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Intracytoplasmic Sperm Injection (ICSI) – What are the risks?

Joseph P Alukal, MD^a and Dolores J. Lamb, PhD^b

*a*Fellow, Division of Male Reproductive Medicine and Surgery, Scott Department of Urology, Baylor College of Medicine

*b*Professor and Vice-Chair for Research, Scott Department of Urology, Department of Molecular and Cell Biology, Baylor College of Medicine

Abstract

In vitro fertilization (IVF) used in combination with intracytoplasmic sperm injection (IVF/ICSI) allows otherwise sterile couples to become parents. Despite the fact that the oldest IVF conceived baby¹ is now over 30 years of age, questions about the safety of assisted reproductive technologies persist. The long term follow-up of the first generation of IVF/ICSI offspring offers a clearer picture of the safety of these technologies; despite these recent studies, however, there is still only an incomplete picture of the risks associated with the usage of these assisted reproductive techniques (ART) to offspring. The risk of multiple gestation continues to be of major concern because of its association with low birth weight, preterm delivery and increased perinatal mortality. Other ART outcomes typically assessed include: 1) congenital abnormality 2) developmental delay or abnormality 3) hormonal dysfunction 4) epigenetic effect. Existing maternal or paternal factors may confound any analysis of ART and spontaneous conception cohorts making it difficult to draw firm conclusions. This review outlines the risks associated with IVF/ICSI as a well defined treatment for couples with severe male factor infertility. Importantly, no discussion of the risks associated with IVF/ICSI can be conducted outside of the context of the existing IVF safety data. As such, both the safety of IVF and IVF/ICSI are considered here. Overall, the total body of data points to the conclusion that ICSI conceived children are at a higher absolute risk of the following conditions: 1) multiple gestation and its associated sequelae, 2) congenital defects (in particular genitourinary defects), and 3) epigenetic syndromes (such as Beckwith Wiedemann). Nevertheless, the absolute incidence of these events remains rare.

Keywords

genetics; in vitro fertilization; intracytoplasmic sperm injection; assisted reproductive technologies; infertility; pregnancy

Introduction

Over the past 30 years, the treatment of infertility has seen the development of revolutionary new assisted reproductive technologies; first in 1978 with the birth of baby Louise Brown, who was conceived by *in vitro* fertilization (IVF),¹ then moving forward with microassisted

Corresponding author for proof and reprints: Dolores J. Lamb, PhD, Scott Department of Urology, Baylor College of Medicine, One Baylor Plaza, N730 Alkek Building, Houston, TX 77030, (713)798-7265, (713)798-1891(fax), dlamb@bcm.tmc.edu.
Coauthor address: Joseph P. Alukal, MD, Scott Department of Urology, Baylor College of Medicine, One Baylor Plaza, N730 Alkek Building, Houston TX 77030, (713)768-1604 (pager), (617)290-3263(cell), alukal@bcm.tmc.edu

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reproduction using techniques such as intracytoplasmic sperm injection (IVF/ICSI),² and finally most recently being refined through the development of pre-implantation genetic diagnosis (PGD) as a technique.³⁻⁶ These highly complex technologies are used with increasing frequency in the treatment of couples around the world; over 1 million babies worldwide have been conceived in this manner.⁷ In fact, in 2003 nearly 3% of children born in Scandinavia and 1.7% of children born in France were conceived using ICSI. During this time, almost 500,000 cycles were performed in Europe (122,872 cycles in the United States), resulting in the birth of almost 300,000 infants.^{8, 9} The number of IVF cycles in the United States has increased 2-fold since 1996 when SART began monitoring the IVF programs.¹⁰

Unlike most therapeutic procedures used in medicine, the assisted reproductive technologies never underwent rigorous safety testing before clinical use. Treatments for infertility overcome natural barriers that prevent fertilization. Because these technologies are used to overcome infertility phenotypes that may have a genetic basis, the possibility exists that unwanted genetic traits may be transmitted to offspring, and while researchers believe that perhaps 75% or more of all infertility has a contributing genetic basis, our ability to diagnose these defects remains limited. For untreated couples, their infertility represents “lethality” within the gene pool, as their condition essentially blocks the transmission of undesirable genetic traits to any offspring. Put simply, large numbers of couples undergo fertility treatments without a complete understanding of the basis of their infertility or the potential long term risks for their offspring.

