

Safety of assisted reproduction, assessed by risk of abnormalities in children born after use of *in vitro* fertilization techniques

Joseph P Alukal* and Larry I Lipshultz

SUMMARY

Assisted reproductive technologies are increasingly used in the treatment of both male and female infertility. The techniques, including *in vitro* fertilization, with or without intracytoplasmic sperm injection as an adjunctive treatment, represent a tremendous step forward for infertile couples who previously had no treatment options. As we move towards the 30th anniversary of the birth of the first baby conceived by *in vitro* fertilization, questions about the safety of these procedures linger. We review here the available literature regarding the safety of assisted reproductive technologies; these data are made far more robust by the inclusion of long-term follow-up data from the first generation of children arising after the introduction of these technologies.

KEYWORDS assisted reproductive technologies, infertility, intracytoplasmic sperm injection, *in vitro* fertilization, pregnancy

REVIEW CRITERIA

PubMed and MEDLINE were searched to identify English-language studies regarding use of assisted reproductive technologies and congenital abnormalities published from January 1950 to July 2007. The search terms were: "assisted reproductive techniques", "*in vitro* fertilization", "intracytoplasmic sperm injection", "congenital malformation", "behavioral disorder", "developmental delay", "hormonal abnormality", "genetic disorder", and "epigenetics". We also searched the abstract handbooks of the European Society of Human Reproduction (ESHRE) and the American Society for Reproductive Medicine (ASRM). Other articles we deemed important that were not returned in the original search were also included.

JP Alukal is a Fellow in and LI Lipshultz is Professor in and the Chair of the Division of Male Reproductive Medicine and Surgery in the Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA.

Correspondence

*Scott Department of Urology, Baylor College of Medicine, One Baylor Plaza, N730 Alkek Building, Houston, TX 77030, USA
alukal@bcm.edu

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INTRODUCTION

Since the first live birth resulting from *in vitro* fertilization (IVF) in 1978,¹ assisted reproductive technologies (ART) have increased in technical complexity and frequency of use. In 2003 alone, 365,103 IVF treatment cycles were undertaken in Europe and 122,872 were performed in the US.^{2,3} These cycles resulted in more than 60,000 live-birth deliveries and almost 100,000 infants.^{2,3} In the US, these numbers represent an increase of more than 100% since the onset of data collection regarding ART in 1996.⁴

As these techniques are used more frequently, questions regarding the safety of ART become ever more important. Retrospective data, both from Europe and the US, demonstrate that ART is generally safe. However, evidence indicates significantly increased risks of multiple gestation, preterm delivery (even in singleton pregnancy), and congenital abnormalities.^{5–7} While these events are rare even in the ART population, the increased likelihood of these events, when compared with that for spontaneously conceived children, is consistent across numerous series.⁸

Unique dilemmas exist with regard to understanding the clinical implications of these data. Are the risks incurred with ART causally related to the procedures themselves, or do they derive from some combination of parental genotype and the nature of the treatment as necessitated by parental phenotype? Specifically, patients with impaired fertility often require the transfer of multiple embryos in order to have a reasonable chance of achieving a live birth; what portion of the risk associated with ART derives from this fact and the resultant likelihood of multiple gestation? Do the procedures necessary for harvesting oocytes from infertile female patients (i.e. ovarian hyperstimulation) also contribute to this risk? Finally, insufficient time has passed since the development of ART to enable the follow-up of multiple cohorts of offspring through to adulthood; what will the evaluation of future generations of ART offspring tell us?

Box 1 A glossary of relevant terminology concerning assisted reproductive technologies.¹⁰

Assisted reproductive technology (ART)

All treatments or procedures that include the *in vitro* handling of human oocytes and sperm or embryos for the purpose of establishing a pregnancy, such as *in vitro* fertilization and transcervical embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy. ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or a sperm donor.

Birth defect

Structural, functional, or developmental abnormalities present at birth or later in life, due to genetic or nongenetic factors acting before birth.

Clinical pregnancy rate

The number of clinical pregnancies expressed per 100 initiated cycles, aspiration cycles, or embryo transfer cycles (denominator, which must be specified when citing rates).

Delivery rate

The number of deliveries expressed per 100 initiated cycles, aspiration cycles, or embryo transfer cycles (denominator, which must be specified when citing rates), including all live births and stillbirths. Each singleton or multiple pregnancy is registered as one delivery.

Embryo transfer

Placement of embryo(s) in the uterus or fallopian tube.

***In vitro* fertilization**

Extracorporeal fertilization for ART use.

