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REVIEW

Long-term health risk of offspring born from assisted reproductive technologies

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Abstract

Since the world's first in vitro fertilization baby was born in 1978, there have been more than 8 million children conceived through assisted reproductive technologies (ART) worldwide, and a significant proportion of them have reached puberty or young adulthood. Many studies have found that ART increases the risk of adverse perinatal outcomes, including preterm birth, low birth weight, small size for gestational age, perinatal mortality, and congenital anomalies. However, data regarding the long-term outcomes of ART offspring are limited. According to the developmental origins of health and disease theory, adverse environments during early life stages may induce adaptive changes and subsequently result in an increased risk of diseases in later life. Increasing evidence also suggests that ART offspring are predisposed to an increased risk of non-communicable diseases, such as malignancies, asthma, obesity, metabolic syndrome, diabetes, cardiovascular diseases, and neurodevelopmental and psychiatric disorders. In this review, we summarize the risks for long-term health in ART offspring, discuss the underlying mechanisms, including underlying parental infertility, epigenetic alterations, non-physiological hormone levels, and placental dysfunction, and propose potential strategies to optimize the management of ART and health care of parents and children to eliminate the associated risks. Further ongoing follow-up and research are warranted to determine the effects of ART on the long-term health of ART offspring in later life.

Keywords Assisted reproductive technologies · In vitro fertilization · Long-term health risks · Adverse perinatal outcomes · Review

Introduction

Assisted reproductive technologies (ART) are defined as all interventions that include the in vitro handling of human oocytes, or embryos for reproduction, such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), preimplantation genetic testing (PGT), assisted hatching, gamete, and embryo cryopreservation [1]. Worldwide, more than 8 million IVF offspring have been born, and over 2.5 million cycles are performed each year, resulting in over half a million births annually [2]. ART accounts for 3.5% of births in 21 European countries, with even 9.3% in Spain

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[3], and 2.2% of births in the US annually [4]. With the rapid growth of ART in China, at the end of 2019, there were 517 assisted reproductive centers and 27 human sperm banks in mainland China [5]. The number of ART newborns in mainland China was 311,309 in 2016, accounting for 1.69% of the total births [6].

Although there is a significant population of ART offspring worldwide, only a limited number of studies provide robust evidence on the long-term health outcomes of adolescents or young adults born to IVF treatment. Most studies have investigated short-term outcomes, and it has been proven that even singletons born from ART have a higher risk of adverse perinatal outcomes, including preterm birth, low birth weight, perinatal mortality, and congenital anomalies [7–10].

According to the developmental origins of health and disease (DOHaD) theory, adverse environments in early life can induce adaptive changes and influence the development of the placenta and fetus, resulting in an increased risk of diseases in later life, such as metabolic disorders and cardiovascular diseases. In 2010, Motrenko proposed the "embryo-fetal origins of diseases" theory, which suggests that abnormal embryo development can cause diseases in later life [11]. In 2014, Huang et al. [12] developed this hypothesis for gamete and embryo fetal origins of adult diseases based on evidence from ART-associated studies. ART is performed at critical stages of gamete maturation and embryo development. Adaptive responses of gametes or embryos to adverse environmental factors make them susceptible to adverse perinatal outcomes and long-term health risks [12]. Increasing evidence suggests that ART offspring are predisposed to increased risks of non-communicable diseases (NCD), such as malignancies, asthma, obesity, metabolic syndrome, diabetes, cardiovascular diseases, and psychiatric disorders [13]

This review aims to summarize current knowledge on long-term health risks of adolescents or adults born from ART, discuss underlying mechanisms, and explore future strategies to optimize the safety of ART.

Long-term health risks of the offspring

Concerns have been raised and research has been conducted on the long-term health outcomes of ART offspring. Researchers have primarily focused on malignancies, asthma, obesity, diabetes, cardiovascular diseases, thyroid disorders, neurodevelopmental disorders, neurological disorders, psychiatric disorders, and reproductive health. Table 1 shows some recent high-quality studies on this subject. The health condition of these young people would provide valuable evidence for both health providers and families who have children born from ART or plan to undergo ART.

Perinatal outcomes

In spite of the relatively insufficient evidence on the long-term health risks of ART, plenty of studies have reported an increased chance of perinatal complications. From a fetal perspective, preterm birth and intrauterine growth restriction (IUGR) are by far the two most discussed outcomes. A large cohort study from Denmark, which, enrolled a total of 20,080 singletons demonstrated a significantly increased risk of preterm birth caused by ART treatment [32]. Another population-based comparative study from the USA suggests a 2.6 times increase in low birth weight in ART singleton infants compared to naturally conceived ones [33]. In addition to these two articles, data from many other studies have also supported the elevated incidence of preterm birth and IUGR in ART [34–36]. From a maternal perspective, hypertensive disorders, placental dysfunction, and cesarean section are considered to happen more often among ART mothers [37, 38]. A study from Sweden, Denmark, and Norway that included all registered ART births from 1988 to 2007 reported an increased incidence of hypertensive disorders following all ART procedures, including IVF, ICSI, and FET [37]. Evidence supporting the susceptibility to placental dysfunction is abundant, including placental previa, placental metabolic alterations, and abnormal placentation [39, 40]. Besides, due to artificial intervention, multiple pregnancies are more commonly seen in ART versus spontaneous conception. Although the main focus of this article is about the future risks of ART children, short-term risks should also be noted considering the potential association between them. Studies have found that low birth weight infants might suffer from a higher risk of obesity, type 2 diabetes, hypertension, coronary heart disease, and other metabolic syndromes in adulthood [41, 42]. For preterm offspring, studies suggested a statistically elevated incidence of mental retardation, cardiovascular abnormalities, and chronic kidney disease [43–45]. Furthermore, mounting evidence has proved that the undesirable physical conditions of mothers during pregnancy could pose a shadow on the long-term health of offspring by intrauterine programming [41, 46]. The most well-known example of this theory may be the Dutch Famine Birth Cohort, in children born to mothers exposed to famine; they are suffering from a greater chance of diabetes in adulthood [47]. This large-scale phenomenon clearly demonstrated the long-lasting effect of suboptimal intrauterine conditions on the future health of offspring. Therefore, we present the worrying outcomes of short-terms here, given that they are very likely to be the hints for far-reaching impact on ART children.