No better example of this phenomenon exists than that of IVF/ICSI as a treatment for severe male factor infertility. Prior to the development of IVF/ICSI, these men simply could *not* have reproduced by any means. Now, a growing percentage of the population of countries in the Western world is comprised of the offspring of these men. What are the health risks these people face? What burden will they create from a public health standpoint? Questions regarding the safety of ART, and IVF/ICSI in particular, become even more important.

Retrospective data suggests that IVF and IVF/ICSI are safe. Health risks to both mother and offspring that are significantly increased with assisted reproduction include multiple gestation, preterm delivery (even in singleton pregnancy), and congenital abnormalities in the offspring.¹¹⁻¹³ The majority of IVF pregnancies proceed uneventfully with the birth of healthy babies. Nonetheless studies consistently identify an increased absolute risk of problems in IVF and IVF/ICSI pregnancies and deliveries.¹⁴

It is challenging to establish the nature of these risks and dissect out whether they are related to the technology itself or the genetic defects of the parents. Other factors cloud this analysis. For example, in the United States, multiple embryos are routinely transferred to increase the ultimate likelihood of a live birth (while also enhancing the chances of a multiple gestation). Whether the altered endocrine milieu resulting from hormonal induction of ovulation by ovarian hyperstimulation contributes further to this risk is unclear. Hormonal induction profoundly changes the normal uterine environment and body homeostasis. Because the oldest child conceived by IVF/ICSI is only about 15 years old, long term studies of multiple cohorts of IVF/ICSI offspring from conception through to adulthood have not been possible. Incomplete follow up due to couples seeking treatment at large fertility clinics far from their homes is a problem. After a pregnancy is achieved, families are again followed by their community physicians. Because we are a mobile society, couples are frequently lost to follow-up for other reasons as well, making long-term follow-up of ART conceived children extremely difficult.

Unfortunately, epidemiological studies regarding the safety of IVF and IVF/ICSI in general are faced with additional challenges, both in retrospective and prospective studies. These challenges include incomplete reporting and inconsistent definition of both congenital

abnormalities and other adverse outcomes. Importantly, the assessment of IVF/ICSI offspring is commonly performed by pediatricians as part of a routine neonatal health exam, yet a medical geneticist may have different criteria for disease. Alternatively, the physician may examine these children more closely than naturally conceived children and inevitably, the closer one looks - the greater the likelihood of finding an abnormality.

The ideal study design to answer the question of whether or not any ART is safe is nearly impossible to achieve. Fertility cannot be directly compared using standard statistics, as it is the combined fertility potential of the couple that ultimately determines whether a couple is fertile or infertile. The identification of an appropriate control population for infertile couples can present a nearly impossible quest. Selection criteria may bias findings because variability between the two groups exists at baseline. Birth defects, as well as adverse ART events, are relatively rare compared to the overall total number of pregnancies and live births each year. The design and implementation of adequately powered research studies is difficult.

Despite these significant limitations, numerous investigations of IVF/ICSI safety have been performed; they are reviewed in this chapter. Although the assisted reproductive technologies include a variety of methods to process oocytes and/or sperm in vitro to enhance the likelihood of fertilization and pregnancy, this chapter focuses specifically on IVF with intracytoplasmic sperm injection (ICSI) regardless of whether the sperm are retrieved from an ejaculate, the epididymis, or the testis. Importantly, no discussion of the risks associated with IVF/ICSI can be conducted outside of the context of the existing IVF safety data. As such, both the safety of IVF and IVF/ICSI are considered here.

Congenital Disorders and/or Hormonal Abnormalities In Offspring of IVF/ICSI

Multiple large studies (Table 1)¹⁵⁻³⁰ consistently show a higher risk of genitourinary, cardiovascular, musculoskeletal, and gastrointestinal defects in IVF and IVF/ICSI offspring. Other older studies with less methodological consistency also report similar findings.^{15, 18, 19, 26, 31} Comparison of the birth defect studies is confounded by differences in the definitions used to classify the birth defects, in the reporting of congenital abnormalities, and in the methods used for statistical analysis. These caveats present challenges for interpretation of this data. Given these issues, it may be difficult to conclude that the increased risk of birth defects seen in the IVF and IVF/ICSI cohorts results from ART.

