

Congenital Anomalies Following Assisted Reproductive Technology

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Introduction

Most of the 5 million children that saw the light thanks to assisted reproductive technologies (ART) were born healthy. The incidence of major congenital birth defects in this group of neonates is low: 4% to 6% [1]. Yet, that is 30% higher than the incidence of congenital anomalies in children born after spontaneous conception [2]. The increased risk for congenital anomalies after ART is not only the subject of ongoing scientific research, but is also an issue in public debates about these techniques [3].

For physicians, it is often unclear whether various ART processes hold different risks and which congenital birth defects can be expected. What concrete associations have been studied?

For scientists, the question whether the subfertility of the couple that conceives after medical intervention accounts for this observation or whether intervening with natural conception itself augments the incidence of congenital birth defects remains unanswered. Should we adapt our techniques and could a decrease in the incidence of congenital anomalies thus be expected?

For future parents, birth defects are one of many concerns when considering ART [4]. They will ask their doctor about it. It is important that physicians can provide a balanced answer.

The following chapter aims to guide the reader through the important questions on the association of congenital anomalies and ART; from bedside to bench and back again.

From Bedside ... : What Do We Know?

In 1978, Louise Brown was the first to be born after in vitro fertilization (IVF). Soon, IVF became widely used and the assisted reproductive technologies developed into a broad spectrum of therapies. At present, different forms of ART can be distinguished along the reproductive cycle (Figure 2.1):

1. In vivo: intra-uterine insemination
2. In vitro:
 - 2.1. In vitro fertilization
 - 2.2. Intra-cytoplasmic sperm injection (ICSI)
 - 2.3. Blastocyst transfer
 - 2.4. Other considerations:
 - 2.4.1. Cryopreservation
 - 2.4.2. Preimplantation genetic diagnosis and extended maturation

Children born after each of these ARTs are sometimes followed-up and sometimes contribute to research programmes.

Lancaster was the first to raise concerns on the possible link between ART and congenital anomalies [5]. His 1987 *Lancet* paper reporting an increased incidence of spina bifida and transposition of the great arteries in neonates born after IVF initiated a still ongoing search to try and understand this link, but other studies over many years have failed to find a link.

The novelty of the technology and the subsequent small patient groups often prevent scientists from reaching significant conclusions. Furthermore, many studies are restricted by the absence of an appropriate control group of spontaneously conceived children.

Additionally, some studies do not use standardized definitions of birth defects. The use of heterogeneous groups might therefore mask associations with specific defects. Moreover, important sampling errors are introduced when comparing clinical data from the ART group with “population” data from local schools, thus excluding the spontaneous conceived children with severe congenital birth defects that may not attend standard schools [6].

Notwithstanding these difficulties in studying the association between ART and congenital anomalies, studies have built up in recent years leading to some good-quality meta-analyses.