Table 1 Studies reporting long-term health outcomes of ART offspring

Disorders	References	Research design and study population	Key findings
Malignancies	Spaan M et. al. [14]	Retrospective cohort study, including 24,268 ART offspring; 13,761 SC offspring; and 9660 naturally conceived offspring of subfertile parents, followed for a mean of 21 years	No significant increase in overall cancer risk in ART offspring, neither compared with naturally conceived children from subfertile women (HR: 1.00, 95% CI: 0.72–1.38) nor compared with the general population (SI, 1.11; 95% CI, 0.90–1.36)
	Spector LG et. al. [15]	Retrospective cohort study, including 146,875 IVF singletons and 2,194,854 SC singletons was followed with a mean (SD) follow-up of 4.5 (2.5) years and 4.7 (2.5) years	An increased risk of hepatic tumors in the IVF group (HR, 2.46; 95% CI, 1.29–4.70); other cancers did not differ
	Hargreave M et. al. [16]	Retrospective cohort study, including 1,085,172 children born in Denmark between 1996 and 2012 and followed up to 2015, with a mean follow-up of 11.3 years	FET was associated with an elevated risk of childhood cancer compared with children born to fertile women (HR, 2.43; 95% CI, 1.44–4.1)
Asthma	Wijs LA et. al. [17]	Meta-analysis, including 14 high-quality studies (Newcastle–Ottawa scale ≥ 7)	An increased risk of asthma in ART offspring (RR, 1.28; 95% CI, 1.08–1.51)
	Kallen B et. al. [18]	Registry study of 2,628,728 children born from 1982 to 2007 in Sweden, including 31,918 ART offspring	An increased risk for asthma in IVF offspring (aOR, 1.28; 95% CI, 1.23–1.34)
Obesity	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	An increased risk of obesity in ART offspring (HR, 1.14; 95% CI, 1.06–1.23; $p = 0.001$)
Diabetes			
Type 2 diabetes	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	No significant increase in the risk of type 2 diabetes in ART offspring (HR, 1.31; 95% CI, 0.82–2.09; $p = 0.25$)
Type 1 diabetes	Norrman E et. al. [20]	Retrospective cohort study, including all 3,138,540 children born in Sweden between 1985 and 2015 with a mean (SD) follow-up of 9.7 (6.4) years for ART children and 16.3 (9.2) years for SC children	An association between FET and type 1 diabetes (aHR, 1.52; 95% CI, 1.08–2.14 frozen versus fresh); no association between ART and type 1 diabetes (aHR, 1.07; 95% CI, 0.93–1.23)
Cardiovascular diseases	Norrman E et. al. [19]	Retrospective cohort study, including 122,429 ART offspring and 7,574,685 SC offspring, with a mean (SD) follow-up of 8.6 (6.2) years for ART offspring and 14.0 (8.6) years for SC offspring	No significant increase of cardiovascular disease in ART offspring (aHR, 1.02; 95% CI, 0.86–1.22; $p = 0.80$)
Thyroid disorders	Sakka SD et. al. [21]	Cohort study, including 106 IVF offspring and 68 SC controls, aged 4–14 years	A significant elevation of serum TSH ($p = 0.046$) in IVF offspring
Neurodevelopmental disorders			
ASD	Davidovitch M et. al [22]	National registry study, including 110,093 males in Israel (born, 1999–2008; ASD, 975, 0.9%)	An association between progesterone hormone treatment and increased risk of ASD (RR, 1.51; 95% CI, 1.22–1.86); no increased risk of ASD for IVF overall
ADHD	Wang C et. al. [23]	National registry study, including 2.4 million children born in Sweden 1986–2012	No significant increase in the risk of ADHD (aOR, 1.03; 95% CI, 0.96–1.10)
ID	Sandin S et. al. [24]	Population-based, prospective cohort study, of 2.5 million births between 1982 and 2007, including thirty 959 (1.2%) IVF offspring, with a follow-up mean of 10 (SD, 6) years	No significant increase in risk of ID among ART offspring (RR, 1.01; 95% CI, 0.83–1.24); ICSI is associated with a higher risk of ID compared with IVF (RR, 1.51; 95% CI, 1.10–2.09)

Table 1 (continued)

Disorders	References	Research design and study population	Key findings
Cognitive development	Norrman E et. al. [25]	Nationwide register-based cohort study, including all singleton of ICSI ($n=6953$), IVF ($n=11,713$), or SC ($n=2,022,995$) in Sweden between 1985 and 2006	No significant difference between ICSI and IVF children for total score (aOR, 1.03; 95% a − 0.22 to 2.28; $p=0.11$)
	Barbuscia A et. al [26]	Longitudinal cohort study of 15,218 children (125 IVF and 61 ICSI), with a follow-up for 11 years	At ages 3 and 5 years, ART offspring have higher verbal cognitive abilities than SC offspring ($p<0.001$) but the effect diminishes by age 11
Neurological disorders			
CP	Goldsmith S et. al. [27]	Registry-cohort study, including 211,660 live births	Prevalence of CP was increased in ART offspring (7.2/1000 live births compared with SC births, 2.5/1000)
Epilepsy	Kettner LO et. al. [28]	Nationwide birth cohort study, including all 565,116 pregnancies in Denmark resulting in live-born singletons, 1995–2003	A slightly increased risk of epilepsy (HR, 1.15; 95% CI, 1.00–1.31) in children born after ovulation induction
Psychiatric disorders			
Anxiety and depression	Wang C et. al. [29]	Nationwide prospective cohort study of 1,221,812 children born in Sweden from 1994 to 2006, with a follow-up to a median age of 18 (IQR, 15–21) years, including 31,565 (2.6%) ART offspring	A significantly higher risk of anxiety (aHR, 1.08; 95% CI, 1.03–1.13) and antidepressants use (aHR, 1.05; 95% CI, 1.01–1.09) in ART offspring compared with all other children
Reproductive health			
Infertility	Belva F et. al. [30]	An ICSI offspring cohort study, including 54 ICSI-conceived young adult men and 57 cross-sectionally recruited SC men controls	Increased risks of low sperm concentrations (aOR, 2.7; 95% CI, 1.1–6.7) and low total sperm counts (aOR, 4.3; 95% CI, 1.7–11.3)
Precocious puberty	Belva F et. al. [31]	Longitudinal cohort study involving 217 singletons ICSI offspring and 223 cross-sectionally recruited SC controls	No difference in menarche, pubic hair development, or genital development in males; ICSI-conceived women were less advanced in breast

Malignancies

Early studies from Australia, Holland, Israel, and Britain showed no increase in the risk of cancer [48–51]. Recently, a study from the Netherlands, which included 24,268 ART offspring, 13,761 SC offspring, and 9660 naturally conceived offspring of subfertile parents, followed for a mean of 21 years, did not observe any increase in cancer risks among ART offspring, neither compared with naturally conceived offspring of subfertile parents (HR, 1.00; 95% CI, 0.72–1.38) nor compared with SC offspring (SIR, 1.11; 95% CI, 0.90–1.36) [14]. A population-based cohort study from Nordic countries, including 91,796 ART offspring and 358,419 spontaneously conceived (SC) offspring, followed for a mean of 9.5 (4.8) years, found no significant increase (HR, 1.08; 95% CI, 0.91–1.27) in overall cancer rates [52].

However, some studies have observed an increased risk of cancer in ART offspring, particularly for certain types of cancer. In a recently published large cohort study from the US, 146,875 IVF singletons and 2,194,854 SC singletons were followed for a mean (SD) follow-up of 4.5 (2.5) years and 4.7 (2.5) years, respectively. Although no significant increase in overall cancer was found in ART offspring, there was an increase in the risk of hepatic cancer and embryonal tumors among ART offspring [15]. It should be noted that most studies in this area are limited by their sample size, and most of these studies did not control for factors, such as parental socioeconomic status, perinatal health status, and maternal smoking, which have been reported to affect the incidence of cancer [53–57].

A cohort study undertaken in Sweden, following up 25,582 ART offspring, including all births between 1982 and 2005, found a moderately increased risk (HR, 1.42; 95% CI, 1.09–1. 87) of cancer in IVF offspring. After adjusting for maternal age, years of unwanted childlessness, parity, and maternal smoking, OR remained statistically significant (OR, 1.45; 95% CI, 1.10–1.91). The increased cancer risk, mostly hematologic cancer, eye tumors, and central nervous system tumors, which are the most common pediatric cancers, is associated with premature delivery, high birth weight, respiratory diagnoses, and low Apgar scores in ART offspring [58]. These factors have already been reported to be associated with pediatric cancer in non-IVF studies [57, 59]. Specifically, high birth weight has been associated with acute lymphoid leukemia (ALL) [60, 61], central nervous system tumors [62], and Wilms' tumors [63, 64]. In summary, disadvantageous maternal characteristics and adverse perinatal outcomes are more likely to be responsible for the increased cancer risk.