Infertility

Failure to conceive after at least 12 months of unprotected coitus.

Intracytoplasmic sperm injection

Injection of a single spermatozoon through the zona pellucida into the oocyte.

Preimplantation genetic diagnosis

Screening of cells from preimplantation embryos for the detection of genetic and/or chromosomal disorders before embryo transfer.

Preterm birth

Birth between 20 and 37 completed weeks of gestation, including live and stillbirths. Each singleton and multiple birth is counted as one birth event.

Unfortunately, there are real barriers to answering these questions through either retrospective or prospective assessments of any sort. Register-based studies considering substantial numbers of children exist, but they are subject to inconsistent

reporting of defects. Conversely, prospective or retrospective individual follow-up studies typically return large volumes of data on individual patients, but they cannot account for participant bias and other causes of incomplete follow-up. Register studies and individual follow-up studies share other additional problems.

First, it is difficult to define an appropriate control population in any study, given the fact that comparing fertile and infertile patients introduces the variability between the two groups that exists at baseline. A more appropriate control group than the commonly used control cohort of age-matched fertile patients would probably consist of infertile patients who conceived spontaneously, but even this is not ideal; the control cohort shows some meaningful difference from the ART cohort, as evidenced by the fact that they did not require ART in order to get pregnant.

Second, powering studies to determine truly significant differences is difficult, given that both ART events and birth defects are rare compared with the overall total number of pregnancies and live births each year.⁹ As a result, many of the data that are generated from these studies are clouded with uncertainty. Given that uncertainty, patients are, rightly, confused as to how to proceed when faced with reproductive choices. This decision is made even more difficult by the emotional burden of infertility and the financial cost of the procedures in question.

In this Review we summarize the existing literature regarding the specific risks associated with ART, including up-to-date resources wherever possible. By definition, ART includes any method of initiating pregnancy that employs the deliberate manipulation of both oocytes and sperm outside the human body. Techniques include IVF, with or without intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer, zygote intrafallopian transfer, and those that are used in conjunction with the above, such as preimplantation genetic diagnosis or usage of donor gametes (Box 1). Importantly, the WHO International Committee for Monitoring ART does not include intrauterine insemination in this category.¹⁰ Consequently, data regarding intrauterine insemination are not included here.

RISK OF MULTIPLE GESTATION

In the US, the incidence of multiple-birth deliveries resulting from spontaneously conceived pregnancies is approximately 1.5%; however, more than 50% of ART pregnancies result in

multiple-birth deliveries.⁴ Multiple gestations incur well-known risks, including preterm delivery, low birth weight, and increased perinatal mortality. Much of the reported differences identified in ART offspring cohorts are thought to derive from the risk of multiplicity.⁵ In contradiction of this theory, however, several meta-analyses have demonstrated an increase in perinatal complications even in singleton pregnancies resulting from IVF.^{11–14} Each of these studies reported significant differences in odds ratios for outcomes, including low birth weight, preterm labor (<36 weeks), and use of hospital resources (i.e. the need for surgery or admission to a neonatal intensive care unit). In all cases, the IVF/ICSI cohort, regardless of multiplicity, had higher likelihoods of each of these conditions.

The overall risk of both adverse pregnancy outcomes and perinatal mortality is consistently higher in ART series than in series of natural conceptions; this effect again is present regardless of multiplicity (although trends suggest improvement in most series after correction for singleton pregnancy; Table 1^{5,15–28}). A study from Finland reported an odds ratio of 1.85 (95% CI 1.40–2.44) for perinatal mortality among all ART births, regardless of single or multiple gestation, when compared with the control group.²² This result was consistent in singleton ART pregnancies, where the odds ratio for perinatal mortality was 1.32 (95% CI 0.88–2.98) when compared with controls. Among IVF children, stillbirths were significantly more common and mortality rates up to age 2 years were twofold higher; again, this finding was independent of single or multiple gestational status.²²

Work by Pinborg and colleagues,^{5,18} from the Danish National Patient Registry, showed a somewhat different picture; multiple studies consistently demonstrated a higher likelihood of adverse outcomes in multiple gestational pregnancies, but found no such trend in IVF/ICSI singleton pregnancies. Importantly, other work from this same group addressed the inconsistencies between their own findings and those in meta-analyses. The authors postulated that vanishing twin syndrome in these pregnancies has had a causal role in the poor outcomes observed by researchers when considering IVF/ICSI singleton pregnancies.²⁰ Another study suggests that this risk relates directly to the number of embryos transferred.

