The health outcomes of human offspring conceived by assisted reproductive technologies (ART)

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Concerns have been raised about the health and development of children conceived by assisted reproductive technologies (ART) since 1978. Controversially, ART has been linked with adverse obstetric and perinatal outcomes, an increased risk of birth defects, cancers, and growth and development disorders. Emerging evidence suggests that ART treatment may also predispose individuals to an increased risk of chronic ageing related diseases such as obesity, type 2 diabetes and cardiovascular disease. This review will summarize the available evidence on the short-term and long-term health outcomes of ART singletons, as multiple pregnancies after multiple embryos transfer, are associated with low birth weight and preterm delivery, which can separately increase risk of adverse postnatal outcomes, and impact long-term health. We will also examine the potential factors that may contribute to these health risks, and discuss underlying mechanisms, including epigenetic changes that may occur during the preimplantation period and reprogram development in utero, and adult health, later in life. Lastly, this review will consider the future directions with the view to optimize the long-term health of ART children.

Assisted reproduction technologies (ART)

ART are defined as all treatments or procedures for initiating pregnancy that include the *in vitro* handling of both oocytes and sperm or embryos, predominantly *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), gamete and embryo cryopreservation, preimplantation genetic diagnosis and preimplantation genetic screening.[1] Briefly, the routine IVF procedure includes three steps: ovarian hyperstimulation, IVF and embryo culture, and embryo transfer. First, high doses of gonadotropins are administered to induce development of multiple follicles. Then, the oocytes are retrieved from the ovaries using a transvaginal ultrasound-guided fine needle and inseminated with the prepared sperm *in vitro* to achieve fertilization. Embryos are cultured in a prepared medium in the incubator for 3 days to reach the eight-cell stage or 5 days to develop to the blastocyst stage. Finally, the embryos are transferred into the uterus or frozen for future transfer. Although IVF is beneficial for most of infertile couples with female infertility, unexplained infertility and some cases of male infertility, ICSI in which a single spermatozoon is injected into the oocyte cytoplasm is required to treat severe male infertility.

Since 1978, more than 5 million children have been born by ART treatment, mostly by IVF and ICSI.[2] Concerns about the potential health implications of ART remain.[3,4] Increasing evidence shows ART treatment is associated with adverse perinatal outcomes, which are related to subfertility of patients, multiple pregnancies and ART technologies.[4–9] As it is well accepted that multiple pregnancies, after multiple embryos transfer, are associated with low birth weight and preterm delivery,[10] which can also impact long-term health risks, this review will limit its focus to the health outcomes of ART singletons born from IVF and ICSI *v.* singletons from natural conception.

Obstetric and perinatal outcome in ART singleton pregnancies

As summarized in Table 1, singleton pregnancies after ART are associated with adverse obstetric and perinatal outcome as compared with spontaneous conception.[4,11,12] These outcomes include an increased risk of low birth weight, preterm birth, small for gestational age, stillbirth, perinatal mortality, admission to a neonatal intensive care unit, *antepartum* haemorrhage, hypertensive disorders of pregnancy, preterm rupture of membranes, gestational diabetes, induction of labour and caesarean section.[8,11–17] It should be noted that vanishing twin pregnancies, which contribute to about 10% of IVF singletons pregnancies, increase perinatal risk in IVF singletons.[18,19] However, whether the procedure of IVF itself, or the underlying parental characteristics or genetics are the main contributors to this increase in obstetric and perinatal risk is not clear. Some studies have shown that IVF singletons have an increased risk of adverse perinatal outcome *v.* their non-IVF siblings.[11] However, other studies have shown

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Table 1. *Obstetric and perinatal outcome in* in vitro *fertilization (IVF) singleton pregnancies*

Participants	Year of birth	Outcomes	References
Siblings born after IVF–ICSI *v.* spontaneous conception (*n* = 7758)	1994–2006	Low birth weight (aOR, 1.4; 95% CI, 1.1–1.7) and preterm birth (aOR, 1.3; 95% CI, 1.1–1.6)	[12]
Siblings born after IVF–ICSI *v.* spontaneous conception (*n* = 545,102)	1999–2007	An association between maternal characteristics and a lower birth weight, ranging from −7 g (95% CI, −40; 26) to −101 g (95% CI, −170; −32)	[23]
ART births (*n* = 4333) *v.* spontaneous conception (*n* = 295,220)	1986–2002	Low birth weight (*P* < 0.001) and preterm birth (*P* < 0.001)	[15]
IVF (*n* = 133), ICSI (*n* = 104) *v.* spontaneous conception (*n* = 338)	1998–2003	Preterm birth (*P* < 0.01)	[17]
IVF/ICSI (*n* = 742) *v.* spontaneous conception (*n* = 16,525)	(1989–2006)	Stillbirth (aOR: 4.08; 95% CI, 2.11–7.93)	[8]
IVF (*n* = 11347) *v.* spontaneous conception (*n* = 571,914)	2002–2006	Low birth weight (aOR, 1.13; 95% CI, 1.02–1.25) and preterm birth (aOR, 1.15; 95% CI, 1.06–1.25)	[168]
ART vanishing co-twin (*n* = 642) *v.* ART singletons (*n* = 5237)	1995–2001	Low birth weight (aOR, 1.7; 95% CI, 1.2–2.2); preterm birth (aOR, 1.3; 95% CI, 1.0–1.7) Child death (aOR, 3.6; 95% CI, 1.7–7.6); small-for-gestational age (OR, 1.50; 95% CI, 1.03–2.20)	[18,19]
Time to pregnancy >1 year (*n* = 3899) *v.* time to pregnancy ⩽ 1 year (*n* = 15302)	1997–2001	Preterm birth (aOR, 1.38; 95% CI, 1.14–1.69)	[20]
Time to pregnancy >1 year (*n* = 5722), infertility treatment (*n* = 4271) *v.* time to pregnancy ⩽ 1 year (*n* = 50,614)	1997–2003	Small-for-gestational age (aOR, 1.24; 95% CI, 1.1–1.4) and (aOR, 1.4; 95% CI, 1.23–1.6)	[21]
Frozen embryo transfer (*n* = 746) *v.* fresh embryo transfer (*n* = 762)	2013–2014	Live birth (rate ratio, 1.17; 95% CI, 1.05–1.31); pregnancy loss (rate ratio, 0.67; 95% CI, 0.54–0.83); and ovarian hyperstimulation syndrome (rate ratio, 0.19; 95% CI, 0.10–0.37), preeclampsia (rate ratio, 3.12; 95% CI, 1.26–7.73)	[24]
Unstimulated IVF (*n* = 190) *v.* stimulated IVF (*n* = 174)	2007–2013	Low birth weight (aOR, 0.11; 95% CI, 0.01–1.0)	[26]
Unstimulated IVF (*n* = 6168) *v.* stimulated IVF cycles (*n* = 584,835)	1991–2011	Preterm birth (aOR, 1.43; 95% CI, 0.91–2.26) Low birth weight (aOR, 1.58; 95% CI, 0.96–2.58)	[27]
IVF births (*n* = 3305) *v.* the general population (*n* = 1,505,724)	1982–1995	Very preterm birth (<32 weeks) (OR, 3.54; 95% CI, 2.90–4.32) Very low birth weight (<1500 g) (OR, 4.39; 95% CI, 3.62–5.32)	[94]

