Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Long-term follow-up of children conceived through assisted reproductive technology*

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Received Dec. 16, 2012; Revision accepted Mar. 5, 2013; Crosschecked Apr. 16, 2013

Abstract: Children conceived via assisted reproductive technologies (ART) are nowadays a substantial proportion of the population. It is important to follow up these children and evaluate whether they have elevated health risks compared to naturally conceived (NC) children. In recent years there has been a lot of work in this field. This review will summarize what is known about the health of ART-conceived children, encompassing neonatal outcomes, birth defects, growth and gonadal developments, physical health, neurological and neurodevelopmental outcomes, psychosocial developments, risk for cancer, and epigenetic abnormalities. Most of the children conceived after ART are normal. However, there is increasing evidence that ART-conceived children are at higher risk of poor perinatal outcome, birth defects, and epigenetic disorders, and the mechanism(s) leading to these changes have not been elucidated. Continuous follow-up of children after ART is of great importance as they progress through adolescence into adulthood, and new ART techniques are constantly being introduced.

Key words: Assisted reproductive technologies (ART), Children, Follow-up
doi:10.1631/jzus.B1200348 **Document code:** A **CLC number:** R711.6

1 Introduction

Assisted reproductive technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), are widely used to solve human infertility, and have provided great benefits for millions of couples who have struggled with infertility disorders. Since the first child conceived by IVF was born in 1978, there has been a consistent growth in the use of ART (Nyboe Andersen *et al*., 2008; de Mouzon *et al*., 2010; Ferraretti *et al*., 2012) and more than 4 million babies worldwide have been born via ART. As the offspring of ART have become a substantial proportion of the population, the safety of ART has gained increasing attention.

The many artificial procedures used during ART contribute to the concern that children conceived by ART might be exposed to greater health risks than naturally conceived (NC) children. First, during the ART process, numerous medications are used to induce ovulation, gametes are recruited, embryos are cultured in an in vitro environment and then frozen and thawed, and large doses of progesterone are used to support the luteal phase. All these artificial procedures may harm the gametes and embryos. Furthermore, ICSI, which can fertilize an egg by directly injecting one sperm to the ooplasm, is more invasive than conventional IVF. ICSI also evades natural selection at the oocyte membrane and both genetically and structurally abnormal sperm will be able to fertilize eggs, which may pass abnormal genetic materials to the children. In addition, transferring more than one embryo significantly increases the rate of multiple

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* Project supported by the National Basic Research Program (973) of China (No. 2012CB944901) and the National Natural Science Foundation of China (Nos. 81070532 and 81200475)

pregnancies, which is associated with a higher rate of prematurity and low birth weights, carrying high risks of morbidity to the children (Liu and Blair, 2002; Alexander and Salihu, 2005; Fauser *et al*., 2005). Evidence suggests that even ART singletons are at elevated health risks (Bower and Hansen, 2005; Henningsen *et al*., 2011; Sazonova *et al*., 2011), which may due to the poor fertility of the parents.

Worldwide, there are many publications on the topic of ART safety. In this review, we try to summarize the current evidence about whether ART-conceived children are at an increased risk of health problems compared with NC children.

2 Perinatal outcomes

2.1 Neonatal outcomes

ART-conceived offspring seem to be at a higher risk of lower birth weight, lower gestational age, premature delivery, prenatal mobility, and hospital admission than NC offspring (Schieve *et al*., 2002; Källén *et al*., 2005). The main reason for this increased risk is multiple pregnancies, mostly caused by transferring multiple embryos. Following ART, multiple birth rates are between 25% and 50% (Martin *et al*., 2009; Sunderam *et al*., 2009; de Mouzon *et al*., 2010). Single embryo transfer (SET) can significantly decrease the multiple birth rates (Gerris, 2009; Källén B. *et al*., 2010b), particularly because the blastocyst culture can dramatically increase the likelihood of embryo implantation (Papanikolaou *et al*., 2008). SET is increasing in popularity in resent years. Compared to conceptions via double embryo transfer (DET), SET can improve neonatal outcome, leading to significantly fewer preterm births and low birth weight infants (Kjellberg *et al*., 2006).