In a multicenter, prospective, randomized, controlled trial, participants received a single embryo followed by another frozen embryo if no live birth resulted from the first transfer, or a double embryo transfer. Pregnancy rates were not substantially lower with the single transfer, while the likelihood of multiple births was dramatically reduced.²⁴

Efforts are now being made throughout Europe to decrease the number of embryos transferred during ART cycles, thereby minimizing the risk of multiple gestations. Multiple large registry studies have shown that widespread use of single-embryo transfer has significantly decreased the incidence of twin, triplet and higher-order deliveries while maintaining an acceptable success rate (Table 1). Even with regard to this important conclusion, there is still some debate about the use of this technique. The argument that obstetric outcomes might not have been improved by single-embryo transfer is outlined in a retrospective analysis of the Finnish Medical Birth Register.²³ This study reported significant increases in odds ratios for gestational hypertension, placenta previa, cesarean section, and preterm birth in the single-embryo-transfer cohort compared with those in fertile controls conceiving spontaneously. The authors in this study do offer the caveat that they used single-embryo transfer in patients who were at high risk of obstetric complications in addition to the cohort normally selected for this therapy—that is, patients with favorable predictors of fecundity, such as being younger than 35 years and with no previous IVF cycles. The authors justify this selection bias by pointing out that single-embryo transfer should ultimately be assessed for safety and efficacy in all female patients presenting for ART and not just in a selected cohort of patients in whom the treatment is more likely to be successful. They argue further that, even in Europe, an inadequate number of women are being offered single-embryo transfer. This conclusion is borne out by the fact that only 15.7% of ART procedures performed in Europe during 2003 used single-embryo transfer.²

RISK OF DEVELOPMENTAL DELAY AND NEUROLOGICAL IMPAIRMENT

Numerous publications have attempted to quantify the developmental delay and neurological status of children conceived using

Table 1 Association between assisted reproductive technology, multiple gestation, single embryo transfer, and poor perinatal outcomes.

Study	Years studied	Study type and sample size	Findings
Dhont <i>et al.</i> , ¹⁵ Belgium	1991–1995	Registry, controlled: 1,263 SC, 426 ART	ART: ↑ multiple pregnancies ↑ incidence preterm birth and low birthweight
Gerris <i>et al.</i> , ¹⁶ Belgium	2000–2001	Prospective, controlled: 367 ART cycles; 136 live deliveries	SET: ↓ neonatal cost
Pinborg <i>et al.</i> , ¹⁷ Denmark	1995–2000	Questionnaire controlled: ^a 2,238 children (634 IVF/ICSI singletons, 472 IVF/ICSI twins, 1,132 SC twins)	IVF: ↑ likelihood of all adverse outcomes in twins
Pinborg <i>et al.</i> , ¹⁸ Denmark	1995–2000	Registry, controlled: 13,800 births (3,438 IVF/ICSI twins, 10,362 SC twins, 168 stillbirths)	IVF: ↑ maternal age, preterm births, low birth and NICU admission. No difference in mortality
Pinborg <i>et al.</i> , ¹⁹ Denmark	1995–2000	Registry, controlled: ^a 18,762 children (10,239 SC twins, 3,393 IVF/ICSI twins, 5,130 IVF singletons)	IVF twins: ↑ use of resources IVF vs non-IVF twins: no difference in term, birth weight, NICU admission All twins vs IVF singletons: ↑ likelihood of adverse outcomes
Pinborg <i>et al.</i> , ²⁰ Denmark	1995–2001	Registry, controlled: 9,557 births (642 with vanished co-twin, 5,237 true IVF singletons, 3,678 IVF twins)	Vanished co-twin: predicts SGA; later vanishing predicts lower birth weight
Tiitinen <i>et al.</i> , ²¹ Finland	1997–2001	Retrospective: ^b 1,871 cycles (1,024 dual embryo transfers, 470 elective SET)	SET: ↓ twin rate without ↓ pregnancy or delivery rates
Klemetti <i>et al.</i> , ²² Finland	1996–1999	Registry, controlled: 4,559 IVF children	IVF: ↑ likelihood of all adverse outcomes, corrected somewhat with multiplicity
Poikkeus <i>et al.</i> , ²³ Finland	1997–2003	Registry, controlled: 499 cycles (269 SET, 230 DET)	SET: ↑ risk of all adverse outcomes
Thurin <i>et al.</i> , ²⁴ Scandinavia	2000–2003	Prospective, controlled: 661 patients (330 SET, 331 DET)	SET: if first pregnancy lost, ↓ in multiplicity
Bergh <i>et al.</i> , ²⁵ Sweden	1982–1995	Registry, controlled: 5,856 children	IVF: ↑ risk of all adverse outcomes
Kallen <i>et al.</i> , ²⁶ Sweden	1982–2001	Registry, controlled: 16,280 children	Multiple births: after change from 3 to 2 embryos transferred, ↓ rate of multiple and preterm births
Schieve <i>et al.</i> , ²⁷ US	1996–1997	Registry, controlled: 42,463 children	ART singletons: ↑ incidence low birth weight, partly accounted for by multiplicity
Gerris ²⁸	2005	Review: ^b 4 RCT (N/A)	SET: ↓ rates preterm birth and SGA, while maintaining birth rates
Pinborg ⁵	2005	Meta-analysis: ^b 26 studies of IVF/ICSI twin status and outcome; 5 studies of SET	SET: ↓ obstetric complications, while maintaining pregnancy rates

