The Consequences of Assisted Reproduction Technologies on the Offspring Health Throughout Life: A Placental Contribution

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The use of assisted reproductive technologies (ART) worldwide has led to the conception and birth of over eight million babies since being implemented in 1978. ART use is currently on the rise, given growing infertility and the increase in conception age among men and women in industrialized countries. Though obstetric and perinatal outcomes have improved over the years, pregnancies achieved by ART still bear increased risks for the mother and the unborn child. Moreover, given that the first generation of ART offspring is now only reaching their forties, the long-term effects of ART are currently unknown. This is important, as there is a wealth of data showing that life-long health can be predetermined by poor conditions during intrauterine development, including irregularities in the structure and functioning of the placenta. In the current review, we aim to summarize the latest available findings examining the effects of ART on the cardiometabolic, cognitive/neurodevelopmental, and behavioral outcomes in the perinatal period, childhood and adolescence/adulthood; and to examine placental intrinsic factors that may contribute to the developmental outcomes of ART offspring. Altogether, the latest knowledge about life outcomes beyond adolescence for those conceived by ART appears to suggest a better long-term outcome than previously predicted. There are also changes in placenta structure and functional capacity with ART. However, more work in this area is critically required, since the potential consequences of ART may still emerge as the offspring gets older. In addition, knowledge of the placenta may help to foresee and mitigate any adverse outcomes in the offspring.

Keywords: assisted reproductive technologies, placenta, epigenetics, metabolism, long-term health, DOHaD, fetal programming

INTRODUCTION

Assisted reproductive technologies (ART) are increasingly used worldwide, to help couples conceive a child, most notably in Europe, where the largest number of ART treatments are performed (De Geyter, 2018; De Geyter et al., 2020). It is estimated that between 1978 - when the first in vitro fertilized child was born - and 2018, over eight million babies were born following ART worldwide (De Geyter, 2018; Sunderam et al., 2019). Though obstetric and perinatal outcomes have improved over the years through the advent of single embryo transfer (Hoyos and Ory, 2021), pregnancies
achieved by ART still appear to bear increased risks for the mother and the unborn child (reviewed in Qin et al., 2015, 2016). Whether the additional maternal and neonatal risks are due to the technology itself or underlying infertility-linked factors remains unresolved (Hoyos and Ory, 2021). When the obstetric and perinatal outcomes of ART and spontaneously conceived (SC) pregnancies were compared using the same mother as control, the results were similar (Ganer Herman et al., 2021), suggesting that health outcomes may be predominantly linked to the parents and not necessarily to ART (Hwang et al., 2018; Molinaro, 2021).

The phenomenon of an adverse in utero environment leading to the development of diseases later in life is well-known and referred to as the "developmental origin of health and disease" hypothesis. This states that the fetus undergoes adaptive changes to maintain homeostasis and to prepare the body for postnatal life. These adaptations depend on numerous factors, such as the type, length, and timing of the insult. Depending on their specific developmental windows, some organs may be programmed differently than others (Barker, 2012). To date, many conditions are believed to lead to fetal programming, including maternal under/overnutrition, smoking, physical inactivity, and psychological stress (Barker et al., 1995; Fleming et al., 2018; Lahti-Pulkkinen et al., 2018), preterm birth (Pandey et al., 2012; Qin et al., 2015; Ombelet et al., 2016; Qin et al., 2016; Hwang et al., 2018; Chang et al., 2020; Cochrane et al., 2020), gestational diabetes mellitus (GDM) (Chaveeva et al., 2011; Pandey et al., 2012; Qin et al., 2015, 2016; Almasi-Hashemi et al., 2019; Petersen et al., 2020), preeclampsia (Chaveeva et al., 2011; Pandey et al., 2012; Qin et al., 2015, 2016; Almasi-Hashemi et al., 2019; Petersen et al., 2020), and infections (Fleming et al., 2018; Hwang et al., 2018; Lahti-Pulkkinen et al., 2018) (Figure 1). Gestational insults lead to a 2–10-fold increase in the susceptibility to cardiovascular disease (CVD), type-2 diabetes mellitus (T2DM), obesity, cognitive dysfunction, and developmental disorders (e.g., autism, Asperger’s, and Rett’s syndromes) (Godfrey and Barker, 2000; Jirtle and Skinner, 2007; Van Den Berg, 2011; Reynolds and Caton, 2012).