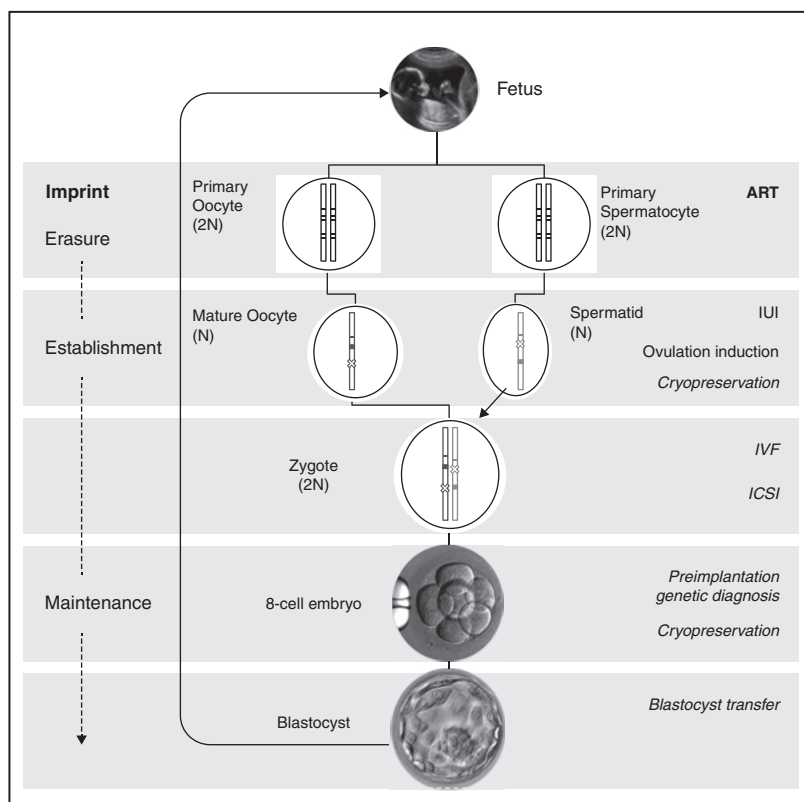


Figure 2.1 Overview of the reproductive and imprinting cycle (paternal genome; maternal genome) with the stage in which ARTs intervene. IUI, intra-uterine insemination; IVF, in vitro fertilization; ICSI, intra-cytoplasmic sperm injection. Stages in italic require in vitro culture.

An increased risk of congenital anomalies has been consistently shown in children born to parents after assisted reproduction. In most cohorts, the combined corrected typical odds ratio for overall risk is 1.3 [7]. Four to six percent of the ART cohort has a congenital birth defect, a moderate increase over the estimated background risk of 3%–4% in spontaneously conceived children [1]. The combined odds ratio of imprinting disorders (see page 18 for further details) in children conceived through ART is around 3 in comparison with spontaneously conceived children [8].

We now discuss ART categories as either birth defects NOS (A) or specific imprinting disorders (B).

(A) Congenital Birth Defects

Intra-uterine Insemination

Intra-uterine insemination (IUI) is widely used for assisted conception in case of sub- or infertility or ejaculatory and coital problems. The technique implies washing of the sperm and ovulation induction. Conception occurs in utero. IUI is regarded as a simple and low-invasive technique compared to other

ARTs allowing “spontaneous” fertilization after minor assistance [9].

The latter possibly explains the paucity of studies into the outcome of children conceived by IUI. Although the technique has been frequently applied to date and several studies on congenital anomalies after IUI are available, only two have a large sample size and appropriate comparison group [10, 11]. Surprisingly, in spite of the minimal invasiveness of the technique, conception through IUI doubled the risk for major birth defects compared to naturally conceived singletons. Even more, this risk was not different from that observed in neonates born to IVF-treated couples.

In Vitro

In Vitro Fertilization

In vitro fertilization (IVF) is the most studied of all ART. Unlike many other ART, not only has the general risk for congenital anomalies in children born after IVF been studied, but also the association of IVF with specific birth defect rates per system (Figure 2.2).

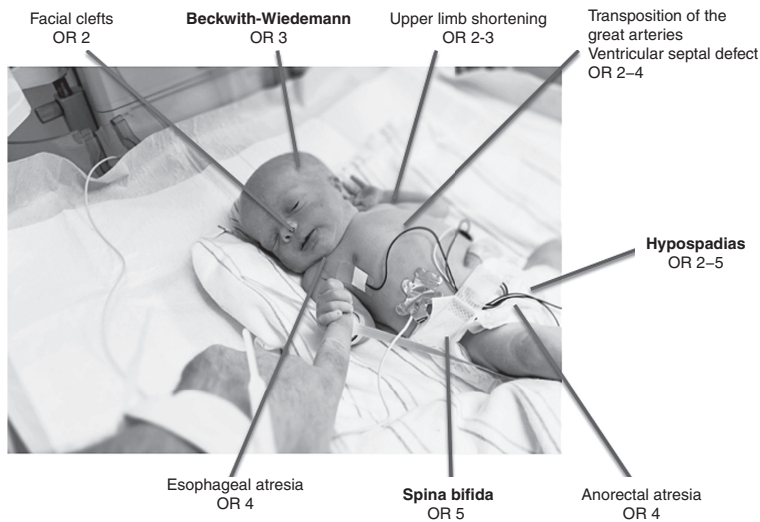


Figure 2.2 Specific birth defects associated with ART consistently reported in literature. For each defect the reported adjusted odds ratio (OR) is shown. Strong associations are bold.

Cardiovascular System When Lancaster raised the first concerns about children born after assisted reproduction, he found a fourfold increased risk for transposition of the great arteries [5]. Tararbit *et al.* broadened the spectrum to other cardiac malformations. They showed not only an adjusted odds ratio of 1.7 for defects of the outflow tracts (e.g. transposition of the great arteries, but also truncus arteriosus and tetralogy of Fallot), but also of ventriculoarterial connections such as pulmonary stenosis in IVF children compared to spontaneously conceived neonates [12]. Cardiac neural crest defects and double outlet right ventricle were also more frequent in the ART group. Reefhuis *et al.* and Källén *et al.* reported more septal heart defects [13, 14].