ICSI, intracytoplasmic sperm injection; CI, confidence intervals; ART, assisted reproductive technologies; aOR, adjusted odds ratio; OR, odds ratio.

that perinatal outcomes from spontaneous conception are also poorer in subfertile women *v.* those with normal fertility,[20,21] and that perinatal outcomes are comparable after IVF or natural conception in subfertile women.[22] A large recent study using siblingship analysis suggested that maternal characteristics such as subfertility and maternal age but not IVF treatment are associated with lower birth weight in IVF children.[23] This discrepancy in the literature requires further study in larger cohorts that control for as many confounders as possible, and also further pre-clinical study. When investigating risk, the type of ART procedure is not always reported, and may contribute to adverse perinatal outcomes. A recent study shows that frozen embryo transfer increases pregnancy rates, improves obstetric and perinatal outcomes, and reduces the risk of ovarian hyperstimulation syndrome in patients with polycystic ovary syndrome.[24] A meta-analysis of 11 studies supports this, reporting that singletons born after the transfer of frozen thawed embryos had better obstetric and perinatal outcome as compared with those after the transfer of fresh IVF embryos.[25] The relative risks (RR) and 95% confidence intervals (CI) of *antepartum* haemorrhage (RR = 0.67, 95% CI, 0.55–0.81), preterm birth (RR = 0.84, 95% CI, 0.78–0.90), small for gestational age (RR = 0.45, 95% CI, 0.30–0.66), low birth weight (RR = 0.69, 95% CI, 0.62–0.76) and perinatal mortality (RR = 0.68, 95% CI, 0.48–0.96) were lower in women who received frozen embryos.[25] The characteristics for each study was shown in the review.[25] This data suggests that suboptimal endometrial development, induced by hormone stimulation, may be a contributor to poorer perinatal outcome after IVF.[7,12] A recent retrospective cohort study suggests natural cycle IVF may decrease the risk of low birth weight *v.* conventional stimulated IVF,[26] but this is not reported universally.[27] The length of embryo culture is emerging as another potential confounder when considering perinatal outcomes, but consideration may also be required as to whether sequential or single-step culture media is employed.[28] Increasing evidence suggests the type of culture medium may also impact birth weight in IVF singletons.[29,30] Although some studies show that blastocyst transfer is associated with a higher cumulative live birth and pregnancy rates,[31] a recent meta-analysis of six studies suggest that blastocyst transfer may increase the risk of preterm birth in IVF singleton pregnancies.[32] Taken together, it seems that parental characteristics, and ART procedures themselves contribute to the adverse perinatal outcomes of singleton pregnancies after ART. Careful further studies are warranted to determine whether cleavage embryo transfer, sequential media with blastocyst transfer, minimal stimulation protocols or natural IVF improve obstetric and perinatal outcomes, and the long-term health outcomes of this on ART children.

Birth defects in ART singleton pregnancies

Major malformations were defined as those causing functional impairment or requiring surgical correction,[33,34] whereas the others were considered minor malformations. The prevalence of major birth defects such as chromosomal and musculo-skeletal defects diagnosed by 1 year of age is two-fold higher in infants conceived by IVF or ICSI than in naturally conceived infants born between 1993 and 1997 in Western Australia.[35] Importantly, this study controlled for parental factors such as maternal age and parity, the gender of the infant and correlation between siblings. In Israel, the percentage of major malformations in infants conceived by ART in 1986–1994 and 1995–2002 was also double that of the general population during the same periods.[36] Similar reports have been observed in Spain,[37] France,[38] Canada[39] and the United States[40,41] (summarized in Table 2).

