived by ART compared with spontaneous conception (Rimm *et al*., 2004; Hansen *et al*., 2005; Lie *et al*., 2005). Although much of the focus has been on the process of ART itself, mounting evidence suggests that parental subfertility may be an important factor (Rimm *et al*., 2004; McDonald *et al*., 2005; Rimm *et al*., 2011). A large population study in Denmark compared congenital abnormality rates among naturally conceived and fertility treatment offspring of subfertile couples, finding no differences in overall prevalence of congenital malformations (Zhu *et al*., 2006). In addition, this study indicates that parental factors are important, as increasing 'time to pregnancy' was associated with a greater risk of congenital abnormalities (Zhu *et al*., 2006).

A recent Swedish study provided further evidence on the important contribution of parental factors to the higher rates of congenital abnormalities in the ART population (Källén *et al*., 2010b). While the overall rate of congenital abnormalities in ART children remained elevated, the incidence of certain types of abnormalities such as neural tube defects, cardiac defects and oesophageal atresia were reduced (Källén *et al*., 2010b). The authors speculated that this reduction might be due to a greater proportion of ART-treated couples with a shorter period of unwanted childlessness (Källén *et al*., 2010b).

Epigenetics and imprinting disorders

There is growing evidence of an increased risk of imprinting disorders in ART children. Epigenetics is the study of heritable changes in phenotype or gene expression that occur independently of alterations in the DNA sequence (Waterland and Michels, 2007). DNA methylation and histone acetylation lead to DNA conformational change and gene silencing. Methylation is the best characterized epigenetic modification of DNA with cytosine guanosine (CpG) islands being particularly vulnerable (Waterland and Michels, 2007). A reduction in gene expression generally occurs when these islands are methylated within unmethylated gene promotor regions. Imprinting involves the silencing of either the maternal or paternal allele and is crucial for many aspects of pre- and post-natal growth and development (Waterland and Michels, 2007). Imprinting occurs at gametogenesis and embryogenesis and imprinted genes undergo de-methylation followed by re-methylation during early embryonic development.

Although environmental influences on gene regulation are increasingly studied in ART offspring, parental factors may lead to epigenetic modification (Horsthemke and Ludwig, 2005). Studies support the assertion that a subfertile couple may have a greater risk of pre-existing methylation defects and consequent imprinting disorders in their offspring (Horsthemke and Ludwig, 2005; Ludwig *et al*., 2005; Hartmann *et al*., 2006; Doornbos *et al*., 2007).

Nonetheless, it is proposed that the process of ART may lead to epigenetic and consequent imprinting changes. While animal studies support the role of DNA methylation changes in ART (Reik *et al*., 2001), there are very few human studies in this area. Fertility medications that cause ovarian stimulation are used in isolation for fertility treatment or as a component of the ART process. Ovarian stimulation is associated with an increased risk of aneuploidy in artificially matured oocytes (Kaleli *et al*., 2005), and may alter the methylation process (Sato *et al*., 2007; Market-Velker *et al*., 2010). However, the risk of alteration of the normal process of methylation is much greater in ART, as it departs more substantially from natural conception. The timing of ART also coincides with critical early embryonic DNA methylation and re-methylation.

Imprinted and epigenetically controlled genes play a key role in implantation and subsequent placental development (Nelissen *et al*., 2011). It is evident from animal and human studies that epimutations of these genes can lead to abnormal placentation and subsequent complications such as abnormal foetal growth (Steinhoff *et al*., 2009; Nelissen *et al*., 2011).

The effect of the culture medium on the growth of ART offspring is well established in animal studies (Young *et al*., 2001). Recent evidence has emerged that different culture media lead to a small but significant change in birthweight in humans (Dumoulin *et al*., 2010). It was suggested that the observed difference (245 g) may partially explain the greater incidence of low birthweight among ART-term singletons (Dumoulin *et al*., 2010). The authors speculated that the weight difference associated with different culture media may be due to distinct epigenetic changes, leading to changes in placental function (Dumoulin *et al*., 2010).

It is recognized that male seminal cytokines play an important role in implantation regulation (Robertson, 2007; Robertson *et al*., 2009), and these cytokines may influence foetal programming (Sjöblom *et al*., 2005). It was found that removal of seminal fluid prior to needle sperm injection into the mouse oocyte led to a reduction in embryo

and offspring size (Sjöblom et al., 2005). When the culture medium of these smaller embryos were treated with a male seminal cytokine (granulocyte-macrophage colony-stimulating factor), embryo and offspring size were corrected to normal (Sjöblom et al., 2005). Work in this area of periconceptual immunology is ongoing, and development of periconceptional immune modulators in human ART is a distinct possibility in the future (Salmassi et al., 2005; Guerin et al., 2009).