Mounting evidence suggests that frozen embryo transfer (FET) may contribute to an increased risk of cancer in ART offspring. Hargreave et al. [16] observed an increased risk of cancer (HR, 2.43; 95% CI, 1.44–4.1) among FET offspring, in a cohort study in Denmark, which included 1,085,172 children born between 1996 and 2012 with a mean follow-up of 11.3 years. A meta-analysis including 11 case–control studies and 16 cohort studies reported an increased pediatric cancer incidence in FET offspring (RR, 1.37; 95% CI, 1.04–1.81) compared with SC controls and 1.28 (95% CI, 0.96–1.69) fresh ET controls, while other ART treatments, such as IVF, ICSI, and fertility drugs were not associated with increased pediatric cancer risks [65]. The increased incidence of malignancies may be related to genetic and epigenetic changes during cryopreservation [66, 67]. In recent years, some studies have suggested that the difference in outcomes between FET and fresh ET may be related to the different endometrial hormone preparation cycles. However, none of the above clinical studies have further classified and discussed different methods of endometrial preparation in the FET and fresh ET groups. In addition, it is worth noting that many of the cases included in these cohort studies received ART treatment 10 or even 20 years ago, when slow freezing was the most used method for cryopreservation. Whereas vitrification has become the mainstream method in replace of slow freezing recently [68, 69], the safety of vitrification warrants further research with longer follow-up periods.

In summary, ART appears to be generally safe in terms of cancer risk. However, it is noteworthy that recent studies have reported increased risks of cancer in offspring conceived using FET. With the widespread use of vitrification, further subgroup studies on FET offspring are necessary to determine any association with cancer. Considering that existing studies addressing cancer risk in ART offspring have focused on childhood cancers, and that cancer development is more common in middle-aged and older adults, more long-term follow-up studies are warranted.

Asthma

As mentioned above, ART-conceived singletons have significantly higher odds of preterm birth and low birth weight, which are associated with the incidence of asthma later in life [70, 71]. Thus, a higher prevalence of asthma is expected in ART offspring than in SC offspring. In a recent meta-analysis of 14 high-quality studies, a significantly increased risk (RR, 1.28; 95% CI, 1.08–1.51) of asthma was observed in ART offspring [17]. A registry study of 2,628,728 children born between 1982 and 2007 in Sweden, including 31,918 ART offspring, discovered an increased risk of asthma, albeit small, in children conceived by IVF (OR, 1.28; 95% CI, 1.23–1.34); the absolute risk increased from 4.4 to 5.6%. However, after adjusting for the duration of involuntary childlessness, the effect was eliminated, and after the removal of anti-asthmatic use in early pregnancy, the risk

was reduced [18], suggesting that parental subfertility and maternal asthma are the main risk factors.

Obesity

Recently, whether ART offspring are more likely to develop obesity than SC offspring has been a topic of concern. A recent large population-based cohort study [19] included 122,429 ART offspring and 7,574,685 SC offspring with a mean (SD) follow-up time of 8.6 (6.2) years for ART children and 14.0 (8.6) years for SC children. The study observed a small but significant increase in the risk of obesity among ART offspring (HR, 1.14; 95% CI, 1.06–1.23; $p = 0.001$). In contrast, a prospective study [72] compared maternally reported length/height and weight in children from the second trimester to 7 years of age, and self-reported height and weight at age 17 years when screening for military conscription. It was found that children conceived by ART grew faster after birth and attained greater height and weight at 3 years of age; however, once the ART offspring entered adolescence, their height, weight, and body mass index (BMI) did not differ from those of SC controls, which may be due to a rapid catch-up growth after birth.

In addition to BMI, previous studies have observed increased peripheral adipose tissue in ART offspring [73–75]. Animal studies have shown increased body weight and body fat content in ART offspring compared with SC offspring. Increased volume and number of lipid droplets and lipid droplet fusion were observed in the hepatocytes of ART-conceived mice, and liver TG content was significantly increased in ART mice, which plays an important role in lipid accumulation in adults [76]. Our previous study found that ART-conceived mice had less monounsaturated fatty acids and more polyunsaturated fatty acids in the adipose tissue in both adult and old mice, while alterations in saturated fatty acids (SFAs) were only observed in adult mice [77].

Fat metabolism often exhibits sexual dimorphism. Belva et al. [78] conducted research on individuals between 18 and 22 years of age and found that male ICSI offspring had higher peripheral fat density (recorded by skinfold) than females. Another study showed that male ICSI offspring had lower HDL levels at 18 years of age than naturally conceived males [79]. Sex-specific metabolism was also observed in IVF mouse offspring. Female IVF offspring had higher body weight and cholesterol levels than female SC offspring, whereas male IVF offspring had increased body fat composition and higher levels of triglycerides and insulin [80].

In summary, current evidence suggests that ART offspring may be at an increased risk of developing unfavorable fat composition and deteriorated metabolic profiles, suggesting a potential risk of subsequent cardiovascular and metabolic diseases in later life. Sexual dimorphism on this topic is noteworthy for future studies.

Diabetes

According to the DOHaD theory, ART offspring are expected to be at a higher risk of metabolic diseases, such as diabetes, with age due to the increased adverse perinatal outcome in early life. However, the abovementioned large cohort study of the Nordic population, including 122,429 children born after ART and 7,574,685 children born after SC, suggested that there was no significant difference in the risk of type 2 diabetes between ART and SC offspring (HR, 1.31; 95% CI, 0.82–2.09; $p = 0.25$); however, the mean follow-up time was only 8.6 years in the ART group and 14.0 years in the SC group [19]. Conversely, as for impaired glucose metabolism, a meta-analysis conducted by our team on 19 studies that included 2112 ART offspring and 4096 SC offspring during childhood to early adulthood indicated a higher fasting insulin level in ART offspring [81]. Another study compared 380 ART offspring and 380 SC offspring aged 6–10 years and found that the fasting blood glucose, serum insulin, and HOMA-IR levels of ART offspring were significantly higher than those of their SC counterparts [82]. As type 2 diabetes is more common among older adults, the current findings of impaired glucose metabolism in ART offspring suggest that more prediabetes and diabetes may be observed in future follow-up studies.

The diet of the offspring may play an important role in accelerating the progression of abnormal glucose metabolism in ART offspring. ART-conceived mice were more likely to develop obesity, fasting hyperinsulinemia, and hyperglycemia when challenged with a high-fat diet (HFD), and insulin-stimulated glucose utilization was 20% lower (steady-state glucose infusion rate) than that in SC mice [83].

For type 1 diabetes, Norrman et al. [20] found an association between ART and type 1 diabetes in a cursory analysis (HR, 1.13; 95% CI, 0.98–1.30), but disappeared after adjusting for known confounders (HR, 1.07; 95% CI, 0.93–1.23). In subgroup analyses, an association was found between FET and type 1 diabetes (aHR, 1.52; 95% CI, 1.08–2.14 and 1.41; 95%, CI 1.05–1.89 for frozen versus fresh, respectively). However, a nationwide birth cohort study including 565,116 singleton pregnancies in Denmark found no association between ART and childhood type 1 diabetes; however, in secondary analyses, ovulation induction was associated with an increased risk of type 1 diabetes (HR, 3.22; 95% CI, 1.20–8.64) [84].

To conclude, an association between type 2 diabetes and ART was not observed, but a trend of impaired glucose metabolism in ART children has been suggested. FET and ovulation induction may be associated with type 1 diabetes. Further studies in older ART populations are warranted to clarify the risk of diabetes.

Cardiovascular disease

The cardiovascular condition of ART offspring has become one of the most concerning problems in recent years. A matched control study on 382 children conceived by ART between 6 and 10 years old based on their sex, age, and maternal age reported that the blood pressure of ART offspring was higher than that of SC offspring, together with the structural and functional disorders of the left ventricle, as well as the prevalence of left ventricular hypertrophy, even after adjustment for early life factors, current lifestyle factors, and the type of ART [85]. A study matched by maternal age found that fetuses from both fresh ET and FET showed larger atria, thicker and more spherical ventricles, and evidence of both suboptimal systolic and diastolic function, with more pronounced changes after fresh ET as compared to FET [86]. Similar results were observed in studies with follow-up until the age of 3 years [87]. In a previous retrospective investigation, we observed that the blood pressure of IVF offspring aged 3–13 years was higher than that of SC offspring [88]. In our meta-analysis including 2112 ART offspring and 4096 SC offspring, a slight but significant increase in both SBP (1.88 mmHg; 95% CI, 0.27–3.49) and DBP (1.51 mmHg; 95% CI, 0.33–2.70) were observed in ART offspring [81]. In addition, suboptimal diastolic function and higher vessel thickness were observed in the ART offspring.