Large meta-analyses have been conducted and show that children born after ART have a 30–40% increased risk of birth defects compared with spontaneous conceptions.[42–47] However, it is not entirely clear if the contributing factor is the ART procedure, or the underlying infertility itself. One Italian study of >7000 infants born after ART or ovulation induction suggested the increased prevalence of birth defects associated with non-spontaneous conception was largely due to confounding factors such as maternal age,[48] which is associated with poorer oocyte quality, mitochondrial dysfunction, aneuploidy and epigenetic alteration.[49] A large Danish longitudinal study found singletons born of infertile couples who conceived naturally (time to pregnancy >12 months) or after infertility treatment including different types of ART or surgeries had a higher prevalence of congenital malformations compared with singletons born of fertile couples (time to pregnancy ≤12 months).[50] Notably, the prevalence of congenital malformations increased with increasing time to pregnancy, suggesting that infertility *per se* was an independent risk factor. On the other hand, amongst infertile couples, infertility treatment was associated with an increased prevalence of genital organ malformations in singletons compared with natural conception.[50] Further, Davies *et al.*[15] compared risks of birth defects among pregnancies in women who received ART treatment, spontaneous pregnancies in women who had a previous birth with assisted conception and spontaneous pregnancies in women with or without a record of infertility. An increased risk of birth defects was significantly associated with infertility *per se*, independently of assisted conception. An increased risk of birth defects was also associated with assisted conception after the multivariate adjustment, however, this association was only observed in births conceived by ICSI but not by IVF, after adjustments.[15] In comparison, two meta-analysis reported the risk of birth defect was not significantly different between children conceived by IVF and ICSI.[45,51]

Ovulation induction alone has also been associated with increased risk of birth defects.[39,52] Evidence shows that exogenous gonadotrophins may impair oocyte and embryo development as well as endometrial receptivity, increase chromosomal aneuploidy, alter epigenetic modifications, thus have detrimental effects on perinatal outcomes and long-term health.[53,54] In Finland, ART singleton girls from ovulation

Table 2. *Birth defects in* in vitro *fertilization (IVF) singleton pregnancies*

Participants	Year of birth	Outcomes	References
ART births (*n* = 1138) *v.* spontaneous conception (*n* = 4000)	1993–1997	Major defects: IVF (aOR, 2.0; 95% CI, 1.5–2.9); ICSI (aOR, 2.0; 95% CI, 1.3–3.2)	35
IVF births (*n* = 31,007) *v.* spontaneous conception (*n* = 278)	1986–1994	Major defects: IVF (OR, 2.3)	36
ART births (*n* = 53208) *v.* spontaneous conception (*n* = 1632)	1995–2002	Major defects: ART (OR, 1.75)	
Cases with major birth defects (*n* = 1905) *v.* controls (*n* = 2722)	1992–2007	ART (aOR, 2.7; 95% CI, 1.8–4.1)	37
Cases with congenital heart defects (*n* = 5493) *v.* malformed controls (*n* = 3847)	1987–2006	ART (aOR, 1.4; 95% CI, 1.1–1.7)	38
ART births (*n* = 319) *v.* spontaneous conception (*n* = 43,462)	2005	ART (aOR, 1.55; 95% CI, 1.01–2.38) The risks of birth defects: non-ART population 1.86%, ovulation induction 2.35%, IUI 2.89%, and IVF 3.45%	39
Cases with major birth defects (*n* = 9584) *v.* controls (*n* = 4792)	1997–2003	Septal heart defects (aOR, 2.1; 95% CI, 1.1–4.0), cleft lip with or without cleft palate (aOR, 2.4; 95% CI, 1.2–5.1), oesophageal atresia (aOR, 4.5; 95% CI, 1.9–10.5) and anorectal atresia (aOR, 3.7; 95% CI, 1.5–9.1)	40
ART births (*n* = 33,601) *v.* spontaneous conception (*n* = 4,421,154)	2000–2010	(aRR, 1.38; 95% CI, 1.21–1.59)	41
Non-spontaneous conception (*n* = 264) *v.* spontaneous conception (*n* = 11240)	2010–2012	Any defect (aRR, 0.95; 95% CI, 0.8–1.1)	48
Spontaneous conception (time to pregnancy >12 months, *n* = 5764) and infertility treatment (*n* = 4588) *v.* spontaneous conception (time to pregnancy ⩽12 months, *n* = 50,897)	1997–2003	Infertility (hazard ratios 1.20, 95% CI, 1.07–1.35), and infertility treatment (hazard ratios 1.39; 95% CI, 1.23–1.57). Among infertile couples, infertility treatment *v.* natural conception (hazard ratio 2.32; 95% CI, 1.24–4.35)	50
ART Births (*n* = 4333) *v.* spontaneous conception (*n* = 295,220)	1986–2002	Any defect: ART (aOR,1.30; 95% CI, 1.16–1.45); IVF (aOR, 1.07; 95% CI, 0.9–1.26); ICSI (aOR, 1.57; 95% CI, 1.3–1.9)	15
IVF births (*n* = 2930), ovarian stimulation (*n* = 3926) *v.* controls (*n* = 26,489)	1996–1998	Any defect: IVF (aOR, 1.30; 95% CI, 1.05–1.61); ovarian stimulation (aOR, 1.17; 95% CI, 0.97–1.41)	52
ICSI births (*n* = 150) *v.* spontaneous conception (*n* = 147)	2001–2003	Major congenital malformation (*P* < 0.05)	58
IVF births (*n* = 3305) *v.* the general population (*n* = 1,505,724)	1982–1995	Malformations (OR, 1.39; 95%CI, 1.25–1.54), and the rates of neural tube defects and oesophageal atresia were higher	94

ICSI, intracytoplasmic sperm injection; CI, confidence intervals; ART, assisted reproductive technologies; aOR, adjusted odds ratio; OR, odds ratio; aRR, adjusted relative risks; IUI, intrauterine insemination.

induction had more major heart anomalies than controls conceived naturally.[52] Similarly, the risks of birth defects were higher in ovulation induction *v.* natural conception, whereas the risk was even higher in IVF *v.* ovulation induction.[39] Taken together, this data suggest that singletons conceived by ART procedures are at increased risk for birth defects. This is at least partly due to the underlying infertility, and parental characteristics, but may be further increased by ovulation induction and ART procedures.