^aNo spontaneously conceived singleton cohort. ^bNo control group. Abbreviations: ART, assisted reproductive technologies; DET, double embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; N/A, not applicable; NICU, neonatal intensive-care unit; RCT, randomized, controlled trial; SC, spontaneous conceptions; SET, single embryo transfer; SGA, small for gestational age.

IVF or IVF/ICSI (Table 2).^{29–40} Initial studies considering young IVF children found no evidence of developmental delay,^{31,39} but one early follow-up study did.²⁹ As time has passed and larger cohorts of patients have become available for evaluation, the majority of long-term follow-up studies have not consistently demonstrated a higher risk of cause-specific neurological impairment.^{30,35,36,38}

A blinded prospective study from Bonduelle and colleagues³² showed no difference between neurological and developmental assessments in IVF and ICSI children. Multivariate regression analysis indicated no greater likelihood of

developmental delay in the IVF or ICSI populations when compared with spontaneous conceptions. Other studies from this investigational group also showed similar neurological function and developmental rates in the IVF and ICSI populations. The most recent series from this group demonstrated a significant trend towards higher intelligence quotient and verbal performance in ICSI children than in controls.^{34,41} The investigators correctly point out that this result may be due partly to differences in the level of maternal education in the ICSI cohort versus those born from spontaneous conceptions.

Table 2 Neurological development in children conceived by assisted reproductive technologies.

Study	Years studied	Study type ^a and outcomes	Findings
Bowen <i>et al.</i> , ²⁹ Australia	1993–1995	Prospective: 253 children (89 IVF/ICSI, 84 IVF, 80 SC)	ICSI vs IVF and SC: ↓ development
Leslie <i>et al.</i> , ³⁰ Australia	1993–1995	Prospective: 287 children (80 IVF, 97 IVF/ICSI, 110 SC)	No significant differences noted
Bonduelle <i>et al.</i> , ³¹ Belgium	1995–1998	Prospective: 332 children (131 IVF, 201 IVF/ICSI)	IVF or ICSI: no differences from control values for singletons; slight ↓ for multiple gestations
Bonduelle <i>et al.</i> , ³² Belgium	1995–2002	Prospective: 646 children (439 ICSI [388 singletons, 61 twins]; 207 IVF [138 singletons, 69 twins])	IVF or ICSI: no differences from control values for singletons; slight ↓ for multiple gestations
Place and Englert, ³³ Belgium	1998–2000	Prospective: 177 children (66 IVF/ICSI, 52 IVF, 59 SC)	No significant differences noted
Leunens <i>et al.</i> , ³⁴ Belgium	2001–2003	Prospective: 304 children (151 IVF/ICSI, 153 SC)	ICSI ↑ intellectual functioning (possibly due to higher maternal education); no differences in motor function
Ponjaert-Kristoffersen <i>et al.</i> , ³⁵ Belgium, Denmark, Greece, Sweden and UK	2005	Prospective: 1,423 children (511 IVF/ICSI, 424 IVF, 488 SC)	No significant differences noted
Koivurova <i>et al.</i> , ³⁶ Finland	1990–1995	Registry: 857 children (299 IVF vs 558 SC; subanalysis performed with cohorts matched for plurality)	IVF: ↑ general health problems and mortality; no differences in psychomotor development
Strömberg <i>et al.</i> , ³⁷ Sweden	1982–1995	Registry: 23,220 children (5,680 IVF vs 11,360 spontaneous singletons; 2,060 IVF vs 4,120 spontaneous twins)	IVF: ↑ neurological problems (cerebral palsy most common) and need for neurological rehabilitation; greater ↑ in IVF twins
Sutcliffe <i>et al.</i> , ³⁸ UK	1997–1999	Prospective: 429 children (208 IVF/ICSI, 221 SC)	No significant differences noted
Neri <i>et al.</i> , ³⁹ US	2002	Questionnaire: 89 IVF/ICSI	No significant differences noted
Neri <i>et al.</i> , ⁴⁰ US	2006	Questionnaire: 339 children (229 IVF/ICSI, 110 IVF)	No significant differences noted

^aAll studies controlled. Abbreviations: ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; SC, spontaneous conceptions.