In this regard, several studies are noteworthy. Hansen et al.^{16, 30} reported a higher likelihood of congenital abnormalities in IVF and IVF/ICSI children based upon a study of the birth registry of Western Australia which focused on 1,138 children conceived using ART (301 ICSI children, 837 standard IVF children). This report was followed by a meta-analysis of 25 existing studies of birth defects in children conceived by ART³⁰. There was a significant increased incidence of birth defects in ART children including cardiovascular, urogenital, musculoskeletal, and chromosomal abnormalities in the IVF cohort. This trend was not statistically significant in the IVF/ICSI cohort with the exception of musculoskeletal and chromosomal abnormalities, perhaps due to inadequate sample size in the IVF/ICSI cohort. Overall, this meta-analysis demonstrates a higher likelihood of birth defects in IVF and IVF/ICSI children.

A cohort of IVF/ICSI offspring has been followed over time by the Bonduelle group²⁸. This group of 8 year old ICSI-conceived children were compared with a group of naturally conceived children.³² Although the IVF/ICSI children were generally healthy with no higher likelihood of requiring surgery, hospitalization, or rehabilitation, there was an increased likelihood of congenital malformation in the IVF/ICSI cohort. Indeed, about 10% of these children had major birth defects, compared to 3.3% of the control group. Minor birth defects were similar in both the IVF/ICSI children and the control group. Using the Western Australian birth registry

(WABDR) system referenced in the Hansen studies, 6/150 IVF/ICSI children had a major malformation compared to 1/147 of the control children.

Again, there remains some controversy. Analysis of the Danish National Birth Registry suggested no greater likelihood of congenital abnormalities for children born to women with significant delay to natural conception (>12 months) compared with those conceived by IVF/ICSI with one exception.²⁰ The incidence of genitourinary tract abnormalities is statistically significantly increased in children conceived by IVF/ICSI. The findings are consistent with other studies that suggest that patients with subfertility are at higher risk of having a child born with congenital abnormalities. This argues that the ART procedures, in and of themselves, do not contribute to this risk.

Throughout the world, the criteria used to define birth defects differ; this presents unique challenges to analysis of ART safety data. This is best illustrated in a study from Kurinczuk and Bower³³ who analyzed an earlier study published by the Bonduelle group. 423 children were followed prospectively after IVF/ICSI in the Bonduelle study³⁴ with no evidence of increased major congenital malformations. Kurinczuk correctly observed that the control population used in this series included spontaneous conceptions pooled from worldwide registries, including the WABDR. Again, the different definitions of birth defects in Australia and Belgium (the location of the Bonduelle group) were not considered in the study; when Kurinczuk re-categorized the IVF/ICSI cohort based on WABDR standards, about a two-fold higher risk of major congenital malformation in the IVF/ICSI cohort was observed. Clearly, standardization of reporting of birth defects should be mandatory for any study of ART safety as these two analyses reached very different conclusions, albeit with the same data.

An increased incidence of genitourinary tract abnormalities (specifically hypospadias in males) is consistently found in ART offspring. Although impaired or abnormal hormonal function is one possible cause of hypospadias, the etiologies of these defects in some IVF/ICSI offspring remains unknown. Genital tract abnormalities in some male parents of IVF/ICSI offspring (e.g., hypogonadism and/or poor testis function) raise the question of whether the congenital genitourinary defect in IVF/ICSI offspring is the consequence of a genetic abnormality inherited from their fathers. A recent Danish series did observe statistically significantly lower testosterone levels in 125 ICSI conceived male offspring, when compared to testosterone levels in age-matched boys who were naturally conceived.³⁵

Possible detrimental effects of oocyte handling and in vitro maturation (IVM) of immature oocytes were considered as possible contributors to the hormonal and developmental effects found in offspring.³⁶ Studies to date show no risk with IVM; however, this area remains an area of active investigation.