Nervous and Musculoskeletal System The link between IVF and an increase in the prevalence of neural tube defects especially with spina bifida is strong. From the first follow-up reports on children born to mothers treated with IVF, a fivefold higher incidence of spina bifida has been described [5, 14]. Part of the explanation for this association might be that parents from IVF children with antenatally diagnosed anomalies are less likely to terminate [14].

An upper limb reduction is the musculoskeletal anomaly most frequently but not consistently cited [10, 14].

Head and Neck The association of IVF with a cleft lip with or without cleft palate has been reported by a large follow-up study after IVF conception in the United States [13], but was not significant in a European setting [15].

Years ago, gastroschisis was linked with ovarian stimulation [16]. In an effort to explain this finding, recent work using animal models suggested that environmental disturbances during gestation might largely impact the DNA repair and chromatin remodeling capacities of the oocyte and preimplantation embryo and cause craniofacial defects in the next-generation offspring [17].

Gastrointestinal System An important association between IVF and birth defects is the occurrence of digestive tract atresias. Esophageal atresia and anorectal atresia have been reported up to four times more frequently in children born after IVF [13, 15].

Genitourinary System The strong association of ART and urogenital anomalies and specifically hypospadias largely depends on the increased risk for disturbances in the external urethral orifice after ICSI conception.

Intra-cytoplasmic Sperm Injection

ICSI is commonly used to overcome male infertility problems. The technique has been consistently shown to be associated with hypospadias. Odds ratios vary between 1.5 [13, 18] and 5 [19].

Given the strong link of hypospadias with ICSI specifically, research has focused on finding an explanation for this increased birth defect risk. Heritable low testosterone could be an explanation. The latter predisposes to hypospadias and also adversely affects spermatogenesis, thus making men with low testosterone more likely to need ICSI.

Low testosterone levels were found in male infants born after ICSI. Yet in adolescence this difference seems to have resolved [20].

Blastocyst Transfer

Blastocyst culture and day 5 embryo transfer for IVF allows more specific selection of embryos for transfer and reduces the risk of multiple pregnancy [21], thus potentially reducing chances of intra-uterine growth retardation and premature births. To our best knowledge, however, there are no studies into the congenital anomalies seen in children born after blastocyst transfer.

Other Considerations

Not only the ART itself but also the in-vitro environment might have an impact on the anomalies seen. Cryopreservation of oocytes or embryos and preimplantation genetic diagnosis change the environment of conception. Does it affect the outcome of children born after these interventions?

Cryopreservation Neither benefit nor harm can be shown for cryopreservation when it comes to the risk for congenital anomalies. Several studies followed a large cohort of children born after oocyte cryopreservation. No apparent increase in birth defects was seen [22]. Data on embryo preservation are also reassuring [23].

Preimplantation Genetic Diagnosis (PGD) and Extended Maturation PGD seeks to reduce the risk for monogenic disorders such as cystic fibrosis, chromosomal aneuploidies, and translocations associated with older maternal age and late-onset diseases with genetic predisposition such as BRCA-breast cancer.

However, this comes with the price of having to remove one or more blastomeres. PGD involves an ICSI procedure and an embryo biopsy. It could thus be considered the most invasive ART commonly used.

Data on the neonatal outcome of children born after PGD are scarce. The UZ Brussels Fertility Clinic has the largest cohort worldwide and recently showed comparable outcomes in neonates born after ICSI or PGD [24]. Follow-up through the PGD consortium showed consistent findings [25]. PGD does not result in an increased risk for birth defects compared to other ARTs.

(B) Imprinting Disorders

An embryo reaches diploidy by receiving one copy of each gene from the mother and one from the father (cf. Figure 2.1). For most genes, both the paternal

and maternal alleles are expressed. For a minority of genes, however, either the maternal or paternal allele will be expressed. An epigenetic process silences the other allele: genetic imprinting. If the maternal allele is imprinted, it is silenced. The paternal allele is then solely expressed. A DNA mutation or an “epimutation” (disrupted methylation) can activate the extra gene, resulting in two active copies of the gene and specific syndromes.

In humans, nearly 100 genes have been identified that require imprinting. They are all vital in normal prenatal growth and development.

For example, imprinting occurs in chromosomal region 11p15. The *IGF2* gene, a growth promoter, is paternally expressed. Methyl tags (methylation) normally silence the maternal *IGF2* gene.