A registry study from Sweden, in which 5,680 IVF offspring were assessed, reached a contradictory conclusion.³⁷ Significantly higher likelihoods of developmental delay and neurological impairment requiring rehabilitation were seen among IVF children than with matched controls. Although these risks decreased when singleton pregnancies were considered alone, there still seemed to be greater risks of these conditions among IVF children. This study identified a fourfold rise in risk with IVF of having cerebral palsy; furthermore, a significantly higher likelihood of this disorder was observed in IVF children from multiple gestational pregnancies. Although a similar trend was also observed in IVF singletons, the difference was not significant. A second study confirmed the risk of cerebral palsy.⁴² Risk was also found to be raised in IVF children regardless of single or multiple gestational status. The authors demonstrated from regression analysis that this

risk can be accounted for by consideration of both preterm delivery and multiple gestational status together. These investigators concluded that this result provides another argument supporting transfer of fewer embryos.

Importantly, Pinborg *et al.*'s analysis¹⁸ of data from the Danish Medical Birth Register also showed a higher likelihood of cerebral palsy in IVF/ICSI children regardless of multiplicity. The authors suggested this effect could be explained by vanishing twin syndrome, which they report in a separate study as having occurred in approximately 10% of IVF singleton births.⁵ The association between vanishing twin syndrome and cerebral palsy is well established in spontaneously conceived multiple gestations; there is no reason to believe that this association would not also be present in ART pregnancies. Again, the authors concluded that this risk represents an additional argument for single-embryo transfer.²⁰

Table 3 Association between congenital malformations and conception by assisted reproductive technologies.

Study	Years studied	Study type and sample size	Findings
Hansen <i>et al.</i> , ⁴⁴ Australia	1993–1997	Registry, controlled: 1,138 ART children (301 IVF/ICSI, 837 IVF, 4,000 SC)	IVF and ICSI: ↑ likelihoods of birth defects even after correction
Westergaard <i>et al.</i> , ⁴⁵ Denmark	1994–1997	Registry, controlled: 2,245 ART, 2,245 SC (cohorts matched for maternal age, parity and multiplicity)	No significant differences noted except poorer pregnancy outcomes observed in ART cohort
Loft <i>et al.</i> , ⁴⁶ Denmark	1994–1997	Registry/questionnaire: ^a 665 respondents	No significant differences noted
Zhu <i>et al.</i> , ⁴⁷ Denmark	1997–2003	Registry/questionnaire, controlled: 64,405 children (SC: 50,897 singletons and 1,366 twins from fertile couples, 5,764 singletons and 100 twins from subfertile couples; ART: 4,588 singletons and 1,690 twins)	ART and subfertile spontaneous conceptions: ↑ congenital malformation with increasing delay to conception
Koivurova <i>et al.</i> , ⁴⁸ Finland	1990–1995	Registry, controlled: 304 IVF, 569 SC	IVF: ↑ adverse pregnancy outcomes before correction for multiplicity and cardiac malformations regardless of multiplicity
Ludwig and Kalainic, ⁴⁹ Germany	1998–2002	Registry, controlled: 3,372 IVF/ICSI, 30,940 SC (Mainz Birth Registry)	IVF/ICSI: ↑ risk of all adverse outcomes; RR 1.25 (95% CI 1.11–1.40)
Katalinic <i>et al.</i> , ⁵⁰ Germany	1998–2002	Registry, controlled: 3,372 IVF/ICSI, 8,016 SC	ICSI: ↑ birth defects after correcting for multiplicity; adjusted OR 1.24 (95% CI 1.02–1.50)
Zádori <i>et al.</i> , ⁵¹ Hungary	1995–2002	Registry, controlled: 221 ART, 221 SC (185 singletons and 36 twins in each cohort)	IVF: ↑ prematurity rate in singletons and risks of adverse outcomes in IVF triplets; differences minimal for any other congenital malformation
Anthony <i>et al.</i> , ⁴³ The Netherlands	1995–1996	Registry, controlled: 4,224 ART, 314,605 SC	ART: ↑ cardiovascular malformation; differences minimal for any other congenital malformation
Wennerholm <i>et al.</i> , ⁵² Sweden	1993–1998	Registry, controlled: 1,008 IVF/ICSI vs all SC in Sweden over same time period (no number given)	IVF/ICSI ↑ risk of any defect after correcting for multiplicity; ICSI: ↑ hypospadias
Ericson and Kallen, ⁵³ Sweden	1982–1997	Registry, controlled: 9,111 IVF, 1,690,577 SC	ICSI: ↑ risk of alimentary atresia, neural-tube defects and hypospadias
Sutcliffe <i>et al.</i> , ⁵⁴ UK	1989–1994	Prospective, controlled: 91 ART (cryopreserved embryos), 83 SC	ART: differences not significant for any congenital malformation
Olson <i>et al.</i> , ⁵⁵ USA	1989–2002	Registry, controlled: 1,462 ART, 8,422 SC	IVF: ↑ birth defects after correction for multiplicity
Rimm <i>et al.</i> , ¹³	2004	Meta-analysis, controlled: 2,8524 IVF vs 2,520,988 SC; 7,234 IVF/ICSI vs 978,078 SC	IVF AND IVF/ICSI: ↑ for all birth defects (all studies examined had design flaws); pooled OR 1.29 (95% CI 1.01–1.67)
Lie <i>et al.</i> , ⁵⁶	2005	Meta-analysis, controlled: 5,395 IVF/ICSI, 13,086 IVF	IVF/ICSI: no significant risk of any single congenital malformation
Hansen <i>et al.</i> , ⁵⁷	2005	Meta-analysis, controlled: 28,638 ART	ART: ↑ any birth defect in all 25 studies or on analysis of 7 well-designed studies