Multiple Gestation, Preterm Labor, and Other Perinatal Complications Are More Common in Pregnancies Resulting from IVF/ICSI

In the United States, the majority of ART conceived pregnancies result in a multiple-birth delivery. In contrast, only 1.5% of naturally conceived pregnancies result in a multiple birth.¹⁰ Multiple gestation is associated with an increased risk of preterm delivery, low birth weight, and increased perinatal mortality. Theoretically, multiple gestation could even account for higher risks of seemingly unrelated conditions in the children conceived by ICSI/IVF, such as the risk of cerebral palsy (to be discussed in detail). As such, it is important to consider the reported differences observed in both IVF and IVF/ICSI cohorts.¹¹

When considering this line of reasoning, it is, at first glance, difficult to account for the fact that several meta-analyses demonstrate an increase in perinatal complications even in singleton

pregnancies resulting from IVF.^{28, 37-39} The IVF/ICSI cohorts in these series, regardless of multiplicity, had higher likelihoods of low birth weight, preterm labor (<36 weeks), and usage of hospital resources (ie, the need for surgery or neonatal intensive care unit (NICU) admission).

The Danish National Patient Registry provided a large body of IVF/ICSI data; in this population, a higher likelihood of adverse outcomes was observed in multiple gestational pregnancies. Importantly, no such trend existed in IVF/ICSI singleton pregnancies.^{11, 40} These authors addressed the inconsistencies between their own findings and the above meta-analyses and hypothesized that vanishing twin syndrome in these pregnancies plays a causal role in the poorer outcome observed in IVF/ICSI singleton pregnancies.⁴¹ This risk relates to the number of embryos transferred. Importantly, a multi-center prospective randomized controlled trial compared outcomes after single embryo transfer (SET) with transfer of multiple embryos. Pregnancy rates were not substantially lower in the SET cohort while the number of multiple births was dramatically reduced.⁴²

SET represents an important intervention in that it allows for an adequate success rate to be maintained with IVF and IVF/ICSI, while it significantly decreases the risks in offspring that derive from multiple gestation.⁴² Remarkably, SET is not routinely performed as standard of care in the United States, and it is still only offered to a minority of women in Europe.⁸ The idea that routine performance of SET can mitigate risks such as preterm labor in IVF/ICSI offspring might seem obvious to most readers, but additional benefits such as a decreased rate of CP in IVF/ICSI offspring would help to minimize the risk of conditions such as neurological and developmental delay in ICSI offspring cohorts. Prospective research with the goal of confirming this hypothesis is vitally important.

Developmental Delay and Neurological Impairment Concerns for Children Conceived by ICSI/IVF May Be Unfounded

Studies suggest that developmental delay and impaired neurological status occur in children conceived using IVF and IVF/ICSI, although these findings are controversial. Two prospective analyses of small numbers of IVF/ICSI children at early age (1-2 years or higher) did not identify a higher likelihood of developmental delay.⁴³⁻⁴⁸

Similarly, Bonduelle and colleagues (2003) found no difference in the likelihood of developmental delay in IVF and IVF/ICSI children compared with natural conceptions.⁴⁹ A statistically significant trend towards higher IQ and verbal performance in IVF/ICSI children when compared to controls was observed.^{32, 50} This may reflect differences in the level of maternal education in the IVF/ICSI cohort versus the spontaneous conceptions cohort.

Again, there is disagreement in the literature. A retrospective study of 5,680 Swedish IVF offspring found statistically higher likelihoods of developmental delay and neurological impairment requiring rehabilitation.⁵¹ Singleton pregnancies were associated with a lower risk. However, children conceived by IVF were at greater risk of these conditions even after considering the risks of multiple gestation. Cerebral palsy (CP) was the major neurological impairment reported in the Swedish IVF children; the IVF children from multiple gestation pregnancies displayed a statistically significantly increased likelihood of CP, whereas a similar but not statistically significant trend was observed in IVF singletons. A higher risk of cerebral palsy was also found in a second more recent study of IVF children regardless of single versus multiple gestational status.⁵² Regression analysis suggests that the complications associated with preterm versus term delivery and multiple gestational status in combination contribute to this risk. These observations again give credence to the ongoing efforts of some groups to mandate the transfer of fewer embryos.⁴²

A retrospective analysis of the Danish National Birth Registry reveals that ICSI/IVF children have a higher likelihood of cerebral palsy regardless of multiplicity.⁴⁰ The vanishing twin syndrome, which occurs in about 10% of IVF singleton births¹¹ and in naturally conceived multiple gestations is associated with cerebral palsy; there is no reason to believe that this association would not be present in ART pregnancies. It is argued that this observation again supports the practice of SET.⁴¹