Growth and development in ART singleton pregnancies

A number of studies have examined the growth patterns of ART children with conflicting results[55–69] (summarized in Table 3). The majority of studies have not observed any differences in the growth of ART children *v.* naturally conceived children. For instance, recent prospective follow-up studies in the United States compared 969 singletons conceived by infertility treatment including ART and ovulation induction

Table 3. *Growth and development in assisted reproductive technologies (ART) singleton pregnancies*

Participants	Age	Outcomes	References
IVF births ($n = 66$) $v.$ spontaneous conception ($n = 66$)	12–45 months	The developmental indices of IVF infants were within the normal range and did not differ from those of their matched controls	56
IVF births ($n = 258$) $v.$ normal references	6–13 years	Surgical procedures, malformation, height and weight, and school performance were comparable	69
Birth from cryopreserved embryos ($n = 158$), IVF births ($n = 160$) $v.$ spontaneous conception ($n = 156$)	0–18 months	Growth features, major malformations and the prevalence of chronic diseases were similar	55
IVF births ($n = 150$) $v.$ spontaneous conception ($n = 280$)	0–3 years	Infant mortality was > two-fold higher. The risk of low height (OR, 1.9; 95% CI, 1.1–3.2). Cumulative incidence of different diseases up to 3 years of age (OR, 2.1; 95% CI, 1.3–3.3) especially regarding respiratory diseases (OR, 3.1; 95% CI, 1.0–9.4) and diarrhoea (OR, 5.7; 95% CI, 2.6–12.7). No statistically significant differences were found in the psychomotor development	64
ICSI births ($n = 150$) $v.$ spontaneous conception ($n = 147$)	8 years	Pubertal staging, neurological examination, remedial therapy or surgery or hospitalization were similar	58
ART births ($n = 69$) $v.$ spontaneous conception (friends and siblings, $n = 71$)	4–10 years	IVF children were taller ($P = 0.001$), with higher levels of serum IGF-II ($P = 0.03$), higher IGF-I to IGF-binding protein 3 ratio ($P = 0.04$), higher high-density lipoprotein ($P = 0.02$), lower triglycerides ($P = 0.02$) and a lower total to high-density lipoprotein cholesterol ratio ($P = 0.01$). There were no differences in body composition	65
ICSI births ($n = 81$) $v.$ IVF births ($n = 81$) and spontaneous conception ($n = 85$)	5–8 years	Outcomes of children conceived by ICSI and IVF were comparable or even more positive for ICSI. Perinatal outcomes were poorer after ICSI than natural conception (prematurity: $P = 0.014$; low birth weight: OR, 7.4; 95% CI, 0.9–62.5)	59
IVF births ($n = 193$) $v.$ spontaneous conception from subfertile parents ($n = 199$)	0–4 years	Significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months, and weight SDS at 6 months of age, a greater gain in weight SDS ($P < 0.001$), height SDS ($P = 0.013$) and BMI SDS ($P = 0.029$) during late infancy (3 months to 1 year) in IVF children	68
ICSI births ($n = 276$) $v.$ spontaneous conception ($n = 273$)	5.5 years	No relevant differences regarding physical examination, the incidence of childhood illnesses, acute or chronic illnesses, accidents, and surgeries up to the age of 5.5 years. However, an increased risk of undescended testicles and urogenital surgeries were observed in ICSI boys	57
IVF births ($n = 143$) and ICSI births ($n = 166$) $v.$ spontaneous conception ($n = 173$)	0–12 years	No significant differences were observed regarding head circumference, height and weight between the three groups at any of the time points	62
Birth from cryopreserved embryos ($n = 43$), IVF births ($n = 72$) $v.$ spontaneous conception ($n = 94$)	3.5–11.0 years	IVF girls were taller, with increased insulin-like growth factor I concentrations compared with controls. More favourable lipid profiles were also evident in IVF children	67
ART births ($n = 433$) and births by ovulation induction/intrauterine insemination ($n = 535$) $v.$ spontaneous conception ($n = 2471$)	0–3 years	No significant differences in growth, motor and cognitive development	60,70
ART births ($n = 4333$) $v.$ spontaneous conception ($n = 295,220$)	<5 years	An increased risk of cerebral palsy (aOR, 2.22; 95%CI, 1.35–3.63)	15
ART births ($n = 3617$) $v.$ spontaneous conception ($n = 35,848$)	>4 years	An increased risk of cerebral palsy (hazard ratio 2.30; 95% CI, 1.12–4.73)	80
ICSI births ($n = 511$) and IVF ($n = 424$) $v.$ spontaneous conception ($n = 488$)	4–6 years	No significant differences in motor and cognitive development	81
ART births ($n = 33,139$) $v.$ spontaneous conception ($n = 555,728$)	4–13 years	No increased risk of autism spectrum disorders	86
349 cases $v.$ 1847 controls	>2 years	No increased risk of autism spectrum disorders	85
4164 cases and 16,582 controls	2–16 years	No increased risk of autism spectrum disorders	87

IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; aOR, adjusted odds ratio; OR, odds ratio; IGF, insulin-like growth factor; BMI, body mass index.