In the current review, we aim to summarize the available knowledge on the effects of ART on offspring cardiometabolic, cognitive/neurodevelopmental, and behavioral outcomes, and to examine placental morphological, functional and epigenetic
factors that may contribute to the developmental outcomes of ART offspring in humans. ART includes procedures like in vitro fertilization (IVF), intra-cytoplasmatic sperm injection (ICSI), surgical sperm retrieval, ovarian hyperstimulation, embryo culture, and embryo freezing. In the current review, we will focus on data reporting the effects of IVF and ICSI, as these are the most used.

**PERINATAL OUTCOME IN NEWBORNS CONCEIVED BY ART**

Many studies have investigated pregnancy outcomes, as well as short- (i.e. perinatal) and long-term (early life, adolescence, and young adulthood) outcomes in people born after ART procedures. Although most children born through ART are healthy, conception by ART has been linked to a variety of health complications and conflicting reports exist (See Figures 1, 2). For example, an increased risk for adverse perinatal outcomes including low birth weight (LBW) and small for gestational age (SGA) was shown when compared to spontaneous conception (SC) pregnancies (Chaveeva et al., 2011; Pandey et al., 2012; Ombelet et al., 2016; Luke, 2020). In contrast, a recent report found no differences and even reduced risk of SGA in ART offspring (Glatthorn et al., 2021). Findings pertaining birth weight were usually analyzed including the maternal weight/height as a covariate. Congenital anomalies also appear to be more prevalent in ART offspring compared to SC pregnancies (Hansen et al., 2012; Donzelli et al., 2015; Qin et al., 2016; Hwang et al., 2018; Valenzuela-Alcaraz et al., 2019; Chang et al., 2020), most notably for the cardiovascular (Valenzuela-Alcaraz et al., 2013, 2018, 2019), gastrointestinal and central nervous systems (Qin et al., 2015; Chang et al., 2020). Importantly, further studies in this area are needed to fully understand the long-term effects of ART on offspring health.
reporting on birth defects showed these were linked to underlying infertility and not necessarily to ART, since children born to subfertile parents show similar health outcomes compared to ART conceived offspring (Davies et al., 2012; Levi Setti et al., 2016; Hwang et al., 2018). Finally, it has been suggested that ART may lead to an increased risk of cancer in the offspring, specifically for hematological malignancies, leukemia, neural and hepatic tumors (Hargreave et al., 2013; Wang et al., 2019). However, further studies showed no increased risk of cancer (Raimondi et al., 2005; Levi-Setti and Patrizio, 2018).

**METABOLIC AND CARDIOVASCULAR HEALTH OF CHILDREN CONCEIVED BY ART**

The cardiovascular changes observed among ART-conceived children starting at the age of 3 years include dilated atria, more globular ventricles, endothelial dysfunction, signs of systolic and diastolic dysfunction, and systemic and pulmonary hypertension (Säkka et al., 2010; Zhou et al., 2014; Liu et al., 2015; von Arx et al., 2015; Guo et al., 2017; Valenzuela-Alcaraz et al., 2019; Zandstra et al., 2020). At the age of 6–10 years, ART offspring also presented a higher risk for metabolic dysfunction, with elevated fasting glucose, insulin, and insulin resistance compared to SC children (Chen et al., 2014; Pontesilli et al., 2015; Guo et al., 2017; Cui et al., 2020). They also presented elevated body fat and skinfolds (Ceelen et al., 2007; Hart and Norman, 2013). In contrast, Scherrer et al reported no differences in lipid profile, basal glucose, glucose and insulin tolerance, blood pressure, or body mass index (BMI) in ART children compared to SC controls. However, they found general and pulmonary vascular dysfunction in a cohort of 11-year-old children born through ART (Scherrer et al., 2012). In addition, despite presenting similar gross body size, the growth patterns of ART children appear strikingly similar to those of SC children who develop T2DM and CVD later in life (Barker et al., 2005; Magnus et al., 2021; Roseboom and Eriksson, 2021). These adverse outcomes persist when looking at singleton ART pregnancies only and thus, cannot be explained by multiple pregnancies after the use of ART (Pandey et al., 2012; Qin et al., 2015; Ombelet et al., 2016; Qin et al., 2016) (Figure 2).