^aNo control group. Abbreviations: ART, assisted reproductive technologies; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; OR, odds ratio; RR, relative risk; SC, spontaneous conceptions.

RISK OF CONGENITAL DISORDERS AND HORMONAL ABNORMALITIES

Multiple studies of congenital abnormalities in both IVF and IVF/ICSI children have been done (Table 3.)^{13,43–57} Most of these studies show a higher risk of genitourinary, cardiovascular, musculoskeletal, and gastrointestinal defects in ART offspring, although the methods of some are inconsistent.^{46,48,49,51,54} Differences in the reporting of congenital abnormalities and the statistical methods employed make the comparison of these studies extremely difficult. As

a consequence, the authors often cannot conclude that increased risk of birth defects seen in the IVF cohort is a direct result of ART itself.

Two large studies from Hansen *et al.*^{44,57} considered this exact question. The first was a registry study of 1,138 children born using ART in Western Australia (301 ICSI children, 837 standard IVF children).⁴⁴ The second was a meta-analysis in which 25 studies were considered.⁵⁷ The results of both studies indicated significant trends towards a higher likelihood of birth defects in ART children. Specific trends

towards higher likelihood of cardiovascular, urogenital, musculoskeletal, and chromosomal abnormalities were significant in the IVF cohort but not in the ICSI cohort, with the exception of musculoskeletal and chromosomal abnormalities; this difference in results was attributed to a small sample size. The meta-analysis also demonstrated a higher likelihood of birth defects in IVF and IVF/ICSI children. The authors pointed out that upon independent review, only 7 of the 25 studies they considered were deemed statistically valid and appropriate for inclusion, but they believed their conclusions remained valid.

Belva *et al.*⁴¹ did a prospective, long-term, follow-up study in a cohort of 8-year-old ICSI children and a group of spontaneously conceived children. Although a statistical trend towards an increased likelihood of congenital malformation in the ICSI cohort was demonstrated, there was no higher likelihood of requiring surgery, hospitalization, or rehabilitation in the ICSI group. The authors noted differences in categorization of major and minor congenital abnormalities between their system and the Western Australian Birth Defects Registry system used in the studies of Hansen *et al.*

Work from Zhu *et al.*,⁴⁷ based on data from the Danish National Hospital Register and the Medical Birth Registry, demonstrated that women in whom natural conception takes longer than 12 months have offspring with an equivalent likelihood of congenital abnormalities to women undergoing IVF/ICSI. The only exception to this trend was the incidence of genitourinary tract abnormalities in IVF/ICSI offspring, which remained significantly higher (hazard ratio 2.32, 95% CI 1.24–4.35). Overall, this trend suggests that patients with subfertility have a higher likelihood of having offspring with congenital abnormalities, and that ART procedures, in and of themselves, do not necessarily contribute to this risk.