In contrast, a population-based cohort study did not find an increased risk of cardiovascular disease in ART offspring after adjustment for factors, such as maternal age and maternal cardiovascular disease [19]. Similar results were observed in children as young as 9 years of age [89] and in 14–17 year-old ICSI-conceived adolescents [90]. For adults, a small study recruited 279 men and women aged 22–35 and observed no increased vascular or cardiac metabolic risk in ART offspring compared with SC offspring from the same source population [91].

Although there is conflicting evidence regarding the risk of suboptimal cardiovascular function, the current data suggests a potential increase in cardiovascular conditions in ART offspring. Future studies should focus on adjusting for confounding factors, such as maternal age and maternal cardiovascular disease. As cardiovascular disorders are more common in older populations, longer follow-up studies are required in this area.

Thyroid disorders

Several small studies have suggested an increased risk of thyroid disorders in ART offspring. A study of 106 IVF offspring and 68 SC offspring, aged 4–14 years, found significantly elevated serum thyroid-stimulating hormone (TSH) compatible with mild TSH resistance of the thyroid in IVF offspring compared with SC controls. Seven IVF offspring, but none in the control group, had persistent elevations in circulating TSH levels, suggesting subclinical primary hypothyroidism or euthyroid hyperthyrotropinemia. Circulating antithyroid antibodies were not detected in either group, indicating that the difference was not due to the presence of antithyroid autoantibodies [21]. In another study, 98 full-term IVF offspring were evaluated by screening for thyroid function. Hyperthyrotropinemia (TSH levels higher than 6.5 mU/L) was diagnosed in 10 IVF offspring at postnatal ages of 2 weeks to 1 month. The control group included 10 randomly selected SC offspring of the same age with hyperthyrotropinemia. A thyrotropin-releasing hormone (TRH) test was performed, and there was an exaggerated TSH response to TRH in all 10 IVF offspring but in none of the controls. Therefore, subclinical hypothyroidism was diagnosed only in the IVF offspring. It is of note that neonatal screening tests in both groups were negative [92].

An increased risk of thyroid dysfunction has been associated with high maternal serum levels of estradiol (E_2) induced by ovarian stimulation. Our previous study found that the mean serum E_2 levels of women undergoing fresh embryo transplantation (ET) were significantly higher than those of women undergoing FET or following natural conception. The levels of thyroxine (T4), free thyroxine (FT4), and TSH were significantly increased in children aged 3 to 10 years conceived through fresh ET compared to SC children, while the levels of T4 and TSH in the FET group did not increase. Furthermore, T4 and FT4 levels in the fresh ET group were positively correlated with maternal E2 levels during the first trimester [93].

In conclusion, IVF may confer susceptibility to subclinical primary hypothyroidism in the offspring, particularly in fresh ET offspring. Therefore, ART offspring should be monitored for thyroid function, even if neonatal screening tests appear normal. Thyroid dysfunction is strongly associated with other metabolic disorders; thus, it is worth noting.

Neurodevelopmental disorders

Autism spectrum disorder (ASD)

Concerns have been raised about whether ART is associated with ASD in offspring, as they share similar risk factors, such as older parental age and infertility [94–96]. Recently, a meta-analysis of 15 studies discovered that ART-born children were at a higher risk of ASD; however, the association was attenuated and did not reach statistical significance, and most studies were restricted to singletons [97]. This is in line with a Danish study including 588,967 children, which found no risk of ASD after adjusting for maternal age, educational level, parity, smoking, birth weight, and multiplicity [98].

Based on the same Danish registry cohort, it was found that children aged 8–17 years born after ovulation induction showed a low but significant increase in the risk of ASD (1.20–1.05% to 1.37–1.5%) [99]. A national registry study of 110,093 males in Israel reported no increased risk of ASD for IVF overall but found an association between progesterone hormone treatment and increased risk of ASD (RR, 1.51; 95% CI, 1.22–1.86) [22]. Furthermore, polycystic ovarian syndrome (PCOS)-exposed offspring were found to have a higher risk of ASD, and this association was stronger in females [100]. These results were consistent with a study that found elevated amniotic fluid steroid hormones in children diagnosed with ASD [101], which suggested that prenatal androgen exposure may contribute to the early abnormalities of the fetal brain and cause ASD.

Additionally, several studies have reported an increased incidence of ASD in offspring with ICSI [24, 102, 103]. Among these studies, the most convincing evidence came from a large cohort study with more than 2.5 million infants that followed up the offspring for a median of 14 years (range, 0.1–26.5 years). This study observed a statistically significant increase in ASD risk following ICSI using surgically extracted sperm (RR, 4.60; 95% CI, 2.14–9.88) compared with that of IVF offspring. Further analysis showed that children conceived using surgically extracted sperm were more likely to have ASD than those conceived using ejaculated sperm (RR, 3.29; 95% CI, 1.58–6.87), indicating that male infertility severity and certain invasive ART procedures may be involved in the development of ASD [24]. Similarly, the prevalence of ASD was found to be associated with any female infertility diagnosis but not infertility treatment in a multi-site case–control study ($n=1538$) [104]. These studies suggest that parental infertility is likely to be responsible for the increased risk of ASD in ART offspring.

In contrast, in a population-based study in Massachusetts, 1539 ART offspring were enrolled. Researchers found that the association between ART or subfertility and ASD was not statistically significant, and the risk of ASD increased by 1% or less through preterm birth [105], suggesting that preterm birth may have an indirect effect on the development of ASD, which was found to be a risk factor for ASD in non-IVF studies [106–109]

Attention deficit and hyperactivity disorder (ADHD)

ADHD is a common, childhood-onset neurodevelopmental disorder. A Swedish study revealed a weak but statistically significant increase in drug-treated ADHD with IVF, after examining the national drug prescription system [110]. However, the outcome lost significance when the duration of infertility was adjusted, indicating that the risk may be due to parental subfertility characteristics. This result was further confirmed by Svahn et al. [111] in a large Danish registry cohort of more than 2 million children. Subsequently, recent research using a Swedish population cohort discovered a lower risk of ADHD in ART offspring by age 15 years [23], which was then attenuated and even slightly reversed after adjustment for parental characteristics and infertility state. Maternal PCOS was found to increase the odds of ADHD in offspring in a national case–control study [112], as mentioned above with regards to ASD, strengthening the evidence that PCOS affects offspring neurodevelopment.

Intellectual disability (ID)

Sandin et al. [24] discovered a small but statistically significant association (RR, 1.18; 95% CI, 1.01–1.36); although, the association was not significant after controlling for singletons (RR, 1.01; 95% CI, 0.83–1.24). However, the use of ICSI was found to be significantly associated with a higher risk of ID than IVF (RR, 1.51; 95% CI, 1.10–2.09) [24]. Similar results were observed in a retrospective population-based cohort study in Western Australia, which found that ICSI conception almost doubled the risk of ID in children (RR, 1.98; 95% CI, 1.12–3.48) [113]. On the other hand, studies [99, 114] have shown that ART offspring are not at a higher risk of ID.

Cognitive development

Most of the literature in this area has methodological limitations, such as selection bias and various criteria [115]. A systematic review of seven high-quality studies concluded that IVF offspring had cognitive development levels comparable to those of the general population after adjustment [115].

A large Swedish national-based register study discovered that SC offspring performed better in the third grade than ICSI offspring; however, no differences were found between groups in the ninth grade [25]. In subgroup analysis, ninth-grade children conceived through ICSI using non-ejaculated sperm scored significantly lower than those conceived using ejaculated sperm [25], indicating that the severity of male infertility may be associated with cognitive development in ICSI offspring. An animal study also showed that, compared with ICSI-conceived mice, SC mice exhibited an overall superior performance at 6 months of age, but the difference disappeared at 12 months of age [116]. Further follow-up studies are required to determine whether the cognitive development of ICSI offspring is impaired.