with or without intrauterine insemination with 2471 singletons conceived naturally, and found the growth and development of children up to 3 years of age was comparable.[60,70] Similar findings have been observed in ART children *v.* the general population up to 13 years of age in European countries.[61,62,69] A study in the United States also reported IVF young adults exhibited normal pubertal development.[71] A handful of studies have found ART children had impaired[63,64] or enhanced childhood growth.[65–67] Notably, some studies recruited children born prematurely, small for gestational age, with low birth weight or from multiple pregnancies, which may confound the results and few of these studies have controlled for subfertility. Ceelen *et al.* examined the growth data from birth to 4 years of age in a small follow-up study that included 233 IVF children aged 8–18 years and 233 spontaneously conceived controls born to subfertile parents. They showed IVF children had significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months, and weight SDS at 6 months of age compared with controls. IVF children demonstrated a catch-up growth during late infancy (3 months to 1 year) *v.* controls, such that no differences were observed in weight, height and BMI after 1 year of age between groups.[68] This is a small study, but potentially of concern, given evidence that rapid catch-up growth is associated with increased risk of disease later in life.[72,73]

A meta-analysis including four studies in singletons reported an increased risk of cerebral palsy for IVF children *v.* those conceived naturally.[74] This risk may be largely due to multiple births, low birth weight and preterm births among ART children.[75–80] In Australia, an increased risk of cerebral palsy was observed in ART infants overall and for ART singletons, even after adjusting for parental and fetal factors.[15] Similarly, Zhu *et al.*[80] found that ART infants had an increased risk of cerebral palsy after controlling for preterm birth and multiplicity, and there was no association between parental subfertility and the risk of cerebral palsy, indicating that the increased risk of cerebral palsy for ART infants was due to the effect of ART treatment.

No differences were observed in the cognitive and motor development in large cohort studies between ART children and controls examined at 3 or 5 years of age who were recruited in Europe,[81] Great Britain[82,83] or the United States.[70] Similarly, a systematic review of 59 studies reported that children born following ART are not at increased risk of severe cognitive impairment compared with naturally conceived children.[84] There is also no increased risk of autism in singletons conceived by ART,[85–88] but studies including IVF multiple births and autism spectrum disorders have shown conflicting results.[85,87] In contrast, Kissin *et al.*[89] found that the incidence of autism diagnosis in ART-conceived children during the first 5 years of life was higher when ICSI was used compared with IVF. Notably, Belva *et al.* showed that 54 ICSI-conceived adults had significantly lower sperm concentration, lower total sperm count as well as lower total motile sperm count but comparable mean levels of follicle-stimulating hormone, luteinizing hormone, testosterone and inhibin B in comparison to 57 spontaneously conceived peers, possibly reflecting inherited

fertility problems.[90,91] Taken together, the available data on the growth and development of ART children is generally reassuring, although an increased risk of cerebral palsy has been observed. This needs to be confirmed in large studies focussing on ART singletons born at term with normal birth weight. In addition, more follow-up studies in adults are warranted to determine if ART is associated with increased risk of impaired cognitive development and psychological adjustment, later in life.

Cancer risk in ART singleton pregnancies

Concerns are turning towards the longer-term health implications of IVF. A number of studies have been undertaken to examine the cancer risk of children conceived by ART procedures (summarized in Table 4). Most of the earlier studies demonstrate that ART procedures are not associated with increased risk of cancers.[92–95] For instance, one data linkage study that included 3528 ART singletons with a median follow-up period of 4 years showed that ART children did not have a significantly increased incidence of cancer.[92] Similar results have been noted in the Netherlands over an average follow-up period of 6 years[93] and in a meta-analysis of 11 cohort studies.[96]

In the last decade, more studies have reported an increased risk of certain cancers in ART children. In Sweden, although there was no overall increase in cancer risk in >16,000 ART children compared with naturally conceived children, more cases of Langerhans histiocytosis were reported.[97] After 5 years, the same group reported a moderately increased risk for all cancers in 26,692 children conceived by ART during the years 1982–2005.[98] Notably, the increased cancer risk was associated with high birth weight, premature delivery and the presence of respiratory diagnoses as well as low Apgar score.[98] A large retrospective Nordic population-based cohort study found ART children had an increased risk for central nervous system tumours and malignant epithelial neoplasms *v.* children born spontaneously between 1982 and 2007.[99] This cohort was matched for parity, year of birth and country, and controlled for maternal age, sex, gestational age and birth defects.[99] Similarly, ART was associated with an increased risk of hepatoblastoma and rhabdomyosarcoma, but not the overall risk of cancer in the United Kingdom.[100] This study also controlled for confounding factors such as sex, age at diagnosis, birth weight, singleton *v.* multiple birth, parity, parental age, type of assisted conception and cause of parental infertility. A meta-analysis, published in 2013, that included 25 cohort and case-control studies reported that children born after ART are at increased risk for all cancers (RR = 1.33; 95% CI, 1.08–1.63), and specifically for leukaemias (RR = 1.65; 95% CI, 1.35–2.01), neuroblastomas (RR = 4.04; 95% CI, 1.24–13.18) and retinoblastomas (RR = 1.62; 95% CI, 1.12–2.35). It should be noted that the majority of these studies did not control for confounders such as socioeconomic status, maternal smoking and perinatal health status, which may affect incidence.[98,101] Further, it is unclear that whether the increased

Table 4. *Cancer risk in assisted reproductive technologies (ART) singleton pregnancies*

Participants	Age	Outcomes	References
IVF births (*n* = 3305) *v.* the general population (*n* = 1,505,724)	0–14 years	No increase in childhood cancer	[94]
ART births (*n* = 3528) *v.* the general population	0–15 years	The standardized incidence ratio (SIR) 1.39; 95% CI, 0.62–3.09	[92]
IVF births (*n* = 332) *v.* the general population	5.2 ± 2.8 years	No increase in childhood cancer	[95]
ART births (*n* = 9484) *v.* spontaneous conception from subfertile parents (*n* = 7532)	1–14 years	No increased risk for childhood malignancies (risk ratio = 0.8; 95% CI, 0.3–2.3)	[93]
IVF births (*n* = 26,692) *v.* the general population	>2 years	The total cancer risk estimate was 1.42 (95% CI, 1.09–1.87)	[98]
ART births (*n* = 61,693) *v.* spontaneous conception (*n* = 351,536)	9.5 ± 4.7 years	An increased risk was observed for central nervous system tumours (adjusted HR 1.44; 95% CI, 1.01–2.05) and malignant epithelial neoplasms (adjusted HR 2.03; 95% CI, 1.06–3.89)	[99]
IVF births (*n* = 62,195) *v.* the general population	0–15 years	An increased risk of hepatoblastoma (SIR, 3.64; 95% CI, 1.34–7.93) and rhabdomyosarcoma (SIR, 2.62; 95% CI, 1.26–4.82)	[100]