**COGNITIVE AND NEURODEVELOPMENTAL HEALTH OF NEWBORNS AND CHILDREN CONCEIVED BY ART**

ART children show similar neurological and psychomotor developmental faculties up to 3 years of age even when born preterm (Roychoudhury et al., 2021) compared with SC children (Nekkebroeck et al., 2008; Carson et al., 2010; Sánchez-Soler et al., 2020). Moreover, singletons and twins of both sexes conceived by ART perform as well as SC children once they reach school (Wagenaar et al., 2008; Punamäki et al., 2016; Normman et al., 2018; Luke et al., 2020). Cognitive function, visual-motor ability, attention, and verbal skills of ART children were similar to SC children (Farhi et al., 2021a). Some reports suggested that ART does exert some negative influences on cognitive development. One study found a 4-fold higher risk of suspected developmental delay and cerebral palsy in IVF versus SC singletons. However, the effect disappeared when only twins were taken into account (Strömberg et al., 2002). Another study found a significant increase in the risk of mental retardation in ART children during their first year of life, but this association disappeared when the analysis was restricted to singletons (Sandin et al., 2013). These findings and high-quality research about whether the use of ART is associated with the diagnosis of autism spectrum disorders (ASD) in the offspring. While most studies were unable to find any associations (Sandin et al., 2013; Lung et al., 2018; Jenabi et al., 2020; Farhi et al., 2021b), a few studies reported a significantly higher risk for ASD in ART offspring (Fountain et al., 2015; reviewed in Liu et al., 2017). However, the effect was substantially reduced when adverse prenatal and perinatal outcomes and demographics were taken into account (Fountain et al., 2015). Remarkably, a further study reported that ART children had a considerably lower risk of developing infantile autism compared to SC children (Maimburg and Vaeth, 2007).

**EFFECTS OF ART ON LONG-TERM HEALTH (ADOLESCENTS AND YOUNG ADULTS)**

Given that the first generation of ART children is now only reaching their early forties, there are, to date, no studies investigating health outcomes in adults of older age, i.e., when developmental programming would be expected to manifest more robustly. However, a few recent studies reporting on the health of adolescents and young adults suggest the long-term health outcomes of ART may be less deleterious than anticipated. Accordingly, the early-life abnormalities in blood pressure, reported in ART children, disappeared by the time they reached adolescence. Fourteen-year-old boys and girls conceived by ICSI had comparable resting systolic and diastolic blood pressure as SC controls (Belva et al., 2007; 2012b). A further comprehensive investigation of metabolic syndrome in a cohort of 18–22-year-olds conceived through ART found similar outcomes to SC individuals for both sexes. Only high-density lipoprotein cholesterol concentrations were lower in ART men (Belva et al., 2018). Another recent study investigated various cardiovascular and metabolic outcomes in an ART cohort including 22–35-year-old men and women (Juonala et al., 2020) and reported no evidence of an altered risk factors, including markers of subclinical atherosclerosis. An investigation including 18–28-year-old adults from ART pregnancies self-reporting on their perceived current quality of life, BMI, pubertal development, and educational achievement found no differences when compared to SC adults (Halliday et al., 2014). Finally, Chen et al reported normal metabolic parameters in 20–21-year-old ART offspring. However, when exposed to a 3-day overfeeding protocol, ART offspring showed
increased systolic blood pressure and reduced peripheral glucose sensitivity, suggesting ART offspring may be at increased risk to develop metabolic diseases when challenged (Chen et al., 2014). In addition, increased peripheral adiposity (Ceelen et al., 2007) and higher systolic and diastolic blood pressure levels were reported in 8–18-year-old IFV offspring (Ceelen et al., 2008a).

Male adolescents conceived by ART showed normal endocrine gonadal function at puberty, but their sperm concentration and quality were significantly lower (Ceelen et al., 2008b; Belva et al., 2019). These findings suggest a possible impact of ART on multigenerational outcomes, which should be further investigated. Pubertal stage and age at menarche were similar in IVF and SC female adolescents, but IVF females presented elevated dehydroepiandrosterone and luteinizing hormone levels (Ceelen et al., 2008b) particularly if they were also SGA (Sakka et al., 2010), indicating impaired hypothalamic-pituitary-gonadal axis.

Finally, the risk of developing psychiatric disorders was studied in a Finish cohort, comparing ART and SC offspring from childhood until young adulthood (Rissanen et al., 2020). This study showed a modest increase in the likelihood of a general psychiatric diagnosis. In addition, ART children received their diagnoses on average 2 years earlier than SC children, probably because ART-treated individuals/couples might be more likely to seek medical help for their children. Of note, the reported effect disappeared with time, and ART young adults ended up displaying a lower cumulative incidence of psychiatric diagnoses than SC offspring regardless of sex (Rissanen et al., 2020).

In conclusion, while the knowledge about health outcomes beyond adolescence for those conceived by ART is still scarce, most of the findings are encouraging and suggest that the abnormalities reported in ART babies and children seem transitory, and are no longer observed when they become older (Halliday et al., 2014; Rissanen et al., 2020; Magnus et al., 2021) (Figure 2).