Several studies have found that PGD offspring have outcomes comparable to those of conventional IVF in terms of cognitive development in children aged 2–5 years [117–119]. However, a study that followed PGD offspring for up to 7.5 years found that one-fifth of children born through PGD showed poorer performance in cognitive and

motor functions [120]. In a mouse model, researchers also found that biopsied embryos were at risk of defective memory function later in life [121].

Our team found that children born after ovarian hyperstimulation syndrome (OHSS) scored lower in intellectual ability tests, which was presumably related to elevated maternal serum estradiol levels in OHSS moms compared with non-OHSS IVF offspring [122]. A 1:1 matched control cohort study based on the gestational age and age of children further examined this association. In this study, 4–7-year-old children conceived through IVF were investigated, and it was discovered that the offspring born to mothers with high serum estradiol levels (> 12,000 pmol/L) on the day that human chorionic gonadotrophin (hCG) was administered scored lower in the language proficiency scale. [123].

Interestingly, a longitudinal cohort study that followed ART offspring for 11 years revealed a positive association between cognitive abilities and ART, which was strong at 3 and 5 years of age but disappeared by 11 years of age [26]. This effect was further explained by the selective characteristics of ART parents, such as older age, better educational background, and higher socioeconomic status, which have been shown to independently influence children's cognitive functioning and adolescent brain development and play a critical role in students' educational achievement [124].

Neurological disorders

Cerebral palsy

Several studies have suggested that ART is associated with a higher risk of cerebral palsy (CP) [96, 125–127]. In Western Australia, a registry-cohort study including 211,660 live births found that the incidence of CP was at least doubled in ART offspring, and the higher risk of preterm and multiple pregnancies due to ART may partly explain the increased prevalence [27]. Subgroup analysis focusing on full-term offspring did not show a higher incidence of CP in ART offspring [27]. After the increasing application of single-embryo transfer combined with surplus embryo cryopreservation, there has been an insignificant declining tendency of CP among ART children [128, 129]. A previous study observed no difference between ART-conceived and naturally conceived preterm offspring at 3 years of age [130].

Kallen et al. [96] analyzed in detail some possible factors that co-varied with IVF and CP. After adjusting for year of birth, maternal age, gestational age, and smoking, they concluded that ART was associated with only a modest increase in the risk of CP, possibly because of increased neonatal morbidity associated with multiple pregnancies. Whether the elevated risk is associated with the use of ART, if adverse obstetric outcomes are controlled, remains to be studied.

Epilepsy

A potential risk for epilepsy has been reported in children conceived through ART [28, 131, 132]. In a Danish registry-based study, parental infertility was found to be associated with the incidence of childhood epilepsy [133]. Similarly, an increased risk of idiopathic generalized epilepsy, which is a subtype of epilepsy generally considered to be of polygenic origin [134], has been found in the offspring of infertile couples with or without fertility treatment [132], suggesting that the severity of parental infertility and ART may increase the risk of epilepsy.

Researchers found a slightly increased risk of epilepsy (HR, 1.15; 95% CI, 1.00–1.31) in children born after ovulation induction. The increased risk was associated with the use of clomiphene citrate and was more pronounced in idiopathic generalized and focal epilepsy [28]. Furthermore, they focused on clomiphene citrate–treated women ($n = 34{,}039$) and revealed a potential dose–response effect [135], suggesting that clomiphene citrate may interfere with neural system development.

Psychiatric disorders—anxiety and depression

An early study in Amsterdam found a higher prevalence of withdrawn/depressed behavior reported by their parents and teachers in children born through IVF at the age of 9–18 years, which raised concerns regarding depression and anxiety levels in ART offspring [136]. However, this result could not be reproduced in a follow-up study by the same group [137]. A Finnish registry-based study in 2019 observed a higher risk of anxiety disorders in ART offspring born between 1998 and 2006 (HR, 1.39; 95% CI, 1.11–1.75) [138]. Similarly, researchers using Swedish national registry data found a significantly higher risk of anxiety and antidepressant use in ART offspring than in all other children (HR, 1.35; 95% CI, 1.20–1.51), but the association was no longer present when restricted to individuals born to couples with known infertility (aHR, 1.02; 95% CI, 0.89–1.17) [29]. In a mouse model, 18-month-old male offspring in the IVF group showed increased anxiety and depression-like behaviors compared to the NC group [139]. A more severe downregulation of the neurotrophin GNDF was observed in the hippocampus of aged mice than in young male mice. Furthermore, fresh embryo transfer was associated with a lower risk of mood disorder compared with non-ART offspring (HR, 0.90; 95% CI, 0.83–0.97), which provides reassuring evidence for the use of fresh embryo transfer [29]. Based on the above studies, longer follow-up studies are needed to elucidate the health risks of ART in psychiatric disorders.

Reproductive health risks

As a treatment for infertility, the reproductive health of ART offspring is of great concern to parents. Our previous study found an increased incidence of de novo Y-chromosome microdeletions in male IVF offspring (5.3% of 19 IVF offspring) and ICSI offspring (16.7% of 18 ICSI offspring) [140]. However, most ART offspring are still relatively young and have not yet entered childbearing age.

ICSI is typically used to treat male infertility. An ICSI offspring cohort study including 54 ICSI-conceived young adult men found that their total sperm count, median sperm concentration, and total motile sperm count were significantly lower than those in SC men [30]. The levels of reproductive hormones in the IVF and SC groups were similar. The antral follicle count and levels of the anti-Müllerian hormone were found to be similar between ICSI and SC young women [141]. Current limited data show that the fertility of female offspring from ICSI and IVF appears to be comparable with that of SC offspring.

Concerns regarding the risk of precocious puberty among ART offspring have been raised. Some researchers have indicated that an altered intrauterine hormonal milieu may affect prenatal development and thus affect the timing and progression of puberty [142, 143]. A study found that girls born after ART had more diagnoses related to early puberty (aHR 1.46, 95% CI: 1.29–1.66) while boys with late puberty (aHR 1.55, 95% CI: 1.24–1.95) [144]. A later study reported no difference in DHEAS concentrations, precocious puberty incidence, or Tanner staging between IVF and SC offspring aged 4–14 years [74]. A Dutch study showed increased DHEAS, LH concentrations, and advanced bone age among IVF girls, but not in boys, compared with controls [145]. A study involving 217 singleton ICSI offspring and 223 SC controls showed that ICSI-conceived women had less advanced breast development than the control groups [31].

Current evidence concerning the effect on the gonadal function of ART offspring is inconclusive; therefore, further long-term follow-up research is warranted, especially for ICSI-conceived boys. Moreover, it is difficult to find a suitable control group to determine whether the genetic background of infertility or ART affects the reproductive health of children. Therefore, animal models and siblings may provide sufficient information.

Potential causes and mechanisms

The long-term health risks of ART offspring can be attributed to various factors including parental genetic background, ovulation induction treatment, ART procedures, obstetric and perinatal complications, postnatal environmental exposure, and multiple pregnancies. In the present review, we focus not only on the mechanisms by which ART treatment itself leads to health risks in offspring but also on the possible role that parental infertility may play. To date, besides parental infertility, studies have explored several possible mechanisms involved in the process, which mainly focused on epigenome alterations, placental dysfunction, and nonphysical hormone levels.

Parental infertility

Increased risk for a variety of diseases in ART children has been consistently reaffirmed by different methods and in diverse populations. However. it is uncertain whether positive associations are accounted for by the ART procedure itself or underlying characteristics of ART patients and increased incidence of multiple births after ART. Couples who perform ART tend to be older and infertile. Male factor infertility was the most common diagnosis of ART patients, followed by diminished ovarian reserve and tubal factor infertility. For both women and men, however, lifestyle factors such as smoking, excessive alcohol intake, and obesity have been associated with higher chances of infertility.