IVF, *in vitro* fertilization; HR, hazard ratio.

risk for cancers is related to underlying subfertility of the parent, or the ART procedure itself.[102] More follow-up studies are needed to determine risk in children, as well as later in life.

Does ART increase the risk of chronic disease?

The long-term health implications of IVF are under-studied. Over the past decade, speculation is increasing that individuals conceived by ART may be at risk of developing metabolic syndrome, type 2 diabetes and cardiovascular disease, later in life.[103–107] To date these studies are small, and this evidence is not conclusive (summarized in Table 5). Discrepancies between studies may be due to differences in the ages investigated, study period, inclusion criteria of subjects, sample size, sampling of the comparison group, dietary intake and/or parental characteristics, as well as the ART technique employed. Ceelen *et al.*[103] reported an increase in body fat as assessed by skinfold thickness in IVF children who were matched for BMI. Post-pubertal IVF children in this study also had a trend towards increased body fat assessed by Dual-energy X-ray absorptiometry (DXA). Importantly, the control group studied were children who were born to subfertile parents and controlled for current size, birth weight, gestational age and parental characteristics.[103] Belva *et al.* reported pubertal ICSI singleton girls had increased central, peripheral and total adiposity assessed by circumferences, skinfolds and BMI, respectively, compared with naturally conceived controls. Furthermore, increased peripheral adiposity was observed in ICSI adolescent singleton boys with advanced pubertal stages *v.* controls.[108] Conversely, one study reported no difference in fat percentage by DXA between IVF children and controls at 4–10 years of age.[65] More studies in ART adults are required to assess the obesity incidence and the amount of liver and visceral fat which is clearly associated with increased risk of type 2 diabetes and cardiovascular diseases.[109]

There is some suggestion that ART may also impair glucose metabolism in the offspring, potentially as a result of increased adiposity. Ceelen *et al.*[104] reported IVF adolescents had elevated fasting glucose levels compared with controls, irrespective of any early life factors or parental characteristics. However, there was no significant difference in fasting insulin levels, and insulin sensitivity as measured by the homeostasis assessment model. Another study reported fasting glucose levels were higher among children aged 5–6 years old conceived through ovulation induction and ART compared with naturally conceived children from fertile couples.[110] Conversely,[105] there was no difference in weight, glucose, insulin, leptin, adiponectin, interleukin-6 or C-reactive protein in IVF children and controls. However, this study may be confounded by neonatal and parental factors. Another study reported IVF children were taller with significantly higher IGF1 serum levels.[65] Reduced peripheral insulin sensitivity was also observed in a small cohort of IVF young adults by using gold standard assessment hyperinsulinemic-euglycemic clamp, without any significant differences in fasting glucose or insulin levels compared with naturally conceived young adults.[107] However, only 14 IVF young adults were studied.

A number of studies suggest that ART may increase the risk of cardiovascular diseases. Celeen *et al.*[104] reported that systolic and diastolic blood pressure levels were elevated in IVF children *v.* children conceived naturally from subfertile couples, after controlling for early life factors and parental characteristics. Sakka *et al.*[105] also reported that children born by IVF had significantly higher systolic and diastolic blood pressure than controls. Elevations in blood pressure in IVF conceived individuals are not universally detected.[111]

Table 5. *Metabolic risk in assisted reproductive technologies (ART) singleton pregnancies*

Participants	Age	Outcomes	Limitation	References
233 IVF singletons (139 pubertal) *v.* 233 singletons (143 pubertal) born from subfertile parents	8–18 years	↑ Body fat, ↑ blood pressure, ↑ fasting glucose	Cross-sectional study	103,104
217 ICSI singletons (116 boys) *v.* 223 singletons (115 boys) born from fertile parents	14 years	↑ Body fat normal blood pressure	Body fat assessed by skinfolds; included participants born prematurely or small for gestation age; not controlling for subfertility	108,111
69 ART singletons (35 IVF, 34 ICSI) *v.* 71 singletons born from fertile parents (friends and siblings)	4–10 years	↑HDL, ↓triglycerides, ↑IGF-2, ↑height. Normal body fat and fasting glucose	Cross-sectional study; not controlling for subfertility; small sample size	65
28 ART singletons *v.* 220 singletons born from subfertile parents and 2240 singletons born from fertile parents	5–6 years	↑Fasting glucose	Small sample size; not controlling for subfertility	110
106 IVF (39 pubertal) *v.* 68 controls (30 pubertal) born from fertile parents	4–14 years	↑Blood pressure, ↑triglycerides, ↑TSH. Normal fasting glucose	Included participants born prematurely, small for gestation age and multiples; not controlling for subfertility	105,116
14 IVF *v.* 20 controls born from fertile parents	17–26 years	↓Peripheral insulin sensitivity	Small sample size; not controlling for subfertility	107
63 IVF singletons *v.* 79 singletons born from subfertile parents	4 years	↑ Blood pressure, ↑body fat	Small sample size; not controlling for subfertility	112
65 ART singletons *v.* 57 controls (friends and siblings)	7–18 years	Systemic and pulmonary vascular dysfunction	Cross-sectional study	106
54 ART singletons *v.* 54 controls (friends)	7–18 years	Right ventricular dysfunction	Cross-sectional study; not controlling for subfertility	115
100 ART fetuses *v.* 100 control pregnancies and 50 ART infants *v.* 50 controls	Fetuses 6 months	Cardiac and vascular remodelling at both time points	Not controlling for confounding factors	114
10 IVF newborns *v.* 10 controls	2–4 weeks	Subclinical hypothyroidism	Small sample size; not controlling for subfertility	117

IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection

There is evidence that the process of ovarian induction may be a contributing factor to increases in blood pressure since systolic blood pressure and subscapular skinfold thickness were elevated in IVF children *v.* children conceived by natural IVF (without ovarian stimulation) and subfertile couples who conceived naturally.[112] Blood pressure was also higher in children born to subfertile *v.* fertile couples.[110] In a more detailed investigation[106] ART children displayed systemic and pulmonary vascular dysfunction, that could not be explained by subfertility or ovulation stimulation because vascular function was not altered in children conceived naturally after ovulation stimulation and in siblings of ART children who were conceived naturally.[113] Further, another group conducted a prospective cohort study and found signs of cardiovascular remodelling in ART fetuses, and ART infants as compared with controls conceived spontaneously.[114] Right ventricular dysfunction has also been detected in children and adolescents conceived by ART.[115] Taken together, ART treatment, ovulation induction and subfertility may all contribute to adverse cardiovascular outcome in childhood,[110] and further pre-clinical studies are necessary to resolve some of the discrepancies reported, as these enable better control of the confounding factors.

Few studies have examined the effects of ART on lipid metabolism. Sakka *et al.*[105] found that IVF children had significantly higher triglycerides, without differences in total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, uric acid, apolipoprotein-A1, apolipoprotein-B or lipoprotein(a) values.[105] Conversely, a group in New Zealand[65,67] found more favourable lipid profiles in a pre-pubertal IVF children with higher HDL levels and lower triglyceride levels than in controls. More prospective follow-up studies in ART adults are required to determine if ART treatment alters lipid profiles in the offspring.

ART may also alter thyroid function. Sakka *et al.*[116] reported thyroid-stimulating hormone (TSH) levels were significantly higher in 106 IVF children *v.* 68 naturally conceived children aged 4–14 years. Seven IVF children, but no controls also had subclinical primary hypothyroidism. It is of note that four of these children were born prematurely with low birth weight, although statistically TSH levels was not associated with birth weight and gestational ages. Similarly, subclinical hypothyroidism was observed in 2–4-week-old IVF infants, born at term.[117] A cross-sectional study in China found that the levels of thyroid hormones including T4, FT4 and TSH were significantly increased in singleton IVF *v.* naturally conceived newborns and children aged 3–10 years old. Notably, the levels of T4 and FT4 of IVF children positively correlated with maternal serum levels of oestradiol during the first trimester of pregnancy.[118] Further, no statistical difference was observed between IVF children born from frozen embryo transfer and naturally conceived individuals.[118] This suggests that a high oestradiol maternal environment, resulting from ovarian stimulation, may increase the risk of thyroid dysfunction in offspring born following IVF. Further study is necessary.

How does ART increase the risk of adverse outcome?

In humans, it is difficult to separate out the effects of ART procedures themselves with the underlying subfertility, paternal characteristics as well as postnatal environmental exposure. Furthermore, if increases in risk are the result of ART techniques, which of these processes increase risk? Animal models suggest that ART procedures contribute to altered fetal and placental growth and development.[119] The developmental origins of health and disease hypothesis[120] proposes that suboptimal periconceptional and perinatal environment can impair fetal and postnatal growth, followed by catch-up growth, predisposes offspring to increased risk of developing hypertension, obesity, type 2 diabetes and coronary heart disease in later life.[121–123] Epigenetic is likely to be the reprogramming mechanism, but there may be other contributing factors including transcription changes, oxidative stress and mitochondrial dysfunction, and endoplasmic reticulum stress as reviewed elsewhere.[124]

Epigenetics is defined as heritable changes in gene expression without alterations in DNA sequence.[125] Epigenetic modifications, including DNA methylation, histone modifications, micro-RNAs and higher-order packaging of DNA around nucleosomes, regulate the temporal and spatial gene expression patterns and are essential in embryonic, fetal and postnatal development.[126] DNA methylation is the most widely studied epigenetic mechanism and occurs through the enzymatic addition of a methyl group to the carbon-5 position of the cytosine of the cytosine–phosphate–guanine dinucleotide sequence. The methyl group interferes with the binding of particular transcription factors to DNA and attracts methyl-binding proteins that also regulate transcriptional repression.[127] Hence, gene expression is generally inhibited by DNA methylation, but is activated by DNA demethylation.[128] DNA methylation also contributes to the preservation of chromosomal integrity and the inactivation of X-chromosome.[129] There are two waves of DNA methylation and demethylation during gametogenesis and early pre-implantation,[130] thus periconceptual manipulation of oocyte or blastocyst during IVF and ICSI treatment may impair the establishment of the DNA methylation in gametes and/or with the maintenance of DNA methylation within preimplantation embryos.[129]

In humans, there is evidence that ART procedures may alter epigenetic modifications during the preimplantation period of development. A high frequency of imprinted methylation errors was observed in ART human preimplantation embryos.[131] Altered DNA methylation and/or gene expression of a number of genes in the fetus, cord blood, placenta, neonatal bloodspots and buccal cell have been reported in ART children.[132–139] Notably, some of these genes whose expression altered by ART have been implicated in imprinting diseases and metabolic disorders such as obesity and type 2 diabetes. Song *et al.*[133] showed ART itself results in significant differences in placental DNA methylation levels by using donor

oocyte from fertile young women compared with fertile control groups. Another study suggests DNA methylation levels of 23 genes can explain around 80% of the variance in infant birth weight and six of these are associated with growth phenotypes in human or mouse models.[140] Therefore, altered DNA methylation in ART offspring may contributes to low birth weight which is a marker of impaired fetal growth and adverse long-term health outcomes.