This change in the ultrastructure of the DNA is a dynamic process. It is erased and re-established through each generation so that genes that are imprinted in an adult may still be expressed in that adult's offspring.

If this imprinting fails, for example through inheriting a maternal chromosome 11 with a deletion of the 11p15 region (DNA mutation) or loss of methylation (epimutation), specific imprinting disorders occur. In this case the child will develop hypotonia, obesity, and hypogonadism, characteristics of the Beckwith–Wiedemann syndrome (BWS).

ART intervenes at the critical moment in setting the genetic imprint (cf. Figure 2.1). In mice, ART procedures have been shown to interfere with normal DNA methylation, parental imprinting status, and imprinted gene expression [26].

In men, the association of ART with specifically BWS has been studied. Although not consistently shown [27–28], several authors suggest an approximately threefold higher prevalence of ART use among children born with BWS compared to the general population [29, 48].

Sutcliffe *et al.* looked into the disease causing mechanism of children with Beckwith–Wiedemann syndrome and Angelman syndrome. In significantly more ART children epigenetic mutations causing the syndromes were found [29].

The association with other imprinting disorders such as Prader–Willi syndrome is less clear.

Clinical Implications

From the information above, clear clinical implications can be deduced.

Clinicians should realize the background risk for congenital anomalies in children conceived after ART is altered. Detailed prenatal screening of specific organ systems, such as for the cardiovascular and genital system, will allow early detection of defects that are known to be increased in children born after ART.

Furthermore, certain fetal ultrasound findings have been shown to have different potential implications in the ART cohort. Wilkins-Haug *et al.* noted the link between omphalocele and an underlying Beckwith–Wiedemann syndrome. Half of the isolated omphalocele fetuses conceived after ART had BWS versus only 4% of the spontaneously conceived fetuses [30].

Hui consistently found an increased nuchal translucency in assisted reproduction pregnancies, possibly because of some delay in fetal development [31]. This needs to be considered when screening for chromosomal abnormalities. Five percent of ART pregnancies screened for Down syndrome by nuchal thickness were false-positive versus only 4% in spontaneously conceived fetuses.

Finally, clinicians should take into account that couples conceiving after ART make thoroughly different choices when it comes to antenatal screening and birth defects. Women will more often attend the first-trimester screening and are followed-up in highly specialized centers, probably explaining the earlier prenatal diagnosis of anomalies [12]. Yet they are far more reluctant towards prenatal diagnosis procedures that might induce miscarriage [32].

... To Bench ...: Is It the Technique or the Parental Subfertility?

The rate of birth defects after ART is augmented. This finding is often brought forward in discussions on the techniques [33]. Various studies have sought to clarify the underlying cause of this association, looking both at the technique applied and the patient cohort the technique is applied to. Although in recent years vast scientific data on the subject have become available, there is no definite answer at present.

Is It the Technique?

It is difficult to contrast different ARTs as all have specific indications and populations vary between centers.

However, data comparing the available options in each stage of the fertilization are available. These

studies all analyze couples conceiving with the help of reproductive therapies.

Firstly, the sperm used for ART can be ejaculated, epididymal or testicular. No difference in the neonatal outcome depending on the origin of sperm could be shown [34].

Secondly, different modes of conception can be applied. In all meta-analyses, no overall difference in ART could be retained [10, 11, 35–37], although caution for specific subgroups must be obeyed [38].

The embryo that is subsequently transferred can be fresh or frozen. Wennerholm *et al.*, Pinborg *et al.*, and Maheshwari *et al.* found the incidence of birth defects was no different after the transfer of embryos that were first cryopreserved or the transfer of fresh embryos [39–41].

Finally, some centers remove one blastomere for performing genetic counseling. Again, this could not be retained as inducing more congenital anomalies.

As studies into the rough technique could not account for the increase in birth defects found, research now increasingly focuses on epigenetic changes during the conception.

We hypothesize that the study of epigenetics is a very promising path into elucidating the potential mechanisms. After all, specific aspects of ART could interfere with epigenetic reprogramming during gametogenesis, and imprinting largely occurs in early embryonic development [26, 42].

Studies into imprinting disorders such as Beckwith–Wiedemann syndrome show data in support of this hypothesis (Figure 2.3).