In addition to chromosomal aberrations, children born to older women may also be at higher risk for gestational diabetes, hypertension, pre-eclampsia, placenta previa, preterm birth, low birth weight, and neonatal mortality [146]. They may also face an increased risk of health problems in adulthood. Studies have found that offspring born to older mothers may have higher adult fasting blood glucose levels [147], as well as higher diastolic and systolic blood pressure levels at the age of 5–7 [148, 149] compared to those born to younger mothers.

A study examined the associations between types of parental infertility diagnoses with autism among ART-conceived children. The incidence of autism diagnosis was lower when parents had unexplained infertility (among singletons) or tubal factor infertility (among multiples) compared with other types of infertility [102]. One possible explanation is that patients with unexplained infertility and tubal factor infertility tend to be younger. A prospective study of 1,221,812 children aged 12–25 years found that adolescents conceived with ARTs had a slightly higher risk of anxiety and antidepressant use; however, the slightly elevated risks were no longer seen when the comparison was made only among individuals whose parents experienced infertility [29], showing that parental infertility is an important confounding factor.

Epigenetic interference

Epigenetic regulation is an important mediating mechanism that regulates the effects of environmental stimuli on individual phenotypes. Two important epigenetic reprogramming

events occur in mammalian embryos: one in primordial germ cell development and the other in the early embryo before and after implantation [150]. Both reprogramming processes involve extensive erasing and reconstruction of the epigenetic spectrum, which includes several interdependent epigenetic layers [151]. The timing of the ART procedure and that of embryo epigenetic reprogramming overlap significantly when both gametes and embryos are extremely susceptible to environmental influence. Exposure to such unphysiological manipulations can lead to perturbations in the reprogramming process, thereby resulting in epigenetic mutations and health risks [152].

Imprinting disorders are a group of rare congenital diseases with common underlying epigenetic etiology [153]. Many studies have reported a significantly increased risk of imprinting disorders in ART offspring [154–156], indicating more potentially extensive epigenetic disruption in ART offspring. An increasing number of studies have found altered DNA methylation in the genes of ART offspring [157–159]. Some of these altered genes are involved in chronic metabolic diseases, such as obesity and type 2 diabetes [160, 161]. However, our previous study assessed the global methylome in fetal tissues. The results showed that the global DNA methylation level was comparable between the IVF-ET and SC groups, although alterations in specific regions warrant further studies [162].

Placental dysfunction

Dysregulation of placental function induces adverse perinatal outcomes and subsequent long-term health risks in offspring, including neurodevelopmental disorders, cancer, and metabolic diseases [163]. Many studies have demonstrated a significantly elevated incidence of placenta-related complications following ART, including placental previa [39], placental accrete [164], and gestational hypertension [165]. Furthermore, ART treatment can result in abnormalities in the morphology and structure of the placenta, which usually presents as increased thickness and a higher rate of hematoma [166]. In addition, placental weight and placental weight/birth weight ratio were significantly increased in the ART group [167], which is an important reference for measuring the susceptibility of the fetus to adult chronic diseases after birth [168].

Nonphysiologic steroid hormone environment

Elevated maternal serum estrogen level

Estrogen affects all aspects of placental function and fetal growth during pregnancy. Elevated maternal serum estrogen levels are common after ovulation stimulation (OS), which is a regular procedure in ART. We previously reported that OS-induced high estrogen levels can persist throughout pregnancy and are associated with dyslipidemia in offspring [169]. Our previous studies also found that high E2 levels in the first trimester can increase the risk of thyroid dysfunction in children aged 3–10 years born from fresh transfer [170]. Our previous research demonstrated that prenatal E2 exposure reduced insulin receptors in the hypothalamus and elevated neuropeptide Y expression in mouse offspring, resulting in insulin resistance and disordered eating. We also found that maternal high E2 levels in early pregnancy altered promoter methylation of hypothalamic Insr in male mouse offspring, and this programming effect impaired insulin signaling in the hypothalamus, thus providing evidence for glucose metabolism disorders in adulthood [171]. This is consistent with the previous finding that exposure to E2 early in life had a lifelong effect on DNA methylation [172].

Our group observed a high rate of attachment of human choriocarcinoma (JAr) cell spheroids to endometrial epithelial cells (EECs) in a high-estrogen environment (10^{-7} M) compared with a relatively low-estrogen environment (10^{-9} M) and subsequently identified 45 differentially expressed proteins that may be involved, suggesting that endometrial receptivity is affected by high estrogen levels [173].

In a retrospective cohort study, pregnancy complications associated with placental abnormalities are significantly increased in the high E2 group [174], suggesting a link between estrogen levels and placental function. Our study found that high maternal E2 levels after ART may also upregulate the expression of imprinted genes in the human placenta through epigenetic modifications [175].

Hyperandrogenism in women with polycystic ovarian syndrome

Another major maternal hormonal abnormality associated with ART is hyperandrogenism, which is primarily induced by PCOS. In our previous study, we found that PCOS is associated with poor IVF outcomes [176] and thus may affect the long-term health of ART offspring.

In our previous study, we included 156 children of mothers with hyperandrogenism and 1060 controls and followed up their glucometabolism for a mean age of 5 years. We found that children of mothers with hyperandrogenism had increased serum fasting glucose and insulin levels and incidence of prediabetes (adjusted RR, 3.98; 95% CI, 1.16–13.58). We also established rat models and found that hyperandrogenism can increase insulin-like growth factor 2 (IGF2) expression, decrease DNMT3a in oocytes, and alter methylation signatures in the pancreatic islets of the offspring, indicating that hyperandrogenism may predispose offspring to diabetes via epigenetic oocyte inheritance [177].

Paternal genome integrity

Genetic factors can explain at least 15% of male infertility [178], and Klinefelter (or 47, XXY) syndrome and Y-chromosome microdeletions (YCMs) are common genetic causes of infertility in men with severe oligospermia or azoospermia as clinical manifestations [179]. PGT of embryos produced by ICSI of sperm from men with Klinefelter syndrome showed a substantially lower rate of normal embryos (54% vs. 77.2%) compared to embryos from couples with sex-linked disorders who underwent PGT to determine fetal sex, and the study further found that chromosomal abnormalities on chromosomes 18 and 21 were at significantly increased risk. In addition, YCM can be transmitted vertically from the father to male offspring via the Y chromosome, which can lead to impaired fertility in the offspring [180]. In addition, some oligozoospermic men with autosomal chromosomal structural aberrations, such as Robertsonian translocations, inversions, and reciprocal translocation, may have an increased risk of aneuploidy or unbalanced chromosomal complements in the fetus [181].

Effects of FET on offspring

Although most studies regard this technology as a very safe medical operation, some studies have reported long-term health risks associated with FET, such as a slightly increased incidence of pediatric cancer [182]. Infants born following FET have been demonstrated to have increased rates of macrosomia or large for gestational age (LGA) [183, 184]. LGA babies have a higher risk of metabolic disease later in life, such as cardiovascular diseases, obesity and overweight, insulin resistance, and type 2 diabetes [185].

Several studies have investigated the mechanism by which FET affects the long-term health of offspring, including epigenetic alterations, cellular impairments, proteomic profile changes, and some other potential mechanisms. In an animal study, researchers found that vitrification significantly decreased the expression of DNA methyltransferase 1o mRNA in mouse MII oocytes [186]. Such alterations strongly indicate possible disruption of the embryonic genome-wide methylation pattern, which may provide insight into the pathogenesis of the unusually elevated morbidity of imprinting-related diseases among ART children. Furthermore, in a clinical study analyzing multi-omics data derived from umbilical cord blood samples of FET offspring, Chen et al. [187] revealed that FET introduced more epigenetic disturbances than traditional ART procedures.