However, multiple pregnancies are just one factor that is attributed to the poor prenatal outcomes, and thus eliminating multiple births will not completely solve the problem. Many studies found that singletons born after ART are still at a higher risk of lower birth weight, younger gestational age, premature delivery, prenatal mobility, and hospital admission compared with NC singletons (Bower and Hansen, 2005; Henningsen *et al*., 2011; Sazonova *et al*., 2011).

Bower and Hansen (2005)'s meta-analysis reviewed all the methodologically sound studies available, examined singletons separately, and found approximately two-fold increases in the risk of perinatal mortality, low birth weight, and preterm birth, an approximate 50% increase in the risk of being small for gestational age, and a 30%–35% increase in birth defects for singletons conceived via ART compared with NC singletons. A recent study with a larger sample by Henningsen *et al*. (2011), compared the perinatal outcomes of 13692 pairs (n=27384 children) of singleton siblings who were conceived via IVF, ICSI, frozen embryo transfer (FET), or spontaneous pregnancy, and found that the mean birth weight was 65 g (95% confidence interval (CI) 41–89), lower in all ART children compared with their NC siblings. In addition, a higher risk of having a low birth weight (LBW; odds ratio (OR) 1.4, 95% CI 1.1–1.7) and a preterm birth (OR 1.3, 95% CI 1.1–1.6) was observed in IVF/ICSI compared with spontaneous conception.

In addition to ART itself, the etiology of infertility or the infertile state of the parents may contribute to the high risk of obstetric outcomes (Romundstad *et al*., 2008; Hayashi *et al*., 2012). A population-based cohort study in Norway (Romundstad *et al*., 2008) assessed the perinatal outcome of 8229 ART-conceived children compared with 120092 NC children and found a significantly lower mean birth weight, a shorter duration of gestation, and increased risks of small for gestational age (OR 1.26, 95% CI 1.10–1.44) and perinatal death (OR 1.26, 95% CI 1.10–1.44) in ART-conceived children. However, when the same comparison was performed in sibling-relationship, all the differences disappeared. Recently, a retrospective cohort study in Japan (Hayashi *et al*., 2012) provided reassuring findings. They compared the perinatal outcomes of singletons who were conceived through ovulation stimulation, intrauterine insemination (IUI), IVF, or through natural conception. Among singleton pregnancies, patients who conceived with ART procedures were at a similarly increased risk for placenta previa, preterm delivery, and low birth weight infant, regardless of the type of ART used.

In addition, Zhang *et al*. (2010)'s study provided another possibility for why ART children have poorer perinatal outcomes. The ART procedure may affect ART offspring via changing the gene expression in the placenta. Zhang *et al*. (2010) used microarray analysis to examine the gene expression profiles of

the placenta from ART patients and NC control patients. They found that some genes involved in the immune response and cell differentiation regulation were differentially expressed, which may affect fetal development. Unfortunately, their control group comprised fertile couples. Hence, we cannot distinguish whether the effect was come from ART itself or infertility.

Currently, whether ART will affect the perinatal outcome for twins is still controversial. Although several studies found that twins born after ART are at a higher risk of lower birth weight, lower gestational age, premature delivery, prenatal mobility, and hospital admission when compared with NC twins (McDonald *et al*., 2005; Kanat-Pektas *et al*., 2008; Hansen *et al*., 2009), some other studies showed the opposite result (Bower and Hansen, 2005; Boulet *et al*., 2008). Boulet *et al.* (2008) compared 1446 ART twins with 2729 non-ART twins and found that the risk of premature delivery, low birth weight, and neonatal death was lower in the ART group than in the non-ART group (adjusted OR 0.75, 95% CI 0.58–0.97; 0.75, 0.58–0.95; and 0.55, 0.35–0.88, respectively) among primiparous deliveries, and there were no differences in the risks among multiparous ART and non-ART twin deliveries. These results are similar to our investigation (unpublished). A potential reason for the differences of perinatal outcomes between ART and NC twins is the high rate of dizygotic twins in ART offspring, which results from DET.

In summary, poorer perinatal outcomes are found in ART-conceived children, even for singletons. Infertility is one possible reason, but currently the true mechanism remains unknown. Thus, studies that address these outcomes and whether there is a way to solve it are urgently advocated.