The Risk of Genetic Disorders Is Increased In Children Conceived By ICSI/IVF

Many patients with idiopathic male infertility are thought to have a contributing, but as yet unidentifiable, genetic etiology. As such, there exists a risk of transmission of these causes to any offspring. The model offered by the transmission of well understood genetic causes of male infertility illustrates this risk. For example, congenital bilateral absence of the vas deferens (CBAVD) is a genital form of cystic fibrosis resulting from a loss of function mutation in the cystic fibrosis transmembrane regulator (CFTR) gene. Men with CBAVD will transmit their mutation to their offspring. Because detection of the mutations causing CBAVD is difficult with routine genetic testing due to the large size of the gene, CFTR mutation screening of the most common disease associated variants is mandatory for female partners of men with CBAVD. Likewise, Y chromosome microdeletions are transmitted to male offspring by ICSI. Because microdeletion of Y chromosomal AZF-a, b, or c regions is associated with male infertility, any male offspring resulting from IVF/ICSI using sperm from these patients will also be affected by these deletions and are expected to be infertile like their fathers.

There is an increased risk of imprinting disorders in IVF/ICSI offspring.⁵³⁻⁵⁶ Imprinting influences gene expression and transmission of phenotypic syndromes or traits in a non-Mendelian manner. These imprinting syndromes, such as Angelman and Beckwith-Wiedemann syndromes, are very rare disorders; they occur following abnormal gene methylation of relevant genes. The methylation pattern of these genes, defined during parental gametogenesis, regulates gene expression in an epigenetic manner in the offspring. Although the absolute risk of these disorders is extremely low, researchers agree that there is an increased incidence of imprinting disorders in children conceived by IVF/ICSI.⁵³⁻⁵⁶ Both defective imprinting of the gamete DNA, as well as the ART procedures themselves, have been implicated as potential causes of these defects.

Childhood malignancies can result from gene defects; some of these gene defects are due to failures of DNA mismatch repair. Maduro, et al., have reported evidence of DNA mismatch repair defects in men with non-obstructive azoospermia⁵⁷, and both mouse models and available human studies have found that defective DNA repair is associated with male infertility. Do ART conceived offspring have a higher incidence of malignancy due to DNA repair defects? Two large retrospective studies did not report evidence of an increased risk of childhood malignancy in IVF and IVF/ICSI conceived offspring.^{58, 59} In one report, a significant increase in the incidence of retinoblastoma in IVF/ICSI conceived children compared to the general population and a control cohort was described; there were five cases of retinoblastoma found in the cohort of IVF/ICSI offspring.⁶⁰ Although the possibility that DNA mismatch repair defects in IVF and IVF/ICSI offspring may put them at higher risk of childhood malignancy, further research must be performed before this relationship can be clearly defined.

Finally, IVF/ICSI offspring are at higher risk of autosomal and gonosomal aneuploidy.^{61, 62} Infertile men have a 10-fold higher incidence of sperm aneuploidy when compared with fertile men. These aneuploidies likely result from meiotic errors during synapsis and homologous recombination in the testis. Regardless of the cause, the clinical implications of

abnormal sperm aneuploidy are incompletely understood and well-designed outcomes studies are required. Simpson and Lamb list possible additional explanations for chromosome aneuploidy in ICSI/IVF children; these include 1) *in vitro* versus *in vivo* selective mechanisms for sperm, 2) transmission of pleiotropic genes causing somatic abnormalities in offspring, whereas these genes had caused oligo/azoospermia in the male parent, 3) physical damage occurring during ICSI, 4) the *in vitro* hormonal milieu, 5) or point mutations resulting from physical and chemical stressors during the *in vitro* process.⁶³

Barriers to Further Research On The Safety and Efficacy of IVF/ICSI

A consistent theme throughout this review is the difficulty faced by investigators in accurately determining the risks of any ART, including ICSI. These challenges include the lack of standardized reporting of congenital abnormalities throughout the world, the lack of standardized methodologies for ovarian hyperstimulation and ART procedures, the lack of comparable study patient cohorts due to the unique and combined characteristics of each couple, and the inability to design and execute prospective, blinded, and well-controlled trials due to obvious ethical constraints. The utility of retrospective trials that focus on questions of ART safety remains controversial. These retrospective trials continue to generate outcomes that alarm patients, despite low absolute risks and poor study methodology.