Somoskoi et al. [188] showed that cryopreservation could affect the mitochondrial distribution pattern, intracellular ROS levels, and energy status in both morula and blastocyst stages [189]. Another study showed that frozen/thawed oocytes had decreased electron density in the mitochondrial matrix and damaged mitochondrial membranes. Mitochondrial impairment can result in elevated oxidative stress and a subsequent cascade of cellular dysfunction, thereby leading to defective embryo development and long-term health risks.

Apart from epigenomic alterations and cellular impairments, Cuello et al. [190] analyzed the gene expression profiles of 30 blastocysts subjected to vitrification. A total of 205 differentially expressed genes were identified in the treatment group, which were mainly involved in the pathways of gap junction, cell cycle, cellular senescence, and signaling for Fox, TFG, MAPK, and p53. Another animal experiment identified 20 differentially expressed proteins in the brain tissue of FET offspring [191], which were mainly related to the development of anatomical structure, signal transduction, transport, cell differentiation, stress response, etc. In addition, our previous animal study demonstrated that FET disrupted the PI3K/AKT signaling pathway in the liver of adult male offspring, resulting in insulin resistance and glucose metabolism dysfunction [192]. Furthermore, our previous study identified 92 differentially expressed genes in the cord blood of neonates born from embryos with blastomere loss during FET, and downstream analysis of these genes predicted the activation of organismal death pathways, which implies a potentially detrimental effect on embryo development [193].

Strategies to optimize the safety of ART

ART has improved significantly from an initial implantation rate of < 5% per replacement embryo to > 50% [194]. A study from our team showed that the blood pressure of children conceived through ART born between 2000 and 2009 was significantly lower than that of children born between 1990 and 1999 [81]. Continuous optimization of ART procedures and management, such as mild stimulation protocol and strict indication for ART application, would benefit both mothers and children.

Control of E2 concentration

In fresh embryo transfer cycles, E2 levels can be 10 to 20 times greater during controlled ovarian hyperstimulation (COH) and persist throughout the first trimester of pregnancy [195], which was found to correlate with increased risks of LBW and SGA in the offspring [196] and may lead to chronic disease later in life. E2 levels on the day of hCG administration can be a useful marker of E2 levels in early pregnancy [195, 196], which can help the OB/GYN take steps in time to avoid prolonged exposure of the gametes/embryos to high E2. In our previous retrospective cohort study, we discovered that high E2 levels on the day of hCG administration led to increased early pregnancy loss and

reduced clinical pregnancies, ongoing pregnancies, and live births [197]. If maternal E2 levels are very high on the day of hCG administration (E2 ≥ 10 460 pmol/L) [195], physicians should seriously consider the risk of adverse outcomes in the offspring and adopt FET, but not fresh embryo transfer.

Oral contraceptive pill pre-treatment for PCOS

For women with PCOS, a blended short-acting oral contraceptive pill (OCP) is usually used before gonadotrophin to restore hormonal balance and synchronize follicular development [198]. An early study demonstrated that OCP pre-treatment could increase implantation and pregnancy rates in women with PCOS [199]. In our previous retrospective study of 500 women with PCOS and 565 controls undergoing ART, we found that OCP pre-treatment for three cycles or more can significantly increase implantation and pregnancy rates and reduce the incidence of small-for-gestational-age ($p < 0.05$) [176]. Besides, a recent propensity score matching study indicated that in women with PCOS, gonadotropin-releasing hormone analogue (GnRH-a) pretreatment was significantly associated with a higher live birth rate and a reduced risk of neonatal PTB [200]. GnRH-a may dispel the intrinsic hormonal abnormalities of PCOS [201]. A meta-analysis of 9 studies, 8327 patients with PCOS, compared endometrial preparations by ovarian stimulation protocols and hormone replacement therapy in women with PCOS before FET, and found a significantly higher clinical pregnancy rate (RR = 1.54, 95% CI = 1.20–1.98) in letrozole group [202]. The stimulation protocol might be better than the HRT protocol in increasing the live birth rate and reducing the miscarriage rate.

Single-embryo transfer

Single-embryo transfer (SET) should be strongly recommended according to current data, although SET is not a priority yet in many areas. SET utilization varies from 8.8% in South Korea to 53.3% in Australia [203]. Compared with single embryo transfer, multiple embryo transfer boosts the live birth rate but also the chance of adverse birth outcomes, while the cumulative birth rates are similar [204–206]. Twin pregnancies are associated with a higher risk of complications such as preterm birth, low birth weight, and neonatal mortality compared to singleton pregnancies. [207] A retrospective study [208] of 2780 live singletons conceived by IVF or ICSI between 1991 and 2015 showed a significant increase in birth weights due to the application of SET. It was observed that 4-year-old twins born after IVF had slightly lower total IQ scores than singletons, and twins were lighter and shorter than singletons [209]. In addition, their risk of hospitalization (OR, 4.9; 95% CI, 3.3–7.0), outpatient visits (OR, 2.6; 95% CI 1.8–3.6), and medical procedures (OR, 1.7; 95% CI 1.2–2.2) was higher from birth up to age 5 [210]. Another cohort study conducted by D.M. Kissin et al. [102] found that the incidence of diagnosed autism was significantly lower in ART singletons compared to ART twins. Although only a small number of studies reported the long-term benefits of SET, multiple births in naturally conceived children pregnancy is a much-reported risk factor. A two-sample Mendelian randomization study conducted by Yi Jiang et al. [211] based on UK Biobank and FinnGen databases has revealed a causal relationship between multiple birth and nervous system disease and various cardiac disorders. Moreover, it is well established that multiple birth is an important risk factor for perinatal outcomes [212], and poor perinatal outcomes may lead to far-reaching impact on the long-term health of offspring. Although the current mainstream view is in favor of SET, more evidence is still needed to support its advantage.

Fresh embryo transfer versus FET

At present, there is no consensus on the relative superiority or inferiority of fresh embryo transfer or FET, although most studies favor FET. A randomized trial conducted in China included 1650 patients with a good prognosis and assigned them to receive fresh or frozen single-blastocyst transfers. The frozen group had significantly higher rates of live births than the fresh group (50.4% vs. 39.9%; RR, 1.26; 95% CI, 1.14–1.41; $p < 0.0001$) [213]. FET is an important method against OHSS [214]. However, not all studies concluded that FET is better than fresh embryo transfer. Coutifaris et al. [215] found that frozen single-blastocyst transfer was associated with a higher incidence of preeclampsia than fresh single-blastocyst transfer.

Compared to the abundant studies focusing on short-term outcomes in FET versus fresh ET, fewer studies demonstrated the comparison of long-term outcomes between these two approaches. Some studies found no significant difference in long-term outcomes between FET and fresh ET. A cohort study from Australia followed 391 cases reported a comparable status of cognition development in FET and fresh ET children [216]. However, some studies indicated worse outcomes in FET offspring. A cohort study in Denmark, which included 1,085,172 children reported that FET children showed a higher incidence of cancer compared to spontaneous children, while other types of fertility treatments did not show such a trend [182]. Besides, fetal pathological events, such as pre-eclampsia, increase cardiovascular risk and are associated with premature vascular aging during adolescence [217]. Further follow-up studies on the advantages and disadvantages of FET and fresh embryo transfer should be conducted.

Vitrification versus slow freezing

Compared to slow freezing, vitrification hardly forms ice crystals outside the cells, which is the most significant source of injury during cryopreservation. However, vitrification requires relatively high concentrations of cryoprotectants, which may induce cellular damage including direct toxicity and osmotic damage.

Regarding the comparison between vitrification and conventional slow freezing, Rienzi et al. [218] demonstrated that embryos, blastocysts, and oocytes all have a better survival rate in the vitrification group than in the slow freezing group. Studies have also suggested that the application of vitrification could elevate clinic pregnancy rates compared to slow freezing [218]. Since vitrification has not been widely used until recent years, studies focusing on the comparison of the long-term effects between vitrification and slow freezing are still in shortage. Thus far, vitrification is generally recommended. However, more studies are warranted to confirm whether it is better for ART offspring in the long run.