2.2 Birth defects

Numerous studies have indicated that children conceived through ART are at a significantly elevated risk of birth defects (Rimm *et al.*, 2004; Bonduelle *et al.*, 2005; Bower and Hansen, 2005; Hansen *et al.*, 2005; Olivennes, 2005; Schieve *et al.*, 2005; Bertelsmann *et al.*, 2008; El-Chaar *et al.*, 2009; Goel *et al.*, 2009; Tararbit *et al.*, 2011; Davies *et al.*, 2012; Mozafari Kermani *et al.*, 2012; Wen *et al.*, 2012). Meta-analyses have shown a 30%–40% increase in the major malformation rates for infants conceived through ART compared with NC children (Rimm *et al.*, 2004; Hansen *et al.*, 2005; Wen *et al.*, 2012). The most recently published meta-analysis by Wen *et al*. (2012) reviewed 46 studies containing 124468 IVF/ICSI children and provided a pooled risk estimation of 1.37 (95% CI 1.26–1.48), which is also evident in subgroup analysis (risk ratio (RR) 1.58 for ICSI and 1.30 for IVF; statistical significance was not reached when ICSI was compared with IVF). Furthermore, they assessed the risk for each organ system and found significantly increased risks in each system (RR 2.01 for the nervous system; 1.69 for the genitourinary system; 1.66 for the digestive system; 1.64 for the circulatory system; 1.48 for the musculoskeletal system; and 1.43 for eye, ear, face, and neck). A population-wide cohort study in South Australia (Davies *et al.*, 2012) also found a significantly elevated risk of birth defects in ART (8.3% [513/6163]) compared with non-ART pregnancies (5.8% [17546/302811]) (OR 1.47, 95% CI 1.33–1.62; multivariate-adjusted OR 1.28, 95% CI 1.16–1.41). Only one multi-center large-scale study from China (Yan *et al.*, 2011), encompassing 15405 ART offspring from seven centers, provides different results. In this study, a total rate of birth defects was 1.23% (189/15405), which was non-significantly different from that in the general Chinese population (1.35%).

Although the processes of ART are well-studied factors, some investigators believe that the condition of infertility is another important reason for birth defects (Zhu *et al.*, 2006; Rimm *et al.*, 2011). Rimm *et al*. (2004) published their first meta-analysis on birth defects. In that analysis, they reviewed 19 studies on major malformations in ART-conceived children and found an overall OR of 1.29 (95% CI 1.01–1.67) for ART-conceived children compared with NC controls. However, when taking subfertility into concern, they found an adjusted OR in their second meta-analysis of 1.01 (95% CI 0.82–1.23) (Rimm *et al.*, 2011). They concluded that ART may not increase the risk of birth defects as much as previously reported. Furthermore, the population-wide cohort study of Davies *et al.* (2012) also found that a history of infertility, either with or without assisted conception, was significantly associated with birth defects. Thus, the infertility situation should be taken into account in future studies.

Intuitively, ICSI is more invasive than IVF and

seems fraught with opportunities to damage the embryo, potentially leading to birth defects. Indeed, one multi-center study (Bonduelle *et al.*, 2005), containing 540 ICSI- and 437 IVF-conceived 5-year-old children from five European countries, found that major malformations were more frequently observed in the ICSI group, in particular in ICSI boys, beyond the neonatal period with the majority of these increased defects due to an excess in urogenital malformations. In addition, Davies *et al.* (2012)'s population-wide cohort study of South Australia found that the OR for birth defects associated with ART, as compared with NC pregnancies, was 1.07 (95% CI 0.90–1.26) for IVF and 1.57 (95% CI 1.30–1.90) for ICSI, after multivariate adjustment; as compared with ICSI, IVF was associated with a reduced risk of birth defect (OR 0.68, 95% CI 0.53–0.87). However, in other recent large studies and meta-analyses, no significant differences were found between ICSI and IVF children (Sutcliffe *et al.*, 2003; Rimm *et al.*, 2004; Lie *et al.*, 2005; Tararbit *et al.*, 2011; Wen *et al.*, 2012).

Whether it is ART procedures or the infertility situation that results in the increased risk of birth defects still remains in debate. Besides long-term follow-up, animal models of ART will be helpful so that the differences of ART population in the causes of infertility as well as in living environments and genetic background could be eliminated.