INTRAUTERINE MECHANISMS–PLACENTAL PROGRAMMING IN ART

Placental Morphology, Gene Expression and Function

In the case of ART, there are limited data available regarding morphological changes in the human placenta (Figure 1). Some studies reported increased placental weight along with reduced birthweight and, thus, an increased placentation weight to birthweight ratio in ART compared to SC pregnancies (Daniel et al., 1999; Haavaldsen et al., 2012; Eskild et al., 2013). Increased placental thickness and elevated rate of abnormal cord insertion were also reported after ART (Daniel et al., 1999; Cochrane et al., 2020). In contrast, other groups were unable to detect any placental differences when comparing IVF and SC newborns (Yanaihara et al., 2018). Of note, many studies investigating morphological changes in the placenta after ART were performed using murine models. The outcomes of these studies with regard to the effects of ART on fetal and placental development have been discussed elsewhere (Hemberger et al., 2020).

Beyond morphological changes, abnormalities in gene expression that could reflect alterations in placental function have also been reported in ART. Using a selective twin-to-singleton fetal reduction strategy and collection of first-trimester placentas in vivo, Zhao et al. showed that 1910 and 1,495 genes were up- and down-regulated, respectively in the placenta by IVF (Zhao et al., 2019). This included alterations in genes involved in biological pathways, like the immune response, transmembrane signaling, carbon, fatty acid and amino acid metabolism, cell cycle, stress control, invasion, and vascularization (Zhao et al., 2019). A second study investigated first-trimester samples from chorionic villus sampling and included a non-IVF fertility treatment group in addition to the IVF and SC groups. Herein the authors reported modest differences in the transcriptome and suggested that underlying infertility, in addition to treatment-related factors, might be key contributors to the observed gene expression abnormalities in the IVF group (Lee et al., 2019). Similarly, a recent study that compared first-trimester maternal plasma metabolomic profiles in women undergoing IVF, non-IVF fertility treatment, and SC (Sun et al., 2019) found elevated circulating levels of several lipid and lipid-related components (e.g., steroid metabolites, and lipids with docosahexaenoic acyl chains, and acylcholines) in the infertile groups of women, especially when IVF was performed. Such changes may have consequences for placental lipid transfer and steroid hormone production, and may contribute to the adverse fetal outcomes associated with ART and infertility (Sun et al., 2019).

At term, IVF placentas show altered global gene expression, leading to an over-representation of certain biological pathways as observed in the first-trimester samples, such as immune response, transmembrane transport, cell cycle control, stress control, invasion, vascularization, and amino acid and cholesterol metabolism (Zhang et al., 2010). Several of the genes whose expression differed the most between IVF and the control groups have been implicated in chronic metabolic disorders like obesity and T2DM, supporting the theory that ART induces fetal programming of metabolic diseases and may do so via alterations in the placenta (Katarì et al., 2009). In contrast, another study reported modest effects in the expression of 108 imprinted genes in the placenta (Litzyk et al., 2017) and suggested that differences in gene expression are more likely associated with infertility rather than the IVF procedure itself.