Optimize culture medium

Some studies have attempted to improve the outcomes by optimizing the culture medium. A study found that the selection of an in vitro culture medium for human embryos was associated with body weight, BMI, truncal adiposity, waist circumference, and waist/hip ratio of the offspring [219]. Sacha CR et al. [220] conducted a study comparing obstetric and perinatal outcomes for deliveries conceived with embryos from single-step versus sequential culture media systems. The study found that single-step culture was associated with increased odds of LGA (aOR, 2.1; 95% CI, 1.04–4.22; $p = 0.038$) [220]. In emerging studies, various research has attempted to determine what should be added to the medium. Supplementing the embryo culture medium with an optimized combination of growth factors and cytokines significantly increased trophectoderm cell number, total cell number, and blastocyst outgrowth area following embryo transfer, fetal weight, and crown-rump length in mice [221]. Velazquez et al. [222] explored the addition of insulin and branched-chain amino acids to the culture medium, which increased the birth weight and early postnatal body weight of offspring. However, male offspring show relative hypertension and female offspring show decreased heart/body weight ratios [222]. A recent study demonstrated that melatonin supplementation in the culture medium can reverse impaired glucose metabolism in IVF mouse offspring [223]. Studies in this area are limited in their application. Stronger evidence is required regarding this topic.

Embryo biopsy

The most commonly used PGT method requires a biopsy of developing embryos to obtain genetic material, which increases the potential risk of embryo development and long-term consequences [224, 225]. Some studies reported a higher incidence of hypertensive disorders and small-for-gestational-age compared to the unbiopsied group [226, 227], whereas others reported no increase [228, 229]. Recently, scientists have explored techniques for noninvasive PGT (niPGT) by correlating the genetic material found in blastocyst fluid and spent blastocyst media [230]. This technique is a potentially more effective and safer way to detect chromosomal abnormalities [231].

Health care for ART parents and offspring

Infertility should be diagnosed and treated promptly, as maternal age affects the outcomes of IVF, pregnancy complications, and neonatal health. Moreover, genetic counseling and health promotion treatment should be considered before ART. Doctors should inform patients of the risks of ART for their offspring [232], to ensure action is taken to limit these risks.

Maternal weight control

Obese women are advised to lose weight prior to conception. Pre-pregnancy obesity of the mother is associated with an increased risk of obesity and overweight at an early age in ART offspring and may also affect the risk of ID in offspring [233]. Similarly, obesity in lactating mothers is associated with faster fat gain in offspring, and infants conceived by overweight or obese mothers exhibit a lower response to human milk insulin than infants conceived by women of normal weight [234]. Additionally, maternal BMI and blood lipid levels are associated with an increased risk of ASD.

Feeding

The diet of the offspring may play an important role in accelerating the progression of abnormal glucose metabolism in ART offspring. Chen et al. [161] found elevated fasting glucose levels, glucose intolerance, and insulin resistance in children fed a high-calorie diet. Similarly, in ART-conceived mice fed with HFD, ART-conceived mice were more likely to develop obesity, fasting hyperinsulinemia, and hyperglycemia, and insulin-stimulated glucose utilization was 20% lower (steady-state glucose infusion rate) than SC mice. It is believed that endothelial dysfunction induced by ART through epigenetic alteration of endothelial nitric oxide synthase would facilitate glucose intolerance and insulin

resistance when challenged with metabolic stress [83]. Our recent study found that in a mouse model, after being fed with HFD, male ART offspring in the FET group performed earlier and had more severely impaired glucose tolerance than the ET group [192].

Evidence has shown that postnatal lifestyle interventions can ameliorate and reverse adverse epigenetic and phenotypic changes induced during pregnancy [235, 236]. Breastfeeding for at least 6 months can protect children from being overweight at the age of 2 years [237]. Formula supplementation, on the other hand, is associated with faster fat gain [234]. Therefore, exclusive breastfeeding is recommended for at least 6 months, especially for infants at risk of developing obesity.

Additional supplements

As demonstrated in this review, ART may be associated with an increased risk of diseases, such as asthma, ASD, and cardiovascular disorders. Some of these conditions can be treated early by the administration of additional supplements. Maternal vitamin D intake can decrease the risk of developing asthma [238]. Prenatal vitamins and folic acid taken during the first month of pregnancy can reduce ASD recurrence in the siblings of patients with ASD [239, 240]. After ART, children are advised to use additional supplements. A prospective, double-blind, placebo-controlled study including 21 ART and 21 control children showed that the administration of antioxidants to ART children improved no bioavailability and vascular reactivity in the systemic and pulmonary circulation [241].

Conclusion

In our review, we summarized the present knowledge on the long-term health risks of ART offspring (Table 1). It is suggested that ART offspring have an increased risk of NCD, such as malignancies, asthma, obesity, metabolic syndrome, diabetes, cardiovascular diseases, and neurodevelopmental and psychiatric disorders. There are still many controversies in this field and much remains unknown, and further research is needed to monitor the long-term health of ART offspring and determine the effects of ART on this population in later life. Evidence indicates that underlying parental infertility, altered epigenome alterations, placental dysfunction, and nonphysical hormone levels may contribute to adverse outcomes in ART offspring. ART procedures should be developed to mimic a natural pregnancy as closely as possible to eliminate these potential risks. Strategies, such as controlling E2 concentration and single embryo transfer, that improve both perinatal and long-term outcomes have been discovered. Attention should also be paid to health education and health care for ART parents and offspring, such as maternal weight control and offspring feeding.

Abbreviations ART: Assisted reproductive technologies; IVF: In vitro fertilization; ICSI: Intracytoplasmic sperm injection; PGT: Preimplantation genetic testing; SC: Spontaneously conceived; IUGR : Intrauterine growth restriction; ALL: Acute lymphoid leukemia; FET: Frozen embryo transfer; BMI: Body mass index; HFD: High-fat diet; TSH: Thyroid-stimulating hormone; TRH: Thyrotropin-releasing hormone; E2: Estradiol; ET: Embryo transplantation; T4: Thyroxine; FT4: Free thyroxine; ASD: Autism spectrum disorder; PCOS: Polycystic ovarian syndrome; ADHD: Attention deficit and hyperactivity disorder; ID: Intellectual disability; OHSS: Ovarian hyperstimulation syndrome; CP: Cerebral palsy; OS: Ovulation stimulation; IGF2: Insulin-like growth factor 2; COH: Controlled ovarian hyperstimulation; OCP: Oral contraceptive pill; SET: Single-embryo transfer; NCD: Non-communicable diseases; DOHaD: Developmental origins of health and disease; HR: Hazard ratio; OR: Odd ratio; CI: Confidence interval; SFA: Saturated fatty acid; HOMA-IR: Homeostasis model assessment-insulin resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; RR: Relative risk; PGD: Preimplantation genetic diagnosis; DHEAS: Dehydroepiandrosterone sulfate; LH: Luteinizing hormone; DNA: Deoxyribonucleic acid; EEC: Endometrial epithelial cell; YCM: Y-chromosome microdeletion; RNA: Ribonucleic acid; MII: Meiosis II; TFG: Trafficking from ER to golgi regulator; MAPK: Mitogen-activated protein kinase; AKT: Protein kinase B; FSH: Follicle-stimulating hormone; LBW: Low birth weight; SGA: Small for gestational age; LGA: Large for gestational age; hCG: Human chorionic gonadotrophin; OB/GYN: Obstetrics and gynecology; IQ: Intelligence quotient; niPGT: Noninvasive PGT; NO: Nitrogen monoxide; GnRH-a: Gonadotropin-releasing hormone analogue

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Declarations

Ethics approval and consent to participate Not applicable.

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