3 Long-term outcomes

3.1 Growth and gonadal development

The growth patterns of ART-conceived children have attracted the attention of many researchers. The vast majority of these studies have not found any differences in the postnatal growth until 12 years old between ART and NC children (Sutcliffe *et al.*, 2003; Bonduelle *et al.*, 2004; 2005; Belva *et al.*, 2007; Knoester *et al.*, 2008; Basatemur *et al.*, 2010; Lee *et al.*, 2010; Woldringh *et al.*, 2011). However, some studies suggest that ART children are taller. Miles *et al*. (2007) found that IVF/ICSI-conceived children aged 5–6 years were significantly taller than NC controls, following adjustment for age and parental height. In addition, it was observed that IVF children with premature and very low birth weight (<1 500 g) were significantly taller than NC ones at 6–10 years of age (Makhoul *et al.*, 2009). Researchers have speculated that pre- or early implantation factors might have contributed to the increased stature of the ART-conceived children (Makhoul *et al.*, 2009). The concern, however, is whether this increased height would lead to health risks in the future because evidence already shows that the rapid weight gain during early childhood (1–3 years) in IVF children is related to higher blood pressure levels (Ceelen *et al.*, 2009).

ART have been created to overcome the problem of infertility. Whether the subfertile conditions would affect the offspring is another question. ICSI is used mainly for overcoming male infertility, and the boys who are conceived by ICSI may inherit the impaired testicular function of their father. For these reasons, the gonadal development of ART-conceived offspring has also gained significant attention. Basically, the gonadal development of ART-conceived children is considered to be normal (de Schepper *et al.*, 2009; Belva *et al.*, 2010; 2011; 2012a). A study assessed 8–14-year-old prepubertal and pubertal ICSI-conceived boys and found that the majority had normal testicular and penile size (de Schepper *et al.*, 2009), serum concentrations of anti-Mullerian hormone (AMH) and inhibin B (de Schepper *et al.*, 2009; Belva *et al.*, 2010), and morning salivary testosterone levels (Belva *et al.*, 2011). A study of ICSI-conceived teenagers (Belva *et al.*, 2012a) showed that there were no differences in the menarche, genital development, or pubic hair development, but breast development was less advanced in the females. However, advanced bone age and increased dehydroepiandrosterone (DHEAS) and luteotropic hormone (LH) concentrations in pubertal IVF-conceived girls (Ceelen *et al.*, 2008b), and decreased serum testosterone levels and altered LH to testosterone ratio in ICSI-conceived boys at birth and at three months of age (Mau Kai *et al.*, 2007) were reported.

3.2 Physical health

There are numerous publications about the physical health of ART-conceived children, and the majority of these studies show that ART-conceived children experience similar childhood illnesses (Koivurova *et al.*, 2003; Pinborg *et al.*, 2003; Place and Englert, 2003; Bonduelle *et al.*, 2004; Belva *et al.*, 2007; 2012b; Knoester *et al.*, 2008; Beydoun *et al.*,

2010) and hospital services (Belva *et al.*, 2007; Knoester *et al.*, 2008) compared with NC children. Place *et al*. (2003) followed 66 ICSI-conceived, 52 IVF-conceived, and 59 NC full-term singletons and prospectively compared the physical health condition of these children, and found no differences among the three groups. Several studies on the physical health of ICSI-conceived children also showed optimistic results (Bonduelle *et al.*, 2004; Belva *et al.*, 2007; 2012b; Knoester *et al.*, 2008). In a recent study by Beydoun *et al*. (2010), the general health outcomes and the chronic diseases of ART-conceived young adults aged 18–26 years were similar to those of the general population, suggesting that young adults conceived through ART appear to be healthy and well adjusted.

However, some reports have suggested that ART children are more likely to have childhood illnesses (Bonduelle *et al.*, 2005; Källén *et al.*, 2005; Koivurova *et al.*, 2007; Ludwig *et al.*, 2009). A multi-center cohort study (Bonduelle *et al.*, 2005) encompassing 540 ICSI, 437 IVF, and 538 NC children suggested a significantly higher risk of childhood illness, surgery, requiring medical care, and being admitted to hospital in ART-conceived children. Ludwig *et al*. (2009) found that, although the physical health of ICSI-conceived children was comparable with the health of NC children at age 5.5 years, there was an increase in urogenital surgeries in ICSI-conceived boys because of a significantly increased risk of undescended testicles.