Most of the patients with BWS born after ART are found to have a loss of methylation of the paternal or maternal DNA, thus having an epimutation. In spontaneously conceived children the syndrome is often caused by Mendelian abnormalities (e.g. DNA mutations, translocations, etc.). Epigenetic changes are found in less than half of the patients.

In addition, epigenetics are more and more cited as disease-causing for the congenital birth defects that ART is associated with, but so far have not previously been linked with the techniques through epigenetic phenomena.

Spina bifida, for example, is strongly related with ART. Its prevalence has been shown to be diminished by periconceptional folic acid supplementation leading to a prevention campaign throughout the Western world. A folate-dependent one-carbon metabolism provides methyl groups for DNA methylation.

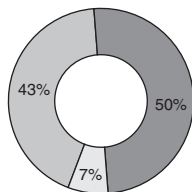
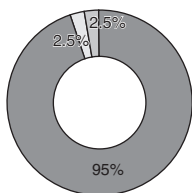
BWS after spontaneous conception**BWS after ART**

Figure 2.3 Of patients with Beckwith–Wiedemann syndrome born after ART, 97% are found to have an epimutation paternally (dark gray) or maternally (medium-dark gray). In spontaneously conceived children, the syndrome is more often caused by Mendelian abnormalities (light gray). Based on [44–47].

Variations in DNA methylation of genes have been shown to interfere with the development of the neural tube. If ART changes the DNA methylation (folic acid-independently), this could be the explanation why even with folic acid supplementation the incidence of spina bifida is still higher in children born after assisted reproduction compared to spontaneously conceived neonates [49].

Is It Parental Subfertility?

Most reports assessing the link between ART and congenital abnormalities compare neonatal outcomes of ART pregnancies with spontaneous pregnancies in fertile couples. The fundamental effect of parental subfertility is not taken into account [13, 14, 35].

Two ways of overcoming this hurdle seem plausible.

Zhu *et al.* and Simpson studied neonates born to subfertile parents that eventually conceived without ART [11, 19]. Children born to these couples were shown to have an elevated, but smaller, risk for congenital anomalies too.

Along with subfertile couples that conceive naturally, assessing the outcomes of fertile couples that conceive after ART, e.g. couples at risk of genetic disorders that opt for PGD, will provide valuable information. It would be a major asset to our understanding of the impact subfertility has in the association with

congenital anomalies. However, the number of such couples is relatively small, and to our knowledge no large studies looking into these couples have been carried out yet [43].

When considering parental subfertility, epigenetics also enter the discussion. Recent research suggests that transgenerational epigenetic inheritance could be present. Epimutations may thus be passed on from subfertile parents to children born after ART [50].

This finding sheds a new light on the duality in this discussion: it shows we will not easily be able to attribute the increased risk to either the technique or the subfertility of the couple itself. It will be impossible to make a full distinction between nature and nurture. Both probably play a major role in ART-related health outcomes.

... And Back Again: What Should We Tell the Patients?

The increased risk for birth defects is a concern for many future parents going through the process of conceiving with ART, and reports in the lay press are often unrefined. It is of major importance that a physician can provide a nuanced answer on the risks and benefits of the techniques.

Is There an Increased Risk for Birth Defects?

Yes, a modest increased overall incidence of congenital anomalies has been consistently shown in the group of children born after ART. The increase is 30%. This augments the risk from 3% (population risk) to 4% in ART children.

However, subfertile couples conceiving without ART have a 20% increase in congenital anomalies. The increased risk for birth defects seems thus to be largely dependent on the subfertility of the couple.

ART such as PGD also enables reducing the risk for monogenic disorders, chromosomal aneuploidies, and translocations and late-onset diseases with genetic predisposition.

What Defects Are We Talking About?

The literature agrees that there is an increased incidence of hypospadias in children after ICSI. Less markedly, cardiac outflow tract defects, spina bifida, and imprinting disorders such as Beckwith–Wiedemann syndrome have also been shown to be associated with ART. Extensive research has been

undertaken to try and understand the mechanisms that underlie this association. Insight in, for example, the methylation of imprinted genes and the epigenetic alterations that in-vitro procedures induce seems to be of major importance. The underlying parental subfertility and its risk for birth defects play an important role as well.

Does It Matter Which ART is Chosen?

Limited information on the difference in outcome between different ARTs is available. However, so far there is no evidence for a major difference between IUI, IVF, ICSI, and PGD. Any intervention in the normal conception increased the risk for anomalies. In specific subgroups, however, significant differences can be found, e.g. the increased risk for hypospadias in ICSI boys.

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