Evidence of subtle changes has emerged from a small cohort of ART subjects who were comprehensively screened for changes in DNA methylation and gene transcription (Katari et al., 2009). A very small study showed hypomethylation of a growth regulating gene (KvDMR1) in 3 of 18 ART children (Gomes et al., 2009). In contrast, work from our centre examined DNA methylation of four likely candidate genes and found no differences between ART and naturally conceived children (Cutfield et al., unpublished data). Further, a more recent study found similar rates of DNA methylation imprints in ART children and controls (Tierling et al., 2010). Overall, however, there are limited data to suggest that ART leads to changes in DNA methylation. Importantly, none of these studies have aligned these changes with alterations in gene expression producing an altered phenotype. Therefore, the impact of ART on phenotype or biochemical profile through DNA methylation has yet to be determined.

Nonetheless, there is increasing evidence of a link between ART and dramatic changes in methylation that lead to rare imprinting disorders, namely Beckwith–Wiedemann Syndrome (BWS) and Angelman Syndrome (AS) (DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003; Halliday et al., 2004; Chang et al., 2005; Bowdin et al., 2007; Doornbos et al., 2007; Paoloni-Giacobino, 2007; Lim et al., 2009; Manipalviratn et al., 2009; Choufani et al., 2010). BWS has an estimated incidence of 1 in 13 700 live births in the general population (Choufani et al., 2010), but the risk is estimated to be 6–9 times higher among ART offspring (Manipalviratn et al., 2009). While this would represent a dramatic increase in relative risk, the actual incidence of BWS in the ART population remains low (~1 in 4000–5500) (Manipalviratn et al., 2009). It is significant that the rate of methylation defects as the cause of BWS in the general population is ~60%, whereas this figure approaches 100% among the ART population (Manipalviratn et al., 2009). AS has an estimated incidence of 1 in 12 000 with imprinting abnormalities the aetiological factor in only 5% of cases (Steffenburg et al., 1996). Thus, the rate of imprinting disorder as a cause of AS in the general population is ~1 in 240 000. In contrast, 5 of the 7 reported cases of AS born after ART had an imprinting defect as the cause (Manipalviratn et al., 2009). However, it is worth noting that subfertility appears to be associated with an increased risk of AS (Doornbos et al., 2007).

There is emerging evidence for an increased relative risk of BWS and AS in the ART population. However, it is possible the case ascertainment of BWS and AS among ART offspring may be higher due to under reporting in the general population, and more intense scrutiny and follow-up of ART-conceived children (Bowdin et al., 2007).It is nonetheless clear that comprehensive, prospective, multi-centre studies are necessary to ascertain if this association is definitive.

Cancer risk

It has been proposed that ART children are at a greater risk of later malignancy, and a possible reason may be a reduction in imprinted gene activity leading to dysregulation in tumour suppression (Lim and Maher, 2010). Although an initial study suggesting an increased incidence of retinoblastoma in ART offspring caused concern (Moll et al., 2003), expansion of this study found this not to be the case (Marees et al., 2009). Other studies indicated that cancer risk among children and adolescents is not increased in ART offspring (Bergh et al., 1999; Bruinsma et al., 2000; Klip et al., 2001; Ericson et al., 2002; Marees et al., 2009). One study detected an increased rate of histiocytosis in ART children (Källén et al., 2005), but there is debate as to whether histiocytosis can be defined as true malignancy (Fadeel and Henter, 2003). Conversely, a very large recent study of over 26 000 ART-conceived children by the same group showed an increased risk of cancer and histiocytosis (odds ratio 1.48); with the cancer risk remaining elevated (odds ratio 1.35) even when children with histiocytosis were removed from the analysis (Källén et al., 2010c). This marginal increase in cancer risk was re-affirmed in a recent long-term review of the same population (Finnström et al., 2011). While both studies suggest an increased risk of cancer in ART children, the authors cautioned that the risk observed is probably not attributable to the ART procedure itself, but rather a result of many other factors such as the recognized increased risk of cancer among children with a history of prematurity (McLaughlin et al., 2006) or asphyxia (Spector et al., 2005).

Further studies on the growing ART population are necessary to determine any associated cancer risks. As the vast majority of the ART offspring population are under 30 years of age, long-term follow-up studies are warranted to determine if an increased cancer risk emerges with age.

Conclusions

There is growing evidence that ART-conceived children are phenotypically and biochemically different from naturally conceived children. However, the mechanism(s) leading to these changes have not been elucidated, and may include parental factors, maternal drug treatment, culture media, as well as egg and embryo manipulation. While there is a possible increase in the risk of imprinted gene disorders in ART offspring, these remain rare. Nonetheless, it is not clear if more subtle changes in DNA methylation can lead to the subtle changes in phenotype observed in some ART offspring. An increased cancer risk among ART children is yet to be conclusively demonstrated, but it is clear that ongoing surveillance is required on this population as it ages.

The population of ART children continues to increase worldwide without a clear understanding of associated long-term outcomes. Further knowledge on ART outcomes will provide health-care professionals, prospective parents and ART offspring with much needed accurate information on any actual risks.

Authors' roles

T.S. wrote the drafts of the paper, with editorial and critical input from J.P., P.H. and W.C.

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