Moreover, healthy children conceived via ART seem to have an elevated risk of suffering from cardiovascular diseases in the future (Ceelen *et al.*, 2008a; Wikstrand *et al.*, 2008; Scott *et al.*, 2010; Scherrer *et al.*, 2012). Ceelen *et al*. (2008a) found the ART-conceived children 8–18-year-old had higher blood pressure and fasting glucose levels compared with age- and gender-matched controls. Scott *et al*. (2010) observed altered glucose parameters in young adult mouse offspring conceived by ART. Furthermore, a recent study (Scherrer *et al.*, 2012) of systemic and pulmonary vascular function in 65 healthy ART-conceived children and 57 NC controls highlighted that ART-conceived children were apparently normal but may have had generalized vascular dysfunction. They found that flow-mediated dilation of the brachial artery was 25% smaller ((6.7±1.6)% versus (8.6±1.7)%), carotid-femoral pulse-wave velocity was significantly faster, carotid intima-media thickness was significantly greater, and the systolic pulmonary artery pressure at high altitude (3450 m) was 30% higher in ART-conceived children than in controls. In addition, Wikstrand *et al*. (2008) assessed the central retinal vessels of 5-year-old ICSI-conceived children and found abnormal retinal vascularization, especially in ICSI-conceived boys. All these study support that ART-conceived children have a different cardiometabolic condition, and emphasize the importance of long-term follow-up of children conceived through ART.

3.3 Neurological and neurodevelopmental outcomes

Neurological sequelae such as cerebral palsy (CP) are more frequently seen in ART-conceived children compared with NC children (Strömberg *et al*., 2002; Hvidtjørn *et al*., 2006; 2009; 2010; Klemetti *et al*., 2006; Romundstad *et al*., 2008; Källén A.J. *et al*., 2010; Zhu *et al*., 2010; Saunders *et al*., 2011). Although multiple pregnancies and premature delivery after multi-embryo transfer have been attributed as causes (Hvidtjørn *et al*., 2010; Källén A.J. *et al*., 2010; Saunders *et al*., 2011), an increased risk of CP in singletons born after ART has also been reported. A population-based retrospective cohort study (Strömberg *et al*., 2002) comprising 5680 IVF children and 11360 matched controls found that IVF singletons had an increased risk of CP at 2.8 (valued as OR, 95% CI 1.3–5.8). Hvidtjørn *et al.* (2009) performed a meta-analysis that included nine CP studies of 19462 ART-conceived children and found that children born after IVF had an increased risk of CP (OR 2.18, 95% CI 1.71–2.77), with a tendency toward an increased risk for CP in IVF singletons compared with non-IVF singletons.

However, the overwhelming majority of the studies on the neurodevelopment of children born at full term after ART consistently show that these children are in a comparable condition to NC children (Källén *et al.*, 2005; Ponjaert-Kristoffersen *et al.*, 2005; Leunens *et al.*, 2006; 2008; Wagenaar *et al.*, 2008; 2009b; Hvidtjørn *et al.*, 2009; Carson *et al.*, 2010; Mains *et al.*, 2010; Tsai *et al.*, 2011). The follow-up studies have all focused on kids and adolescents through 18 years of age because of the short duration of ART's history. Although the available

evidence provides encouraging results, further and long-term follow-up studies are necessary.

3.4 Psychosocial development

Wagenaar *et al*. (2009a) accessed the behavior and socioemotional functioning of 9–18-year-old (mean age 13.6 years) ART-conceived children through parent and teacher assessments. There were trends towards fewer externalizing behaviors and increasingly withdrawn behaviors or depressive symptoms in the ART-conceived group, as reported by both the parents and teachers. However, no significant differences were found reported by the children themselves (Wagenaar *et al*., 2011).

Studies on ICSI-conceived children also show reassuring results. Leunes *et al*. (2006; 2008) followed 8–10-year-old children for cognitive and motor development and found similar results compared with NC children. Using the Infants-Junior Middle School Students' Social-Life Abilities Scale, the social adjustments of 86 ICSI- and 165 IVF-conceived children of 4–6 years of age were recently compared in China (Xing *et al*., 2011). No significant differences were found between the ICSI and IVF groups for communication, self-dependence, locomotion, work skills, socialization, or self-management. A publication by Knoester *et al*. (2007) also showed that child behaviors are comparable after ICSI, IVF, and natural conception. However, the prevalence of autism/autism spectrum disorders (ASDs) seemed higher after ICSI (Knoester *et al*., 2007).