Placental Epigenetics

ART procedures occur during a developmental time that is critical for epigenetic reprogramming. Hence, perturbations due to technical manipulations during this sensitive time may lead to changes in the epigenetic profile of the conceptus (Monk et al., 2019). This is relevant for the finely-tuned expression of imprinted genes, of which about 100 have been identified in humans. These genes are stimulated by fetal signals and have an impact on transplacental nutrient allocation, placental growth, and vascularization, directly affecting fetal growth and long-term health (Reik et al., 2003; Morison et al., 2005). They are a subset of epigenetically-regulated genes that are selectively expressed from the maternal or paternal allele (Thamban et al., 2020). Imprinted genes are, by definition, functionally haploid and are thereby potentially more susceptible to mutations (Fowden et al., 2011). Epigenetic modifications might occur either at the DNA level via...
methylation/hydroxymethylation, at the protein level via histone modifications, or at the mRNA level via short and long non-coding RNAs (Ghai and Kader, 2021). Imprinted genes are abundantly expressed in fetal and placental tissues, and DNA methylation of imprinted genes is established in a parent-specific manner during gametogenesis. Several studies highlighted an altered epigenetic status in gametocytes from infertile couples, raising the possibility of a heightened risk of imprinting defects and somatic epigenetic changes in ART-conceived children (Van Montfoort et al., 2012; Lazaraviciute et al., 2014; Choux et al., 2015, 2018; Cortessis et al., 2018). Specifically, the DNA methylation level of several imprinted genes was altered in ART compared to SC placentas (Van Montfoort et al., 2012; Choux et al., 2015, 2018). Furthermore, these differences were associated with gene expression differences at both imprinted and non-imprinted genes (Katari et al., 2009). Thus, aberrant methylation of imprinted genes may be an indicator of more global epigenetic instability (Denomme and Mann, 2012). Specifically, H19/IGF2, LINE-1Hs, ERFVDR-1, and KCNQ1OT1 are affected in ART through changes in placental DNA methylation (Turun et al., 2010; Nelissen et al., 2013; Choux et al., 2018; Dong et al., 2019). Expression of H19 is linked with fetal and placental growth suppression (Gao et al., 2012) and was significantly higher (Turun et al., 2010; Nelissen et al., 2013, 2014; Sakian et al., 2015; Chi et al., 2020), while IGF2 expression, which increases fetal and placental growth (Sakian et al., 2015; Chi et al., 2020) was significantly lower in ART compared with SC placentas (Nelissen et al., 2014; Sakian et al., 2015; Chi et al., 2020). However, changes in DNA methylation do not always correlate with alterations in transcriptional levels (Rancourt et al., 2012). A further study reported no significant differences in gene expression despite methylation changes between placentas from ART and SC pregnancies (Litzky et al., 2017). Finally, a recent longitudinal study that assessed genome-wide changes in DNA methylation in blood collected from newborns and adults conceived by ART showed that variations observed at birth largely resolved by the time offspring reached adulthood and found no evidence of any impact on development and general health (Novakovic et al., 2019).

An additional factor to consider when assessing impacts of ART is the medium used during embryo culture, since using media that lacks essential amino acids may affect placental DNA methylation and can cause aberrant imprinting in the embryo (Menezo et al., 2010; Eskild et al., 2013). Moreover, the available evidence further indicates that subfertility itself is a risk factor for imprinting diseases and that methylation errors are already present in sperm and oocytes. Thus, the unequivocal proof of a causal relationship between imprinting diseases and IVF or ICSI treatments is still lacking (Vermeiden and Bernardus, 2013) (Figure 1).

CONCLUSION

In the current review, we aimed to examine the latest available findings examining the effects of ART on behavioral and health related outcomes in the offspring throughout the lifespan, including the potential contribution of the placenta. It is hypothesized that ART may affect the development of gametes and embryo, and epigenetic adaptations aiming to protect the fetus may exacerbate vulnerability to diseases in the offspring. In fact, a combination of genetics, the intergenerational and the current environments in addition to the ART procedure are all involved in disease causation (Hochberg et al., 2011). The long-term effects remain to be seen once the first-generation of ART offspring reaches an older age (i.e. > 65), a time-point where fetal programming effects may still emerge.

Depending on later life outcomes, the need to identify those at risk from an early stage will be imperative to treat and prevent their development throughout the lifespan in individuals conceived by ART. Though it is difficult to establish the mechanisms underlying the changes observed among ART newborns and children, it is plausible that the placenta could play a key role in the process. Since placental size and shape are indicative of its efficiency and function, and the imprinted genes in the placenta appear to regulate nutrient allocation, the observed changes could cause potential epigenetic adaptations in the fetus that may further exacerbate disease susceptibility.

In addition, each step utilized in ART (i.e. ovarian stimulation, in vitro culture, culture media, cryopreservation technique) could represent a risk for the pregnancy (Palomba et al., 2016) and in turn, the placental response to environmental stress can further define the outcome for the offspring (Litzky and Marsit, 2019). E.g., the use of fresh versus thawed embryos in IVF can affect weight, height, and circulating growth factor and lipid profiles in the resulting children (Green et al., 2013). In addition, evolving laboratory procedures used for ART, and sometimes inappropriate choice of control groups, make comparisons between studies difficult. Since ART is predominantly used on infertile couples, distinguishing between the effects of ART procedures and those of underlying infertility is challenging. Further disregarded aspects that should be considered when assessing the long-term impacts of ART include, e.g. differences in lifestyle and the high anxiety and stress levels experienced by couples that are unable to conceive naturally (Litzky and Marsit, 2019).

Altogether, our work highlights the need for further study into the role of potential confounding factors when assessing the short- and long-term effects of ART for the offspring, and whether these effects could be passed to the next generation. While there is a need for additional studies to investigate the effects of ART on the offspring when they are >65 years, based on the currently available literature, ART offspring until around 40 years of age do not appear to be at greater risk of developing persistent life-long health complications.

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