Animal models also support that ART alters epigenetic modifications in preimplantation embryos and offspring, thereby altering embryonic growth, fetal and placental growth, growth trajectory, increases risk of metabolic and cardiovascular diseases later in life, and shortens lifespan.[107,141–149] Metabolic profiling in mice serum and microarray analysis of pancreatic islets and insulin-sensitive tissues (liver, skeletal muscle and adipose tissue) indicated systemic oxidative stress and mitochondrial dysfunction, which is associated with increased expression of thioredoxin-interacting protein (*TXNIP*) and enrichment for H4 acetylation at the *Txnip* promoter in blastocysts and adipose tissue in adult mice.[148] As *TXNIP* plays an important role in regulating peripheral glucose metabolism and integrating cellular nutritional and oxidative states with metabolic response, the data suggest that IVF results in epigenetic and gene expression changes in blastocysts that persist in adulthood.[148] Rexhaj *et al.*[149] reported that ART mice offspring show endothelial dysfunction, increased arterial stiffness and arterial hypertension as well as shortened life span fed with a high-fat diet. Moreover, male ART mice transmit vascular dysfunction to their progeny and the methylation of imprinted genes such as *H19* in the aorta is altered in ART mice and their progeny. Further, ART mice display increased DNA methylation of the promoter of the *eNOS* gene, decreased *eNOS* expression in the aorta and decreased plasma nitric oxide concentration. Importantly, all these alterations can be normalized by administration of the deacetylase inhibitor butyrate or addition of antioxidant melatonin to culture media, suggesting that altered epigenetic modification by ART causes vascular dysfunction in mice.[149,150]

Placenta plays an important role in fetal development by transporting nutrients and oxygen, adapting morphologically and functionally to adverse environmental stress and minimizing their impact on the fetus.[151,152] Placenta size can predict cardiovascular diseases and insulin resistance.[153,154] ART may also impair placental development and function and thus fetal growth in utero.[141,142,155,156] Increased placental thickness and placental haematomas as well as pathological findings were reported in ART pregnancies.[157–159] IVF impairs placental nutrient transport and metabolism in mice.[141,144,156] Placental weight and placental:fetal weight ratio was significantly higher in ART pregnancies than in naturally conceived pregnancies in humans and mouse models.[119,155,156,160,161] This was associated with reduced methylation levels and altered genomic imprinting and developmental gene expression by ART treatment in the placenta in mice and humans.[132,138,141,142,144,155] These altered DNA methylation levels may impair a number of biological

processes and functions during IVF placentation, including actin cytoskeleton organization, haematopoiesis, placental growth and vascularization, energy metabolism and nutrient transport.[144,162] Improper adaptive responses of placenta throughout pregnancy may result in adverse outcomes such as abortion, preeclampsia or intra-uterine growth restriction.[162] Although successful placental adaptation leads to normal progress of the pregnancy, the memory of epigenetic adaptation mechanisms established during pregnancy increases the risk of developing metabolic diseases later in life.[162] Taken together, impaired placental function and development due to altered DNA methylation may play a key role underlying the adverse outcomes in ART offspring. However, more studies are needed to examine whether other epigenetic mechanisms such as histone modifications and micro-RNAs during the development of preimplantation embryos are altered by ART.

Future directions to improve health of ART children

The perinatal outcomes in children born after ART have improved over time, mainly as a result of single embryo transfer and frozen thaw embryo transfer.[163,164] As already practiced in many countries, single embryo transfer clearly reduces many of the risks associated with ART procedures, including improved perinatal outcomes, without compromising live birth rates.[165,166] The impact of hormone stimulation on perinatal and longer-term outcomes is under increasing scrutiny. The available evidence indicates that frozen embryo transfer may improve outcomes for both patients, and especially women with polycystic ovary syndrome and infants.[24,167] However, large well-controlled trials to determine if freeze-all protocols have benefits to the general infertile population and the later metabolic health in ART children are still needed. Further pre-clinical and large epidemiological studies from around the globe, that collect data to control for as many potential confounders as possible, are needed to compare ART techniques including frozen embryo transfer, stimulated *v.* unstimulated IVF cycles and the embryo culture lengths for not only the optimal perinatal outcomes, but for long-term health.

Conclusion

Concerns remain over the health and development of ART babies. Multiple pregnancies, due to multiple embryo transfer, increase the health risks, but ART singletons are also at increased risk of adverse obstetric and perinatal outcomes, increased risk for birth defects as compared with singletons conceived spontaneously. Further studies are needed to confirm if ART singletons have an increased risk of cancers, and cerebral palsy. Although accumulating data suggests that individuals conceived by ART may also have an increased risk of ageing-related chronic metabolic disorders, the evidence to date is obtained from pre-clinical studies, or small human cohorts. Thus large scale, well-controlled epidemiological studies are necessary. Greater work is also necessary to identify whether the increase in obstetric, perinatal and health impacts observed in

ART children are the direct result of the ART procedure itself, or a result of the underlying subfertility of the parents. Although evidence suggests that altered DNA methylation and impaired placental development may contribute to the adverse outcomes in ART children, more studies are needed to examine whether altered epigenetic regulations are the underlying mechanism or the consequence of aberrant embryo development. As genetics and many parental characteristics cannot be altered, careful further study to identify the optimal ART procedures that maximize both perinatal and long-term health outcomes are necessary.

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