ASDs are a group of neurobehavioral disorders that are defined by social and communication deficits and repetitive and stereotyped behaviors. Studies about the relationship of ART and ASD have shown inconsistent results (Hvidtjørn *et al.*, 2009), as do the most recent two large investigations. A population-based follow-up study in Denmark (Hvidtjørn *et al.*, 2011) assessed the risk of ASD in ART-conceived children born between January 1995 and December 2003 and found no significant increased risk. Their follow-up time was 4–13 years (median 9 years), 0.68% (225/33 139) of children born after ART and 0.61% (3 394/555 828) of NC children had a diagnosis of ASD. After adjusting for maternal age, educational level, parity, smoking, birth weight, and multiplicity, the adjusted hazard rate ratio (HRR) was 1.13 (95% CI 0.97–1.31). However, another large study from Israel (Zachor and Ben Itzchak, 2011) showed the opposite result. Five hundred and seven children diagnosed with ASD were assessed, and the rate of ART in children with ASD was significantly higher (10.7%) than that in a large Israeli population (3.06%). Based on the inconsistency between the studies available, a well-designed prospective study that assesses the rate of ASD in a large cohort of newborns conceived after ART is very important.

3.5 Risk for cancer

Several studies have revealed the risk of epigenetic disorders in ART-conceived children (Laprise, 2009) and studies have demonstrated the relationship between epigenetic disorders and cancer (Choo, 2011). Whether ART will increase the risk of cancer in the offspring is another current issue. However, due to the rarity of cancer in children, assessing the risk of cancer in ART-conceived children is not easy. Hence, just a handful of studies exist on this topic, and these studies display divergent results.

A Dutch study reported an increased risk of retinoblastoma in children conceived by IVF between 1995 and 2002 (RR 4.9, 95% CI 1.6–11.3) (Moll *et al.*, 2003), but the expanded study between 2002 and 2007 showed no significantly elevated risk (RR 1.29, 95% CI 0.16–4.66) (Marees *et al.*, 2009). In addition, a nation-wide study from France revealed that retinoblastoma is not associated with ART, but is associated with the infertility situation (Foix-L′Hélias *et al.*, 2012). In a case-cohort study by McLaughlin *et al*. (2006), a 9-fold increase in risk of hepatoblastoma (HB) was observed for those patients with reported or inferred parental infertility treatment. Another case-control study (Puumala *et al.*, 2012a) showed no significant association for any of the measures of parental infertility, or its treatment after adjusting for birth weight and other potential confounders. Recently, a report from nation-wide hospital-based case-control studies in Greece and Sweden has been published. The results suggest that IVF is associated with increased risk of early onset acute lymphoblastic leukemia (ALL) in the offspring. Increased risk of leukemia was observed with 3.8 years earlier in IVF offspring than in the control (OR 2.21, 95% CI 1.27–3.85) (Petridou *et al.*, 2012). Meanwhile, a moderately increased risk of cancer in IVF children (RR 1.42, 95% CI 1.09–1.87) has also been observed

by Källén B. *et al.* (2010a) through a follow-up study of 26 692 children who were born after ART between 1982 and 2005.

In brief, the evidence of cancer risk in children after ART is still very limited. Further studies and long-term follow-up are necessary to determine whether ART had an impact on cancer occurrence.

4 Epigenetic abnormalities

Epigenetics refers to stably heritable phenotypes "resulting from changes in a chromosome without alterations in the DNA sequence" (Wu and Morris, 2001). Two important regulators of imprinted gene expression are DNA methylation and histone modification. Genomic imprinting is an epigenetic mechanism that regulates DNA methylation, resulting in expression of either the maternal or paternal allele (Koerner and Barlow, 2010). Most imprinted genes are involved in fetal growth and development, while others control behaviors. Imprinting disruption can cause various developmental defects and diseases (Tomizawa and Sasaki, 2012).