The difficulties brought about by inconsistent recording of birth defects are elucidated in the work of Kurinczuk and Bower.⁵⁸ These authors considered a study published in 1996 by Bonduelle *et al.*,⁵⁹ in which 423 children were followed prospectively after ICSI. The researchers had concluded that an increased incidence in major congenital malformation was not associated with ICSI. Kurinczuk and Bower point out, correctly, that the control population used in this series comprised spontaneous

conceptions pooled from worldwide registries, including the Western Australian Birth Defects Registry. Differences in the reporting of birth defects in Australia and Belgium (the location of Bonduelle *et al.*'s study) are not accounted for; recategorization of the IVF/ICSI cohort based on Western Australian Birth Defects Registry standards yields a nearly twofold higher risk of major congenital malformation in the IVF/ICSI cohort. These contradictory conclusions, obtained from the same data, illustrate the need for standardized reporting of birth defects.

The fact that reported series have consistently shown a higher incidence of genitourinary tract abnormalities in ART offspring, specifically hypospadias in males, points to the possibility of impaired or abnormal hormonal function in IVF/ICSI offspring. Again, confounding variables exist. Specifically, a notable number of male parents of IVF/ICSI offspring have a history of genitourinary tract abnormalities, such as hypogonadism, poor testis function or both. As such, it is difficult to say that any observed hormonal dysfunction in IVF/ICSI offspring is not due at least partly to genetic abnormalities inherited from the fathers. A Danish series of 125 ICSI-conceived male offspring, for example, had significantly lower testosterone levels than boys who were naturally conceived.⁶⁰

Other research questions include whether hormonal and developmental effects on offspring are due to the methods used to handle and induce *in vitro* maturation of oocytes.⁶¹ Although initial studies show no risk with *in vitro* maturation, further research is obviously warranted.

RISK OF GENETIC DISORDERS

Two specific risks exist with regard to genetic disorders in IVF/ICSI offspring. First, the risk of passing genetic causes of male infertility onto male offspring is well understood. For example, patients with cystic fibrosis transmembrane regulator gene (*CFTR*) mutations and associated congenital bilateral absence of the vas deferens will, by definition, pass this mutation on to their offspring. Screening for *CFTR* mutations is mandatory for female partners of male patients with congenital bilateral absence of the vas deferens. An additional example is male infertility secondary to deletion of the Y chromosomal AZF-a, b, or c regions. Again, by definition, any male offspring resulting from IVF/ICSI using sperm from these patients will also be affected by

these deletions. Second, growing evidence indicates an increased risk of imprinting disorders in IVF/ICSI offspring.^{62–65} These disorders, which include Angelman and Beckwith–Wiedemann syndromes, result from abnormal methylation of relevant maternal alleles. Again, the absolute risk of these disorders is extremely low, and it is almost impossible to conclude whether this trend is due to genetic contributions from IVF/ICSI parents or the ART techniques themselves.

Development of childhood malignancies is in part determined by genetic contributions. Two registry-based studies (Klip *et al.*⁶⁶ in The Netherlands, and Bruinsma *et al.*⁶⁷ in Australia) could not find any evidence of an increased risk of childhood malignancy in ART-conceived offspring. However, a Dutch report described five cases of retinoblastoma diagnosed between 2000 and 2002 in a cohort of IVF/ICSI offspring.⁶⁸ This latter finding represents a significant increase over the natural incidence of the condition in both a control cohort and the general population.

Finally, higher degrees of chromosomal aneuploidy, both autosomal and gonosomal are known to occur in IVF/ICSI offspring than in naturally conceived offspring.^{69,70} Simpson and Lamb⁷¹ have outlined some possible explanations for this difference: *in vitro* versus *in vivo* selective mechanisms for sperm; transmission of pleiotropic genes causing somatic abnormalities in offspring, whereas these genes had caused oligo/azoospermia in the male parent; physical damage occurring during ICSI; the *in vitro* hormonal milieu; or point mutations resulting from physical and chemical stressors during the *in vitro* process. Regardless of the cause, the clinical implications of elevated sperm aneuploidy are as yet incompletely understood. It is incumbent upon physicians and other health care professionals to educate patients about the potential impact of these risks.