As described above, reporting of congenital abnormalities is not consistent and sometimes inaccurate. It is difficult to determine whether these children are healthy or not. Simpson and Lamb outline the shortcomings of IVF outcomes research and focus in part upon the baseline control incidence of congenital abnormalities in the overall population of 1% to 2%. These numbers are derived from the report of “congenital abnormalities” identified in the neonatal nursery and stop when the child leaves the hospital. Thus, significant underreporting of existing conditions in the spontaneous conception cohort may occur.⁶³ Using the WABDR criteria consistently estimates a higher incidence of birth defects in the ART population than is self-reported by ART practitioners, leading Hansen to conclude that practitioner reporting of birth defects is inaccurate.⁶⁴ The question of whether birth defects can be accurately defined and recorded is of utmost importance to ART safety research. In addition, other defects may become evident with aging, such as those that manifest themselves only in adulthood (i.e., congenital malformation of the kidney). Because of the challenges in accurately reporting defects in both the ART cohort and the spontaneous conception cohort, the conclusions that are reached using these data become difficult to interpret.

Buck Louis et al.⁶⁵ suggest that standardization of clinical and developmental endpoints is necessary for meaningful data to be generated. Prospective study design with standardization of study protocols would reduce the confounding effects, although the data remain highly complex. Indeed, one of the most basic problems with ART research is that the ideal study design for answering whether we can differentiate ART treatment effects from underlying fecundity impairments requires a randomized clinical trial. In everyday clinical practice this approach is impossible because it would require administering ART to fertile couples.

In conclusion, despite these unique and perhaps insurmountable challenges, research on the safety of IVF and IVF/ICSI remains a high priority from a public health perspective. The ever-increasing use of these techniques mandates that we continue research on ART safety, regardless of its limitations. ART research can generate meaningful conclusions; the best example of this is the trend towards SET. Outcomes research showed that SET limits multiple gestation pregnancies with the associated advantage of lowering the risk of poor perinatal outcomes while maintaining an acceptable pregnancy rate. This single and very powerful example proves that ART safety research can generate clinically essential data.

Conclusions

Research on the safety of IVF/ICSI is crucial, especially given the increasing popularity of this treatment and the vast numbers of IVF/ICSI procedures performed each year throughout the world. IVF and IVF/ICSI, in general, are associated with multiple gestation and an increased risk of congenital abnormalities (including hypospadias). IVF/ICSI in particular, carries an increased risk of endocrine abnormalities, as well as epigenetic imprinting effects. Conversely, SET appears to diminish at least some of the above risks. Regardless, the absolute risk of any of these conditions remains low.

Patients are only able to make informed decisions about these procedures after proper education about these risks. Infertile couples should receive this information before they embark upon the financially costly and emotionally burdensome process of ART. The healthcare profession has a responsibility to counsel the couple about the health risks to both the mother and to any future offspring. Patients should be reminded, again, that while the absolute risks of any of the conditions described above are still negligible, there can be no guarantees of a perfectly healthy baby; no such guarantee exists, of course, for fertile couples either. Further research is required, not only to define IVF/ICSI outcomes, but also to better understand the molecular basis of the infertility phenotypes necessitating these treatments. Efforts must also include standardized reporting of birth defects, proper study design, and responsible analysis of the results. Complete information on defective reproductive processes as well as outcomes based research data will allow physicians to determine the real risk of IVF/ICSI.