Since 2002, several reports have raised concerns that children conceived by ART are at an increased risk of having imprinting disorders, especially some rare and severe imprinting-related diseases, such as Beckwith-Wiedemann syndrome (BWS), Angelman syndrome (AS), and retinoblastoma (Laprise, 2009). There is biological plausibility for such a concern because of the synchrony between ART procedures and crucial imprinting events. Moreover, in animal models, ART affects gene imprinting (Doherty *et al.*, 2000; Khosla *et al.*, 2001; Zaitseva *et al.*, 2007; Li *et al.*, 2011b; Wang N. *et al.*, 2012; Wang L.Y., 2013), particularly for large offspring syndrome (LOF) in sheep and cattle, which is reminiscent of BWS in humans (Young *et al.*, 1998). However, some studies have suggested that the subfertile condition of the parents may also be responsible for imprinting disorders (Horsthemke and Ludwig, 2005; Ludwig *et al.*, 2005). However, other studies show no correlation between ART and genomic imprinting disorders, including BWS and AS (Bowdin *et al.*, 2007), Prader-Willi syndrome (PWS) (Sutcliffe *et al.*, 2006), and retinoblastoma (Lidegaard *et al.*, 2005). The precise risk of imprinting disorders, such as BWS and AS, caused by ART is difficult to estimate because of the rarity of the conditions. Therefore, further and unremitting investigations are needed.

Some epigenetic modifications appear to be dynamic throughout life and could contribute to aging and multifactorial adult-onset diseases (Feinberg, 2007; Petronis, 2010). Thus, ART may result in subtle abnormalities in phenotypically normal children that could present later in life. Katari *et al*. (2009) evaluated more than 700 genes in cord blood and placental samples and found a modest change in the methylation level of CpG sites from ART-conceived children. Furthermore, a fraction of the differences were associated with altered gene transcription, and several of these genes have been implicated in chronic metabolic disorders, such as obesity and type II diabetes. Gomes *et al*. (2009) assessed qualitative and quantitative methylation at KvDMR1 using peripheral blood or umbilical cord blood and placentae of 18 children conceived by IVF or ICSI, and found hypomethylation at KvDMR1 in 3 of 18 clinically normal children conceived by ART and a discordant methylation pattern in the 3 corresponding dizygotic twins. Turan *et al*. (2010) compared two populations of children conceived either in vitro or in vivo, and found aberrant methylation of the differentially methylated region (DMR) of the maternal imprinting control regions (ICR) at the IGF2/H19 locus in samples from in vitro conceptions, although evaluation of the mRNA transcripts did not correlate with these aberrations.

Nevertheless, several recent studies (Tierling *et al*., 2010; Li *et al*., 2011a; 2011c; Puumala *et al*., 2012b; Rancourt *et al*., 2012) have shown no significant epigenetic differences between ART-conceived and NC offspring. Using bisulfite-based technologies, Tierling *et al*. (2010) analyzed 10 DMRs of maternal peripheral blood, umbilical cord blood, and amnion/chorion tissue of 185 phenotypically normal children. The results did not reveal any significant differences at nine DMRs among the conception groups in either maternal peripheral blood, umbilical cord blood, or amnion/chorion tissue. In addition, Li *et al*. (2011a) evaluated 29 pairs of IVF-conceived twins and 30 pairs of NC twins and examined two maternally methylated regions (KvDMR1 and PEG1) and one paternally methylated region (H19/IGF2 DMR). No significant increase in imprint variability at these

DMRs was found, except for slightly more variable levels of methylation in IVF cases than in spontaneous cases.

Altogether, ART is likely to cause some epigenetic changes in the offspring, which might be the molecular basis of complex traits and diseases (Gomes *et al*., 2009). However, it is still unclear whether the small differences observed in several studies represent a real difference between ART-conceived and spontaneously conceived children. Thus, larger studies with long-term follow-up are needed to fully answer these questions.

5 Conclusions

In conclusion, most children conceived by ART are healthy. The main risks for these children are poorer perinatal outcome, birth defects, and epigenetic disorders. However, whether ART procedures or subfertility itself had led to these changes is still unresolved. Currently, the first IVF-conceived people are now more than 30 years old, and some of them have conceived children. A mouse model study (de Waal *et al*., 2012) showed that although ART can influence the epigenetic outcome of its offspring, there are no lifelong or transgenerational effects. However, a mouse study may not allow for meaningful conclusions to be drawn in the human case. Thus, the health situation for next generation of ART-conceived children is an important question. In brief, there are still a number of unanswered questions, and further, well-designed studies on the topics described above are urgently needed.

Compliance with ethics guidelines

Yue-hong LU, Ning WANG, and Fan JIN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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