BARRIERS TO FURTHER RESEARCH

Almost all ART studies are hampered by barriers to accurate determination of the risks of ART. These main barriers are as follows: inconsistent reporting of congenital abnormalities; the lack of standardized methods for ovarian hyperstimulation and ART procedures; the inability to obtain comparable patient cohorts due to differing characteristics of each couple; and the inability to design and execute prospective, blinded, controlled trials due to

ethical constraints deriving from the limited window of maternal fertility. Debate continues regarding the utility of further retrospective trials that examine these questions. This utility might, at first glance, seem limited given that these trials continue to generate results that alarm patients, despite low absolute risks and inconsistent study methodologies.

At the most fundamental level, the reporting of congenital abnormalities is limited by inaccuracy. Since this most basic question—*are the children in question healthy or not?*—is difficult to answer, further research might not seem warranted. Simpson and Lamb⁷¹ outline the shortcomings of IVF outcomes research; these authors specifically focus upon the baseline control incidence of congenital abnormalities of 1–2% in the overall population. Given that reporting of congenital abnormalities stops when neonates are sent home on day 2 or 3 of life, there may be substantial under-reporting of existing conditions in the spontaneous conception cohort. Hansen and colleagues⁷² observed that the Western Australia Birth Defects Register consistently reports higher incidences of birth defects in the ART population than is reported by ART practitioners. The authors concluded that practitioner reporting of birth defects is too inaccurate to be useful. The question of whether or not these defects can be accurately tallied and recorded is vitally important to ART safety research. Additional questions include the identification of those birth defects that manifest themselves only in adulthood (e.g. congenital malformation of the kidney). If the approximate numbers of defects in both the ART cohort and the spontaneous conception cohort are inaccurately counted in the first place, the conclusions that are reached using those data are difficult to believe.

Buck Louis *et al.*⁷³ offer an excellent summary of the questions and conflicts that arise from considering ART and its effects on child health. They suggest that standardization of clinical and developmental endpoints is necessary for meaningful data to be generated. They conclude that longitudinal studies of the effects of ART are feasible and warranted, and that standardization of study protocols using prospective data collection can at least minimize confounding effects in what are already complicated data. These guidelines provide a meaningful starting point for the discussion of further research goals in the field of ART safety. On the other hand,

they clearly state one of the most basic problems with research into assisted reproductive techniques; namely that the “ideal study design for answering this question [Can we differentiate ART treatment effects from underlying fecundity impairments?] is a randomized clinical trial ... [this] is not possible given that it would require administering ART to fecund couples.”

In summary, research into the safety of ART is vitally important, but it is hampered by unique challenges, some of which might be insurmountable. The importance of this research from a public-health standpoint, especially in an era of ever-increasing use of these techniques, mandates that we continue this research, regardless of its limitations. ART safety research, even with its limitations, can generate meaningful conclusions, illustrated by single-embryo transfer, for which outcomes data on single-embryo transfer translated into meaningful information for practitioners: single-embryo transfer limits the risk of poor perinatal outcomes associated with multiple gestation pregnancies and single-embryo transfer still provides appropriate success in terms of delivery rate. This example alone illustrates clearly that ART safety research can generate clinically essential data.

CONCLUSIONS

Research into the safety of ART continues and remains crucial given the increasing numbers of procedures being performed each year. Existing data illustrate the relationship between ART and multiple gestations, congenital abnormalities (including hypospadias), hormonal abnormalities, and epigenetic effects. Overall, the absolute risk of these conditions is still low but is not insubstantial.

Patients deserve to be properly educated about the risks of ART procedures, especially before they commit themselves to a financially costly and emotionally burdensome process. The obligation to state clearly the health risks to the mother and to any future offspring before ART use falls to health-care professionals, and is imperative. Patients should, however, be reminded that, with the millions of these procedures that are being performed, the absolute risks of any of the conditions described above are still negligible. In order to educate patients properly, research efforts must continue. These efforts must include standardized reporting of birth defects, proper study design, and responsible analysis of the results.

KEY POINTS

- Births resulting from use of assisted reproductive technologies (ART) are increasing in frequency but questions about the safety remain unanswered
- The existing data for ART paint an incomplete picture of the risks to offspring; safety issues are still being studied
- ART follow-up studies typically assess several outcomes—congenital abnormalities, developmental delays or abnormalities, hormonal dysfunction, and epigenetic effects, and the role of multiple gestation on birth outcomes—but accounting completely for the risks related to the latter can be difficult
- Single-embryo transfer mitigates the risk of multiple gestation, thereby substantially decreasing the risk of congenital and developmental abnormalities
- The absolute risk of the above disorders is higher in ART children than in spontaneously conceived children but is still low for the majority of the conditions considered, and correction for maternal and/or paternal factors can make the differences nonsignificant

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Competing interests

The authors declared no competing interests.