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Table 1

Congenital Malformation in ART Children

Author	Country	Year	Study Type	Control	Outcomes	Sample Size	Findings
Hansen32	Australia	1993-97	registry	yes	congenital malformations	1138 ART children (301 IVF/ICSI, 837 IVF alone, v. 4000 SC children)	Significantly higher likelihoods of birth defects with IVF and ICSI even after correction (OR 2.0 for both)
Westergaard33	Denmark	1994-97	registry	yes	Congenital malformations, Pregnancy outcomes	2245 ART children children v. 2245 naturally-conceived children (cohorts matched for maternal age, parity, and multiplicity)	No difference in risk of Congenital malformation (poor pregnancy outcomes observed in ART cohort, however)
Lof34	Denmark	1994-97	registry/questionnaire	no	Congenital malformations, genetic abnormality, and pregnancy outcome in ICSI conceptions	665 questionnaires returned	No statistically significant differences identified, >90% responder rate to questionnaire
Zhu35	Denmark	1997-2003	registry/questionnaire	yes	time to pregnancy, treatments for infertility, congenital malformations	64405 children(50897 singletons and 1366 twins from fertile couples, 5764 singletons and 100 twins naturally conceived by subfertile couples, 4588 singletons and 1690 twins by ART.)	A higher likelihood of congenital malformation in both ART group and subfertile group with delay to spontaneous conception (>12 months); as delay increased so did likelihood of malformation
Koivurova 36	Finland	1990-95	registry	yes	Congenital malformations and pregnancy outcomes	304 IVF children v. 569 SC children	All adverse pregnancy outcomes were significantly higher in the IVF group but corrected largely after consideration of multiplicity. Cardiac malformations higher in IVF cohort regardless of multiplicity
Ludwig37	Germany	1998-2002	registry	yes	Congenital malformations	3372 IVF/ICSI children v. 30940 spontaneous conceptions	RR 1.25 (95% CI 1.11-1.40); used separate population (Mann Birth Registry) for control cohort
Katalinic38	Germany	1998-2002	registry	yes	Congenital malformations and pregnancy outcomes	3372 IVF/ICSI children v. 8016 naturally-conceived children	Adjusted OR 1.24 (95% CI 1.02-1.50); higher incidence of birth defects in ICSI cohort after correcting for multiplicity, true control cohort
Zádori39	Hungary	1995-2002	registry	Yes	Pregnancy outcomes, neonatal complications (including birth defects)	221 ART pregnancies (185 singleton and 36 twin v. identical SC cohort)	A minimal difference between cohorts, except for a significantly higher prematurity rate in IVF singletons; IVF triplets had much higher risks of adverse outcomes in a separate analysis
Anthony40	The Netherlands	1995-96	registry	yes	Congenital malformations	4224 ART children v 314605 SC children	Risk of any congenital malformation only slightly higher, corrects

Author	Country	Year	Study Type	Control	Outcomes	Sample Size	Findings
Wennerho Im41	Sweden	1993-98	registry	yes	Congenital malformations	1008 IVF/ICSI children v. all SC in Sweden over comparable time period (no number given)	after accounting for confounders[Crude OR 1.20 (95% CI 1.01–1.43), corrected OR 1.03 (95% CI 0.86–1.23)]; cardiovascular malformation higher in ART cohort regardless
Ericson42	Sweden	1982-1997	registry	yes	Congenital malformations	9111 IVF children v. 1690577 SC children	Adjusted OR (after correcting for multiplicity) 1.19 (95% CI 0.79–1.81); only hypospadias higher in ICSI cohort (RR 3.0)
Sutcliffe43	UK	1989-1994	prospective	yes	Congenital malformation and neurological development in cryopreserved embryos	91 ART children (cryopreserved embryos) v. 83 SC children	Unadjusted OR 1.47, adjusted 0.89; higher risk of alimentary atresia, neural tube defects, and hypospadias in ICSI cohort even after correction
Olson44	US	1989-2002	registry	yes	Congenital malformation and mortality	1462 ART children v. 8422 SC controls	Incidence were higher in the ART cohort, although no numbers reached statistical significance
Rimm16	NA	2004	meta-analysis	yes	compiled 16 IVF studies, 7 ICSI studies	28524 IVF v. 2520988 SC children; 7234 IVF/ICSI children v. 978078 SC children	Adjusted OR 1.30 (95% CI 1.00–1.67), higher incidence of birth defects in IVF cohort even after correcting for multiplicity
Lie45	NA	2005	meta-analysis	yes	compiled 4 prospective well designed studies (out of 22)	5395 IVF/ICSI children v. 13086 IVF children (pooled from all four studies)	Pooled OR of 1.29 (95% CI 1.01–1.67) (statistically significant); all studies examined had design flaws
Hansen46	NA	2005	meta-analysis	yes	compiled 25 studies (only 7 well-designed per author's criteria)	28638 ART children	Pooled RR 1.12 (95% CI 0.97–1.28), no significant increase for any single category of defect with ICSI
							On both analyses (all 25 studies or limited just to the 7 appropriate studies) pooled OR of malformation was significantly higher; 1.40 (95% CI 1.28–1.53) on analysis of 7 well-designed studies

in vitro fertilization, IVF; ICSI, intracytoplasmic sperm injection, ICSI; confidence interval, CI; odds ratio, OR; relative risk, RR